043
RAM
12734

STABLE ISOTOPE SYSTEMATICS IN PLANT CELLULOSE: IMPLICATIONS TO PAST CLIMATE

by

R. RAMESH

A THESIS
SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

TO THE
GUJARAT UNIVERSITY

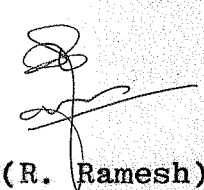
DECEMBER 1984

043
B12734

PHYSICAL RESEARCH LABORATORY
AHMEDABAD 380 009
INDIA

CERTIFICATE

I hereby declare that the work presented in this thesis is original and has not formed the basis for the award of any degree or diploma by any University or Institution.



(R. Ramesh)
Author

Certified by:



Professor K. Gopalan
Thesis Supervisor

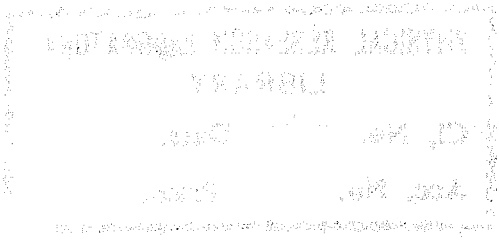
December, 1984

TO

MY PARENTS

AND

MY DARLING YOUNGER BROTHER



CONTENTS

			Page No.
Statement			I
Acknowledgements			(I)
CHAPTER I		INTRODUCTION	1
	1.1	Introduction	1
	1.2	Theoretical basis	4
	1.3	Statement of the problem	8
	1.4	Field collection of samples	11
	1.5	Climatic setting and Forests of Kashmir Valley	15
	1.5.1	Climatic setting	15
	1.5.2	Forests	16
CHAPTER 2		CRITICAL REVIEW OF EARLIER WORKS	24
	2.1	Introduction	24
	2.2	Development of Experimental Techniques	27

2.2.1	Cellulose Extraction	27
2.2.2	Removal/Replacement of Exchangeable Hydrogens in Cellulose	29
2.2.3	Preparation of hydrogen for δD analysis	32
2.2.4	Preparation of CO_2 from cellulose for $\delta^{18}O$ analysis	34
2.2.5	Preparation of CO_2 for $\delta^{13}C$ Analysis	36
2.3	Hydrogen Isotopes in Tree Rings	37
2.3.1	Rationale behind the approach	37
2.3.2	Earlier results on field and laboratory measurements	39
	Plant water relationship	39
	Tree rings and Climate	43
	Biochemical fractionation factor	48
	Results on components other than cellulose	51

2.4	Oxygen Isotopes in Tree Rings	51
2.4.1	Rationale behind the approach	51
2.4.2	Earlier results of laboratory and field measurements	53
	Plant water relationship	53
	Tree rings and climate	56
	Biochemical fractionation factor	58
	Results on components other than cellulose	61
2.5	Carbon Isotopes in Tree Rings	61
2.5.1	Rationale behind the approach	61
2.5.2	Earlier results of field and laboratory measurements	62
	Intra ring variations	66
	Juvenile effect	66
	Pollution effect	67
	Canopy effect	67

CHAPTER 4 RESULTS AND DISCUSSION 100

4.1 Results 100

4.2 Discussion 101

4.2.1 Intraring isotopic variations 102

4.2.2 Coherence of the isotopic record among trees from a site 109

4.2.3 Inter-correlations among ring width and isotopic records 118

4.2.3.1 Correlation between ring width and δ values 119

4.2.3.2 Correlation between δD and $\delta^{18}O$ values 124

4.2.4 Correlation between climatic parameters and isotopic records 129

4.2.4.1 δD record in tree rings and climatic parameters 131

4.2.4.2 $\delta^{13}C$ record in tree rings and climatic parameters 134

STATEMENT

In the past decade, there has been a growing interest to assess the δD, $\delta^{13}C$ and $\delta^{18}O$ records in tree rings as potential paleo-environmental indicators. It is known that δD and $\delta^{18}O$ of precipitation are related to the temperature at the site of precipitation. A tree nurtured by this water might record the temperature-induced δD and $\delta^{18}O$ variations of precipitation in its wood. The tree's isotopic record might thus lead to a paleotemperature reconstruction, which could serve as a useful constraint on climatic models. Similarly the $\delta^{13}C$ record in trees could give valuable clues about past changes in $\delta^{13}C$ and concentrations of atmospheric CO_2 and/or climate.

The first chapter of the thesis gives a brief introduction to the principles involved in the field of isotope dendroclimatology and a description of our efforts in India in this direction. The Himalayan region, with its extensive temperate forests has so far remained unexplored for its dendroclimatic potential. In collaboration with Dr. M.K. Hughes, Liverpool Polytechnic, U.K., a lot of discs and cores from fir, deodar, spruce and pine trees from this region have been collected. Based on this collection, ring width chrolonogies are now available for 5 sites in the Kashmir region. This chapter also describes the climatic setting of the sample locations.

The second chapter summarises the earlier work in this field. Most studies to date deal with spatial correlations rather than year to year variations at a single site. The effect of circumferential variations, early wood-late wood variations, coherence of the isotopic signal from several trees in a site have not received due attention yet. Simultaneous measurement of $\delta^{13}C$, δD and $\delta^{18}O$ in a given tree, which might lead to better resolution of competing effects has not been done; while seeking a relationship with climatic parameters, mainly temperature and rainfall have been individually considered, thus overlooking possible effects of other parameters. In some cases, the weather station has been as far as 500 km from the tree site. It is obvious that for a better comparison, this distance should be minimal.

This work aims to assess the fidelity with which trees record climatic changes on annual scale and to study the effects of intra ring variations and coherence of the signal from several trees in a given site. Since many of the fractionation processes in the biosynthetic pathways are poorly understood, it has not been possible to estimate the various fractionation factors theoretically. So an empirical approach of relating the isotopic variations with available meteorological data has been adopted.

The third chapter of the thesis describes the experimental methodology employed in this work. The major

part of the experimental work was to construct sample extraction systems, test and calibrate them. The procedure involved isolation of cellulose from wood powder from each annual ring, its subsequent nitration (to remove the exchangeable hydroxyl hydrogens) and combustion to obtain H_2O and CO_2 for measurement of isotope ratios. The water is quantitatively reduced to H_2 with hot uranium ($\sim 800^{\circ}C$) and collected by means of a uranium pump. For $\delta^{18}O$ measurement of cellulose the latter is combusted with $HgCl_2$ and the resulting CO converted to CO_2 by high voltage electrical discharge. The HCl produced is removed by treatment with benzoquinoline. The yields of the various gases were close to 100%. The blanks were less than 1 μMole compared with typical gas contents of 500 μMoles. The nitrogen content of cellulose nitrate was consistently greater than 13.2% - the accepted standard value. The isotope ratios were measured using VG micromass 602D Mass Spectrometers with an overall precision of $\pm$ 2 °/oo for δD, $\pm$ 0.2 °/oo for $\delta^{18}O$ and $\pm$ 0.1 °/oo for $\delta^{13}C$.

The results are presented in the fourth chapter and their implications discussed. δD and $\delta^{13}C$ were measured in annual rings along 3 radii of a silver fir disc for a period of 30 years (1903-32). $\delta^{18}O$ was measured along 2 radii. The tree had a lobate growth and analysis was performed on the compressed side and the elongated side. Though the isotope values are not identical along the three radii, the pattern

of variation is similar. This indicates that in the case of silver fir, the circumferential variation does not affect seriously the interpretation based on measurements along a single radius for the purpose of trend analysis. Also, δD and $\delta^{13}C$ values of another fir tree from the same site for the same period were found to follow the same pattern. δD and $\delta^{13}C$ values of 4 trees (two firs, a deodar and a pine) from a single site for a period of 10 years (1923-32) were also compared. This time, to avoid problems of intraring variation, only the early wood part was sampled. It is seen that there is 79% common signal in δD and 84% common signal in $\delta^{13}C$ among these trees. While there seems to be a coherence in the signal even among different species, it seems that the tree physiology plays a non-trivial part in determining the δ values of the tree, which could account for some noise in the signal.

Significant anti-correlations between δD and ring width have been found, indicating that the climatic parameters might have opposing effects on them. This is true for $\delta^{13}C$ as well. δD and $\delta^{18}O$ are related with a slope of 7.8 ± 0.8. This is consistent with the value calculated on the basis of recent literature data on δD, $\delta^{18}O$ of plant cellulose and environmental water.

The $\delta^{13}C$ results are found to be consistent with Francey and Farquhar's (1982) model for carbon isotopic fractionation in C$_3$ plants.

Multiple linear regression of the isotopic records (δD, $\delta^{13}C$ and $\delta^{18}O$) with mean growing season temperature, maximum temperature, humidity, amount of precipitation, wind velocity and cloud amount yielded significant multiple correlations ($\geq .8$) in the case of δD and $\delta^{13}C$.

Individually, δD is found to be significantly influenced by temperature, humidity and rainfall; $\delta^{13}C$ is influenced by precipitation, humidity, wind velocity and cloud amount; $\delta^{18}O$ is sensitive only to precipitation, humidity and cloud amount.

The "amount effect", which perhaps exists for precipitation in Kashmir, has been detected in tree ring cellulose. The sign of the various correlation coefficients are as expected from empirical considerations. Since an accurate evaluation of the dependence of δ values on climatic parameters needs more extensive measurements, the above results are to be considered as first approximation in indicating the exact relative influences of the difference climatic parameters on the isotopic records in tree ring cellulose.

In the fifth and final chapter, the results are summarised and recommendations for future work are made. While the results point to definite effect of climate on isotopic variations in trees, extra climatic parameters also seem to play a role in affecting such a relationship. Therefore, it

is necessary to select trees from climatically stressed sites to optimise the climatic signal. Teak trees on the west coast of India offer such a possibility.

ACKNOWLEDGEMENTS

The past six years of my life at the Physical Research Laboratory have been very rewarding for my growth both as a scientist and as a person. A great many people have helped me in various ways throughout the course of this work. Though only a few are mentioned below by name, I take this opportunity to thank all my friends and well wishers at PRL and elsewhere, who have always been excessively kind to me, more than what I deserve.

It was a great pleasure working with Prof. K. Gopalan and Dr. S.K. Bhattacharya, my thesis advisors, I am indebted to them for their constant help and encouragement. Dr. S. Krishnaswamy's untiring efforts to obtain assistance from the Indian Forest Department for the collection of samples for this work are gratefully acknowledged. I am beholden to Dr. B.L.K. Somayajulu, without whose efforts it would not have been possible for me to visit and know about laboratories in the other parts of the world. I can never forget Prof. D. Lal's kindness, who was a constant source of inspiration. Thanks are also due to Prof. P.R. Pisharoty for helping me to obtain the meteorological data from the Indian Meteorology Department.

Dr. R.V. Krishnamurthy deserves special thanks. His company has not only contributed to my science, but also

my physical and mental well being. He was the one whom I could readily go to at hours of crises and I felt his absence very much during the course of writing this thesis.

Dr. M.K. Hughes taught me a great deal about trees and his kind assistance is gratefully acknowledged. My association with Dr. G.B. Pant has been very rewarding. I am greatly benefitted by working with Dr. Jim White, who instilled a great deal of confidence in me. I am grateful to Prof. Austin Long for many useful discussions and his hospitality during my visit to Tucson. I also thank Dr. A.T. Wilson and Dr. S. Leavitt from whom I could learn much.

Prof. M.J. DeNiro deserves special thanks for his advice, encouragement and guidance to make some modifications in the sample extraction line for measurement of nitrogen content. He also taught me the procedure for measurement of $\delta^{18}O$ in organic samples, for which I remain grateful.

I am indebted to Dr. R.K. Pant who was always willing to help despite his health.

I thank Prof. D.P. Agrawal for accommodating me in the guest house at Kashmir during my field trips.

I owe a special word of thanks to Dr. S. Kusumgar, who always kept me cheerful.

Profs. M.N. Rao and N. Bhandari reviewed this work from time to time and offered suggestions for improvements, for which I remain thankful to them.

I can never adequately thank Mr. M.P.K. Kurup and Mr. K.K. Sivasankaran for their excellent glass blowing support, without which this work would have been almost impossible.

I record my gratitude to other senior colleagues, Drs. T.R. Venkatesan, J.N. Goswami, P.N. Shukla, V.K.B. Kota, C.A. Sastry, A.R. Prasanna, N. Venkatramani, Rajesh Pandey, U.C. Joshi and to Drs. M.M. Sarin, C.M. Nautiyal, N. Hussain, N.M. Ashok, A.N. Sekar and R. Jha.

I also thank my friends Messrs. A. Jayaraman, Surendra Pal, Navin Juyal, M. Bhaskaran, A. Narayan, D. Sengupta, V. Ranganathan, P.M. Sanghvai, A. Sarkar, B.P. Pande, B.S. Kotlia and K.C. Sahu.

I acknowledge with thanks the help rendered from time to time by Messrs. J.R. Trivedi, D.K. Kankane, R.A. Jani, J.T. Padia, R. Rangarajan and Mrs. R. Jadeja.

I thank Mr. Ranpura for his excellent job of photography and the staff of Library and Computer Centre. Thanks are also due to Messrs. A.R.S. Pandian, H.S. Mazumdar and P.G. Oza for electronics support.

I am grateful to Mr. S.T. Qadri and Mr. M.S. Khan of Jammu & Kashmir Forest Department for their assistance in collecting the samples.

Finally, I thank my friend V.T. Viswanathan for giving this thesis its looks.

CHAPTER I

INTRODUCTION

1.1 Introduction

There has recently been a growing concern to understand the factors governing the climate on earth and its long and short term variations. A first step towards this goal is to study the behaviour of earth's climate in the past. Of particular importance are the short term variations in the immediate past viz., annual and seasonal changes in the past few centuries. A detailed knowledge of these short term climatic variations can serve as useful constraints on models aimed at predicting climate in the near future. Apart from this, such information can be used to gain insight into the causal mechanisms bringing about changes in climate.

Instrumental weather records provide this data to some extent. Unfortunately, such records are not only limited to a few regions on earth, but date back, at best, only to a century or so. This has resulted in a search for natural systems serving as proxy indicators of past climate. Of the various approaches that have been successfully employed to extract this information, the most important is the stable isotope systematics in time sequential natural systems.

The stable isotopes of a light element like hydrogen, carbon or oxygen, get partitioned between coexisting substances in any physico-chemical process. This fractionation, which is due to the finite differences between the masses of isotopes, has been shown to be temperature dependent in simple inorganic systems (Urey, 1947). With the development of high precision mass spectrometers capable of measuring such small, temperature induced variations (McKinny, et al, 1950), an experimental study of the systems recording past climates became possible (Epstein, et al, 1953). Since then, there has been a great deal of palaeoclimatic work based on stable isotope systematics. Well known examples are the study of $^{18}O/^{16}O$ ratios of $CaCO_3$ tests of marine invertebrates (Emiliani, 1955), of glaciers and polar ice-sheets (Dansgaard, et al, 1971) sedimentary carbonates and speleotherms (Hendy, 1971).

The most attractive of the various natural systems explored so far from the palaeoclimatic point of view are the annual growth rings of trees. This is because of the following reasons:

1. Tree rings form every year. Any growth ring can be reliably assigned an absolute year - the year of its formation. Instances are not rare where even specific months of growth have been identified within a given tree ring. Thus they are potentially capable of providing a climatic record of high resolution viz., a year or less.

2. Trees have been known to live for several thousands of years. For example, wood from Pinus aristata (Bristle cone pine) trees can be obtained which goes back beyond 9000 yr. BP. (Wilson and Grinsted 1975). Impressive lengths of tree ring chronologies running several throusand years back in time are available for some regions like the south western USA (Fritts, 1976) and Europe. By using methods of cross matching, one can develop such long sequences of tree rings for places where long living trees are not available. Wood from old buildings, buried logs etc. can be used for this purpose. In short, tree ring chronologies running continuously for a few thousand years back in time are not difficult to obtain. This is because trees grow over a wide variety of geographical locations and

can easily be sampled by taking a couple of cores using increment borers. An additional advantage is that the different species of trees can be sampled and the results cross-checked. The spatial coverage of trees on earth enables us to infer variations in a given climatic zone.

The variations in ring widths and ring densities of trees have extensively been used in palaeoclimatic studies (Fritts, 1976; Hughes et al, 1982). However, the use of stable isotopes in tree rings as possible indicators of past climate is a relatively recent development (Yapp and Epstein, 1982a, 1982b; Burk and Stuiver, 1981). This thesis is an attempt to follow up the preliminary work in this field, especially in the context of a monsoon dependent country like India.

1.2. Theoretical Basis

The theoretical basis for expecting a relationship between climatic parameters and stable isotope ratios in tree rings is discussed below.

A tree manufactures glucose from the water and carbon-di-oxide available to it using solar energy. Subsequent to this photosynthesis, a number of other compounds are synthesised and some are deposited as wood in the trunk of the tree.

The main constituents of wood are cellulose (~50%), lignin (~30%), hemicellulose (~15%) and gums and resins (~ 5%). Cellulose which makes up the cell walls of wood, is a long chain polymer of β-(1→ 4) linked D glucopyranose units:

$$C_6H_{11}O_5 - \left[C_6H_{10}O_5 \right]_n - C_6H_{11}O_6$$

where n can be as high as 10,000. Lignin is an amorphous polymeric material, which forms, together with cellulose and other carbohydrates of the cell wall, a tissue of high strength and durability. Hemicelluloses are mainly non-cellulosic carbohydrates like xylan, araban, galactan etc. (Browning, 1975).

It is conceivable that the D/H and $^{18}O/^{16}O$ ratios in any of these compounds reflect those of the water taken up by the tree for their synthesis, with possible modifications because of the ambient climate. To elaborate, it is well known that the D/H and $^{18}O/^{16}O$ ratios in precipitation depend on the temperature at the site of precipitation (Dansgaard, 1964). A tree samples this precipitation through the soil moisture. Depending on the site, the ground water may or may not contribute to this soil moisture. Subsequently in the leaf, the site of photosynthesis, evapotranspiration takes place leading to an enrichment of the isotopic ratio of the water taken in by the tree. The extent of this

enrichment depends on the ambient temperature and humidity (Epstein, et al, 1977). The photosynthates produced from this leafwater have a characteristic isotopic composition dictated by that of the leaf water and the ambient temperature. The biosynthetic process which follows to produce the various constituents of wood and the translocation of these to the trunk of the tree might introduce additional modifications in the isotopic ratios (D/H and $^{18}O/^{16}O$), depending on the ambient climate. Similarly, the $^{13}C/^{12}C$ ratio of the constituents of wood could reflect the changes in $^{13}C/^{12}C$ ratio or the concentration or both of the atmospheric carbon-di-oxide. This is because the assimilation rate of CO_2 and its conductance through the stomatal pores is controlled by the local temperature, humidity and water availability (Francey and Farquhar, 1982). Thus, in principle, one can expect a correlation between the D/H, $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios in constituents of wood and the ambient climatic parameters. These ratios are usually measured relative to that of a standard and are expressed as parts per thousand or per mil.

$$\delta D = \left[\frac{(D/H)\ Sample}{(D/H)\ Standard} - 1 \right] \times 1000\ ^{o}/_{oo}$$

$$\delta^{18}O = \left[\frac{(^{18}O/^{16}O)Sample}{(^{18}O/^{16}O)Standard} - 1 \right] \times 1000\ ^{o}/_{oo}$$

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)Sample}{(^{13}C/^{12}C)Standard} - 1 \right] \times 1000\ ^{o}/_{oo}$$

The standard for δD and $\delta^{18}O$ measurements is SMOW (Standard Mean Ocean Water) (Craig, 1961) and for $\delta^{13}C$ measurement, PDB (Cretaceous belemenite (Belemnitilla americana) from Peedee formation of South Carolina, USA) (Craig, 1953).

The major problem in this regard is that the various biosynthetic pathways are complex and the effect of climate on each of them is poorly understood. Therefore, it has not yet been possible to evaluate theoretically the likely fractionation factors involved and their temperature dependence. (Some approximate calculations were made by Libby (1972), but they did not agree with the experimental measurements). Whether a bio-organic process like the synthesis of wood can be treated as one under equilibrium chemical thermodynamics is another important question. (Urey, 1947).

To identify a climatic signal, then, one has to take the empirical approach, both for samples collected from the field and controlled growth chamber experiments. Tree ring samples chosen from stations for which instrumental weather records are available can be used to establish a calibration between stable isotope ratios and climatic parameters. This approach has been adopted in the present work. The objectives of this work are described in the next section.

1.3. Statement of the Problem

In the early years of development of isotope dendroclimatology, isotope ratio measurements were made on whole wood from tree rings (Libby and Pandolfi, 1974; Schiegl, 1974). Later, two major problems hindering the meaningful interpretation of results from whole wood measurements were identified, viz., the inherent chemical heterogeneity of the plant material and the post depositional exchange of the hydroxyl hydrogens with the ambient water (Epstein, et al, 1976). The need for isolating a single chemical component from wood, viz., cellulose in its nitrated form was demonstrated. Epstein et al (1976) achieved this by nitration of whole wood followed by isolation of cellulose nitrate. Wilson and Grinsted (1975), on the other hand, extracted cellulose first and exchanged the hydroxyl hydrogens of cellulose with a standard water of known isotopic composition. However, it was shown by Epstein (1978) that the latter method was not reliable and needed further testing before it could be safely employed. Similarly, some limitations in Epstein et al's (1976) nitration procedure were pointed out by DeNiro (1981), which led Yapp and Epstein (1982a, 1982b) to reassess their earlier work by repeating the measurements with a new procedure called the "renitration method". Thus the experimental techniques involved in isotope dendroclimatology have been perfected only recently.

Subsequent focus has been mainly on spatial correlations of oxygen and hydrogen isotopic variations in tree rings with climate (Yapp, 1980; Burk, 1979; Arnold, 1979). The reason for this is that the variations of oxygen and hydrogen isotopic compositions of precipitation with latitude, longitude and altitude is better known from IAEA data than the variations at a single place for long periods.

Interpretation of carbon isotope ratios in tree rings has been a subject of debate. As will be discussed in detail in the next chapter, it is very difficult to delineate the effects of climate, changing atmospheric CO_2 levels and variations in $^{13}C/^{12}C$ ratios of the atmospheric CO_2 on the carbon isotopic record of tree rings. However, more recently two groups (Leavitt and Long, 1983; Peng, et al, 1983) have claimed to have deconvoluted the tree ring based $^{13}C/^{12}C$ record of atmospheric CO_2.

Growth chamber experiments have been going on simultaneously, with a view to study individual steps in the route that water and CO_2 take to form plant material. These have resulted in controversial information as discussed by Long (1982).

Much of the work to date which does not suffer from problems of analyses of whole wood and post depositional exchange, has been on bunches of five to ten rings, thereby averaging out year-to-year variations. Existence of

variations, large compared to experimental errors, in isotope ratios along the circumference and the radial direction of a single growth ring has been known (Epstein and Yapp, 1976; Tans and Mook, 1980; van der Straatten, 1981). Therefore conclusions based on isotopic analysis of a single radial strip of a single tree at a given site must be taken with caution, until it is established in each case that intra-ring isotopic variations do not seriously hinder a meaningful interpretation of the tree ring isotopic record. Also, it is necessary to establish that several trees in a given climatic regime have a coherence in their isotopic signal, relatable to the common forcing mechanism, viz., climate. While seeking a relationship with climatic parameters, mostly temperature and in a few cases humidity, have been considered, thus overlooking the possible effects of other climatic parameters. The station from where the weather records are taken, has been as far as 500 km. from the tree site. Obviously, for a reliable comparison, this distance should be minimal. Simultaneous measurement of the isotope ratios of all the three elements, (H, C and O), which could lead to better resolution of competing effects, has not been done so far.

This thesis attempts to approach the problem keeping in mind the considerations outlined above, which have not received due attention yet. The major objective of this work is to assess the fidelity with which trees record climatic

changes on annual and regional scales. For this the δD of non-exchangeable C-H hydrogens and the $\delta^{13}C$ and $\delta^{18}O$ ratios of cellulose from annual growth rings of some Himalayan trees have been determined with careful chemical preparation and mass spectrometric measurements. The main emphasis of this thesis is to obtain an empirical relationship, if any, between regional climatic parameters and annual variations of stable isotope ratios in tree rings rather than on the reconstruction of past climate. Possible noises due to intra-ring isotopic variations and the coherence of the climatic signal from several trees at a given site have also been investigated. To resolve the effects of various climatic parameters on isotopic variations, appropriate statistical techniques like multiple regression have been tried.

1.4. Field Collection of Samples

Palaeoclimatic research in general, and tree ring studies in particular, are still in their infancy in India. The Himalayan region, containing extensive temperate forests, is one of the most important climatic zones of the world. Yet, it has not received much attention from the dendrochronological and dendroclimatological points of view. Hence I decided to focus my attention on this area.

There are no institutions in India involved in "traditional" dendrochronology. Neither the Forest Research Institute at Dehra Dun nor the various research divisions of

the Indian Forest Department have been interested in this type of work. Records about the availability of old trees are generally not maintained. Well documented tree ring samples are difficult to obtain. This necessitated my personal visit to the forest areas for collection of samples. This was facilitated by my training in timber identification and wood anatomy at the Forest Research Institute, Dehra Dun.

In May, 1980, I undertook a reconnaissance trip to the Kashmir Valley. With the kind assistance of Mr. S.T. Qadri, the then Divisional Forest Officer, Research Division, Jammu and Kashmir Forest Department, I could collect discs of some conifers. Increment borers and chain saws were not available, making the collection rather difficult. So I had to restrict the sampling to areas where commercial felling of trees was going on. These were mainly in Gulmarg forest compartment #63 located near Tangmarg (74° 25' E, 34° 04' N, 2650 msl) and Lidder forest compartment # 33B near Pahalgam (75° 20' E, 34° 03' N, 3220 msl). The details of the samples collected from these two places are listed in Tables 1.1 and 1.2. The sampling locations along with the nearby meteorological stations are shown in fig. 1.1. Samples analysed in this work are restricted to some of the samples collected during this first field trip. Since samples were from live trees, dates could be easily assigned by ring counting, after polishing the surface of the discs. This was made easier because the rings

Table 1.1

Details of samples collected from Gulmarg forest compartment # 63 near Tangmarg (74° 25' E, 34° 04' N, 2650 msl) during May, 1980

Sample No.	Date of Sampling	Species Sampled	Common Name	Ht. at which tree was sampled	Average radius	Age of the tree
CD 1	20	Cedrus deodara	deodar	2'	8.5"	80
AP 1	22	Abies pindrow	silver fir	2.5'	15"	90
AP 2	22	"	"	1.5'	11"	120
AP 3	22	"	"	2'	14"	100
PW 1	22	Pinus wallichiana	blue pine	3'	2'	100
PW 2	22	"	"	2'	14"	106
PW 3	24	"	"	2'	9.5"	70

All these trees were located within a ~ 50 m radius.

Table 1.2

Details of samples collected from Lidder forest compartment # 33B near Pahalgam (75° 20' E, 34° 03' N, 3220 msl) during May, 1980

Sample No.	Date of Sampling	Species Sampled	Common Name	Ht. at which tree was sampled	Average radius	Age of the tree
LAP 1	29	Ables pindrow	silver fir	3'	14"	300
LAP 2	29	"	"	3'	14"	300

The distance between the trees was ~ 20 m.

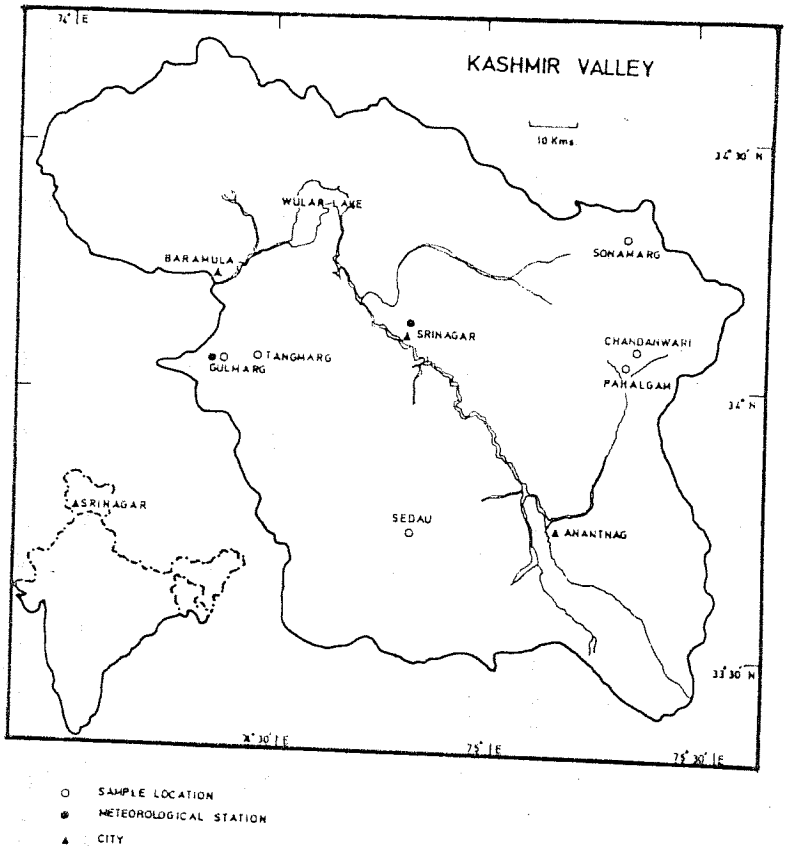


Fig. 1.1 : Map of Kashmir Valley showing the sample locations and meteoroligical stations.

were fairly wide in general, ranging from 0.2 to 1 cm. There were no missing rings, double rings or fire damage in any of the samples collected.

Subsequently in September and October 1980, more samples were collected from different sites in the Kashmir valley. This time, I was accompanied by Dr. M.K. Hughes of the Liverpool Polytechnic, who had been involved in dendroclimatological work in the UK for quite some time. Dr. S.K. Bhattacharya of our laboratory was also in this team. Dr. Hughes had brought along with him increment borers which enabled us to collect cores from Abies pindrow (Silver fir), Picea smithiana (spruce) and Pinus wallichiana (blue pine) at sites ranging from closed forest (ca. 2500 msl) to more open sites close to the upper limit of conifers (ca. 3250 msl). Some of the important sites were Gulmarg, Kilanmarg, Sonmarg, Yusmarg, Sedau and Pahalgam. It was possible to build seven tree ring chronologies of suitable quality for use as proxy climatic records from material taken during this second field trip. The dating of the tree rings sampled during the first field trip was verified with the ring width chronologies for Gulmarg provided by Dr. M.K. Hughes after the second field trip.

Sampling of Himalayan trees for dendroclimatic studies was extended to regions other than Kashmir in July, August 1982. Dr. M.K. Hughes and Dr. G.B. Pant of Indian

Institute of Tropical Meteorology, Pune, accompanied me during this field trip. Sites near Sonmarg, Pahalgam, Chandanwari in Kashmir, Kulu, Manali and Pulga in Himachal Pradesh were some places where useful sampling could be done. Cedrus deodara (deodar) samples as old as 250 years from Manali in Himachal Pradesh and Picea smithiana (spruce) discs from Pahalgam in Kashmir dating 400 years BP. were the important collections of this third field trip. Reconnaissance of the Garhwal and Kumaon Himalayas was also made. Unfortunately sampling in these places and in Chakrata near Dehra Dun was made difficult by the monsoon rains and the resulting landslides blocking the approaches to some of these places. Dr. M.K. Hughes and Dr. G.B. Pant are working on ring width and ring density chronologies and we at PRL, Ahmedabad, concentrate on isotopic analysis of the samples.

Samples collected during the first field trip, which have been used for this work, have been collected from relatively open forests and hilly terrains remote from permanent water sources. And only samples from Gulmarg have been analysed because Gulmarg has a meteorological station functioning since the beginning of this century, though only during the summer months. The only other meteorological observatory in Kashmir which has been functioning for such a long period is in Srinagar. But conifers are not generally available here for sampling. However, fast growing species like chinar

(Platanus orientalis) as old as 200 years are available here. These could be used for extracting high resolution climatic information. These trees are protected and sampling could not be done. However, some discs collected from a few chinar trees which fell during a storm are available with the Research Division of the Jammu and Kashmir Forest Department.

The species of trees studied in this work are not generally found in other parts of the world and have not been used earlier for dendroclimatic studies. Of the various trees, Abies seems to have the greatest promise, being a slow growing and durable species. The samples are usually stored wrapped in polythene sheets after drying them in air for several days.

In what follows, a general description of the climatic setting and forests of Kashmir Valley is given in brief.

1.5. Climatic Setting and Forests of Kashmir Valley

1.5.1. Climatic Setting

The valley of Kashmir (33° $30'$ - 34° $30'$ N, 74° - 75° $30'$ E) is flanked by the Himalaya on the north east and the Pir Panjal on the south west. It is climatically very different from the rest of the Jammu and Kashmir state.

Winter here lasts upto March and is often severe. The mid-Mediterranean depressions (also called the Western

disturbance) blow over the Afghan frontier after passing over Iran and precipitate in the valley and its surrounding mountains as snow. Spring begins after March and lasts till May. This is the rainiest season of the year. The hottest months are July and August. Summer rains are generally absent because the monsoons are blocked by the Panjal mountains extending about a distance of 80 km.

To the north and east of the valley, the mountains rise higher till the valleys of the Sind, the Lidar, the Vaishav and the Rambiara are reached. The monsoon dies out to the south of the Pir Panjal ranges of mountains and the summers have, therefore, very little in common with the general winds and pressure pattern over the mainland India. Bright warm sunshine and bracing winds make the summer hot during the day. The influence of the south west monsoon reaches at most the Uri gorge in the north of Kashmir and generally not beyond. Humidity is high in July and August and combined with high temperature, causes discomfort. The lakes and waterways which are many in the Valley of Kashmir make the atmosphere of the surrounding areas damp and oppressive. This state of depression lasts till September but October is the healthiest month (Raina, 1977).

1.5.2. Forests

The most thickly forested region of the Jammu and Kashmir state is the Kashmir valley, the forest area being

11095 Km^2. The districts of Baramulla (of which Gulmarg is a part) and Anantnag have respectively 71% and 60% of their area under forests. Species of trees have strong regional dependence, punctuated by altitudinal zones, though in general conifers account for 85% of the total forest area.

The northern slopes of Pir Panjal are covered with thick coniferous forests. The four districts of Doda, Udhampur, Baramulla and Anantnag have verdant forests constituting of 55% fir (mostly Abies pindrow) and spruce (Picea smithiana), 22% deodar (Cedrus deodara), 18% Kail (blue pine or Pinus wallichiana) and 5% Chir pine (Pinus roxburghii) (Raina, 1977).

The forests of Kashmir Valley can be classified into montane, temperate and alpine. The montane and temperate forests are found between 1500 and 3200 m. They have a lower zone in which broad leaved varieties are preponderant and a higher zone (2100 - 3200 m) in which the conifers predominate. The alpine forests occur at an average elevation of 3200 m and above. Birch is the most common tree type in this zone, though stunted junipers and rhododendrons are also found (Raza, et al, 1978).

This work is restricted to three species of trees: Abies pindrow, Cedus deodara and Pinus wallichiana.

As mentioned before, Abies pindrow is the most suitable for dendroclimatic work because of its longevity

and slow growth rate. This is by far the most important species in the fir forests of the Himalayan region. This species grows gregariously over the whole length of its distribution in the western Himalaya extending from Sikkim westward to Pakistan. It generally grows between 2300 m to 3050 m above sea level, outstretching this zone under favourable conditions of moisture and aspect. In shape and appearance, the fir forests are majestic, the grandeur of which is a feast to the eyes.

This fir is shade loving. That explains its choice for cooler sites and sheltered depressions where soil is also generally deep and fertile. The soil on which fir grows must have the capacity to remain moist so as to release it during the periods of drought. In general, the precipitation where it grows varies from 1000 mm to 2500 mm the bulk being received during the monsoons which last from July to September. Heavy snow falls during winter also contribute to the total precipitation. But, in the Kashmir Valley, where the monsoons do not gain access, the precipitation is around 1000 mm and is mainly contributed by snow during winter and spring rains. The maximum temperature in the shade seldom exceeds 25° C (Sufi, 1970).

The wood of Abies pindrow is creamy white to pale yellow, turning light brown on exposure. It is fairly soft and light with an average weight of 450 Kg/m^3 at 12% moisture

content. It is somewhat lustrous when fresh, medium fine and even textured with straight grain. The wood is non-porous with distinct growth rings visible in the tangential, radial and cross sections of the tree. Resin canals are absent and there is no heartwood - sapwood separation. Transition from early to late wood is mostly gradual. (early wood is the "spring" wood which is generally lighter in colour than the late wood or "summer" wood. The cells are smaller in size in the latter and the walls are heavily lignified). The rays are very fine, not visible to the eye, but seen under the lens as fine closely spaced lines. These are the features which enables one to identify this species anatomically (Rao and Juneja, 1971).

The needles (leaves) of this species persist for three to six years and sometimes in part for seven or eight years. The old needles fall chiefly in May and June. The new shoots appear in April-May, their bright green colour contrasting strongly with the dark green of the older foliage; the young needles and shoots are soft, not stiff like the older ones.

The leaves of seedlings persist for at least 3-4 years, and sometimes in part for 5 years. The season's growth commences in April or early May and is practically completed by the end of June. The growth of natural seedling is slow.

The first year's growth is very little more than the length of the hypocotyl, that is, a maximum of a little over 2". The second year's growth ordinarily varies from 0.4 - 1.3" and that of the third year from 0.5 - 2.8", according to vigour. Subsequent growth is somewhat faster, but even under favourable conditions a height of barely 3' is attained in 10 years (Troup, 1921).

Cedrus deodara, commonly known as deodar, occupies all types of soils and terrains and is found usually from about 2900 m above the sea level. But in the Pir Panjal range, it is conspicuous by its absence, except in some well-defined areas like Yarwan and some toes of the Karewas. In general, the majority of the best deodar forests are found where rainfall varies from 1000 to 1750 mm, mostly contributed from snow in the winter. The temperature and moisture requirements are intermediate between those of fir and blue pine (Fotidar, 1970).

Deodar has a distinct sap wood, white to creamy white and a heart wood light yellowish brown turning pinkish brown on exposure, occasionally with dark brown lines or streaks along the grain. The wood is soft to moderately hard, moderately heavy, with an average weight of 545 Kg/m^3 at 12% moisture content. It looks dull, with an oily feel and a characteristic pungent odour. The texture is medium fine and even, with straight grain.

Deodar is a non porous wood and growth rings are distinct on all the three (radial, tangential and cross) surfaces. Transition from early wood to late wood is mostly gradual. Resin canals are not always present, but when present, are arranged in long, tangential rows, showing up as dark brown lines on the longitudinal surfaces. Rays are very fine, not visible to the eye, but seen under a lens as fine, numerous, closely spaced lines. Deodar is identified by the above anatomical characteristics (Rao and Juneja, 1971).

In deodar trees, the new shoots appear in March or early April, small pale green opening buds marking their first appearance. The old leaves are shed for most part in the hot season, chiefly in May, but leaf shedding may also take place to some extent in the autumn, about the time the cones ripen. The persistence of the leaves varies. On vigorous saplings, they do not persist so long as on older trees with slow growing branchlets. In the former case, they may fall the year following their first appearance, while in the latter case, they may persist, in part at least, as long as six years; this applies both to the whorls of leaves on the arrested branchlets and to the solitary leaves, which are spirally arranged on the normal shoots (Troup, 1921).

Pinus wallichiana, commonly known as blue pine or kail, is a large evergreen tree with bluish feathery foliage.

This seeks drier and hotter aspects. This grows faster than fir and deodar and is found generally from 1800 m to 2500 m above sea level (Fotidar, 1970).

Blue pine has a white to pale yellowish white sap wood distinct from the light pinkish red to light red heart wood with resinous odour. The wood is soft, light, average weight being 515 Kg/m^3 at 12% moisture content. It is fine textured and straight grained. It is a nonporous wood and growth rings are distinct on all the three surfaces (radial, tangential and cross). Transition from early to late wood is gradual. Resin canals are of the vertical type, scattered, large and distinct to the eye, appearing as prominent dark lines or resin streaks on longitudinal surfaces. Rays are very fine, not visible to the eye, but seen under the lens as fine, numerous, closely spaced lines. These are anatomical features by which blue pine is identified (Rao and Juneja, 1971).

The new shoots of blue pine appear in March or early April, according to locality; by August to September, the needles attain full size. The needles persist for one year and two or three months, or, in part at least, for a second year. The normal season for the shedding of the needles is the summer, chiefly during May, June and July. In June-July, the trees often have a somewhat base appearance, many of the old needles having fallen, while the new ones

have not yet expanded fully and are in tufts resembling tar brushes. The needles are usually shed earlier in the case of less vigorous trees, at high elevations and on exposed ridges, than in the case of vigorous poles in favourable situations. Sometimes, partial shedding of leaves takes place at seasons other than the usual one, particularly from October to December (Troup, 1921).

None of the three species of trees described above have a clear cut line of demarcation between early and late woods. The precise months corresponding to the diameter growth of these trees are not known. However, in the case of conifers, it is known that the diameter growth starts almost at the same time as the shoot growth. (Kramer and Kozlowski, 1960). Therefore, it can reasonably be assumed that, in general, March-April is the season for the beginning of cambial activity in these trees. Depending on site and species, cambial activity may last till September-October.

CHAPTER 2

CRITICAL REVIEW OF EARLIER WORKS

2.1. Introduction

Variations in stable isotope ratios of many datable, stratified, natural systems have been shown to reflect climatic conditions prevalent during deposition (Gray, 1981). Application of stable isotope techniques to tree rings with a view to extract spatial and temporal climatic information on long and short term bases is a decade old. This chapter is an attempt to review the earlier results in isotope dendroclimatology. Two such reviews have already been published (Long, 1982; Wigley, 1982). A more general review of isotope palaeoclimatology concentrating on the past 5000 years, and covering biological materials such as tree rings and peak deposits is also available (Gray, 1981).

The emphasis here is on focussing the attention on more recent work in this field and assessment of the progress made so far, rather than giving an exhaustive summary of the earlier work.

Preliminary isotope works on tree-rings (Schiegl, 1974; Libby, et al., 1976; Farmer and Baxter, 1974; Freyer and Weisberg, 1975; Pearman, et al., 1976) were on whole wood. These results suffer from two complications: Chemical heterogeneity of wood and post depositional exchange. These are elaborated in the next two paragraphs.

Wood is composed of cellulose (~ 50%), lignin (~ 30%), hemicellulose (~ 15%), resins and lipids (~ 5%). The percentages of these components vary from year to year. It has been found that lignin/cellulose ratios in trees are not climate dependent (Gray, 1981). Also, in the same annual ring, the different chemical components have different isotopic ratios. For example, lignin is found to be lighter in $\delta^{13}C$ than cellulose by about 3 °/oo (Wilson & Grinsted, 1977) and in $\delta^{18}O$ by about 10 °/oo (Gray and Thompson, 1977). Hemicellulose is heavier in $\delta^{13}C$ relative to cellulose by about 1 °/oo (Freyer, 1979). The lipid and resin fraction is depleted by about 100 °/oo in δD relative to the cellulose. The chemical heterogeneity of wood, coupled with the varying isotopic ratios of its components makes any climatic interpretation of whole wood δ values difficult at best and

impossible at worst. It was soon realised that for meaningful interpretation, a single component has to be isolated from wood for climatic studies (Epstein, et al., 1976). Since cellulose is the major component and is present in most photosynthesising plants, it is the most preferred component for such studies (Yapp, 1980).

Though chemically cellulose is preserved for long periods after the death of the tree, isotopically there is an uncertainty due to post-depositional exchange. The carbon and oxygen isotopes in cellulose do not seem to suffer from this problem (Long, 1982). However, the hydrogen atoms in cellulose which are bound to oxygen (called the hydroxyl hydrogen or OH-hydrogen accounting for 30% of the total hydrogen in cellulose), exchange with the water which comes into contact with it subsequent to the deposition of cellulose in the ring. The rest 70% hydrogen atoms which are bound to carbon (called the CH hydrogen) do not undergo such an exchange (Epstein, et al., 1976). So it is necessary to eliminate or replace the OH hydrogen for δD analyses aimed at deciphering the climatic record. This can be done in two ways as will be discussed in section 2.2.2.

Apart from cellulose, lignin and hemicelluloses also contain such exchangeable hydroxyl hydrogens (Grinsted and Wilson, 1979). This poses an additional problem for whole wood δD analyses. However, the lipid fraction has

been shown to be almost free of OH hydrogens and almost all of the hydrogen in it is bonded non-exchangeably to carbon (Northfelt, et al., 1981).

Therefore, in this chapter, only those results will be discussed which are based on isotopic measurements of a single component, lignin or cellulose. The discussion is divided in four parts:

(i) Development of experimental techniques

(ii) Hydrogen isotopes in tree rings

(iii) Oxygen isotopes in tree rings

(iv) Carbon isotopes in tree rings

The concluding section points out the limitations in the earlier work and discusses the experimental strategy for the present work.

2.2. Development of Experimental Techniques

2.2.1. Cellulose Extraction

The methods of cellulose extraction are standard (Green, 1963a). The wood powder is extracted with organic solvents, delignified by chlorination and hemicelluloses removed by treatment with hot alkali (see section 3.2 for details). Most workers follow this procedure. (Grinsted and Wilson, 1979; Epstein, et al., 1976; DeNiro, 1981;

Gray and Thompson, 1977; van der Stratten, 1981; White, 1983; Leavitt and Long, 1983; Bender and Berge, 1982). Freyer (1979) uses a variant of this method. The wood samples are treated with 5% NaOH for 2 hours at 60°C in order to convert the wood into a reactive form and also to resolve hemicellulose, resins and tannic acids. After washing, the samples are treated with a buffered sodium chlorite solution (7% NaCl O_2, 1% K_2HPO_4) which is renewed after 1 day. During the oxidation procedure the samples are mechanically crushed to facilitate the conversion of lignin and accompanying matter into a soluble form. The pure bleached cellulose residue, is washed and dried for isotopic analysis.

Although the various constituents of wood have different chemical structures, the definitions are operational. For example, while pure cellulose is composed of only linked glucose units, pure cellulose cannot be isolated from whole wood. (White, 1983). Rather, the term alpha cellulose is used to describe the predominantly cellulosic fraction which can be extracted from whole wood. Alpha cellulose contains traces of xylose and other sugars (Timell, 1957). The difficulty of separating pure compounds from whole wood and the necessity of isolating as pure a compound as possible for isotopic analyses, necessitates that the purification procedures be as rigorous and complete as possible (White, 1983). The results from the two methods described above have

not been compared and it is not possible to say which method is superior. For this work the method of Green (1963a) has been used (see section 3.2).

2.2.2. Removal/Replacement of Exchangeable Hydrogens in Cellulose

As discussed earlier 30% of the hydrogen in cellulose is bonded to oxygen and this hydroxyl (OH) hydrogen exchanges with the ambient water. After the deposition of wood in the tree, this OH hydrogen exchanges with the sap water which flows through the tree. Subsequently, during laboratory treatment, it exchanges with the chemicals used. The remaining 70% hydrogen which is bound to carbon is non-exchangeable (Epstein, et al., 1976). It is therefore necessary to either replace the exchangeable OH hydrogen with known hydrogen or remove it completely.

Two methods have been tried so far. One is the nitration method (Epstein, et al., 1976) and the other is the equilibration method (Grinsted and Wilson, 1979).

There are three different ways of nitrating cellulose. The whole wood can be nitrated with a mixture of nitric, phosphoric acids and P_2O_5 and cellulose nitrate extracted by dissolution in acetone and precipitation with water (Epstein, et al., 1976); the cellulose can be extracted first using the method of Green (1963a) and subsequently nitrated

using the above acid mixture; (see section 3.2 and 3.3 for details); or the cellulose can be nitrated by using a mixture of nitric acid and acetic anhydride (DeNiro 1981; White, 1983).

These three methods have been investigated in detail by DeNiro (1981). He has found that the nitration of whole wood is unsatisfactory because, in the case of woody plants, some amount of lignin nitrate is also associated with cellulose nitrate and is not removable by acetone dissolution. This in turn leads to errors in the δD value of cellulose nitrate prepared in this way. (This has been confirmed by the findings of Gray and Song (1984), more recently). However, he notes that a second nitration of the cellulose nitrate thus prepared minimises greatly the contamination of nitrated lignin. This has led Yapp and Epstein (1982a) to reinvestigate their earlier conclusions using a second nitration of the previous samples and some more. DeNiro (1981) has also observed that it is important to determine the nitrogen content of each sample. As long as the nitrogen content is at least 13.2%, there is no significant difference between the δD values of incompletely nitrated samples and those of the completely nitrated samples. At a nitrogen concentration of 13.2%, approximately 95% of the hydrogen is carbon bound.

He has further noted that nitration of cellulose by a mixture of nitric acid, phosphoric acid and phosphorous pentoxide yields a cellulose nitrate of the same δD value

as the one obtained from nitration using the nitric acid and acetic anhydride mixture. Also the infrared spectra of these two cellulose nitrates are identical and show the absence of OH groups. The trace amounts of hemicelluloses present in the cellulose do not seem to affect the δD values of the cellulose nitrate.

Both the abovementioned methods using two different nitration mixtures produce cellulose nitrate of nitrogen contents consistently higher than 13.2% and of similar δD values. But in the present work the nitration by nitric acid, phosphoric acid and P_2O_5 mixture was preferred. This is because the cellulose nitrate prepared by this method is stable and very little degradation of the cellulose structure occurs during the nitration. Additionally the acid mixture swells the cellulose appreciably so that nitration is uniform. The nitric acid and acetic anhydride mixture does not have this swelling effect on cellulose (Green, 1963b). Though Yapp and Epstein (1982a) have shown that the "renitration method" (involving two nitration steps of the whole wood) produces cellulose nitrate having δD value same as that of cellulose nitrate produced by nitration subsequent to cellulose extraction, it is not preferable to use the "renitration method" because of possible degradation of the cellulose structure due to excess contact with the nitrating acid mixture.

The other method of getting rid of the problem of exchangeable OH hydrogen is to equilibrate the cellulose with a standard water of known isotopic composition (Grinsted and Wilson, 1979). Initial results (Wilson and Grinsted,1975) obtained using this method have been criticised by Epstein and Yapp (1976). Subsequently, Epstein (1978) analysed a different section of the same tree (Pinus radiata) used by Wilson and Grinsted (1975) using the nitration method. He has found that the relationship between δD of the cellulose nitrate and precipitation is opposite to that determined by Wilson and Grinsted (1975). Epstein notes that the method of equilibration needs further testing before it could be safely employed. However, since it has been shown by DeNiro (1981) that Epstein et al's (1976) original nitration method could lead to erroneous δD values, the samples have to be reanalysed using either the "renitration" method or by extraction of cellulose and nitrating it using one of the acid mixtures discussed earlier. This must be the first step to resolve this contradiction. Once the equilibration method is established, both nitration and equilibration methods could give valid but different climatic signals as observed by Long (1982).

2.2.3. Preparation of Hydrogen for δD Analysis

The procedure is to combust the cellulose nitrate in an oxygen atmosphere; the water thus obtained is reduced to hydrogen by passing through hot uranium (see section 3.4 for details). The oxygen for combustion can be obtained from either a static or dynamic atmosphere of oxygen gas (Epstein, et al., 1976) or from cupric oxide (Stump and Frazer, 1973).

When the cellulose nitrate is combusted using oxygen gas, oxides of nitrogen are also produced. These have to be reduced to nitrogen by passing over copper fillings kept at 400°C. This is necessary because it is difficult to separate cryogenically some oxides of nitrogen and CO_2. (For example, boiling point of NO is -89.2°C, for CO_2 this is -78.5°C).

But when the oxygen for combustion is derived from copper oxide as in the Stump and Frazer method (1973), the oxides of nitrogen produced during combustion are reduced by the remaining copper back to nitrogen. And by using a charcoal finger this nitrogen can be collected and measured to calculate the nitrogen content. Ths Stump and Frazer method (1973) as modified by Northfelt et al., (1981) has been adopted in the present work because of this convenience (see section 3.4 for details).

Another method is available for extraction of hydrogen from organic samples (Gray, et al., 1984). Milligram quantities of water (with added carbon) or an organic material can be pyrolysed in a nickel vessel at 1150°C and the hydrogen can be allowed to diffuse through the walls of the vessel, collected and analysed. This method has been found to be quite reliable for compounds with high O/C ratios (citric acid, cellulose nitrate and glycine) and less suitable for samples with relatively low O/C ratios (cellulose and dextrose).

This is because the latter compounds tend to form tars during the early stages of combustion. These tars condense on to the cooler part of the combustion chamber and never get combusted completely.

The δD values in the methods described above have been found to be quite reproducible.

2.2.4. Preparation of CO_2 from Cellulose for $\delta^{18}O$ Analysis

The general method is to convert all the oxygen in cellulose to carbon dioxide and measure the $\delta^{18}O$ of this carbon dioxide. Four such methods have been developed (Rittenberg and Ponticarvo, 1956; Hardcastle and Friedman, 1974; Thompson and Gray, 1977; Brenninkmeijer and Mook, 1981). The Rittenberg and Ponticarvo method has since been modified (Wilson and Grinsted, 1977; Burk, 1979). The method, developed by P. Thompson and S. Epstein at California Institute of Technology and reported by Thompson and Gray (1977), has been used for simultaneous extraction of hydrogen and CO_2 for δD and $\delta^{18}O$ analyses respectively (Gray, et al., 1984).

In the first method (Rittenberg and Ponticarvo, 1956, modified by Wilson and Grinsted, 1977 and Burk, 1979), the cellulose is combusted at 550° with $HgCl_2$. The sample is pyrolysed to C, CO, CO_2 and HCl. The HCl is removed by absorbing it in quinoline and the CO is converted to CO_2 by

12734

electrical discharge. (see section 3.5 for details). The total CO_2 is then recovered for $\delta^{18}O$ analysis.

In the second method (Hardcastle and Friedman, 1974), the cellulose sample is pyrolysed at 1250°C in a quartz tube and the products are passed through hot diamonds (1250°C). The resulting H_2 is pumped away and CO and CO_2 are collected. The CO to CO_2 conversion is done by electric discharge as before.

In the third method (Brenninkmeijer and Mook, 1981), the sample is heated in a nickel bomb with nickel catalyst powder at 950°C. H_2 diffuses out through the walls of the tube, leaving a mixture of C, CO and CO_2. On cooling the tube to 350°C, the CO gets converted to CO_2 by the catalytic action of nickel. This CO_2 is then purified and collected. This method avoids the electric discharge step.

In the fourth method (Thompson and Gray, 1977), the sample is pyrolysed at 1125°C in a nickel vessel and the hydrogen diffuses out through the walls. The CO is converted to CO_2 by electrical discharge. In a later version of this method (Gray, et al., 1984) the hydrogen is also collected and analysed for δD. This method has the advantage that both δD (see section 2.2.3) and $\delta^{18}O$ of the organic sample can be determined simultaneously.

The $\delta^{18}O$ values of samples determined using any of the above methods have been found to be quite reproducible. However, the $HgCl_2$ method is quite convenient in practice. Batches of 6 samples can be processed in a day and with a little modification, the hydrogen and CO_2 extraction line can be used for this purpose (see section 3.5). Hence this method has been adopted in this work.

2.2.5. Preparation of CO_2 for $\delta^{13}C$ Analysis

The standard method is that of Stump and Frazer (1973). The cellulose is combusted with CuO at 800°C when all the carbon is quantitatively converted to CO_2. This is cryogenically separated from the other products of combustion and collected for $\delta^{13}C$ analysis.

Since cellulose and cellulose nitrate have the same carbon isotopic composition, the CO_2 from the combustion of cellulose nitrate can as well be used for $\delta^{13}C$ measurement as done by Yapp and Epstein (1977) and Northfelt, et al., (1981). This saves a lot of time when one is interested in measuring both δD and $\delta^{13}C$ of organic substances. This method has been adopted in this work. As will be seen in section 3.6, the method is quite reproducible.

2.3 Hydrogen Isotopes in Tree Rings

2.3.1. Rationale behind the approach

In general δD and $\delta^{18}O$ ratios in meteoric waters reflect the temperature at the site of precipitation - the lower the temperature the smaller the isotopic ratio. These ratios have been shown to fluctuate in response to seasonal temperature changes, geographic temperature changes and long term climatic changes such as glacial and interglacial. At present the temperature distribution over much of the surface of the earth is correlated with the pattern of δD and $\delta^{18}O$ variations in meteoric waters. Thus, natural systems which record the variations with time in δD or $\delta^{18}O$ values of meteoric waters at a particular site should preserve a record of past climatic variations (Epstein, et al., 1976).

One such system is series of annual growth rings in trees. But one has to face a number of complications in attempting to decipher such a climatic signal. Firstly, the chemical heterogeneity of the plant material and post depositional exchange problems have to be solved by isolating a single chemical component and removing the exchangeable hydrogens, as emphasised in section 2.1. Secondly, the water which is used by the tree for its growth may be a combination of the rain water and the ground water. While the ground water at a given place may be reasonably constant in its isotopic composition for a long time, the rain water isotopic ratio changes

not only with the temperature at the site but also with the temperature history of the airmass which brings it to the site, (Epstein and Yapp, 1976). It is therefore important to ascertain whether the tree in question sampled meteoric water alone or a mixture of ground water as well. Thirdly, the evapotranspiration at the site of photosynthesis (leaves) may involve equilibrium and kinetic fractionations of the isotopes of oxygen and hydrogen in water. The former is temperature dependent whereas the latter is practically temperature invariant (Gat, 1981). Fourthly, the biosynthesis of the various components may lead to further fractionation of the isotopes either in the same direction as the previous steps or in the opposite directions. Knowledge about the isotopic fractionation in each of the steps of biosynthesis leading to the formation of the various components of wood is still rudimentary. Finally, apart from temperature, other environmental factors like humidity, wind velocity, solar radiation etc. may influence the isotopic values of the chemical components of wood in a direct or indirect way.

When the system is so complex, two approaches can be made. By controlled laboratory experiments one can study the fractionations in each of the various steps. Or, by measuring the isotopic ratios in trees from places with well documented weather records, a gross correlation between the two can be attempted. Both these methods have to be tried with well established and valid experimental techniques.

2.3.2 Earlier results on field and laboratory measurements

Plant water relationship:

Yapp and Epstein (1982a) have measured the δD of 7 aquatic plants using the "renitration method". When they compare these with the δD values of the associated environmental water, they see that δD of the cellulose carbon bound hydrogen is depleted with respect to that of the water in all cases. The net fractionation factor is defined as

$$(1) \qquad \alpha_{net} = \frac{1 + 10^{-3} \ \delta D_{CN}}{1 + 10^{-3} \ \delta D_{W}}$$

where δD_{CN} and δD_{W} are the δD values for cellulose nitrate and the environmental water respectively. The values of α_{net} range from 0.961 to 0.988 and the range of temperatures of the environmental waters is $15^{o}C$ to $27^{o}C$. Of the seven samples, five collected from waters with temperatures in the range of 15 to $17^{o}C$ show similar α values (0.961 to 0.973).

Additionally, 42 plants representing a wide variety of species and local climates have been analysed by them. The δD_{CN} when compared with δD_{W} of the environmental waters show a linear relationship (best fit line) of the form.

$$(2) \qquad \delta D_{CN} = 0.87 \quad \delta D_{W} - 11$$

with a correlation of 0.89. While this represents a fairly high degree of correlation, a large

degree of scatter is evident from their data. Part of this scatter is explained due to (i) species effect (ii) the difference between the δD_W analysed and that of the water ingested by the plants. Additionally, apart from temperature, humidity can be one of the parameters that affect δD_{CN}. Yapp and Epstein (1982a) plot α_{app} with humidity (h) which gives a relation

$$(3) \quad \alpha_{app} = \frac{1 + 10^3 \ \delta D_{CN}}{1 + 10^3 \ \delta D_W} = -0.124\ h + 1.089$$

with a correlation coefficient of -0.79. Again a lot of scatter is present. They explain this scatter to be due to many factors. For example (i) the assumed constancy of the equilibrium (α_{eq}) and kinetic (α_k) fcationation factors at the leaf may not be valid, (ii) the value of α_{net} may depend upon species, (iii) the isotopic steady state of the leaf water (when the evapotranspired) vapour has the same δD as that of the input water to the tree) assumed may not be strictly valid and (iv) the equilibrium between the atmospheric water vapour and the environmental water (assumed to be the input water to the tree) may not have been attained.

Notwithstanding these possible perturbations, Yapp and Epstein (1982a) get a linear relationship to a reasonable approximation which confirms that the dominant control on the isotopic variation of plant cellulose CH hydrogen is of the variations of the δD values of source water, where the source water is that present in the leaf.

They also derive an equation of the form (under the assumptions discussed above)

$$(5) \quad \delta D_{CN} = A\, \delta D_W + 1000\,(A-1)$$

where $A = \alpha_{eq}\ \alpha_{net}\ \alpha_k - \alpha_{net}(\alpha_{eq}\ \alpha_k - 1)\ H$

They see that the value of A does not change from unity by more than about 7% over a relative humidity range 0.20 to 0.90. Thus relative humidity has little impact on the slope of equation (5). For the same values of various α's, the intercept, 1000 (A-1) varies only by one per mil for an incremental change in the relative humidity of 0.01. This implies that unless growing season relative humidity changes by more than a few percent from year to year at any single site, the δD variations of the nitrated cellulose extracted from a sequence of tree rings can directly reflect the δD variations of the soil water passing through the tree, and thus, quite possibly, the variations of local precipitation. Because of the relationships between the isotopic composition of precipitation and such climatically important variables as temperature, the preceding conclusions have important implications for the climatic interpretation of δD variations in tree ring sequences (Yapp and Epstein, 1982a).

White (1983) has modelled sap water movement through trees and leaf water enrichment by evapotranpiration. He has also proposed an overall model linking the δD of source water

and δD of cellulose nitrate. The important variables in this model are temperature, relative humidity and the δD values of source water and water vapour in air. How these latter two parameters are described in nature determines the climatic response of cellulose nitrate δD values.

For white pines at Mohonk Lake, the model for cellulose nitrate δD values predicts that the δD values of the source water for the tree is the primary variable. Variations in temperature and relative humidity at this site do not significantly affect the cellulose nitrate δD values. He has supported these conclusions with both interring and intraring samples from white pine trees.

He has also observed that the climatic information in δD of cellulose nitrate depends on the site conditions. While for a dry site the trees track the changes in δD of the growing season precipitation, for wet and intermediate sites the trees record the climatic information in ground water δD value and the growing season rain δD value, in addition to responding to changes in the relative contributions of the ground water and growing season rain to the tree.

Calculating the δD of leaf water (δDs) from his models, he gets a relationship for the δD of cellulose nitrate and δD_S as

$$(6) \quad \delta D_{CN} = 0.82 \delta D_S - 12 \quad (r^2 = 0.844)$$

which is quite similar to equation (2) obtained by Yapp and Epstein (1982a).

Tree rings and Climate

Epstein and Yapp (1976) have measured the δD values of CH hydrogen in cellulose from tree rings in a Scots pine from Scotland and a bristlecone pine from California. The 40 year running averages of the δD data of the two pines correlate linearly over the period 1841-1970 A.D., which is the total growth period of the Scots pine. The long term δD trend of the Scots pine correlates well with the 1841-1970 winter temperatures of Scotland. The long term δD trend of the bristlecone pine correlates qualitatively with the long term climatic trends estimated by LaMarche from the tree ring width data and by Lamb from many sources of climatic data. δD analyses have also been made on early and late woods from the same annual ring. The δD values of these woods differ to various degrees and the sign of the difference can also vary. Their data show that the early and late wood δD values for CH hydrogens could differ by as much as 52 $^{o}/oo$. They note that caution must be exercised regarding the interpretation of δD record in trees considering the large difference between early and late woods. These measurements were based on the whole wood nitration method of Epstein, et al., (1976) and have to be confirmed by either the "renitration method" or nitration of cellulose as discussed in section 2.2.2.

Yapp and Epstein (1982b) make a comparison of the geographical variation of the δD values of trees with the associated average annual temperature, which reveals an overall correlation of many sites and species of trees. The spatial linear temperature coefficient for δD values from 20 widely separated tree samples in North America is 5.8 °/oo per °C. This compares with a temperature coefficient of 5.6 °/oo per °C, obtained from annual percipitation δD values at 11 North American IAEA sites. The similarity of these two coefficients is consistent with the idea that the δD variations in trees reflect the δD variations of the local precipitation. These conclusions are supported by the spatial distribution of the δD values, which decrease from coast to the interior and from south to north. This pattern of variation is analogous to that observed for meteoric waters over North America.

From their studies, it seems that site conditions are important in determining the δD values recorded by trees and some sites can produce scatter in plots of annual temperatures against the tree δD values on a spatial scale. However, their work indicates that favourable sites are not uncommon.

The geographical temperature-tree δD correlation for seven widely separated North American trees has been shown to be remarkably consistent over the interval 1931-70. This

consistency is maintained inspite of the differences in both species and relative ages of the seven tree samples involved.

In another type of studies van der Straatten (1981) has measured the δD of CH hydrogens of cellulose from late woods of an American oak. He gets the following relationships:

$$\frac{d\ \delta D\ (\text{tree ring})}{d\ \delta D\ (\text{precipitation})} = +\ 0.7\ (r = 0.69)$$

$$\frac{d\ \delta D\ (\text{tree ring})}{d\ (\text{temperature})} = +\ 3.5\ {}^{o}/oo\ /{}^{o}C\ (r = 0.31)$$

He also finds a singificant anticorrelation between ring widths and δD values (-0.45). This anticorrelation has been explained as follows: It has been assumed that (i) The early wood is enriched in δD compared to the late wood and (ii) the variations in the ring widths are mainly due to annual variations in the amount of late wood. It is possible that while sampling the late wood got contaminated with early wood. This contamination might be more for the narrow rings than the broad rings.

But his conclusions are tentative because he has observed a large variation of δD within a single ring ($\sim$16 ${}^{o}/oo$). Also the two radial directions of his tree do not show parallel variations in δD. He has therefore emphasised the need for sampling the entire circumference of the tree for isotopic analysis.

More recently, Gray and Song (1984) have found that the δD of CH hydrogens in cellulose from trees from Edmonton show a temporal correlation with mean annual temperature at growth site. For three white spruce trees, they obtain

$$\delta D_{CN} = (7.3 \pm 2)t - (155 \pm 5)\ ^{o}/oo$$

$$\delta D_{CN} = (13 \pm 1)\ t = (156 \pm 4)\ ^{o}/oo$$

and $$\delta D_{CN} = (15 \pm 2)\ t = (165 \pm 8)\ ^{o}/oo$$

where t is temperature in ^{o}C.

Though the intercepts agree, the slopes differ. They attribute this difference to that in the δD values of the soil moisture used by these trees and the relative contributions of summer and winter precipitations to the soil moisture. These results are for 5 year blocks of rings covering a period of 70 to 80 years. They see that in all the three trees the younger rings (first 15 to 20 years) are more depleted in δD relative to the rest of the years and this difference is of the same magnitude as the overall δD variations shown by the mature rings. They explain this "juvenile" effect to be due to physiological factors controlling the tree growth.

There are a number of changes which occur in the structure and chemical composition of a cell wall associated with the development from juvenile stage to maturity. For

example the average cell length increases through the successive growth rings from the centre of the tree outward until a more or less constant value is reached. There are also changes in the chemical composition as the tree grows. For example, the fraction of cellulose increases through the juvenile growth period. Thus, as the tree ages, the wood formed becomes more uniform in structure and composition. Due to the isotopic "competition" between chemical constituents of wood, changes in structure and chemical composition may alter the isotopic composition of the wood giving rise to the difference in δD observed in mature and juvenile wood. However, this does not explain the lack of this effect with $\delta^{18}O$ values in the same trees.

They have also measured δD of nitrated cellulose from trees that grew in various sites in North America. Average value of δD obtained from tree ring series ranging from 15 to 60 growth rings (to eliminate seasonal and annual fluctuations), when regressed against mean annual temperature t, for the sites yielded a relationship

$$\delta D_{CN} = 6.0\ t - 130\ ^{o}/_{oo}$$

They combine their data with Epstein et al's (1976) data (after suitable corrections for the whole wood nitration method) and get a relationship

$$\delta D_{CN} = 5.5\ t - 130\ ^{o}/_{oo}$$

which compares well with the relationship

$$\delta D_W = 5.6\ t - 100\ ^{o}/oo,$$

which is the spatial relationship between δD of meteoric water and mean annual temperature. This slope of 5.5 $^{o}/oo$ per ^{o}C compares well with Yapp and Epstein's(1982b) slope of 5.8 $^{o}/oo$ per ^{o}C.

Biochemical fractionation factor

The biochemical fractionation (α_B) factor is defined as

$$\alpha_B = \frac{R_{CN}}{R_{SW}}$$

where R_{CN} = D/H in cellulose nitrate and R_{SW} = D/H in source water. In terms of δ notation, this relation can be approximated as $\varepsilon_B = \delta D_{CN} - \delta D_{SW}$ where $\varepsilon_B = (\alpha_B - 1)\ 10^3$.

The source water for terrestrial plants is the leaf water and for submerged aquatic plants is the environmental water. The temperature dependence of this biochemical fractionation factor has been determined by many workers.

Wilson and Grinsted (1975) estimate this to be -5 $^{o}/oo$ per ^{o}C, on the basis of eleven sections of two annual growth rings of Pinus radiata. However, their method of determination of δD is uncertain as discussed in section 2.2.2.

The most exhaustive study to date of ε_B values is that of DeNiro and Epstein (1981). From measurements of δD_{CN} and δD_{SW}, they calculate the ε_B values for different species of algae, tunicates (aquatic animals which form cellulose) and vascular plants growing in natural salt water habitats. The range of ε_B values are:

+ 50 °/oo to - 170 °/oo for algae

0 °/oo to - 100 °/oo for tunicates

0 °/oo to - 20 °/oo for vascular plants.

In addition, they have studied the effect of temperature on the value of ε_B for different species of fresh water vascular plants grown in controlled conditions. The ε_B values range from -4 °/oo to + 75 °/oo and have apparent temperature effects which vary from + 4 °/oo per °C to - 5 °/oo per °C. Even when considering only the results for vascular plants which are most comparable to trees in terms of their physiology, it is apparent that ε_B is highly variable between different plant species both in terms of its magnitude and temperature dependence.

Yapp and Epstein (1982a), using aquatic plants, give seven values of ε_B which range from -12 °/oo to -39 °/oo. The water temperatures in this case range from 15 to 27°C. These plants have been originally analysed by Epstein, et al., (1976), who cautiously note an apparent temperature effect on ε_B of about + 2 °/oo per °C.

DeNiro and Epstein (1979), (growing wheat plants in a controlled environment) provide ε_B values of -11 °/oo and -9 °/oo, which are consistent with the magnitude and possible temperature effect on ε_B, reported by Yapp and Epstein (1982a).

White (1983) has reported ε_B values ranging from -75 °/oo to -53 °/oo for white pines from eastern USA. The temperature coefficient in this case is + 1.6 °/oo per °C. These ε_B values are significantly lower than the values for vascular plants discussed earlier. However, these are the first reliable values to be calculated using tree-ring measurements.

van der Stratten (1981) has grown wheat and maize plants in controlled environments and found the temperature coefficients to be -1.39 $\pm$ 0.35 °/oo per °C for wheat and -1.45 $\pm$ 0.72 °/oo per °C for maize.

Whether different species of trees have different ε_B values and different temperature effects on this fractionation factor is not clear at present. (White, 1983). This is because while laboratory experiments show large variation in ε_B as outlined above, Epstein and coworkers, using a variety of species of plants and trees, have obtained fairly good correlations between δD_{CN} and temperature. A species effect in trees is all the more expected. This uncertainty must be considered when comparing δD values of different

species of trees.

Results on components other than cellulose

Apart from the cellulose fraction of wood, the saponifiable lipid fraction in the sapwood part of a California redwood (Northfelt, et al., 1981) has also been studied. The δD values of this fraction is found to be related to the δD values of the meteoric water used by the tree. This conclusion, however, is based on an indirect method, i.e., the δD values are compared with that of cellulose nitrate from the same rings and they are found to be linearly related. This result, must be viewed with caution because only 4 measurements have been made so far and possibility of diffusion of the saponifiable lipid fraction between rings is not ruled out.

2.4 Oxygen Isotopes in Tree Rings

2.4.1 Rationale behind the approach

The reason for expecting a climatic signal in $\delta^{18}O$ of plant cellulose is similar to that for δD, explained in section 2.3.1. Further, it is known that δD and $\delta^{18}O$ of meteoric water are linearly related (Craig, 1961) as

$$\delta D = 8 \ \delta^{18}O + 10$$

Consequently one can expect that $\delta^{18}O$ of plant cellulose might be linearly related to the $\delta^{18}O$ of precipitation, (as in the case of δD) which in turn is relatable to climatic temperature.

The mechanism of oxygen intake is simple in the case of aquatic plants, for which the only possible sources of oxygen for cellulose synthesis are the water and CO_2 dissolved in the aquatic medium. The dissolved CO_2 is almost certain to be in isotopic equilibrium with the aquatic medium because equilibration can take place in a matter of hours (Epstein, et al., 1977). Thus the $\delta^{18}O$ of the dissolved CO_2 varies directly as the $\delta^{18}O$ of the waters. It follows that the $\delta^{18}O$ of cellulose of aquatic plants should have an approximately one to one correlation with the $\delta^{18}O$ of the water, regardless of the oxygen isotopic fractionation that might be associated with the synthesis of cellulose.

Non-aquatic plants obtain their CO_2 from the atmosphere and the $\delta^{18}O$ of atmospheric CO_2 is not related to the $\delta^{18}O$ of meteoric water. Atmospheric CO_2 has a $\delta^{18}O$ value of about 41 $^{o}/oo$ and is nearly uniform isotopically throughout the world (Bottinga and Craig, 1969). Thus, the $\delta^{18}O$ of that part of the cellulose oxygen originating from the atmospheric CO_2 would not be sensitive to climate unless the CO_2 isotopically equilibrates with the water in the plant cells or there is a temperature sensitive oxygen isotopic fractionation

associated with the fixation of atmospheric CO_2. If some of the cellulose oxygen is derived from the environmental water, the $\delta^{18}O$ of the cellulose in terrestrial plants would be sensitive to climate, but to a lesser degree than cellulose in aquatic plants.

Another complicating factor in the terrestrial plants is the evapotranspiration of water through their leaves. This effect increases the $\delta^{18}O$ of water used for synthesis of cellulose compared to the meteoric water. In this section are described both the laboratory and field measurements carried out by earlier workers to investigate this problem.

2.4.2 Earlier results of laboratory and field measurements

Plant water relationship

For aquatic plants the $\delta^{18}O$ of plants and the water media are related by

$$\delta^{18}O_C = 1.027 \quad \delta^{18}O_W + 27 \ ^{o}/oo$$

as determined by Epstein et al.,(1977). For terrestrial plants they found that this relationship is complicated by evapotranspiration effects. δD and $\delta^{18}O$ of water extracted from leaves and pine needles of plants grown in a semi arid region in California show a linear relationship with a slope of 2.5, which is lower than 8, the slope of meteoric water collected throughout the world. This had led them to

the following conclusions: "Possibly, the major use of the $\delta^{18}O$ of terrestrial plant cellulose in climatic studies may be as an indicator of evapotranspiration, which itself is a function of relative humidity and the amount of rainfall in the growth area, rather than of temperature". They developed two models for the fixation of CO_2 in plants. One model (A) assumed isotopic equilibration of CO_2 with plant water and the other model (B) assumed no isotopic equilibration with leaf water. Significantly, both models assumed that during fixation of CO_2, one molecule of CO_2 is fixed along with one molecule of H_2O which was conceptually written as

$$\text{ribulose 1,5 di phosphate} + H_2O + CO_2 \rightarrow 2(\text{3-phosphoglycerate})$$

thus implying that 2/3 of the oxygen in cellulose comes from CO_2 and 1/3 from water. This equation, however, does not account for the evolution of oxygen by photosynthesis. This model contradicts the classical picture of photosynthesis. In a later paper, DeNiro and Epstein (1979) showed that for wheat plants, the oxygen in CO_2 is completely equilibrated with the oxygen of the water during synthesis of cellulose, by growing them with two isotopically different CO_2 gases and the same water. ($\delta^{18}O$ values of CO_2 was 127 $\pm$ 25 $^{o}/oo$ in one case and 979 $\pm$ 115 $^{o}/oo$ in another case whereas $\delta^{18}O$ of water was -8.2 $^{o}/oo$). A more detailed study of biochemistry involved in photosynthesis has led to the view that the oxygen in CO_2 is virtually the sole source of oxygen for

cellulose (Burk, 1979). But since this gets equilibrated with the leaf water completely, it is the $\delta^{18}O$ of leaf water which is reflected by the $\delta^{18}O$ of cellulose.

Ferhi, et al., (1979) have grown bean plants (Phaseolus vulgaris) in controlled growth chambers and studied the variation of the $\delta^{18}O$ of plant cellulose vis-a-vis the variations in temperature, humidity and $\delta^{18}O$ of water input to the plants. They did not see any significant effect of temperature on $\delta^{18}O$ of cellulose. They obtained the following empirical relationships.

$$\delta^{18}O \text{ (cellulose)} = 0.15 \quad \delta^{18}O \text{ (soil water)} + 26.68$$

and

$$\delta^{18}O \text{ (cellulose)} = -10.92\ h + 32.12$$

where h is the relative humidity. They have developed a model based on that of Craig and Gordon (1965) to explain the variations in $\delta^{18}O$ of plant cellulose with variations in $\delta^{18}O$ of the input water (δ_i), $\delta^{18}O$ of the atmospheric water vapour (δ_v), equilibrium (ε_e) and kinetic (ε_k) enrichment factors , leaf water $\delta^{18}O$ and humidity (h).

$$\delta^{18}O \text{ (cellulose)} = 0.376 \left[(1-h)\ \delta_i + \varepsilon_e + \varepsilon_k + h(\delta_v - \varepsilon_k) \right] + 23.56$$

This model seems to explain their results fairly well. They also argue, based on their results, that it is likely that CO_2

does not completely equilibrate with water in the leaf. This statement contradicts the conclusion of DeNiro and Epstein (1979). More controlled experiments should be done to check the validity of Ferhi et al's (1979) conclusions.

Tree rings and climate

$\delta^{18}O$ of cellulose extracted from fifteen segments of two annual growth rings of Pinus radiata that grew in New Zealand were determined by Wilson and Grinsted (1976). They found that the $\delta^{18}O$ of cellulose varied in the opposite direction to δD. They attributed this small variation ($\sim 3^{o}/oo$) to the changing $\delta^{18}O$ of the precipitation which nurtured the tree. They noted that cellulose oxygen was enriched in $\delta^{18}O$ by about 37 $^{o}/oo$ compared to the local precipitation.

Gray & Thompson (1976) have reported the $\delta^{18}O$ values of cellulose obtained from 5 year blocks of a white spruce (Picea glauca) which grew in Edmonton, Canada. They obtained a linear relationship between the $\delta^{18}O$ of cellulose and the mean annual temperature (September to August) t, as:

$$\delta^{18}O = (1.3 \pm 0.1)\ t + (20.5 \pm 0.2)\ ^{o}/oo$$

Since the temperature coefficient (1.3 $^{o}/oo$ per ^{o}C) was higher than that for ($\delta^{18}O$, t) relationship for meteoric water for this area (~ 0.3 $^{o}/oo$ per ^{o}C) by a factor of four, they

ascribed the $\delta^{18}O$ variation in cellulose to the changes in mean annual temperature at the site. Their correlation coefficient was 0.97. Subsequently, Gray and Thompson, (1979) analysed more trees from this area and did not find as high a correlation.

However, based on the analyses of $\delta^{18}O$ from 5 year blocks of tree rings and peat producing plants from the North American continent representing a diversity of climatic regions, they obtained a relationship of the form

$$\delta^{18}O \text{ (cellulose)} = 0.54\ t + 22.9\ ^{o}/_{oo}$$

where t is the mean annual temperature for the different sites from which the plants and trees were collected. The correlation coefficient is 0.98. (Gray and Thompson, 1979). This spatial correlation is similar to that observed for δD (discussed in section 2.3.2).

Such a spatial correlation has also been reported by Burk and Striver (1981):

$$\delta^{18}O \text{ (cellulose)} = 0.41\ t + 22.97 \quad (r^2 = 0.95)$$

This result is based on measurement of $\delta^{18}O$ of cellulose from a variety of species of conifers that grew at various latitudes (32.7°N to 64.8°N). This relationship compares well with that for precipitation from these areas:

$$^{18}O(\text{precipitation}) = 0.43\ t - 11.75$$

$$(r^2 = 0.98)$$

Burk (1979) has also demonstrated that the ^{18}O of cellulose from trees from various altitudes vary linearly with the ^{18}O of the precipitation (which also varies linearly with altitude). He has calculated a mean temperature lapse rate of $5.2^{\circ} \pm 0.5^{\circ}C$ per km, which agrees well with the accepted mean value of $5^{\circ}C$/km for the region (Mt. Rainier, Washington).

In addition, he noted that there was an intraring variation of ^{18}O of the order of $\pm$ 0.4 $^{\circ}$/oo in Douglas Fir and trees of the same species at the same site under similar conditions may vary in ^{18}O by about $\pm$ 0.5 $^{\circ}$/oo. Therefore, he has indicated cautiously that the conclusion are only first approximations and need further careful study.

Biochemical fractionation factor

The biochemical fractionation factor for ^{18}O

can be defined in a similar fashion to that for δD.

$$\alpha_B = \frac{1 + 10^{-3} \ \delta^{18}O \text{ (cellulose)}}{1 + 10^{-3} \ \delta^{18}O \text{ (source water)}}$$

where the source water is leaf water for terrestrial plants and environmental water for submerged aquatic plants.

Epstein et al (1977) have reported values of 1.027 and 1.028 for three different species of aquatic plants. DeNiro and Epstein (1979), from their growth chamber experiments on wheat plants, obtained a value of 1.028. From the tree ring measurements, Burk and Striver (1981) obtained values ranging from 1.026 to 1.027 for a temperature range of 9.2 to 21.2°C.

DeNiro and Epstein (1981) have measured the $\delta^{18}O$ of cellulose from marine plants (algae and higher vascular forms) and animals (tunicates) collected in their natural habitats and from fresh water vascular plants grown in the laboratory under controlled conditions. They have obtained α_B values of 1.027 $\pm$ 0.003 for marine specimens and 1.027 $\pm$ 0.002 for the fresh water vascular plants. Within the temperature range of 15° to 25°C, α_B was found to be constant for the fresh water plants. For marine plants, because of the uncertainty about the temperatures at which they grew, the

temperature dependence of α_B could not be ascertained. The available data thus suggest that the process which determines the oxygen isotopic relationship between the cellulose and water used in its synthesis are similar in all plants. However, the basis for this relationship is unknown. DeNiro and Epstein (1981) suggest that the isotopic exchange during hydration of the carbonyl groups of intermediates of cellulose synthesis might be a possible cause. To establish this, data on magnitude and temperature dependence of oxygen isotopic fractionation that occurs during carbonyl hydration reactions of intermediates of cellulose synthesis is needed.

Recent work by Sternberg and DeNiro (1983) offers support to this hypothesis. They found that the equilibrium isotopic fractionation between the carbonyl oxygen of acetone and water at 15°, 25° and 35° was 1.028, 1.028 and 1.026 respectively, very similar to α_B, the oxygen isotope fractionation factor between cellulose and the source water.

It is apparent from the relative constancy of α_B that it seems possible to reconstruct the mean leaf water $\delta^{18}O$ of trees from the measured $\delta^{18}O$ of cellulose. Knowing the $\delta^{18}O$ of the precipitation sampled by the tree, the difference

$$\delta^{18}O \text{ (leaf water)} - \delta^{18}O \text{ (precipitation)}$$

may be interpreted in terms of ambient temperature and humidity.

Another result of interest here is from DeNiro and Epstein (1981). They have compared δD and $\delta^{18}O$ of fresh water plants grown in the laboratory. They did not obtain a linear relationship between the two and have suggested the possibility that one or more of the factors which determine the isotopic ratios for one element (oxygen or hydrogen) operate independently of those which determine the isotopic ratios of the other element.

Results on components other than cellulose

Gray and Thompson (1977) have measured the $\delta^{18}O$ of whole wood and holocellulose fraction of wood from a white spruce in Edmonton area. They have calculated the $\delta^{18}O$ of lignin using mass balance. They have found that the lignin fraction is consistently depleted in $\delta^{18}O$ relative to the holocellulose fraction by about 10 $^{o}/_{oo}$. They noted no difference between the $\delta^{18}O$ of cellulose and hemicellulose fractions. While the $\delta^{18}O$ of cellulose correlated well with mean annual temperature, neither the whole wood values nor those of the lignin fraction showed any such correlation. However, they have not given any convincing explanation for this behaviour.

2.5 Carbon Isotopes in Tree Rings

2.5.1 Rationale behind the approach

Most of the trees fall under the C_3 category of plants, which fix the atmospheric carbon by the Calvin cycle

of photosynthesis. The typical value for the $\delta^{13}C$ of these trees is -27 °/oo with a range of -37 to -20 °/oo. In general, plants seem to preferentially intake ^{12}C compared to ^{13}C. The main source of carbon for the trees is the atmospheric carbon dioxide, which has a $\delta^{13}C$ value (at present) of about -7 °/oo (Wigley, 1982).

One can expect that the $\delta^{13}C$ record in trees might reflect the changing $\delta^{13}C$ of the atmospheric CO_2. On the other hand, it is possible that they track the changing CO_2 concentration in the atmosphere. It is also likely that the carbon isotope fractionation in trees might be influenced by changes in climatic parameters like temperature, rainfall, humidity etc., because these factors affect the trees metabolism. As will be seen in the following discussion, it is very difficult to delineate these effects from one another and give a unique interpretation for the $\delta^{13}C$ record in tree rings. Both controlled laboratory experiments and field measurements have been undertaken to study the various aspects of the problem.

2.5.2 Earlier results of field and laboratory measurements

Laboratory experiments were done mainly on non-woody plants. Growth chamber experiments on trees is cumbersome and time consuming. *In vivo* studies of carbon isotopic fractionation in non woody plants and, *in vitro* studies on

CO_2 fixing enzymes have been summarised by Long (1982). The temperature coefficients in the former case ranged from -0.046 to -1 $^{o}/_{oo}$ per ^{o}C, while in the latter case from -0.03 to + 1.2 $^{o}/_{oo}$ per ^{o}C. Hence no definite conclusions could be reached.

Freyer and Weisberg (1973) measured $\delta^{13}C$ of cellulose from 5 year blocks of rings from four free-standing trees grown at different sites on the French Atlantic coast for the period 1900 to 1970. The range was from -21 $^{o}/_{oo}$ to -24 $^{o}/_{oo}$. They have interpreted this general decrease as due to fossil fuel effect (the burning of coal, petroleum etc. (fossil fuels) introduce CO_2 into the atmosphere whose $\delta^{13}C$ is around -25 $^{o}/_{oo}$; thus due to the progressive input of this fossil fuel CO_2, the $\delta^{13}C$ of the atmospheric CO_2 decreases with time. This is termed as the fossil fuel effect) on which climatic influences are superposed. Six more trees were analysed (Freyer and Weisberg, 1974) and they found the trend to be similar.

Wilson and Grinsted (1977) have measured $\delta^{13}C$ of cellulose and lignin extracted from over three annual rings of Pinus radiata grown in New Zealand and found a similar pattern of variation in both the components, of the order of 2 $^{o}/_{oo}$ (though $\delta^{13}C$ of lignin was found to be about 3 $^{o}/_{oo}$ lighter than that of cellulose). They attributed this variation to the changing temperature (seasonal) and obtained a temperature coefficient of 0.2 $^{o}/_{oo}$ per ^{o}C.

To investigate whether this result could be applied to the study of past climate, they (Grinsted and Wilson, 1979a) measured the $\delta^{13}C$ of cellulose from a specimen of Agathis australis which grew during the last millenium in New Zealand. The short term fluctuations were found to be related to climate (when compared with Lamb's Central England Temperature curve and $\delta^{18}O$ variations of a New Zealand speleotherm). They attributed this "climatic effect" to (i) the temperature dependence of carbon isotopic fractionation during photosynthesis and (ii) the changes in $\delta^{13}C$ of atmospheric CO_2 induced by long term changes in sea surface temperature. However, MacIntyre (1979) has presented some arguments to show that no measurable carbon isotope redistribution occurs between sea and air for any plausible change in the sea surface temperature.

Grinsted, et al., (1979) have also measured $\delta^{13}C$ of two bristlecone pines from California which grew during the last 1000 years. The overall range observed was greater than 2 $^{o}/_{oo}$. The variations correlated well with Lamb's 50 year mean high summer temperatures in Central England. (Interestingly, the same samples were measured for the determination of δD by Epstein and Yapp (1976). No correlation was found between the δD and $\delta^{13}C$ variations). If we compare the results of Agathis australis and Bristlecone pine we note that the peaks in $\delta^{13}C$ curve for the former correlate with troughs (A, B & C) in the $\delta^{13}C$ curve for the

latter. This contrasting behaviour of the trees from both hemispheres is puzzling.

Arnold (1979) measured $\delta^{13}C$ in leaf cellulose isolated from living juniper twigs collected around the perimeters of several trees at each site, for nine different sites in Arizona where meteorological records were available. He compared the average $\delta^{13}C$ for each site with the average spring temperature and precipitation and found an excellent correlation with a function involving both parameters. Long (1982) has claimed that based on this study $\delta^{13}C$ of any juniper site in Arizona should be predictable from growing season temperature and precipitation measurement with an accuracy of $\pm$ 0.1 $^{o}/oo$, while temperature alone gives a predictability of about $\pm$ 0.5 $^{o}/oo$. In another study, Leavitt and Long (1983a) found that $\delta^{13}C$ of cellulose in modern Pinus edulis leaves sampled from a variety of locations showed a linear response (with less correlation) to elevation and climate, though the coefficients were similar to those found for juniper leaves. Second order polynomial relationships of $\delta^{13}C$ with elevation and temperature, however, emerged significant.

While various workers have interpreted the $\delta^{13}C$ variations either in terms of temperature effects or in terms of $\delta^{13}C$ changes in the atmosphere, factors described below tend to complicate this interpretation.

Intraring variations

In the same annual ring, variations of the order of 1 °/oo along the circumference have been observed (Freyer and Weisberg, 1974; Mazany et al., 1980; Francy, 1981). Also along the radial direction, between the early and late woods a difference of about 2 °/oo has been seen (Wilson and Grinsted, 1977). Tans and Mook (1980) have reported circumferential variations as large as 4 to 5 °/oo, but in their study circumferential patterns of consecutive years correlates well. Harkness and Miller (1980) noted an intraring "noise" of ± 0.4 °/oo.

Juvenile effect

The rings corresponding to the first few years of growth have been found to be depleted in ^{13}C relative to the rest of the rings (Craig, 1954; Jansen, 1962; Freyer and Weisberg, 1973; Freyer, 1979; Harkness and Miller, 1980; Francey, 1981; Freyer and Belacy, 1983). This effect is explained by some authors as follows. The atmosphere near the ground in a forest can be depleted in ^{13}C. This is because of the root and mycorrhizal respiration utilizing photosynthates which are depleted with respect to the atmosphere. Therefore young plants growing in such a situation (and hence the rings near the centre in trees) are depleted in ^{13}C. Once the tree reaches maturity, the foliage

values vary diurnally as well as seasonally and are more negative in times and places of greater plant respiration. For example, the respired CO_2 atmosphere of very dense forests have a $\delta^{13}C$ as low as -39 °/oo. Trees growing in such forests, therefore, may not sample the free atmosphere and may have less $\delta^{13}C$ values than free standing trees.

Pioneer effect

During the later half of the nineteenth century, there was rapid development of agriculture in the new lands of North America, New Zealand, Australia, South Africa and in Eastern Europe. This "pioneer revolution" had hardly begun in 1850, yet large areas had been clared and/or ploughed by 1900. The change was almost synchronous all over the world and has led to the release of very large quantities of CO_2. Like the fossil fuel CO_2, this is also depleted in $\delta^{13}C$ having a value of about -25 °/oo. By measuring the $\delta^{13}C$ of a bristlecone pine Wilson (1978) has estimated that due to this "pioneer" effect, the $\delta^{13}C$ of the atmosphere changed by 0.9 °/oo and the CO_2 concentration changed by about 10% in the late nineteenth century.

This additional CO_2 from forest clearing and soil organic reservoir is indistinguishable isotopically from the fossil fuel CO_2 except by ^{14}C activity measurements (fossil fuel has no measurable ^{14}C activity). One needs to be careful therefore in interpreting $\delta^{13}C$ records of the

trees from 1850 to the present.

This effect has also been seen by Farmer and Baxter (1974) and Stuiver (1978).

Ring width effect

Craig (1954) noted that many narrow rings of Sequoia gigantea were depleted in ^{13}C relative to broad rings. Freyer and Weisberg (1974) also observed a positive correlation between ring width and $\delta^{13}C$ whereas Bender and Berge (1982) did not observe any correlation between the two. Grinsted and Wilson (1979a) have reported that they did not find any relationship between $\delta^{13}C$ and ring width. But inspection of their data show that even when "only the peaks and troughs" are compared, there is a good correlation from 900 to 1350 A.D. and there is a good anticorrelation from 1350 to 1900 A.D.

Tans and Mook (1980) have found a significant anticorrelation (-0.3) between $\delta^{13}C$ variations and ring width variations in the case of Dutch trees. Mazany et al., (1980) observed significant anticorrelations (-0.8 and -0.65) between $\delta^{13}C$ variations and regional tree ring width indices for white fir and ponderosa pine from New Mexico. However, the correlation between $\delta^{13}C$ and the ring width of the individual trees were found to be insignificant. They have explained this effect as follows. When climate is favourable for growth, the rings are broad. As a result of increased plant

productivity, the plant respiration is more and the resultant CO_2 depleted in $\delta^{13}C$ is sampled by the tree and the $\delta^{13}C$ of the broad rings becomes less. However, this explanation has been questioned by Francey and Farquhar (1982) on the basis that the respired CO_2 has been found to rapidly mix with the atmosphere and consequently significant modification of the local $\delta^{13}C$ may not take place.

Another interesting explanation for the anticorrelation between ring width and δ values has been provided by Wigley, et al., (1978). If δ_W is the $\delta^{13}C$ value of the whole ring, δ_L that of the late wood, δ_E that of the early wood and W, L and E the respective widths of the three, then by using mass balance we can write

$$\delta_W = \delta_E + (\delta_L - \delta_E)L/W$$

$$\text{or } \delta_W = \delta_L - (\delta_L - \delta_E)E/W$$

In many species of trees, much of the variation in ring width is in either the early wood or the late wood part of the ring. Depending on whether early wood is lighter or heavier in $\delta^{13}C$ relative to the late wood, one may find an anticorrelation or correlation provided the late wood is approximately constant, as in the case of most conifers. Wigley et al., (1978) further suggest that variations in whole ring isotopic data may be significantly influenced by the ring width variations and such isotopic data need not be independent climatic indicator.

It should be noted that the "distinctive" things about early wood and late wood are physical in nature and these physical differences provide a convenient choice to use them as guides for subdividing rings for isotopic analysis (Epstein, et al., 1976). In fact, the physical traits of early wood and late wood grade into one another, albeit sometimes with a large gradient (Fritts, 1976). The same appears to be true of intra ring isotopic variations (Wilson and Grinsted, 1977). The apparent two component isotopic nature of early wood and late wood is more an artifact of the imposed two-component sampling method than anything else. Hence, the assumptions made by Wigley, et al., (1978) are either unproven or contradicted by available data (Yapp, 1980).

In order to minimise the effects described in the preceding paragraphs, a large number free standing trees have to be analysed for obtaining the $\delta^{13}C$ trend. This has been done by Freyer and Belacy (1983), for about 50 Northern Hemisphere trees. While forest trees show large non-systematic behaviour, free standing trees show smaller fluctuations relatable to climate in the past 500 years. After industrialization in 1850 A.D., the $\delta^{13}C$ records of these trees show a systematic decline in the $\delta^{13}C$ trend of about 2 $^{o}/oo$. The experimentally measured decline in $\delta^{13}C$ of atmospheric CO_2 from 1956 to 1978 A.D. by Keeling, et al., (1979) has been observed in the $\delta^{13}C$ record of about 50 Northern Hemisphere

trees. Interestingly even a free standing pine shows juvenile effect and intra ring variations.

Francey (1981) has reported the $\delta^{13}C$ values of seven trees from Tasmania and they do not show a decreasing trend in $\delta^{13}C$ as opposed to Northern Hemisphere trees. He, therefore, has questioned the interpretation of the tree $\delta^{13}C$ record based on global atmospheric behaviour and suggested that a solution must be sought in the mechanism of fractionation in plants.

Recently, Francey and Farquhar (1982) have proposed a simple model for the carbon isotope fractionation in C_3 plants, which seems to explain the various available data satisfactorily. According to this model,

$$\delta^{13}C_p = \delta^{13}C_a - a - (b-a)\, C_i/C_a$$

where

$\delta^{13}C_p$ is the $\delta^{13}C$ of the plant

$\delta^{13}C_a$ is the $\delta^{13}C$ of the atmospheric CO_2

C_a is the CO_2 concentration in the atmosphere

C_i is the CO_2 concentration in the leaf intercellular space

a is the difference between the diffusivity of $^{13}CO_2$ and $^{12}CO_2$ in air (~ 4.4 $^{o}/oo$)

b is the biochemical fractionation factor (~ 30 ‰).

Also if A is the assimilation rate of CO_2 in the leaf and g is the conductance of CO_2 in the boundary layer and stomatal pores,

$$C_i = C_a - A/g$$

Therefore, factors which reduce CO_2 assimilation rate (A) through effects primarily on the mesophyll capacity for photosynthesis (e.g. very low light intensities and deficiency of certain mineral nutrients) will increase C_i/C_a and reduce $\delta^{13}C_p$; factors which reduce CO_2 assimilation rates primarily through the reduction of supply of CO_2 through the stomata will decrease C_i and increase $\delta^{13}C_p$. (e.g. large vapour pressure deficits). Also in some situations a change in an external parameter may affect both A and g with no marked effect on C_i/C_a and thus $\delta^{13}C_p$ (Farquhar, 1980).

The seasonal variations (of 1 to 2 ‰, Wilson and Grinsted, 1977) can be explained by observed seasonal changes in C_i. The juvenile effect (which has been observed even in the case of free-standing trees, thereby casting doubts on the explanation based on cyclic enrichment of ^{12}C through plant and soil respiration) can be explained in this model as due to changes in A and g through shading in the case of forest trees and due to external factors affecting

the mesophyll capacity for photosynthesis, which could vary during early tree development, in the case of free standing trees.

The observed anticorrelation between ring width and $\delta^{13}C$ values can be explained using this model as follows: In general tree rings are broad in wet cool years and narrow in warm dry years. Water stress can cause stomatal closure and reduced CO_2 uptake. Low humidity has been shown to reduce g and C_i, so that in conditions of high temperature and low precipitation we could expect narrow rings, lower C_i and hence higher $\delta^{13}C_p$.

The intra ring variations can be explained as due to variations in stomatal conductance g and assimilation rate A because of difference in structure and shading in leaves. This, however, does not explain the intra ring variations in free standing trees.

The absence of declining trend in $\delta^{13}C$ in Tasmanian trees can also be explained using this model. The fossil fuel release increases C_a and decreases $\delta^{13}C_a$. Increasing C_a would increase $\delta^{13}C_p$ when all other parameters are constant while decreasing $\delta^{13}C_a$ would decrease $\delta^{13}C_p$ when all the other parameters are constant. A cancellation of the two effects in certain situations is not unlikely.

There are many complexities in the uptake and use of CO_2 in a tree which must be better understood before the

model for fractionation to explain the tree records can be used with confidence. Francey and Farquhar's (1982) model illustrates a plausible scenario and if true, can be used for probing physiological behaviour in trees over long time scales.

Tans and Mook (1980) have interpreted their $\delta^{13}C$ record from Dutch trees in terms of past biospheric growth and shrinkage (after suitably adjusting the data for climatic and fossil fuel effects). Leavitt and Long (1983) have reported a similar study for juniper trees from Arizona, and found that the biosphere acted as a CO_2 source till about 1965 but has become a sink afterwards. However, they cautiously note that other interpretations cannot be excluded because of the noise associated with their reconstruction of the atmospheric $\delta^{13}C$. Peng, et al., (1983) have used Farmer & Belacy's (1983) $\delta^{13}C$ record for northern hemisphere trees and using model calculations, have deconvoluted the tree ring based $\delta^{13}C$ record. They have obtained interesting results on the history and magnitude of forest soil (CO_2) reservoir changes over the past 150 years. They have concluded that the data in hand are still not adequate to give a firm answer to the question of the magnitude of the forest-soil contribution to the build up of atmospheric CO_2.

2.6 Experimental Strategy for the Present Work

In most of the earlier work many of the complicating factors affecting the isotope-climate relationship have not received due attention. While excellent spatial correlations between δ values and one or more of climatic parameters have been demonstrated, this does not help much in reconstructing the palaeoclimate of a given region. In a few studies which attempted to do this, the results are inconclusive because of the following factors.

Firstly bunches of five to ten rings have been analysed thereby averaging year to year variations. Secondly the station from which the weather records have been obtained to compare with the tree ring record has been far away (as much as 500 km) from the actual growth site. Because large differences in the microclimate between two regions even 50 km away cannot be ruled out, this could cause an additional uncertainty. Thirdly, large intra ring variations cast doubt on the validity of interpretations of results based on a single radial strip of a tree. Since various workers have chosen different species of trees, it is difficult to compare the results. Fourthly, it is important to ascertain whether the isotopic record obtained from a single tree is a representative record of the many trees of the same species in the site. The amount of coherence between the records of different species of trees in a site have to be ascertained. Only recently such efforts are being made (see, for example, Leavitt and Long, 1984).

Fifthly, while seeking a correlation with climate, only one or two parameters (temperature and in a few cases rainfall or humidity) have been tried out. It is likely that the various climatic parameters like temperature, rainfall, humidity, wind velocity and cloud amount (affecting solar insolation) affect the tree ring record in a combined way. Only multiple regression studies could reveal the relative importance of these variables. Such studies have not been done till todate.

Sixthly, in most of the earlier work proper emphasis on appropriate site selection with a view to maximise the climatic signal has been seriously lacking.

Finally, a simultaneous study of all the three elements (hydrogen, carbon and oxygen) in tree rings which could lead to the resolution of various competing effects has not been undertaken so far.

The experimental strategy for the present work was planned bearing in mind the above considerations. The trees were selected from the Gulmarg area which is free from local and global pollution effects. The selected site was from a slope on a hilly terrain far away from permanent sources of water including ground water. Thus the only water sampled by the trees is that of the precipitation. Trees were selected at random and without any bias. Details of the trees selected is given in table 1.1. The meteorological station was at

a distance of about 8 km. Continuous records of temperature, precipitation, humidity, wind velocity and cloud amounts were available from 1903 to 1960. Individual rings for the years 1903-1932 were analysed for this work. δD, $\delta^{13}C$ and $\delta^{18}O$ were measured using standard procedures discussed in section 2.2. This particular choice of the years were from two considerations. Firstly, a continuous meteorological record was available for this period. Secondly, rings were quite broad in this period (2 to 11 mm) thus could be separated easily without cross ~~examination~~ contemination. The first 10 to 20 rings were not analysed because of possible juvenile effects.

Three radial directions of a tree (AP#2) were analysed for δD and $\delta^{13}C$ and two radial directions were analysed for $\delta^{18}O$. δD and $\delta^{13}C$ of another tree of the same species (Abies pindrow # 3) were analysed for the same period.

Early woods of 4 trees from the same site (within a radius of about 50 m) were analysed for the δD and $\delta^{13}C$ for ten years (1923-1932). Again, the broad rings were chosen (mean $\sim$ 6.2 mm) to enable easy separation of early wood.

Finally, maximum precautions were taken during the chemical preparation of cellulose, its subsequent nitration, gas extraction and mass spectrometric measurements to ensure that the measurements were precise. Inter-laboratory comparisons were made to check for possible systematic errors.

The experimental results are presented in the next chapter.

CHAPTER 3

EXPERIMENTAL TECHNIQUES

3.1. Introduction

Very few laboratories in India are involved in stable isotope research. Our laboratory (PRL) was the first to start research programs in this field. The stable isotope laboratory in PRL started functioning since 1980.

Most of the experimental procedures involved in isotope dendroclimatology have been perfected only in the past three years, as discussed in section 2.2. This called for a great deal of effort on my part to be continuously in touch with laboratories abroad and develop and perfect these techniques independently in our laboratory.

This chapter deals with the experimental procedures employed in the present work. The description is in six parts:

(i) Separation of wood from annual growth rings and isolation of alpha cellulose.

(ii) Nitration of the alpha cellulose.

(iii) Extraction of hydrogen and carbon dioxide from cellulose nitrate for δ D and δ ^{13}C analyses.

(iv) Extraction of carbon dioxide from cellulose for δ ^{18}O measurements.

(v) Mass spectrometric measurements, their analytical precision and reproducibility.

(vi) Interlaboratory comparisons of standards.

3.2. Separation of Wood from Rings and Isolation of Alpha Cellulose

Tree ring samples for this work were collected from the field in the form of discs. Discs were shipped to PRL where radial strips were cut out from these discs along the selected directions. These strips were polished, rings counted and corresponding years ascertained by counting the rings and knowing the date of felling. These dates were later verified using the master chronology provided by Dr. M.K.

Hughes of Liverpool Polytechnic, U.K.

Subsequently, using a scalpel, hammer and chisel, individual growth rings for the years of interest were separated. Care was taken not to contaminate wood of one year with that of the next. In cases where only earlywood was sampled, the light coloured portion of the ring was chipped out. Again, the dark coloured late wood contamination was eliminated by leaving out a little portion of the early wood along with the late wood. This could be done only when the rings were wide enough (.3 to 1 cm), so that enough sample could still be recovered. Thus, the widths of the rings were one of the factors which decided the time span for which measurements were carried out.

Subsequent to the separation of whole ring or early wood from each growth ring, the wood chips were powdered in a Wiley mill. The mill was thoroughly cleaned between powdering of two consecutive samples to avoid possible cross contamination. The 40-60 mesh size fraction was sieved out and stored in plastic vials with screw cap for further treatment.

For the extraction of cellulose, wood samples were processed in batches of nine. About 500 mg of the wood powder was kept immersed in a 1:1 mixture of pure benzene and methanol (ANALAR grade) for 24 hours. This was followed by a similar treatment with acetone (ANALAR grade) for 24 hours. This extraction process removes all the extraneous compounds in

wood which are soluble in organic solvents, like resins, gums and lipids. Out of the three species of trees taken for study viz., Abies, Pinus and Cedrus, Abies was found to be almost resin free and extraction for one or two hours was found adequate. The other two species were highly resinous, needing more extensive pretreatment with the organic solvents. The solvents were replenished after every eight hours. After extraction, the wood samples were boiled in 50 ml of water for two hours to expand their structure and remove water-soluble impurities.

The subsequent delignification procedure followed was that of Green (1963a). The wood powder suspended in water was heated and kept at a constant temperature of 70°C. To this about 0.1 ml of ANALAR grade glacial acetic acid was added, followed by 150 mg of pure sodium chlorite. Chlorine dioxide evolved, which bleached the wood by removing the lignin into solution. Continued additions of acetic acid and sodium chlorite at every hour for six hours left the wood completely free of lignin (The cellulose thus obtained, known as the holocellulose is a mixture of alpha cellulose and hemicelluloses). The heating was terminated, the solution filtered through fritted glass funnels and washed thoroughly with distilled water.

To remove the hemicellulose from the holocellulose, the sample was then treated with 17% sodium hydroxide solution

for about an hour. The hemicelluloses got dissolved in this process leaving a relatively pure compound called the alpha cellulose. This might contain trace amounts of hemicelluloses which are not removable by sodium hydroxide treatment. The solution was filtered again through fritted glass funnel, and the sample washed with distilled water several times. The sample was then freeze dried and stored in a plastic vial with screw cap in a dessicator.

3.3. Nitration of Cellulose

The procedure followed was that of Green (1963b). Phosphorous pentoxide (121.2 g) was slowly added to cold 90% fuming Nitric acid (300 g) (Sp. gr. 1.5) in a flask kept in an ice bath. The acid mixture was continuously stirred by means of a teflon-coated magnetic stirrer. This took about 6 hours. The final composition of the mixture was 64% nitric acid, 26% phosphoric acid and 10% dissolved phosphorous pentoxide. This amount of acid mixture ($\simeq$ 200 ml) was used for every batch of 10 cellulose samples.

About 100 mg of pure dry alpha cellulose was added to about 20 ml of acid mixture, stirred and kept at $5^{o}C$ for 12 hours in a refrigerator (The individual beakers were covered with glass plates and the batch of 10 beakers was kept in a crystallising dish with a tight lid). The solution was then filtered, washed with distilled water and freeze-

dried. This dry material was then dissolved in 200 ml of ANALAR grade acetone and precipitated by adding a large amount (2 litres) of cold water. The white fluffy precipitate of cellulose nitrate was pressed into a compact square shape using metallic tongs and freeze-dried after washing with methanol. The dry cellulose nitrate samples were then stored in plastic vials with screw caps in a dessicator.

As has been explained in section 2.2, it is important to check the degree of nitration of the cellulose. When all the exchangeable hydroxyl hydrogens of cellulose are replaced by NO_2 groups from the acid, the cellulose is fully nitrated and the degree of nitration is 3. The nitrogen content of the cellulose nitrate sample then, should be 14.14%. But in general such a high nitrogen content is not obtainable by the usual methods for nitration. Some amounts of hydroxyl hydrogens are still left unreplaced by the nitrate groups or during the washing of the cellulose nitrate, the nitrates are again replaced by hydrogens from the water. Such under-nitration, when limited to small amounts, have been found not to affect the reproducibility of the δ D measurements of cellulose nitrate (Yapp, 1980; DeNiro, 1981). The accepted minimum value for the nitrogen content is 13.2% (DeNiro, 1981). Our nitration procedure yielded nitrogen contents which were consistently within the range of 13.3 to 14.0%.

3.4. Extraction of Hydrogen and Carbon Dioxide from Cellulose Nitrate

For δD and $\delta^{13}C$ analyses, hydrogen and carbon dioxide were extracted from cellulose nitrate samples by a procedure developed by Stump & Frazer (1973) and modified by Northfelt, et al., (1981).

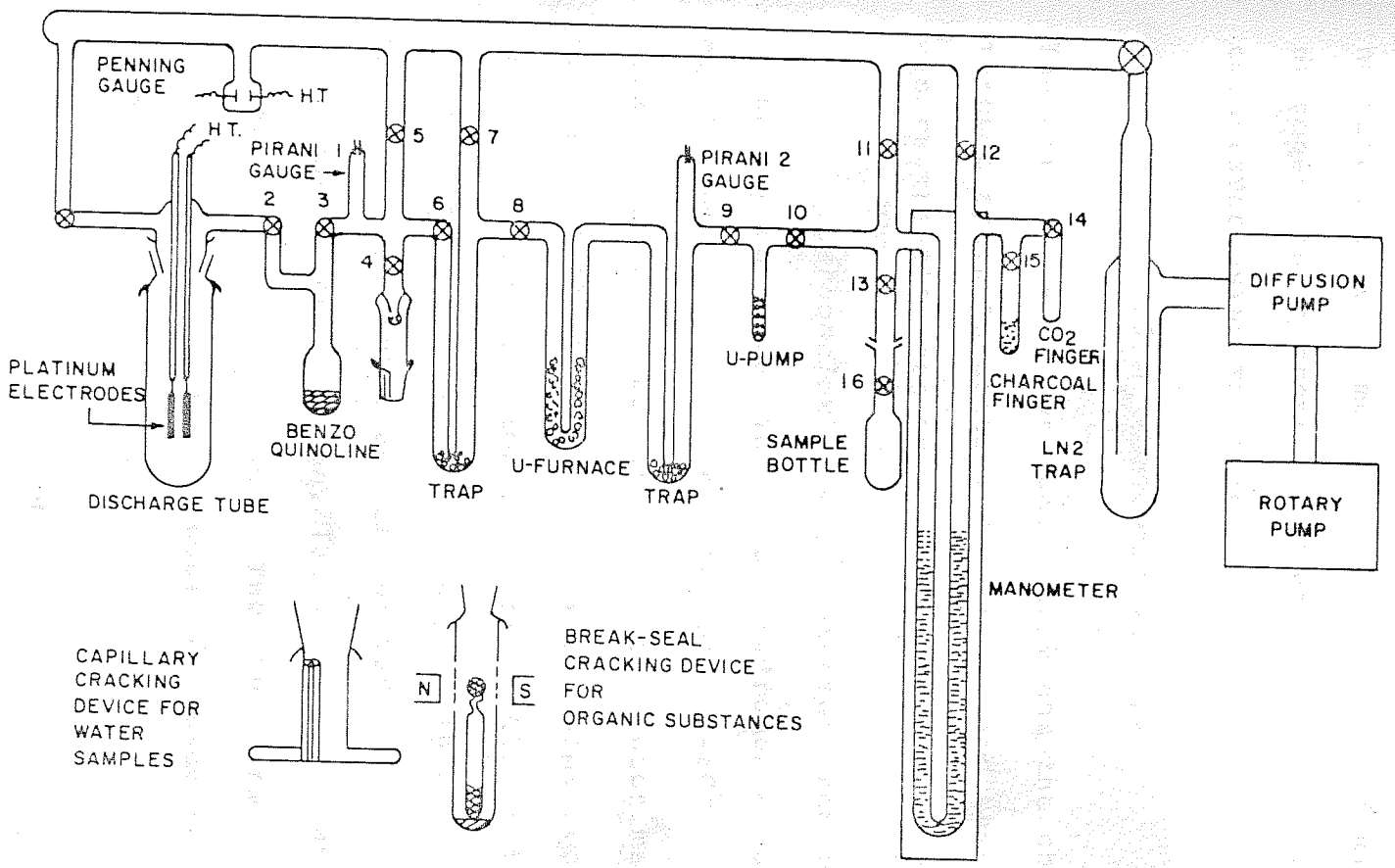
The important considerations were that the extraction of the gases must be non-fractionating, quantitative and without any contamination of hydrogen and carbon from extraneous sources like atmospheric water vapour and organic carbon that might possibly be associated with the chemicals used. To ensure this, the quartz tubes (10 mm OD, 8 mm ID, 10 cm long) with break seal tips were preheated for 3 hours in a furnace at 650° C, before the samples were loaded in them (The furnace, which was specially designed and fabricated for this purpose in our laboratory, was capable of maintaining the temperature within $\pm$ 5° C at the set value). A stainless steel jacket which could accommodate eight such tubes at a time, enabled the uniform heating of the tubes.

Many local brands of cupric oxide were tried out and found unsatisfactory for this work. Pure E-merck grade cupric oxide, imported from Germany, was used. This CuO, in wire form, was baked at 600°C to remove any traces of organic carbon that might be present.

About 25 mg of pure dry cellulose and about 1 g of CuO were taken in the quartz tubes and evacuated to less than 10^{-4} torr to remove any possible contamination due to atmospheric water vapour, which the cellulose nitrate might had absorbed subsequent to its removal from the dessicator. The desorption of this water was facilitated by heating the samples by a hot air gun to about 70°C.(Evacuation for 6 hours).

The tubes were than sealed off and put in the furnace at 650°C for 6 hours. Batches of eight samples were processed at a time. Weight of each sample was noted accurately (to 0.1 mg) to calculate its percentage carbon and hydrogen contents.

The gas extraction line designed and fabricated in our laboratory for separation and purification of gases is shown in fig. 3.1. The sample tube, with the combustion products was contained in a glass tube connected to the line, with a stainless steel ball resting on the tip of the break seal. After evacuation to less than 10^{-4} torr, the seal was broken and the carbondioxide and water were condensed in the U tube with liquid nitrogen around it (-196°C) and the nitrogen (one of the combustion products) absorbed in charcoal kept at -196°C in a quartz finger. After the complete absorption of nitrogen, the charcoal finger was torched and the pressure of the released nitrogen measured using the pre-calibrated mercury manometer. Knowing w , the weight of the cellulose



GAS PREPARATION LINE FOR ISOTOPIC ANALYSIS OF WATER AND ORGANIC SAMPLES

Fig. 3.1 : Schematic diagram of the gas preparation system.

nitrate sample in mg and n, the number of micro moles of nitrogen, the nitrogen content of the cellulose nitrate was calculated using the formula

$$\% \ N_2 \text{ content} = 2,8 \ n/w$$

The nitrogen was then pumped away and the charcoal finger torched again to degas the charcoal and keep it ready to absorb the nitrogen from the next sample.

Carbondioxide was separated from the water in the U tube by replacing the liquid nitrogen around it with an alcohol-acetone slurry maintained at $-80^{\circ}C$. The carbon dioxide was collected in the glass finger meant for this purpose and the amount of CO_2 was manometrically measured. Again, the complete condensation of CO_2 in the glass finger was monitored by the Pirani gauge. (This pirani gauge was designed, fabricated and calibrated in our laboratory, Bhattacharya and Gopalan, 1981). If n was the number of micromoles of CO_2 produced, then the carbon content of the cellulose nitrate samples could be calculated using the formula

$$\% \ C \text{ content} = 1.2 \ n/w$$

(the theoretical value for cellulose nitrate is 24.24%). The CO_2 was then collected and stored in leak tight O ring tapped glass bottles for $\delta^{13}C$ measurement.

The uranium furnace used for the reduction of the water fraction to hydrogen was degassed at 800°C for one hour for each set of eight gas extractions. The uranium finger which served as a pump for hydrogen was also degassed in between two gas extractions at 700°C.

The water fraction was passed through the uranium filings, kept at 800°C. The hydrogen produced was quickly absorbed by the uranium in the uranium finger, kept at 50°C. When most of the hydrogen had been absorbed the remaining water in the U tube is driven through the uranium furnace by torching the U tube to about 150°C. This, combined with the rapid absorption of the hydrogen by the uranium pump forced the reaction to completion, as monitored by the pirani gauge.

Subsequently the hydrogen was liberated from the uranium pump by heating it to 700°C. When the hydrogen was completely liberated, its amount was manometrically measured as n micromoles. The hydrogen content of the sample could be calculated using the formula

$$\% \text{ H content} = 0.2\ n/w$$

(theoretical value for fully nitrated cellulose = 2.36%).

Only 67% of the hydrogen produced was collected and the rest pumped out subsequently while the uranium pump was still maintained at 700°C. It was found important to allow

5 to 10 minutes between the total liberation of hydrogen and its collection in the bottle to ensure isotopic homogeneity between the gas produced and the gas collected. Memory effects could be minimised by keeping the extraction line and uranium pump clean.

Since the molecular weights of cellulose nitrates prepared from plant materials vary, it was preferable to report % C, H and N contents rather than yields of CO_2, H_2 and N_2 as suggested by DeNiro (1981; personal communication, 1982). The calculation of yields involved the assumption that the molecular weight of cellulose nitrate was 297, whereas the % C, H, N could be calculated based entirely on the weight of the sample and the amount of gas produced.

Usually 6 samples and a standard (also cellulose nitrate) were processed in a set. The reproducibility of the % C, H, N, δD and $\delta\ ^{13}C$ of this standard acted as a check on the overall procedure. Blanks were measured periodically and were found to be less than a micromole compared to typical gas contents of 300 micromoles for H_2 and 500 micromoles for CO_2. Nitrogen content of each of the cellulose nitrate samples processed was measured and ranged from 13.3 to 14.0%. It has been observed that as long as the N_2 content of the cellulose nitrate is at least 13.2%, there is no significant difference between the δD values of completely nitrated and undernitrated cellulose (DeNiro, 1981).

3.5. Extraction of CO_2 from Cellulose for $\delta^{18}O$ Measurements

The method followed was that of Rittenburg and Ponticarvo (1956) as modified by Burk (1979). About 10 mg of pure dry cellulose along with 375 mg of mercuric chloride was taken in a quartz tube with a break seal trip. After evacuation to less than 10^{-4} torr for 6 hours, this tube was sealed and kept in a furnace at 550°C for 6 hours, at the end of which the cellulose was completely pyrolysed to C, CO, CO_2 and HCl.

The tube was then broken in vacuum using the line shown in fig. 3.1 and CO_2 and HCl were frozen in the U tube with liquid nitrogen. The carbon monoxide which did not freeze was slowly allowed into the discharge chamber, to convert it to carbon dioxide.

The lower portion of the discharge tube was surrounded by liquid nitrogen so that the CO_2 condensed, thereby forcing the reaction to completion, as monitored by the pirani gauge.

The discharge chamber was made of a glass tube (4.5 cm dia) with platinum electrodes (3 cm x 1.5 cm x .1 mm) connected to a high voltage transformer (primary 220V, secondary 12 kV, 20 mA) by insulated nickel wires. The input voltage to the transformer was controlled by a 15A, 220V variac to get appropriate output voltage. When small amount of CO was let into the tube, the voltage was gradually

raised to 1 kV. The discharge started with a characteristic blue colour. As the discharge died down, the voltage was further increased. Thus the discharge was continued till there was no more discharge at about 4 kV. More CO was let in and the same procedure was followed repeatedly till the discharge became intermittent and ceased ultimately. The pressure at this stage was less than 10^{-2} torr. The entire discharge process took about half an hour.

Some CO got absorbed in the carbon deposit on the walls of the tube, kept at -196°C. It was necessary to remove the liquid nitrogen trap and heat the tube to release the CO and do the discharge again. The carbon deposit on the walls of the tube was cleaned between two consecutive samples. In a later improved version the discharge chamber was a spherical bulb of dia 12.5 cm provided with a cold finger.

When all the CO was converted to CO_2, the mixture of CO_2 and HCl in the U tube was transferred quantitatively to the tube containing reagent grade benzoquinoline, by replacing the liquid nitrogen around the U tube with an acetone-alcohol slurry maintained at -80°C. After all the CO_2 and HCl was collected, the benzoquinoline was gently heated to 90°C, when it melted and absorbed the HCl. The tube containing benzoquinoline was allowed to cool for about 15 minutes and then the slurry was kept around the tube. The CO_2 was then transferred to the CO_2 finger, and the quantity of CO_2

produced was measured manometrically, as n micromoles

$$\% O = 3.2 n/w$$

(theoretical value for cellulose = 49.38%).

The CO_2 was then collected and stored for $\delta^{18}O$ measurement as described earlier. The carbon deposit on the platinum electrodes was cleaned in between samples by torching them to red heat in air. The benzoquinoline was melted, cooled to -80°C and pumped in order to degass it. Every batch of benzoquinoline (replaced after 7 samples) was initially melted and degassed several times to ensure its purity. In between two consecutive samples also this degassing was done.

Again, 6 samples and a standard (also cellulose) were processed in a set and the reproducibility of O% and $\delta^{18}O$ of the standard cellulose acted as a check on the overall procedure. Blanks were measured periodically and found to be less than a micromole compared to typical gas contents of 170 micromoles.

3.6. Mass Spectrometric Measurements, Analytical Precision and Reproducibility

$\delta^{13}C$ and $\delta^{18}O$ were measured using a VG micromass 602D mass-spectrometer, relative to a tank CO_2 which served as an internal standard.

This tank CO_2 was periodically calibrated with CO_2 prepared from NBS-20 calcium carbonate standard by reaction with 100% H_3PO_4 at 25°C. Knowing the $\delta^{13}C$ and $\delta^{18}O$ of the NBS-20 standard relative to PDB, the δ values with respect to PDB were calculated. All $\delta^{13}C$ values reported in this thesis are relative to PDB standard.

$$\delta^{13}C^{X}_{PDB} = \delta^{13}C^{X}_{TC} + \delta^{13}C^{TC}_{PDB} + 10^{-3}\ \delta^{13}C^{X}_{TC}\ \delta^{13}C^{TC}_{PDB}$$

where X = sample, TC = Tank CO_2.

In this case $\delta^{13}C^{TC}_{PDB} = -21.96‰$, a value close to the $\delta^{13}C$ values of the samples measured.

The tank CO_2 was also calibrated by CO_2 equilibrated with V-SMOW water standard at 25°C and all $\delta^{18}O$ values reported in this thesis are relative to V-SMOW (Gonfiantini, 1984). In this case, $\delta^{18}O^{TC}_{SMOW} = +20.78\ ^{o}/oo$, a value close to that of the samples measured.

δD measurements were carried out on a second VG Micromass 602D mass-spectrometer. Automatic electronic correction for H_3^+ contribution was available in this machine. All measurements were made relative to the laboratory H_2 standard prepared in a large quantity (5 litres at 400 torr), from a laboratory water standard called SAB, collected from Sabarmati river, Ahmedabad. The hydrogen gas standard which was thus prepared was in turn periodically calibrated with H_2 prepared from V-SMOW and all δD values reported in this

thesis are with respect to V-SMOW (Gonfiantini, 1984).

In this case,

$$\delta D^{SAB}_{SMOW} = -20\ ^{o}/oo \quad (n = 13),$$

a value which falls within the range of δD values for the samples measured.

Apart from these laboratory standards, one more bottle of tank CO_2 and one more bottle (5 litres) hydrogen gas (400 torr) from SAB water standard were used as check standards to test the long term performance of the machines.

Six sets of reference and sample isotopic ratios were printed out in a set of isotope measurements. A microcomputer fabricated in our laboratory was attached to the machine, which enabled quick calculation of the δ values and the standard deviation for each run. This standard deviation (1σ), which was the precision of the mass spectrometric measurement, was better than $\pm$ 0.01 $^{o}/oo$ for $\delta\ ^{13}C$, $\pm$ 0.02 $^{o}/oo$ for $\delta\ ^{18}O$ and $\pm$ 0.5 $^{o}/oo$ for δD. When the same gas was run several times (e.g. the laboratory check standards) the reproducibility of the measurements were same as the precision of individual runs, given above.

The C, H and N percentages and $\delta\ ^{13}C$, δ D values for the laboratory standard cellulose nitrate AP330E are listed in Table 3.1. Apart from AP330E, many other samples

Table 3.1

Standard cellulose nitrate sample

AP330E **

Date	% C	% H	% N	δD_{SMOW} °/oo	$\delta {}^{13}C_{PDB}$ °/oo
1982					
May 29	24.19	2.32	13.7*	-54	-25.5
June 3	24.39	2.31	13.7*	-59	-25.8
4	24.25	2.31	13.8*	-55	-25.8
4	24.33	2.30	13.8*	-54	-25.8
4	24.33	2.26	13.9*	-58	-25.8
7	24.25	2.24	13.8*	-54	-25.8
15	24.20	2.25	13.9*	-58	-25.7
23	24.38	2.25	13.8*	-55	-25.6
1983					
Jun 23	24.53	2.30	14.0	-54	-25.7
29	24.35	2.32	14.1	-54	-25.4
July 8	24.51	2.25	13.6	-53	-25.7
8	24.27	2.33	13.8	-52	-25.7
8	23.83	2.35	14.1	-55	-25.8
Aug. 5	24.23	2.20	13.7	-52	-25.8
17	24.67	2.24	13.7	-56	-25.8
22	24.58	2.24	13.8	-56	-25.7
26	24.42	2.27	13.8	-58	-25.6
29	25.37	2.32	13.9	-54	-25.9
Sep. 1	24.51	2.28	13.7	-54	-25.8
5	24.64	2.28	13.8	-53	-25.9
13	24.48	2.29	13.8	-55	-25.8
1984					
May 29	24.50	2.30	13.8	-54	-25.6
29	24.51	2.30	13.9	-54	-25.8

Table 3.1 (continued)

Date	% C	% H	% N	δD_{SMOW} °/oo	$\delta^{13}C_{PDB}$ °/oo
Means[1]	24.42 ± 0.28	2.28 ± 0.37	13.8 ± 0.1	-55 ± 2	-25.7 ± 0.1
Theoretical value for fully nitrated cellulose $[C_6 H_7 O_2 (ONO_2)_3]_n$	24.24[2]	2.36	14.14	-	-

* The nitrogen content for these cases were calculated assuming 100% yield of CO_2, see text for details.

** This standard, weighing about 700 mg, was prepared by nitration of cellulose extracted from the early wood of the year 1930 from Abies pindrow disc # 3, in April 1982. This has since been stored in a plastic vial in a dessicator and measured periodically.

1 Uncertainties given as one sigmas.

2 The actual values are little more than the theoretical value because the latter is for fully nitrated cellulose; slight undernitration will result in a reduction in molecular weight of the cellulose nitrate.

were run more than once and the overall reproducibility (including the cellulose extraction, nitration, combustion and measurement) for δD and $\delta\ ^{13}C$ measurements were found to be $\pm\ 2^{o}/oo$ and $\pm\ 0.1\ ^{o}/oo$.

The charcoal finger for measurement of nitrogen content was installed in November 1982. In the case of measurements carried out earlier, the nitrogen content was calculated by assuming the molecular weight of cellulose to be 162 and 100% conversion of carbon to CO_2. If C was the carbon content of the cellulose nitrate (expressed as a fraction) and x the degree of nitration (which can vary theoretically from 0 to 3), the molecular weight of the cellulose nitrate could be written as (162 + 45 x), the formula being $C_6\ H_{10-x}\ O_{5-x}\ (ONO_2)_x$.

$$\therefore\ C = 72/(162 + 45\ x)$$

$$\text{and } x = (72-162C)/45\ C$$

Once x , i.e., the degree of nitration was calculated, the nitrogen content could be inferred to be $14x/(165 + 45\ x)$. The runs in pre-November 1982, for which this method was used to calculate the N content are shown with asterisks in Table 3.1.

The mean C content and H content for AP330E standard were $24.42 \pm 0.28\%$ and $2.28 \pm 0.37\%$ respectively.

The standard cellulose nitrate, AP330E was prepared in April 1982 and had since been stored in plastic vials in a dessicator. It can be seen from Table 3.1 that even after a lapse of two years, the cellulose nitrate had not degraded and had yielded the same δD and $\delta^{13}C$ values as the newly prepared sample.

Similarly for oxygen isotope measurements of cellulose samples, a cellulose standard kindly provided by Prof. W.G. Mook, Groningen University, Netherlands, was used. The oxygen percentage and $\delta^{18}O$ values are shown in Table 3.2. The mean oxygen content was $49.27 \pm 0.37\%$ and $\delta\,^{18}O_{SMOW} = 23.3 \pm 0.2\ ^{o}/oo$.

The reproducibility (overall) over a period of one year was $\pm 0.2\ ^{o}/oo$ for this standard, as well as for many other samples which have been separately analysed more than once.

All experimental errors are given as one standard deviations in this thesis; the experimental results reported have been obtained with maximum controls and checks:

(i) Cellulose nitrate and cellulose standards were run along with every batch of samples.

(ii) Replicate measurements were made for most of the samples.

Table 3.2

Standard Cellulose Provided by Prof. W.G. Mook*

Date	% O	$\delta^{18}O_{SMOW}$ ‰
1983		
Mar. 28	48.91	+ 23.35
Apr. 4	48.80	+ 23.16
5	49.20	+ 23.20
1984		
Jan. 19	49.50	+ 23.44
19	49.50	+ 23.64
Feb. 14	48.82	+ 22.73
Mar. 20	49.55	+ 23.35
31	48.96	+ 23.12
Apr. 6	49.69	+ 23.36
11	49.77	+ 23.42
Means	49.27 ± 0.37	+ 23.3 ± 0.2

* The sample was in the form of fine white powder and was stored in a bottle with an air tight screw cap, kept in a dessicator.

(iii) Blanks were measured periodically.

(iv) Pure chemicals were used and the extraction line was cleaned periodically to ensure minimum blanks. Also grease-stop cocks were kept minimum (only 2; the rest were Kontes high vacuum stop cocks with viton 'O' rings).

(v) Calibration of the gas standards were checked periodically.

(vi) The machine performance was checked routinely (capillary balance, zero enrichment, through valve leaks and cross-mixing in the change over valve etc.).

3.7. Inter-laboratory Comparison of Standards

In addition to the tests listed in the last section, it was necessary to check our measurements for systematic errors. This could be best done by measuring the δ values of cellulose, wood, cotton or cellulose nitrate samples prepared in other well known laboratories and comparing the values. Also samples prepared in our laboratory were sent to different laboratories for measurement.

Table 3.3 gives a list of such inter-laboratory comparisons. It can be seen that our values agree very well with the values quoted by the respective laboratories.

Table 3.3

Interlaboratory Comparison of δ values ($^{o}/_{oo}$)

	Sample	Source	Quoted Value	PRL Value
1.	Eucalyptus wood powder (cellulose extracted and nitrated at PRL)	M.J. DeNiro UCLA	δ D = -55 ± 2 (4)[1]	-54 ± 2 (3)[a]
			$\delta^{13}C$=-26.3 ± 0.1(4)	-26.3 ± 0.1 (3)
2.	Sigma cellulose powder (Nitration done at PRL)	M.J. DeNiro UCLA	δ D = -91 ± 6(4)[2]	-93 ± 6 (5)
			δ D = -94 ± 2[3]	
3.	Cotton (Nitrated at PRL)	W.G. Mook Groningen	δ D = -59.5[4]	-57 ± 2 (2)
4.	Glucose (not nitrated)	M.J. DeNiro UCLA	δD = +10 ± 4[5]	+ 16 ± 2 (3)
			$\delta^{13}C$=-9.78±0.1(6)[6]	-9.58 ± 0.1 (1)
5.	Cellulose powder (not nitrated)	W.G. Mook Groningen	$\delta^{18}O$ = +23.5[7]	+23.3 ± 0.2 (10)
6.	AP330E cellulose nitrate	PRL Ahmedabad	δD = -57 ± 2(3)[8]	-55 ± 2 (23)
			$\delta^{13}C$=-25.2±0.1(1)[1]	-25.7±0.1(23)
7.	AP331E cellulose nitrate	PRL Ahmedabad	$\delta^{13}C$=-25.5±0.1(1)[1]	-25.6±0.1(2)

Table 3.3 (continued)

a	No. of measurements given in paranthesis.
1	M.J. DeNiro, personal communication. (1981)
2	M.J. DeNiro (1981).
3	Epstein *et al* (1977).
4,7	W.G. Mook, personal communication. (1983)
5.	Krishnamurthy & DeNiro (1981).
6.	Krishnamurthy (1984).
8.	Measured at CALTECH, Krishnamurthy, personal communication. (1984)

The uncertainty in the case of commercial glucose and cellulose are a little higher than the normal. This may perhaps be due to the inhomogeneity of these samples.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

The experimental results presented in this section (Table 4.1 to 4.8) can be classified broadly into two categories; one dealing with annual growth ring studies and the other related to early wood analyses. Both these studies pertain to samples AP # 2 (*Abies pindrow* # 2), AP # 3 (*Abies pindrow* # 3), CD # 1 (*Cedrus deodara* # 1) and PW # 3 (*Pinus wallichiana* # 3) (see table 1.1 for details), all collected from a single site, viz., Gulmarg forest compartment # 63, near Tangmarg, Kashmir ($74^{o}25'E$, $34^{o}04'N$, 2650 msl). The early wood results cover the period 1923-32 (A.D.). The

reasons for this particular choice of the period have been explained earlier (see section 2.6).

Table 4.1 contains the ring width data for AP # 2 along 3 radii R_1, R_2 and R_3 and the mean values. The δD and $\delta^{13}C$ values of nitrated cellulose extracted from these rings along the three radii are listed in tables 4.2 and 4.3 respectively, with the mean values for each year. The $\delta^{18}O$ values of cellulose from two radii R_1 and R_2 and the means for each year, from the same tree specimen, are shown in table 4.4

Ring width and δD, $\delta^{13}C$ values of nitrated cellulose from the annual rings of an adjacent tree of the same species, AP # 3, are listed in table 4.5.

Tables 4.6, 4.7 and 4.8 respectively contain the ring widths and δD, $\delta^{13}C$ values of cellulose nitrate prepared from the early wood part of the growth rings from 4 trees. Two were of the same species (Abies pindrow # 2 and # 3) and two were of different species (Cedrus deodara # 1 and Pinus wallichiana # 3). All trees grew within a radius of 50 m.

4.2 Discussion

The implications of the results presented in the last section will be discussed in four parts. The first part deals with the effect of intraring isotopic variations on the meaningful interpretation of the tree ring isotope record,

Table 4.1

Ring widths (mm) along 3 radii R_1, R_2 and R_3* of *Abies pindrow* # 2 (AP # 2) and their mean values for the period 1903-32 (A.D.). Uncertainty is ± 0.1 mm.

Year (A.D.)	Ring width (mm) along R_1	R_2	R_3	Mean (mm)
1903	7.5	5.2	7.0	6.6
1904	8.2	4.6	5.5	6.1
1905	5.0	4.0	4.5	4.5
1906	6.4	3.0	6.3	5.2
1907	6.1	3.0	6.5	5.2
1908	4.7	3.4	8.0	5.4
1909	3.3	2.9	6.0	4.1
1910	6.0	4.2	8.5	6.2
1911	3.9	2.6	4.2	3.6
1912	4.0	3.0	4.2	3.7
1913	3.0	2.9	4.2	3.4
1914	4.1	2.8	5.0	4.0
1915	3.0	2.0	4.5	3.2
1916	6.2	3.4	6.0	5.2
1917	7.1	3.4	8.0	6.2
1918	4.7	3.0	5.5	4.4
1919	6.3	3.6	6.7	5.5
1920	5.8	3.4	6.3	5.2
1921	6.7	4.2	5.0	5.3
1922	5.7	3.4	6.0	5.0
1923	6.6	4.0	7.0	5.9
1924	5.7	3.6	6.0	5.1
1925	4.0	2.7	4.0	3.6
1926	6.4	2.4	4.5	4.4
1927	5.2	2.4	4.2	3.9

....Contd.

Table 4.1 (continued)

Year (A.D.)	Ring width (mm) along R_1	R_2	R_3	Mean (mm)
1928	4.2	2.0	3.5	3.2
1929	7.4	2.3	6.0	5.2
1930	7.6	3.0	7.0	5.9
1931	8.0	4.2	7.5	6.6
1932	5.2	3.2	5.5	4.6

* R_1, R_2 and R_3 denote the directions shown in Fig. 4.1

Table 4.2

δD_{SMOW} (°/oo) values along 3 radii R_1, R_2 and R_3 of *Abies pindrow* # 2 (AP # 2) and their mean values for the period 1903-32 (A.D.). Uncertainty is ± 2 °/oo, one standard deviation.*

Year (A.D.)	δD_{SMOW} (°/oo) along			Mean (°/oo)
	R_1	R_2	R_3	
1903	-89	-83	-89	-87
1904	-63	-54	-63	-60
1905	-42	-35	-40	-39
1906	-60	-46	-59	-55
1907	-87	-68	-87	-81
1908	-70	-55	-68	-64
1909	-64	-55	-56	-58
1910	-76	-53	-63	-64
1911	-49	-28	-37	-38
1912	-63	-46	-51	-53
1913	-53	-35	-45	-44
1914	-70	-50	-56	-59
1915	-51	-36	-41	-43
1916	-66	-54	-72	-64
1917	-86	-67	-68	-74
1918	-50	-33	-32	-38
1919	-73	-42	-57	-57
1920	-51	-18	-30	-33
1921	-56	-30	-38	-41
1922	-39	-14	-51	-35
1923	-80	-53	-51	-61
1924	-56	-38	-32	-42

....Contd.

Table 4.2 (Continued)

Year (A.D.)	δD_{SMOW} (‰) along			Mean (‰)
	R_1	R_2	R_3	
1925	-45	-21	-15	-27
1926	-67	-38	-31	-45
1927	-49	-25	-19	-31
1928	-43	-27	-18	-29
1929	-81	-59	-66	-69
1930	-67	-56	-56	-60
1931	-71	-55	-66	-64
1932	-52	-40	-51	-48

* All δD values listed here are for nitrated cellulose.

Table 4.3

$\delta^{13}C_{PDB}$ (°/oo) values along 3 radii R_1, R_2 and R_3 of *Abies pindrow* # 2 (AP # 2) and their mean values, for the period 1903-32 (A.D.). Uncertainty is ± 0.1 °/oo, one standard deviation.*

Year A.D.	$\delta^{13}C_{PDB}$ (°/oo) along			Mean (°/oo)
	R_1	R_2	R_3	
1903	-24.1	-23.5	-23.5	-23.7
1904	-23.7	-23.2	-23.2	-23.4
1905	-23.0	-22.8	-22.6	-22.8
1906	-22.9	-22.9	-22.7	-22.8
1907	-23.4	-22.9	-23.1	-23.1
1908	-23.2	-22.7	-23.1	-23.0
1909	-23.7	-23.0	-23.1	-23.3
1910	-23.5	-23.3	-23.2	-23.3
1911	-21.8	-22.3	-21.9	-22.0
1912	-22.5	-22.7	-22.2	-22.5
1913	-21.7	-22.0	-21.7	-21.8
1914	-22.3	-22.1	-22.2	-22.2
1915	-21.9	-22.5	-22.3	-22.2
1916	-22.4	-22.9	-22.9	-22.7
1917	-22.6	-23.2	-22.9	-22.9
1918	-21.8	-22.3	-22.1	-22.1
1919	-22.3	-22.8	-23.0	-22.7
1920	-21.9	-22.2	-22.5	-22.2
1921	-22.4	-22.6	-22.4	-22.5
1922	-22.5	-22.7	-23.5	-22.9
1923	-23.0	-23.3	-23.3	-23.2
1924	-23.0	-23.2	-23.3	-23.2

....Contd.

Table 4.3 (Continued)

Year (A.D.)	$\delta^{13}C_{PDB}$ (°/oo) along			Mean (°/oo)
	R_1	R_2	R_3	
1925	-23.1	-23.1	-23.0	-23.1
1926	-23.5	-23.4	-23.8	-23.6
1927	-22.6	-22.7	-23.7	-23.0
1928	-22.0	-22.2	-22.7	-22.3
1929	-23.4	-23.2	-23.6	-23.4
1930	-23.6	-23.7	-23.6	-23.6
1931	-24.0	-24.0	-23.8	-23.9
1932	-23.6	-23.8	-22.5	-23.3

* All $\delta^{13}C$ values listed above are for nitrated cellulose.

Table 4.4

$\delta^{18}O_{SMOW}$ (°/oo) values along 2 radii R_1 and R_2 of *Abies pindrow* # 2 (AP # 2) and mean values for the period 1903-32 (A.D.). Uncertainty is ± 0.2 °/oo, one standard derivation*.

Year (A.D.)	$\delta^{18}O_{SMOW}$ (°/oo) along R_1	$\delta^{18}O_{SMOW}$ (°/oo) along R_2	Mean (°/oo)
1903	26.6	27.0	26.8
1904	29.8	30.0	29.9
1905	30.9	31.1	31.0
1906	29.0	29.4	29.2
1907	26.8	26.6	26.7
1908	29.1	28.4	28.8
1909	27.9	28.9	28.4
1910	26.3	28.1	27.2
1911	30.6	31.3	31.0
1912	27.1	28.7	27.9
1913	29.7	29.4	29.6
1914	28.4	27.4	27.9
1915	28.3	29.7	29.0
1916	27.2	27.5	27.4
1917	24.0	25.1	24.6
1918	29.3	30.0	29.7
1919	26.8	27.5	27.2
1920	32.1	32.1	32.1
1921	31.2	31.4	31.3
1922	31.2	30.9	31.1
1923	27.7	27.5	27.6
1924	30.4	28.7	29.6
1925	30.1	28.6	29.4
1926	27.9	27.0	27.5

....Contd.

Table 4.4 (Continued)

Year (A.D.)	$\delta^{18}O_{SMOW}$ (‰) along R_1	R_2	Mean (‰)
1927	29.9	29.1	29.5
1928	32.4	33.0	32.7
1929	29.1	29.6	29.4
1930	29.0	29.8	29.4
1931	29.2	29.6	29.4
1932	29.7	30.4	30.1

* $\delta^{18}O$ values listed above are for alpha-cellulose.

Table 4.5

Ring width (mm), δD_{SMOW} (°/oo), $\delta^{13}C_{PDB}$ (°/oo) values along a single radius of Abies pindrow # 3 (AP # 3) for the period 1903-32 (A.D.). Uncertainties are ± 0.1 mm, ± 2 °/oo and ± 0.1 °/oo respectively, one standard deviations*.

Year (A.D.)	Ring width (mm)	δD_{SMOW} (°/oo)	$\delta^{13}C_{PDB}$ (°/oo)
1903	7.8	-73	-24.3
1904	8.2	-52	-24.1
1905	4.5	-24	-23.2
1906	5.7	-42	-23.5
1907	6.8	-65	-23.6
1908	9.2	-71	-24.4
1909	4.5	-33	-24.2
1910	5.8	-55	-24.8
1911	4.1	-15	-22.9
1912	5.6	-31	-23.7
1913	4.0	-37	-23.8
1914	5.4	-49	-24.0
1915	5.8	-21	-23.1
1916	10.2	-53	-24.6
1917	8.7	-52	-25.2
1918	5.6	-20	-24.4
1919	7.8	-45	-24.7
1920	5.2	-13	-24.2
1921	9.0	-41	-24.3
1922	8.4	-20	-24.6
1923	8.8	-55	-25.0
1924	6.8	-44	-24.8
1925	4.8	-20	-25.3

....Contd.

Table 4.5 (Continued)

Year (A.D.)	Ring width (mm)	δD_{SMOW} (‰)	$\delta^{13}C_{PDB}$ (‰)
1926	6.0	-46	-25.1
1927	5.4	-30	-24.5
1928	6.4	-41	-23.8
1929	10.8	-67	-25.4
1930	11.0	-52	-26.1
1931	11.0	-49	-26.3
1932	8.0	-35	-25.8

* δD and $\delta^{13}C$ values listed above are for nitrated cellulose.

Table 4.6

Ring widths (mm) of four trees from a single site: (Gulmarg forest compartment # 63) Abies pindrow # 2 (AP # 2), Abies pindrow # 3 (AP#3), Cedrus deodara # 1 (CD # 1) and Pinus wallichiana # 3 (PW # 3) for the period 1923-32 (A.D.). Uncertainty ± 0.1 mm, one standard deviation.

Year (A.D.)	Ring width (mm)			
	AP # 2	AP # 3	PW # 3	CD # 1
1923	6.5	8.8	8.4	6.1
1924	6.2	6.8	6.8	4.7
1925	4.2	4.8	6.9	6.0
1926	6.5	6.0	7.1	3.0
1927	4.3	5.4	6.4	3.6
1928	3.4	6.4	6.6	3.9
1929	6.1	10.8	10.7	1.3
1930	8.1	11.0	5.5	2.6
1931	8.5	11.0	7.3	3.4
1932	5.1	8.0	7.8	2.1

Table 4.7

δD_{SMOW} ($^{o}/oo$) values of cellulose nitrate from 4 trees from a single site: (Gulmarg forest compartment # 63), Abies pindrow # 2 (AP # 2), Abies pindrow # 3 (AP # 3), Cedrus deodara # 1 (CD # 1) and Pinus wallichiana # 3 (PW # 3) for the period 1923-32 (A.D.). Uncertainty is $\pm$ 2 $^{o}/oo$, one standard deviation. In all cases only early wood was measured.

Year (A.D.)	δD_{SMOW} ($^{o}/oo$)			
	AP # 2	AP # 3	PW # 3	CD # 1
1923	-54	-43	-60	-50
1924	-37	-25	-35	-26
1925	-30	-21	-34	-39
1926	-45	-30	-26	-22
1927	-29	-17	-36	-42
1928	-39	-33	-34	-30
1929	-49	-56	-43	-23
1930	-47	-56	-46	-34
1931	-50	-47	-36	-36
1932	-27	-42	-33	-18

Table 4.8

$\delta^{13}C_{PDB}$ ($^{o}/oo$) values of cellulose nitrate from 4 trees from a single site (Gulmarg forest compartment # 63) for the period 1923-32 (A.D.): Abies pindrow # 2 (AP # 2), Abies pindrow # 3 (AP # 3), Cedrus deodara # 1 (CD # 1) and Pinus wallichiana # 3 (PW # 3). Uncertainty is $\pm$ 0.1 $^{o}/oo$, one standard deviation. In all cases only early wood was measured.

Year (A.D.)	$\delta^{13}C_{PDB}$ ($^{o}/oo$)			
	AP # 2	AP # 3	PW # 3	CD # 1
1923	-22.8	-24.6	-23.8	-21.5
1924	-23.2	-25.2	-24.0	-21.5
1925	-23.5	-24.7	-23.5	-22.1
1926	-23.2	-24.8	-24.4	-22.0
1927	-23.2	-24.9	-24.6	-21.6
1928	-23.2	-23.8	-23.8	-21.5
1929	-22.9	-24.8	-24.2	-21.9
1930	-24.3	-25.8	-24.6	-23.1
1931	-23.8	-25.6	-24.4	-23.2
1932	-23.7	-25.6	-23.4	-22.7

the second with the coherence of this record among trees in a given site, the third with intercorrelations among the tree ring and isotopic records and the fourth part describes the statistical treatment of the data and the available instrumental weather records from the site to discern the relationship between the isotopic record in tree rings and climatic variables.

4.2.1 Intraring isotopic variations

It has been shown that ring width and ring density are affected by non climatic parameters as well (Fritts, 1976; Hughes, et al., 1982). Therefore, to maximise the climatic signal, dendroclimatologists sample at least 10 trees in a site with two cores per tree. If it were shown that isotopic variations in trees are prone to much less noise (than ring widths and ring densities) due to non-climatic effects, then such extensive sampling need not be done. This is a major potential advantage of isotope dendroclimatology. However, because of the large amount of time and expense involved in isotope dendroclimatology, various workers have analysed bunches of rings instead of individual rings, very often along a single radial direction of a single tree at a site. Because of the large intra ring isotopic variations discussed in detail in section 2.5.2, such single radius results are of doubtful validity. One way to overcome this problem is to sample the entire circumference of each ring (Leavitt and Long, 1983). But this is a laborious process. Another way is to choose

certain radii along different directions and measure the isotopic variations. If the isotopic variations are parallel, one can conclude that intraring variation may not seriously interfere with a meaningful interpretation of the isotopic record.

When one is interested in observing the trend (like in cases where the atmospheric CO_2 record is sought), the difference between the absolute δ values along the different radial directions, if any, does not matter as long as the trends are parallel. However, when one is interested in relating the various climatic parameters with isotopic variations with a view to obtain fractionation factors, their temperature dependence etc., to be used in predictive equations, this difference in δ values between the different radii should be considered. If such differences are large and unsystematic, then the only option is to analyse the different radial sections and take mean values for such purposes. This would also mean that many trees from the same site should be studied in order to obtain a site representative record.

In the present work, to assess the importance of intra-ring isotopic variations a fir tree (Abies pindrow # 2) was chosen. The tree had a lobate growth. To allow for maximum dispersion in the data, the longest, the shortest and an intermediate radius were selected for measurement of δ values. R_3 is the longest radius ($\sim$ 35 cm), R_1 is the intermediate

radius (~ 32 cm) and R_2 is the shortest (~ 20 cm). Thirty individual rings corresponding to the years 1903-32 were analysed from each radius. The δD and $\delta^{13}C$ results are shown in figs 4.1 and 4.2 respectively. $\delta^{18}O$ was measured only along 2 radii (R_1 and R_2) and the results are shown in fig 4.3. The ring width values along the three radii are shown in fig. 4.4, for the sake of comparison. The schematic diagram of the cross section indicating the radii selected are shown in each of these figures.

The similarity in the pattern of variations in δD, $\delta^{13}C$ along the three radii and $\delta^{18}O$ along the two radii is strikingly clear. But the absolute δ values are different in each case. Inspite of the asymmetric growth of the tree, the variations in δ values along the different radii closely resemble each other, whereas the ring width variations do so only to a lesser extent.

The maximum difference between any two radii is 33 °/oo for δD, 1.3 °/oo for $\delta^{13}C$ and 1.8 °/oo for $\delta^{18}O$. For some of the years, however, the δ values along the different radii agree within experimental error. For e.g., along R_1 and R_3, δD values agree for the years 1903-8 and 1932; along R_2 and R_3, δD values agree for the years 1909, 17, 18, 23 and 30, and $\delta^{13}C$ values are in agreement for the years 1903-7, 9, 10, 14-16, 18-19, 21, 23-25, 30 and 31. $\delta^{18}O$ values along R_1 and R_2 agree within error for the years 1903-7, 13, 16, 20-23 and 31.

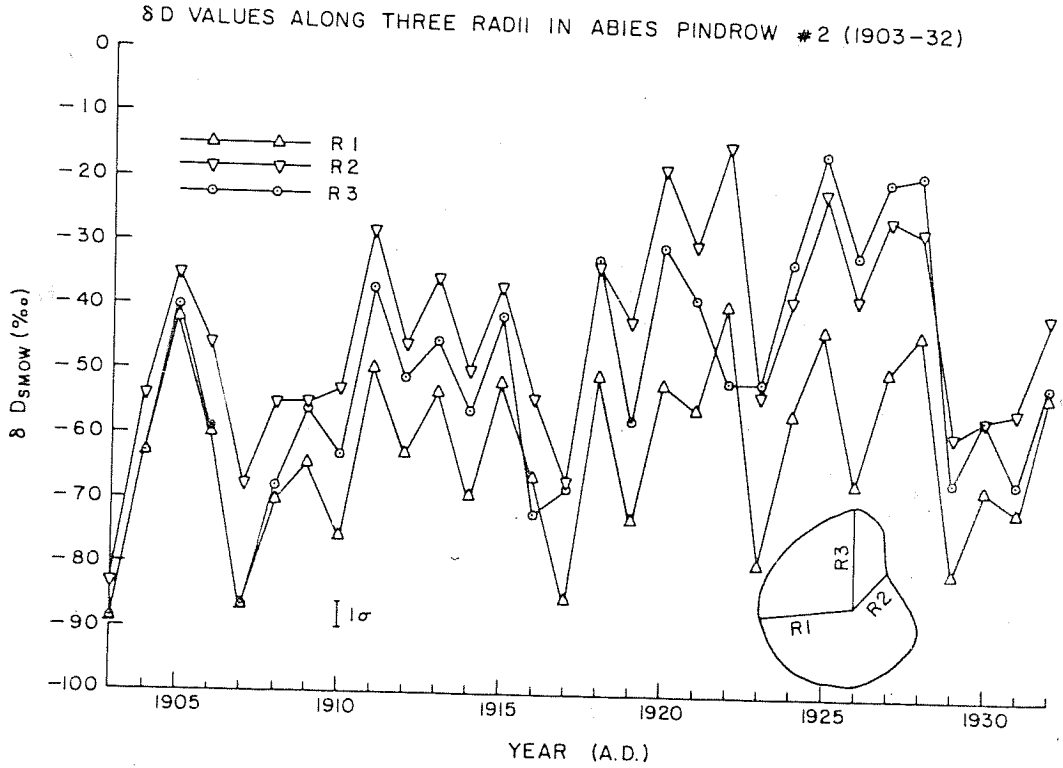


Fig. 4.1 : δ D values along three radii in Abies pindrow # 2

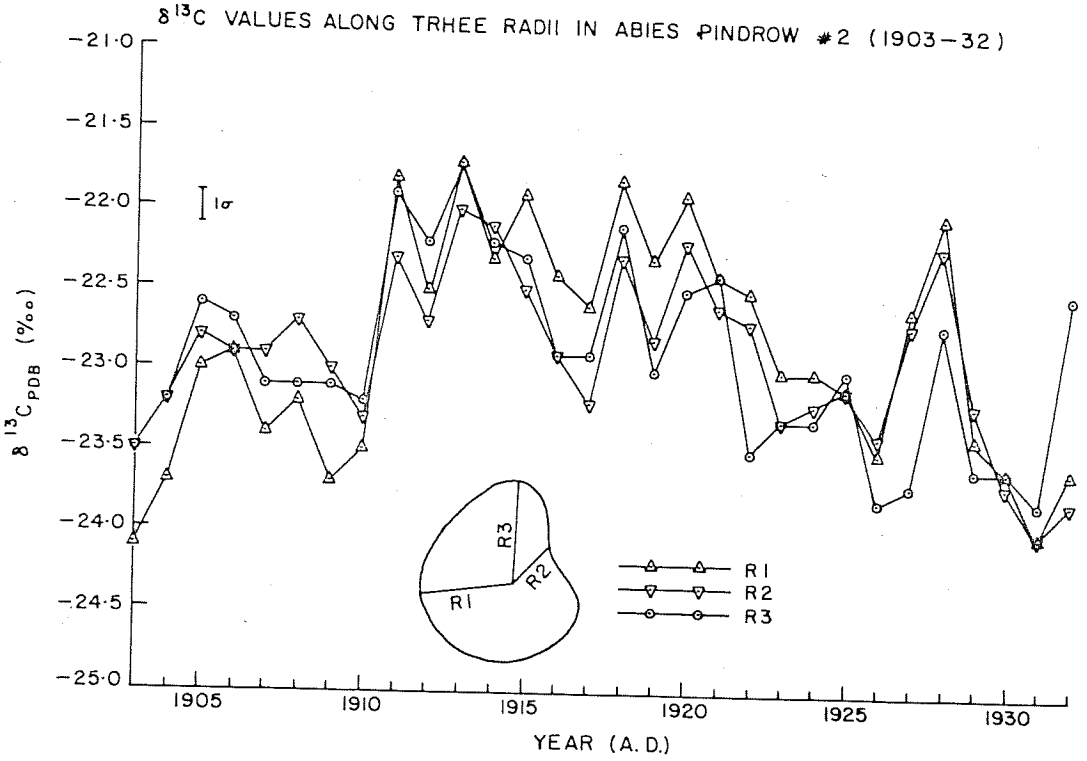


Fig. 4.2 : $\delta^{13}C$ values along three radii in Abies pindrow # 2.

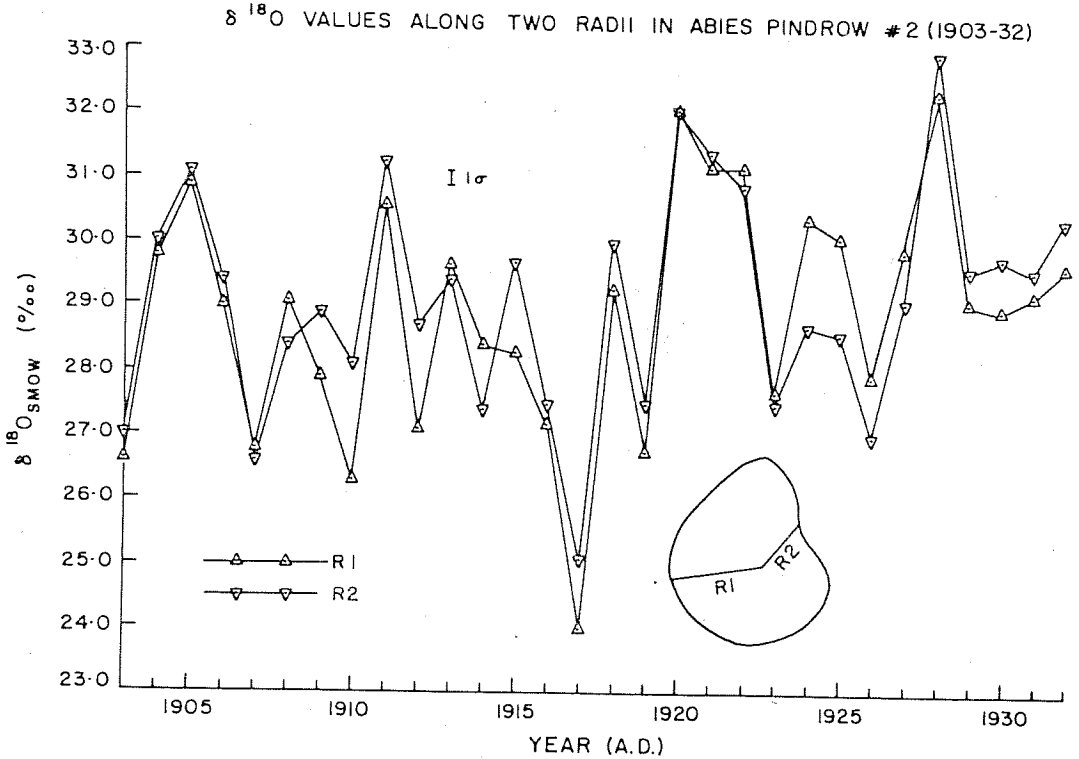


Fig. 4.3 : $\delta^{18}O$ values along two radii in Abies pindrow # 2.

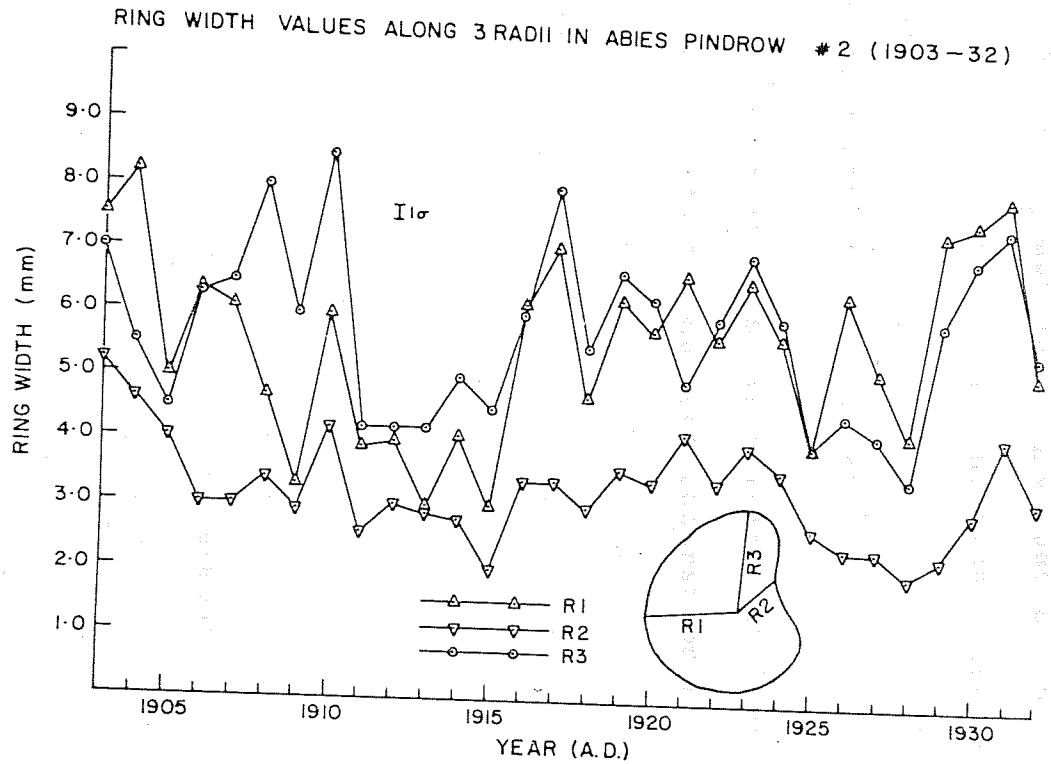


Fig. 4.4 : Ring width values along three radii in Abies pindrow # 2.

It is apparent that the intraring isotopic variations are not same for different years. These variations do not seem to bear any systematic relationship with the intraring width variations. For example, only 4 (1906, 12, 16 and 25) out of 30 years show an agreement in the ring width along the radii R_1 and R_3 but the δ values are not in agreement for these years in general. Also, no significant correlation was found between the difference in ring widths and the differences in δ values along any of the radii.

The reason for this intraring isotopic variation is not clear. With the current understanding of the behaviour of stable isotopes in tree rings, any explanation for this effect can only be speculative. One plausible cause for this effect is described here.

Fritts (1976) has discussed in detail the asymmetric growth of trees. The possible causes for the asymmetric growth may be due to lateral redistribution or asymmetric production of a growth regulating hormone, a growth inhibitor or an auxin-destroying enzyme. Depending on how and when such a redistribution takes place some part of the cambium may be more active during a growing season and some part may be less active or even dormant. As a result, the cells growing at various spots along the circumference of a growth ring may use food material produced at slightly different times of the growing season. The isotope ratio measured in the laboratory

at these different spots is integrated over the growing season. This integrating period, if slightly different for these different spots, could result in different δ values. Since this process is dependent on the harmone activity, it may vary from year to year (depending on crown vigour) resulting in a non-uniform intraring variation for the different years.

Using the procedure for analysis of variance outlined by Fritts (1976), the common signal among the different radii were calculated. For δD, the common signal was 94.7% and for $\delta^{13}C$ this was 89.3%. Thus the intraring variation has introduced a "noise" of about 5% in δD and 11% in $\delta^{13}C$. The common signal in $\delta^{18}O$ along the two radii was 94.6%. Again a "noise" of 5% has been introduced because of intraring variations. Considering the lobate growth of the tree, it is likely that this "noise" may be less in less asymmetric trees, where the harmone control on growth has been more uniform alomg the circumference of the cambium.

While in some of the rings δ values along different radii agree, in most of the rings they do not. This result has an important implication. White (1983) has studied the circumferential variability of δD in two white pines from Mohonk lake area. Six equally spaced samples from a single annual ring corresponding to the year 1977 were analysed and their δD values found to agree within experimental error, in each of the two trees. The symmetry structure

of the sections of these trees was not described. Based on these two sets of data, White (1983) has concluded that δD values do not vary circumferentially in white pine. While this may be true, our results show that some caution must be exercised in making such conclusions (at least for rings from an asymmetric section of a tree) based on analysis of a single growth ring. The extent of intraring variation may be different not only from tree to tree of the same species, but also from year to year in the same tree. So it is necessary to sample at least 30 rings (for statistics) in a sequence and verify whether the intraring variation (which may be absent in a ring or a few rings) is really absent in the tree.

To ascertain the similarity of the trends in δ values among different radii, the following procedure was adopted: Apart from AP # 2, one more tree of the same species, (Abies pindrow) AP # 3, was analysed for δD and $\delta^{13}C$ values along a single radius. This tree had a lesser degree of asymmetry in growth. Individual rings corresponding to the same 30 year period (1903-32) were analysed. The mean δ values for each year was calculated from the 3 radial sections of AP # 2 and single radial section of AP # 3. These mean values were taken, to a first approximation, to be representative of the site. It is noted here that our emphasis in this procedure is to identify the trend in δ values and not the absolute values themselves. The choice of mean δ as site representative is valid since the trends are seen to be

similar for the two trees from figures 4.5 and 4.6.

The correlation coefficient between the ε values for each of the radii and the mean ε values were calculated. These are listed in table 4.9.

All the correlation coefficients are significant at 0.01 level. With the available degrees of freedom (28), the correlations are indistinguishable from each other at 0.05 level. For comparison, similar correlation coefficients were calculated for ring width variations as well. These values are also shown in table 4.9. It is seen that the correlation coefficient between the ring width along R_2 and the mean ring width is significantly different.

Since in the case of δ values the correlation coefficients between the different radii and the means are not significantly different, it appears that for trend analysis, measurement along a single radius will suffice in the case of Abies pindrow from the site. It is possible, however, that since only two trees (4 radii) were analysed, such high correlations have been obtained. Therefore, it is necessary to verify this result by analysing more trees from this site. But it must be pointed out that the results seem to be encouraging.

In the case of $\delta^{18}O$, only two radii of AP # 2 were measured and there is a high degree of correlation

Table 4.9

Correlation coefficient between	δD	$\delta^{13}C$	$\delta^{18}O$	Ring width
AP # 2 R_1 and mean	.938	.874	.975	.896
AP # 2 R_2 and mean	.964	.936	.971	.637
AP # 2 R_3 and mean	.916	.856	-	.796
AP # 3 and mean	.911	.844	-	.883

'mean' denotes a set of 30 data points obtained by averaging the corresponding values for each year along the three radii of AP # 2 and one radius of AP # 3. All correlation coefficients are significant at 0.01 level.

between the two. It appears that for the purposes of trend analyses, measurement along one radial direction would do for this tree. Since the δD and $\delta^{13}C$ trends are similar between the two trees AP # 2 and AP # 3, there is good reason to believe that this might be true for $\delta^{18}O$ variations as well.

However, in the case of ring width variations, even for trend analyses, a single radius measurement would not suffice, as is already known.

Summarising, it appears that for Abies pindrow from this site, δ value measurements along a single radius would do for trend analysis, while multiple radial measurements are required for the purposes of climatic calibration. This result, though obtained from a limited number of trees, is strengthened by the fact that a tree with extreme asymmetry was chosen. In normal cases, the agreement might be even better.

4.2.2 Coherence of the isotopic record among trees from a site

If the δ values of growth rings of a tree are controlled mainly by ambient climate, one would expect a coherence in the time variation of δ values among trees in a given climatic regime. There has been no serious effort to verify the degree of such a coherence. Recently Leavitt and Long (1984) have found a coherence in $\delta^{13}C$ variations

among 8 Pinus edulis trees from Arizona. But the measurements were made on 5 year blocks of rings (17 samples for each tree). White (1983) has made an inter-tree comparison of δD values of white pines and hemlocks. Though individual rings were analysed, only 5 rings were measured. 2 white pine trees and 4 hemlocks were studied. The years corresponding to these 5 rings have been chosen arbitrarily (1959, 66, 70, 75 and 77). In another site, 3 white pines were compared. Individual rings for 11 years (1970-1980) were compared for 2 trees while for the third tree only 5 measurements were made. Though White (1983) found a remarkable agreement between δD values of the trees, the arbitrary selection of the rings and the limited number of measurements cast some doubt on extrapolating this high degree of coherence to all the rings.

In the present work two experiments were carried out to determine the coherence of the δD and $\delta^{13}C$ records in trees from a given site.

In the first experiment 30 individual rings (1903-32) were analysed from each of the two trees of the same species, AP # 2 and AP # 3. These trees were separated by about 10 m in the field and hence one can reasonably expect them to have experienced the same microclimate. In the first case (AP # 2) measurements were made along 3 radii and the mean value for each year was calculated. This was compared with the single radius measurements of AP # 3.

Results for δD and $\delta^{13}C$ are shown in figures 4.5 and 4.6 respectively. For the sake of comparison, the mean (of 3 radii) ring widths of the two trees for the period 1903-32 are shown in fig. 4.7.

It is clear from the figures 4.5 and 4.6 that the δD and $\delta^{13}C$ records in the two trees are indeed coherent. The correlation coefficients between the δ values of the two trees are 0.84 for δD and 0.68 for $\delta^{13}C$. These are not distinguishable at 0.05 level. Using the procedure for analysis of variance outlined by Fritts (1976), the common signal was calculated at 91.9% for δD and 83.4% for $\delta^{13}C$ between the two trees.

Considering the maximum estimate of "noise" due to intraring variations in a given tree (5 $^{o}/_{oo}$ for δD and 11% for $\delta^{13}C$, see previous section), this coherence among a couple of trees is quite good.

It is seen that while the δD and ring width curves for the 3 radii overlap at some points, the $\delta^{13}C$ curves are distinctly different. $\delta^{13}C$ values of AP # 2 are in general higher than those of AP # 3 by a mean of 1.6 $^{o}/_{oo}$ (a minimum of 0.4 $^{o}/_{oo}$ to a maximum of 2.5 $^{o}/_{oo}$). This is a very interesting finding in the light of the fact that the ring widths of AP # 3 are in general, much higher than those of AP # 2. An explanation for this can be found from Francey and Farquhar's (1982) model, discussed in chapter 2.

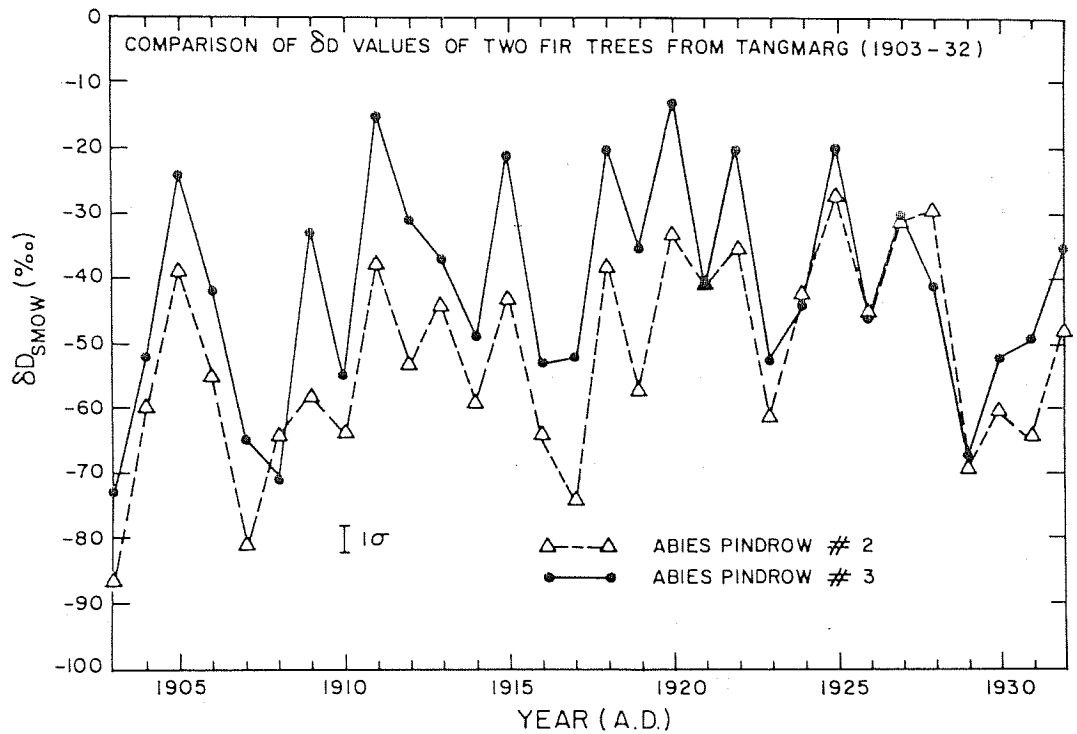


Fig. 4.5 : Comparison of δD values of two fir trees from Tang Marg (1903-32).

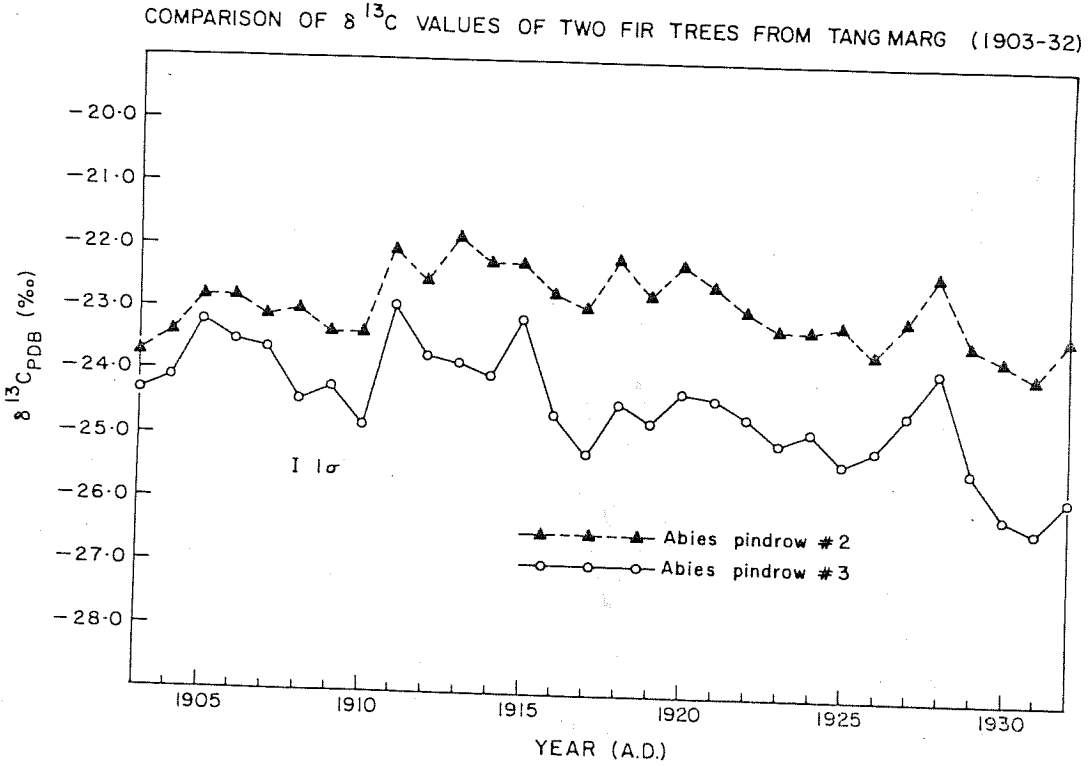


Fig. 4.6 : Comparison of $\delta^{13}C$ values of two fir trees from Tang Marg (1903-32).

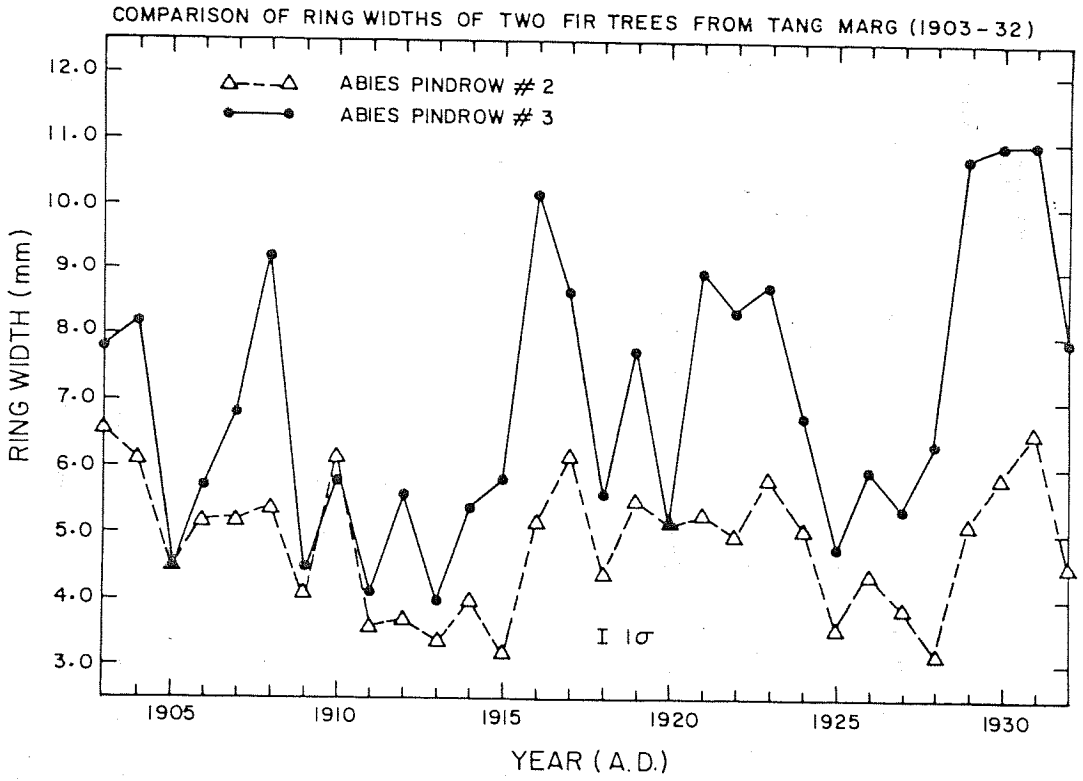


Fig. 4.7 : Comparison of ring width values of two fir trees from Tang Marg (1903-32).

The growth rate of AP # 3 is on the average of about 44% more than that of AP # 2 in the period for which the analysis is made. If one can assume a linear relationship between growth rate and CO_2 assimilation rate (Farquhar, 1980) then, the CO_2 assimilation rate for AP # 3 was on the average more than that of AP # 2 by $\sim$44%. This might have something to do with the setting of this particular tree AP # 3) vis-a-vis the other trees in the site, perhaps giving rise to increased competition for nutrients and moisture.

According to Farquhar (1980), the $\delta^{13}C$ of cellulose is given by

$$\delta^{13}C = -11.1 - 27.6\ C_i/C_a \qquad (4.1)$$

$$\text{and } C_i = C_a - A/g \qquad (4.2)$$

where C_i is the mole fraction of CO_2 inside the leaf and C_a outside the leaf (i.e., atmosphere), A the assimilation rate and g the stomatal conductance of CO_2. Typical values of A and g are $\sim$ 20 Mole/m^2/sec and $\sim$ 0.5 Mole/m^2/sec. (Farquhar, 1980) respectively at $\sim$ 18^{o}C which is the mean growing season temperature for these trees. One can write two different equations for AP # 3 and AP # 2 as

$$\delta^{13}C\ (AP\ \#\ 3) = -11.1 - 27.6\ \frac{C_i}{C_a}\ (AP\ \#\ 3) \qquad (4.3)$$

$$\delta^{13}C\ (AP\ \#\ 2) = -11.1 - 27.6\ \frac{C_i}{C_a}\ (AP\ \#\ 2) \qquad (4.4)$$

C_a is the same for both trees since they are only $\sim$ 10 m apart.

Also since the trees are in the same site and the water availability is identical for both of them, their stomatal conductances (g) might be expected to be the same. This is because g is mainly controlled by the transpiration rate, which again depends on water availability. Hence one can write

$$C_i \text{ (AP \# 3)} = C_a - \frac{A \text{ (AP \# 3)}}{g} \qquad (4.5)$$

$$C_i \text{ (AP \# 2)} = C_a - \frac{A \text{ (AP \# 2)}}{g} \qquad (4.6)$$

$$\text{Now} \quad A \text{ (AP\# 3)} = 1.44 \; A \text{ (AP \# 2)} \qquad (4.7)$$

$$\therefore \quad C_i \text{ (AP \# 3)} - C_i \text{ (AP \# 2)}$$

$$= -\frac{1}{g} \left[A \text{ (AP \# 3)} - A \text{ (AP \# 2)} \right]$$

$$= -\frac{0.44 \; A \text{ (AP \# 2)}}{g}$$

Substituting typical values of A and g we obtain

$$C_i \text{ (AP \# 3)} - C_i \text{ (AP \# 2)} \simeq -17.6 \qquad (4.8)$$

Subtracting (4.3) from (4.4) one obtains

$$\delta^{13}C \text{ (AP \# 2)} - \delta^{13}C \text{ (AP \# 3)} = \Delta^{13}C$$

$$= -\frac{27.6}{C_a} \left[C_i \text{ (AP \# 2)} - C_i \text{ (AP \# 3)} \right]$$

Using (4.8) it can be seen that

$$\Delta^{13}C \approx \frac{27.6}{C_a} \times 17.6$$

C_a can be taken as $\sim$ 300 ppm during the period under consideration. Hence

$$\Delta^{13}C \approx 1.6\ ^{o}/oo$$

which is in excellent agreement with the data. Inspite of the fact that our assumption of a linear relationship between growth rate and CO_2 assimilation rate may be an oversimplification, it is significant to note that the results are consistent with Francey and Farquhar's (1982) model. Additionally, it can be seen that when the growth rates of the two trees are identical (1905 ring) the difference in the $\delta^{13}C$ values is a minimum (0.4 $^{o}/oo$) and when the growth rates are different to a maximum extent (1929-32) there is also a large difference in the $\delta^{13}C$ values ($\sim$2.5 $^{o}/oo$).

In a second experiment to determine the extent of coherence in the isotopic signal among trees in a site, δD and $\delta^{13}C$ values of 4 trees were compared for the period 1923-32. Two trees were of the same species (Abies pindrow # 2 and # 3) and two trees were of different species (Cedrus deodara # 1 and Pinus wallichiana # 3), all of them from the same site (see table 1.1 for details). The trees grew within a circle of radius $\sim$50 m and can be expected to have experienced similar climatic conditions.

Because large differences in δ values are known to exist between early wood and late wood parts of a single ring (Epstein and Yapp, 1976), it was decided to sample only the early wood part. Rings corresponding to the years 1923-32

were broad enough in the four trees to facilitate easy sampling of pure early wood. To eliminate late wood contamination, a little portion of the early wood towards the late wood was left out.

The results for δ D and $\delta^{13}C$ are presented in figures 4.8 and 4.9 respectively. The whole ring widths of these four trees for the 10 year period has also been presented in the figure 4.10 for comparison.

The ranges of δ D values are 27 $^{o}/_{oo}$ for AP # 2, 39 $^{o}/_{oo}$ for AP # 3, 32 $^{o}/_{oo}$ for CD # 1 and 34 $^{o}/_{oo}$ for PW # 3. The mean values are -40.7 ± 9.7 $^{o}/_{oo}$ (1σ) for AP # 2, -37.0 ± 13.9 $^{o}/_{oo}$ for AP # 3, -32.0 ± 10 $^{o}/_{oo}$ for CD # 1 and -38.3 ± 9.4 $^{o}/_{oo}$ for PW # 3. It is seen that the means agree within one sigma indicating that the trees have sampled waters of more or less similar isotopic composition, as expected.

The ranges in $\delta^{13}C$ values are 1.5 $^{o}/_{oo}$, 2 $^{o}/_{oo}$, 1.7 $^{o}/_{oo}$ and 1.2 $^{o}/_{oo}$ for AP # 2, AP # 3, CD # 1 and PW # 3 respectively -23.4 ± 0.5 $^{o}/_{oo}$ (1σ), -25.0 ± 0.6 $^{o}/_{oo}$, -22.1 ± 0.7 $^{o}/_{oo}$ and -24.1 ± 0.4 $^{o}/_{oo}$. It is to be noted that the mean difference of 1.6 $^{o}/_{oo}$ between the whole rings of AP # 2 and AP # 3, found earlier, is still maintained in the early wood $\delta^{13}C$ values. This can be explained, as before, using Francey and Farquhar's (1982) model. Comparing the growth rates of the four trees we see that CD # 1 is the one slowest grown. AP # 2 has grown 60% faster, PW # 3 has grown 100%

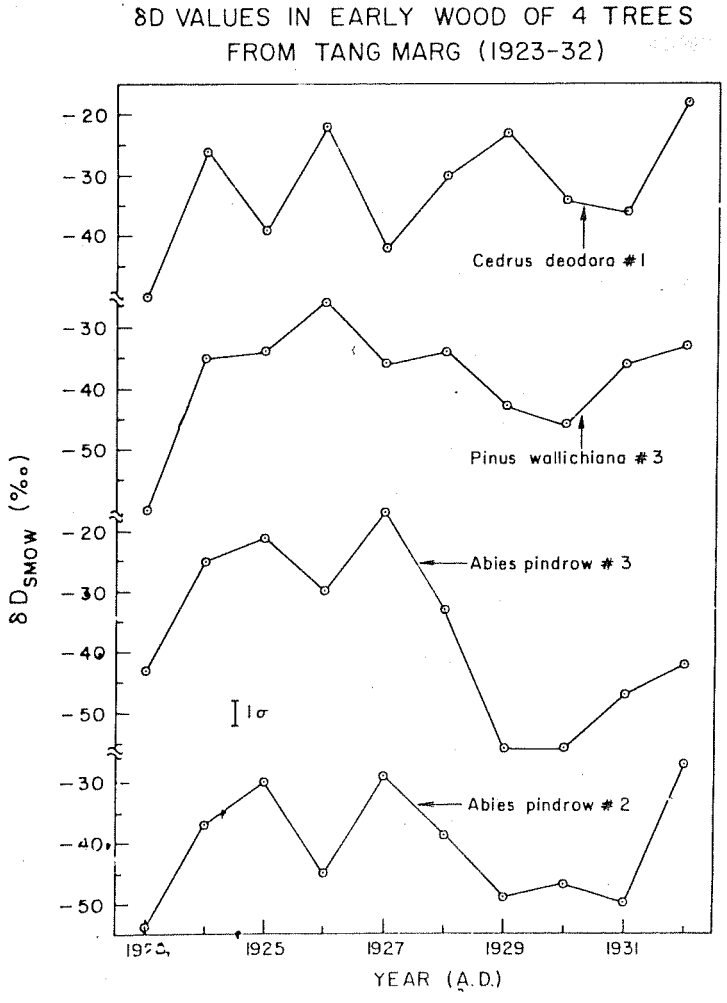


Fig. 4.8 : δ D values of Early woods of 4 trees from Tang Marg (1923-32).

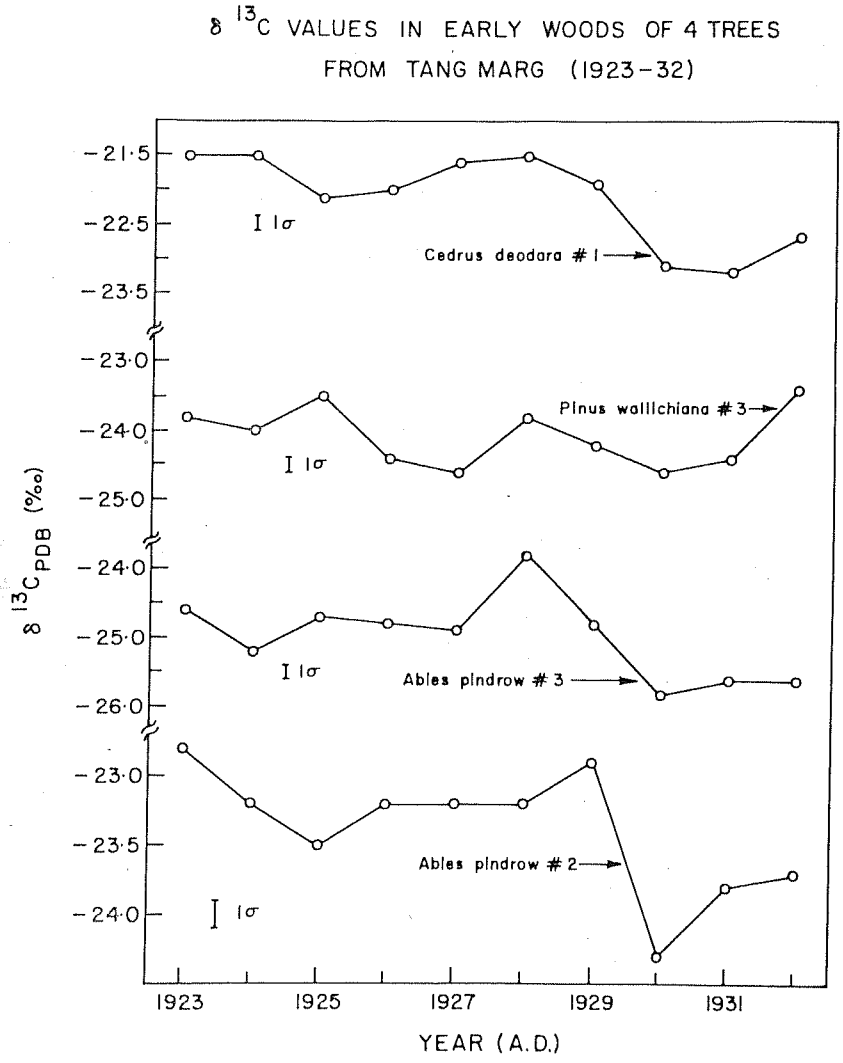


Fig. 4.9 : $\delta^{13}C$ values of Early woods of 4 trees from Tang Marg (1923-32).

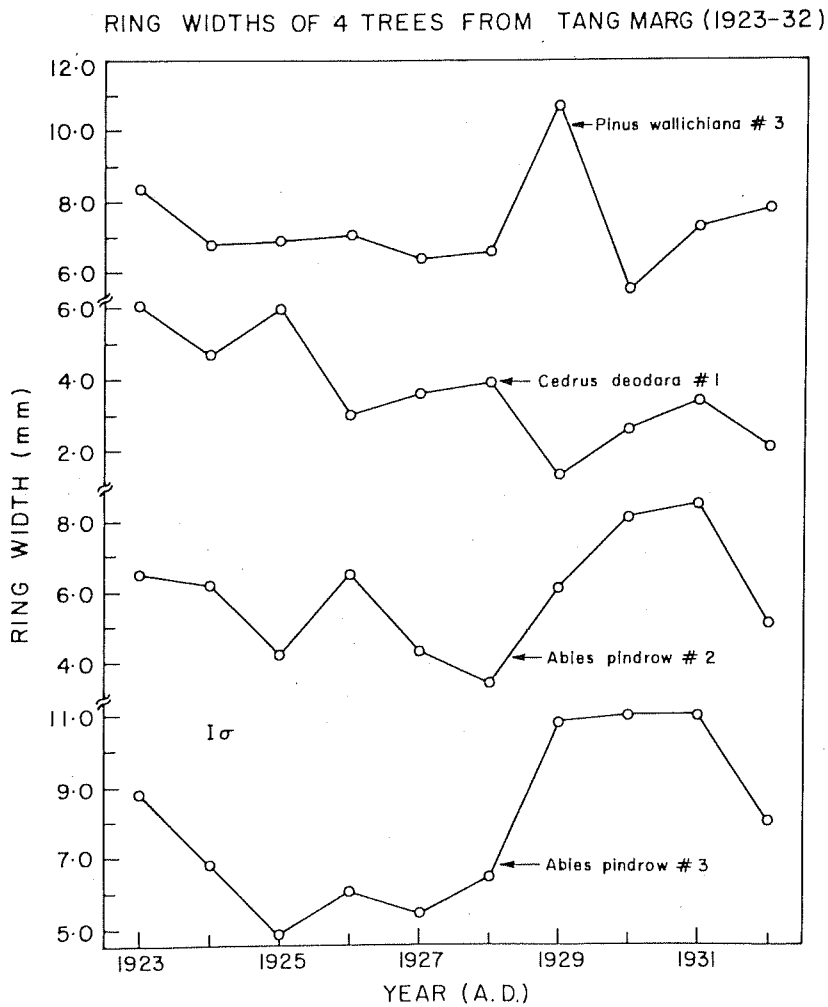


Fig. 4.10 : Ring width values of 4 trees from Tang Marg (1923-32).

faster and AP # 3 has grown 115% faster, relative to CD # 1. Consequently, as expected from Francey and Farquhar's model, the mean $\delta^{13}C$ value is maximum for CD # 1, next for AP # 2, followed by AP # 3 and the least for PW # 3.

The multiple correlation coefficients have been calculated between the δ values of the four trees for the 10 year period and are given by

$R_D = 0.75$ for δD and

$R_C = 0.87$ for $\delta^{13}C$.

These are significant at 0.05 level.

Using the procedure for analysis of variance outlined by Fritts (1976), the common signal among the four trees have been calculated to be 78.6 $^{o}/_{oo}$ for δD and 83.6 $^{o}/_{oo}$ for $\delta^{13}C$.

Apart from the "noise" due to intra ring isotopic variations discussed earlier, some more "noise" is present when the different species are compared. It is possible that some "noise" might have been introduced by the sampling procedure, where the percentage of early wood sampled was not constant (DeNiro, personal communication, 1982). It is not possible in practice to sample a constant percentage of early wood. There are two reasons for this. Firstly, the amount of early wood varies from ring to ring. Secondly, to avoid late wood contamination a small portion of early wood has to be left

out. Also, since in all the species studied, there is no clear-cut boundary between early wood and late wood, only the relative colouration was used as the criterion for selecting early wood.

To check the extent of "noise" introduced by the sampling procedure it was decided to study the circumferential variation in early wood alone. Again, the early wood was sampled using the same procedure. The early wood of the ring corresponding to the year 1923 from Pinus wallichiana # 3 was sampled at 9 different places as shown in fig. 4.11. The δD and $\delta^{13}C$ values are also shown. The mean values are -60 ± 5 °/oo (1σ) for δD and -24.0 ± 0.3 °/oo for $\delta^{13}C$. It may be recalled that the maximum difference between the whole ring values among the different radii was 33 °/oo for δD and 1.3 °/oo for $\delta^{13}C$ (see section 4.2.1). While one may not be justified either in comparing the intra ring variation of one species with that of the other or in extrapolating the 1923 early wood intra ring variation of PW # 3 to the other rings sampled, the above result at least indicates that no serious error has been introduced by the sampling procedure. That is to say, by sampling a non-constant percentage of early wood the "noise" in the signal may not have reduced,but certainly has not increased.

The extra "noise" (apart from that due to intra ring variation) in the signal when the different species are compared may be due to two possible reasons. Firstly, there

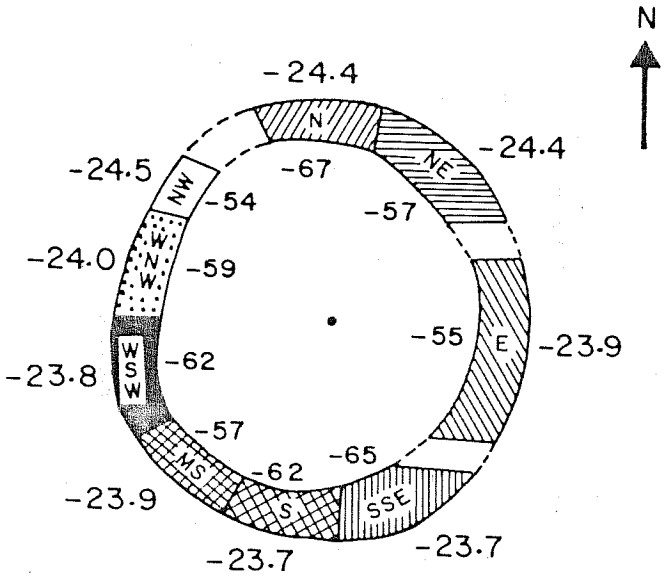


Fig. 4.11 : Intra ring variation in Pinus wallichiana early wood (1923).

may be a species effect. The biochemical fractionation factor for δD and $\delta^{13}C$ may be different for different species of trees. Alternatively, the "growing seasons" for the different species may be slightly different. The stimulus to start the cambial activity (cell division and growth) comes from the harmones like auxin, which are produced as soon as the shoot growth starts (Kramer and Kozloski, 1960). Depending on whether such stimulus comes earlier or later in the trees, the "growing seasons" could be slightly different. The δ values measured could thus be integrated over slightly different periods leading to some "noise" in the common signal.

Secondly, both the species effect and the differences in the "growing seasons" could be responsible for the "noise".

Summarising, it appears that the isotopic signal (δD and $\delta^{13}C$) among different species of trees in a given site are coherent to a large extent, and this signal is conceivably relatable to climate. It is a common practice in dendroclimatology to assume that the external agents forcing the pattern of variability common to trees in a region relate to climate (Hughes, et al., 1982).

Though this result is based on a limited data set, it is to be pointed out that such a detailed study of the intra ring variations for individual growth rings and the

of different species at a site
coherence of the isotopic signal among trees has not been attempted by earlier workers.

4.2.3 Inter-correlations among ring width and isotopic records

The mean values (average of three radii) of ring width, δD and $\delta^{13}C$ for individual rings of AP # 2 are shown in fig. 4.12. The ring width, δD and $\delta^{13}C$ values of AP # 3 are plotted in fig. 4.13. It is apparent from the figures that there is a positive correlation between δD and $\delta^{13}C$ and δD and $\delta^{18}O$ and a negative correlation between δ values and ring width. When the time series from the two trees are averaged by taking the means of the corresponding years, the following correlation coefficients are obtained:

δD	vs.	$\delta^{18}O$	= 0.67
δD	vs.	$\delta^{13}C$	= 0.47
δD	vs.	ring width	= -0.68
$\delta^{13}C$	vs.	ring width	= -0.70

All these correlation coefficients are significant at 0.05 level. The correlation coefficient between $\delta^{18}O$ values and ring width is not significant at this level.

The common signal in the δD, $\delta^{18}O$ and $\delta^{13}C$ records is 61%.

The above results indicate that there is a common mechanism influencing both the ring width and the isotopic

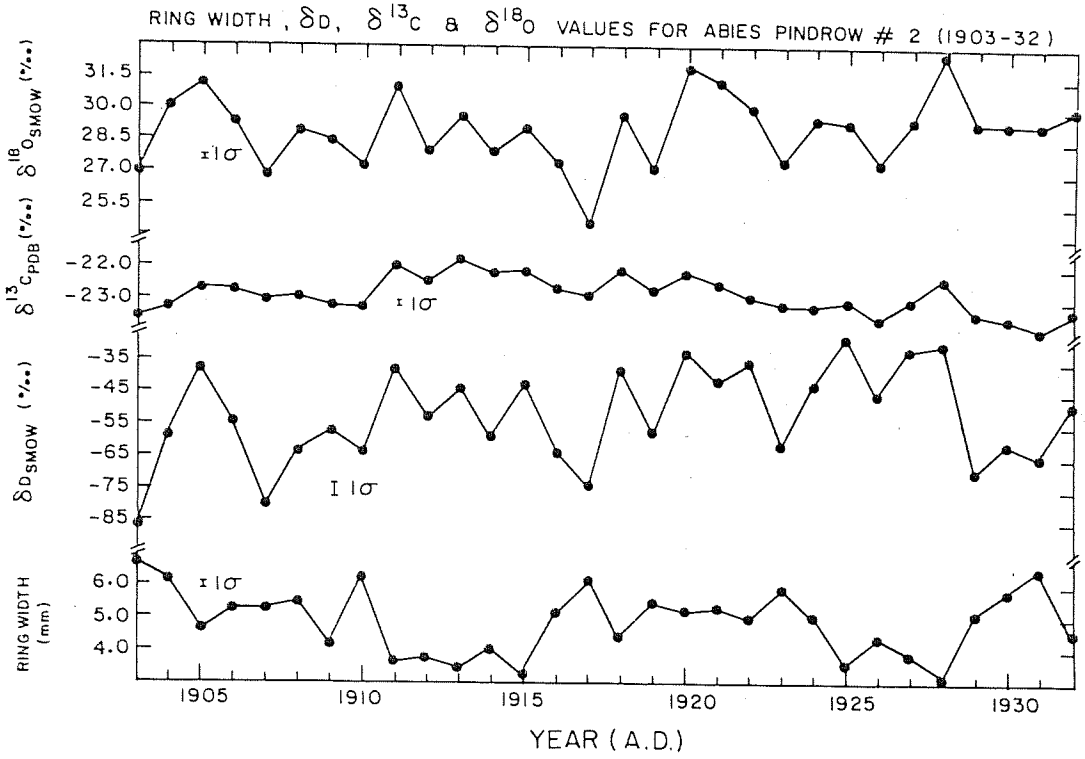


Fig. 4.12 : Ring width, δD, δ^{13}C and δ^{18}O values for Abies pindrow # 2 (1903-32).

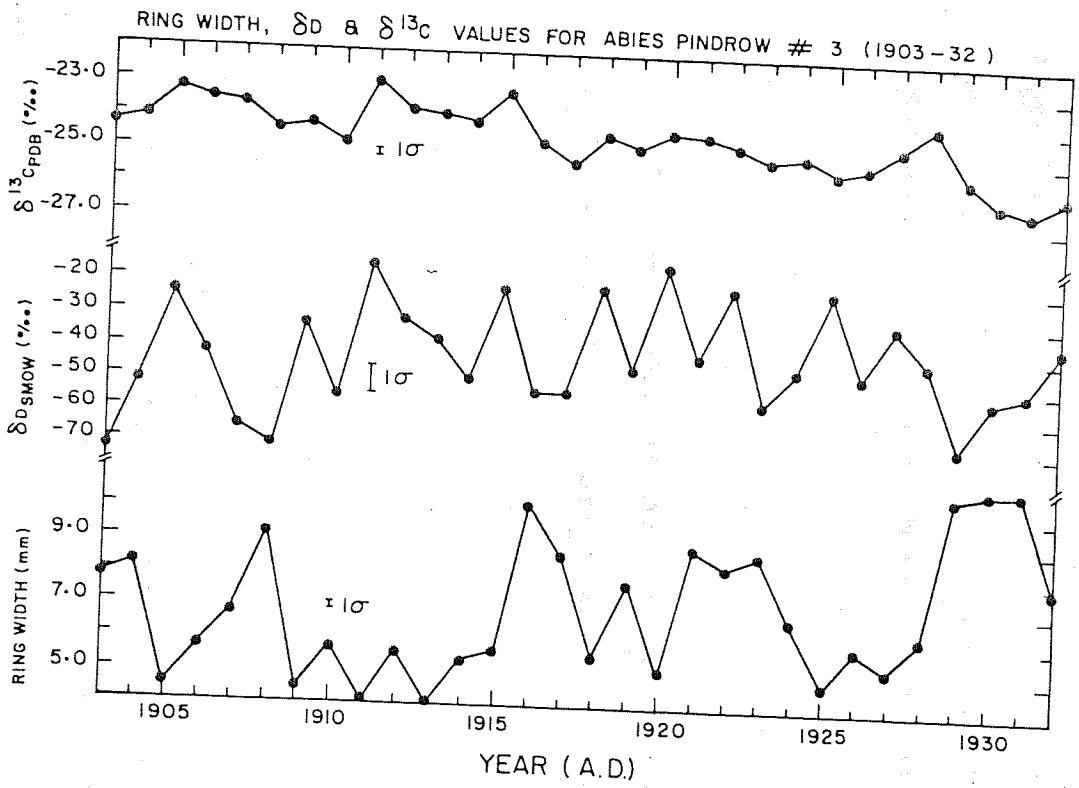


Fig. 4.13 : Ring width, δ D and δ^{13}C values for Abies pindrow # 3 (1903-32).

ratios to a greater or smaller, but significant extent. This mechanism is most likely to be local climatic variations.

4.2.3.1 Correlation between ring width and δ values

This subject has already been discussed in detail in section 2.5. Based on the data of the present work, the applicability of Wigley et al's (1978) model to explain the observed anticorrelation between δD and $\delta^{13}C$ and ring width can be checked. In the case of Abies pindrow (conifer) the late wood width can be assumed to be reasonably constant and that the variations in ring width are mainly due to the variable contributions of early wood. The observed anticorrelation between ring width and δ value can be explained by Wigley et al's (1978) model only if the late wood is isotopically lighter than the early wood. This is obvious from the equation

$$\delta_W = \delta_E + (\delta_L - \delta_E)L/W$$

where δ_W is the whole ring δ value, δ_E and δ_L are the early and late wood δ values respectively. L is the late wood width (assumed to be constant) and W is the whole ring width (Wigley, et al., 1978).

For the 10 year period 1923-32, both early wood (δ_E) and whole ring (δ_W) have been analysed for their δD and $\delta^{13}C$ values for two Abies pindrow trees, AP # 2 and AP # 3. The δ_E vs. δ_W plots (with a reference line y = x)

are shown in fig. 4.14 for δD and fig. 4.15 for $\delta^{13}C$. It is clear from the figures that the required condition $\delta_L < \delta_E$ is not always met. Therefore, it is not possible to explain the observed anticorrelation between ring width and δ values using Wigley, et al's (1978) model.

It appears, therefore, that Wigley et al's (1978) contention that δ values may in part be controlled by ring width variations and need not be independent indicators of climate is not valid. The basic assumption in their model is that isotopically the tree ring cellulose can be considered to be a two component system. However, Wilson and Grinsted (1975, 77) and Epstein (1978) have found that if a tree ring is sampled on a finer scale than just early wood and late wood, there is a more or less continuous isotopic variation throughout the ring. Thus, the apparent two component isotopic values of early wood and late wood is more an artifact of sampling (Yapp, 1980). An anti-correlation of δ values with ring width would not, in itself, be of sufficient evidence to say that ring width variations are controlling δD and $\delta^{13}C$ variations.

Mazany et al's (1980) explanation (for $\delta^{13}C$) for this effect based on plant productivity, respiration and modification of the $\delta^{13}C$ of the local atmosphere is also unlikely to be valid (Francey and Farquhar, 1982). This is because CO_2 is known to mix rapidly with the free atmosphere,

especially during photosynthesising hours and sparsely vegetated areas. Consequently, a difference in $\delta^{13}C$ between the local and global atmosphere, large enough to produce this effect is unlikely to be maintained.

So one has to look for an alternative explanation for the observed anticorrelation between δ values and ring width. One possible explanation could be that one or more of the climatic parameters have effects on the ring width and δ values in opposite directions. For example, as will be shown in the next section, δD and $\delta^{13}C$ values are negatively correlated with rainfall and humidity while ring width values correlate positively with them. This is expected because during periods of high rainfall and humidity the moisture available to the tree is more; growth is more and consequently the ring is broad. But the δD of the water available to the tree is expected to be less because of possible amount effects (to be discussed in the next section) and less evapotranspiration. Assuming the biochemical fractionation factor (which is unknown for δD, see section 2.3.2) to be constant with respect to variations in rainfall and humidity, this would mean that the δD of the broad ring would be less.

On the other hand during periods of low humidity and rainfall, the tree might be moisture-stressed and the ring will be narrow as a consequence. Also, by the above arguments the δD of the tree ring will be high.

Similar explanation for the $\delta^{13}C$ - ring width effect has been offered by Francey and Farquhar (1982, see sec. 2.5 for details).

The explanation given here is also similar to that proposed by Yapp (1980).

Another result of interest here is that the early wood δ values could be either more or less than the late wood δ values for the different rings in the same tree. This is apparent from fig. 4.14 and 4.15. It is worth comparing this result with that of Epstein and Yapp (1976), who have measured the δD of early and late woods from 10 rings of Douglas Fir, 3 rings of Ponderosa Pine and 4 rings of Montery Pine. In the case of Douglas Fir, they found that the late wood was consistently depleted in δD relative to the early wood, while the opposite was true in the case of Montery Pine. Two rings of the Ponderosa Pine had equal δD values for early wood and late wood while in the third ring the late wood was again depleted in δD relative to early wood.

Normally since the late wood is formed during summer or late summer, the precipitation sampled by the tree will be heavier in δD and consequently, the late wood δD value should be more than that of the early wood. This is seen in the case of Montery Pine. But the opposite effect seen in the case of Douglas Fir and Ponderosa Pine have been explained by Epstein and Yapp (1976) as follows: A small

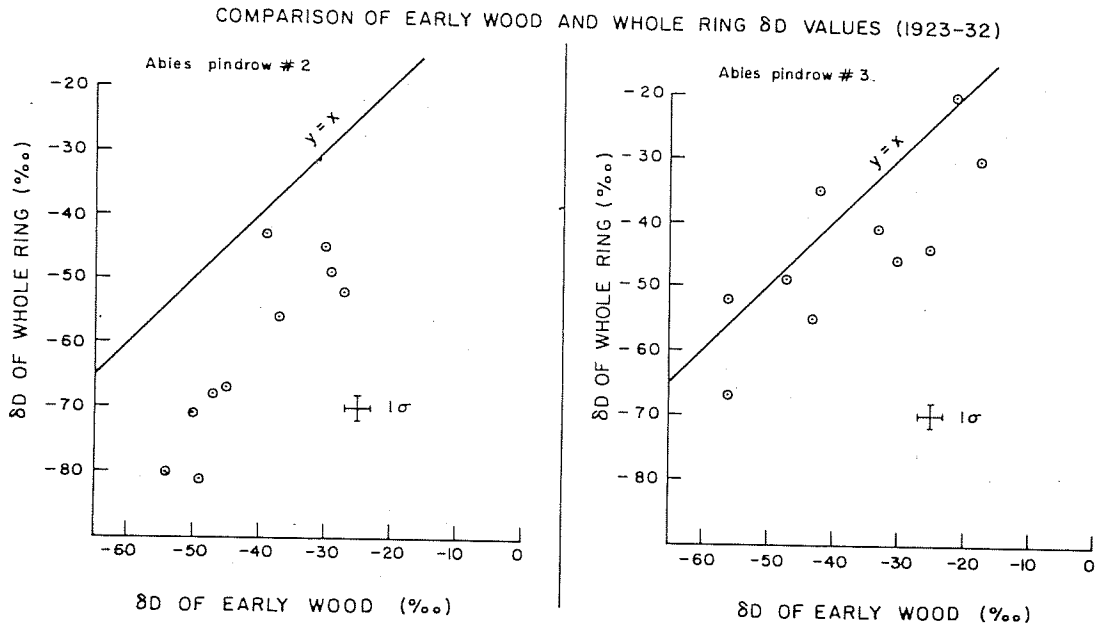


Fig. 4.14 : Comparison of early wood and whole ring δ D values (1923-32).

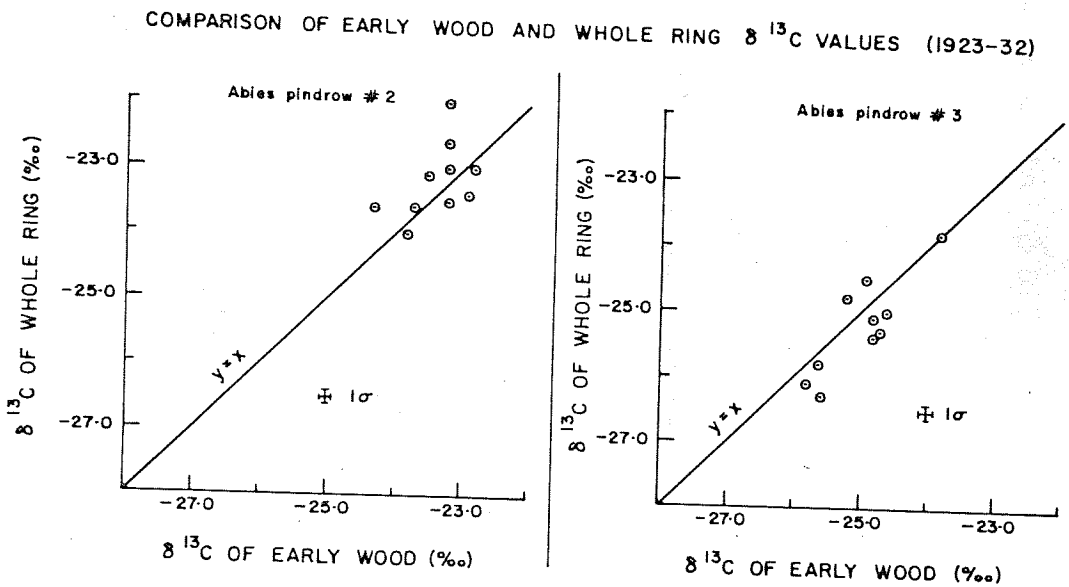


Fig. 4.15 : Comparison of early wood and whole ring $\delta^{13}C$ values (1923-32).

portion of the starch manufactured by the tree during the previous summer could be stored and re-used for the growth during the spring of the current year. Consequently, the early wood could be enriched in δD. Also, if the late wood is synthesised utilizing the winter and spring precipitation, it could be depleted in δD.

This explanation is questionable. The stored photosynthates are used for fresh shoot growth and not for the diameter growth. (Kramer and Kozlowski, 1979). This is evidenced by the findings of Burk (1979) as well. Therefore, it appears that Epstein and Yapp's (1976) explanation may not be valid.

However, Epstein and Yapp (1976) have offered another explanation which seems more plausible:

The source of water for the trees during the formation of early wood might be a mixture of warm rain and isotopically lighter, melted snow. For the formation of the late wood, on the other hand, the only source of moisture must be isotopically lighter, late melting snow, which might have possibly been preserved in the shade of the tree, or stored in the deeper strata of the soil.

However, with the limited data on hand this explanation cannot be tested and still remains a speculation.

It appears from the results of the present work that the early and late wood differences in δ values are not explainable by such simplistic assumptions and some complex physiological process might be responsible for such an effect.

4.2.3.2. Correlation between δD and $\delta^{18}O$ values

The δD values of cellulose nitrate and $\delta^{18}O$ values of cellulose prepared from individual rings along two radial sections (R_1 and R_2) of AP # 2 are plotted in fig. 4.16. A linear trend is obvious, but some scatter is also evident.

The best fit line for the radius R_1 is given by

$$\delta D = (7.00 \pm 0.29)\ \delta^{18}O - (264.80 \pm 8.40)$$

$$n = 30,\ R = 0.79$$

and for the radius R_2 is given by

$$\delta D = (8.62 \pm 0.41)\ \delta^{18}O - (294.95 \pm 11.94)$$

$$n = 30,\ R = 0.64$$

The procedure followed to obtain the least square fits is that of Williamson (1968).

The scatter is more in the case of shorter radius (R_2) than in the case of the longer radius (R_1). Also, the slopes and intercepts of the two equations representing the two radii are distinctly different. Inspite of the scatter,

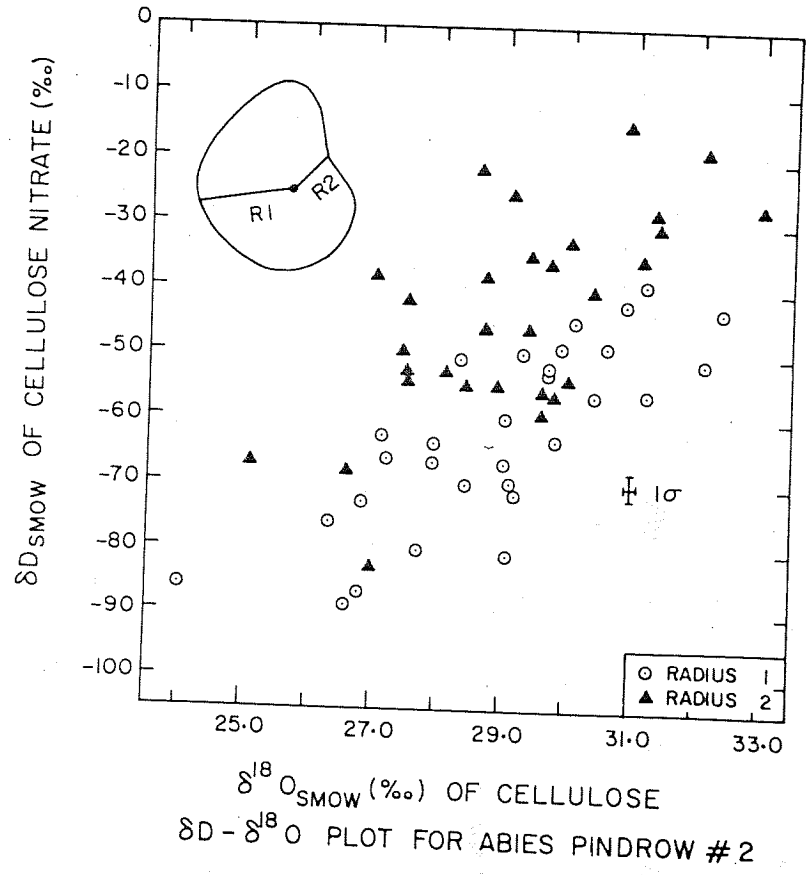


Fig. 4.16 : $\delta D - \delta^{18}O$ plot for Abies pindrow # 2.

the correlation coefficients in both cases are quite good and significant at 0.05 level.

The expected relationship between the δD of cellulose nitrate (δD_{CN}) and the $\delta^{18}O$ of cellulose ($\delta^{18}O_C$) extracted from plant material can be derived empirically using the currently available data in the literature. When submerged aquatic plants are considered, the isotopic fractionation is limited to the biochemical step alone. Yapp and Epstein (1982a) have reported the δD_{CN} and δD of the associated environmental water (δD_{EW}) for different species of aquatic plants. Using their data, the following relationship was obtained:

(a) $$\delta D_{CN} = 1.11 \ \delta D_{EW} - 22.9$$

Likewise, the $\delta^{18}O_C$ and $\delta^{18}O_{EW}$ for some aquatic plants have been reported by Ferhi et al (1983). Using this data the following relationship was obtained:

(b) $$\delta^{18}O_C = 0.62 \ \delta^{18}O_{EW} + 25.8$$

This is slightly different from what has been obtained by Ferhi et al (1983):

(c) $$\delta^{18}O_C = 0.65 \ \delta^{18}O_{EW} + 25.4$$

This is because in contrast to usual unweighted regression the least square method of linear regression proposed by Williamson (1968), where each datum point is weighted with the uncertainty, has been used here, to obtain both equations (a) and (b).

The global relationship between average δD and $\delta^{18}O$ of precipitation has been reported by Craig (1961):

(d) $$\delta D = 8 \delta^{18}O + 10$$

However, for continental stations, the slope of the above equation is generally less than 8. van der Straaten and Mook (1983) have reported a slope of 6.8 ± 0.8 for 33 continental stations. Krishnamurthy (1984) has measured δD and $\delta^{18}O$ of North Indian ground waters and has obtained the following relationship.

(e) $$\delta D = (6.75 \pm 0.21)\ \delta^{18}O + (1.79 \pm 0.18)$$

Additionally, he has analysed some water samples from the Kashmir valley, collected from lakes, rivers and ground water. These data fall on a cluster. Since δD and $\delta^{18}O$ of precipitation from Kashmir are not available, it is assumed here that the relationship between δD and $\delta^{18}O$ for Kashmir precipitation is same as (e):

(f) $$\delta D = 6.8\ \delta^{18}O + 2$$

Since the interest here is to compare the empirically derived relationship between δD_{CN} and $\delta^{18}O_C$ with the observed results for Abies pindrow # 2 from Kashmir, the relationship between δD and $\delta^{18}O$ of precipitation for continental stations has been chosen rather than the world meteoric line equation (d).

If the environmental water (soil moisture in this case) sampled by the plant isotopically represents the precipitation then equation (f) can be rewritten as

(g) $$\delta D_{EW} = 6.8\ \delta^{18}O_{EW} + 2$$

Combining (a), (g) and (b), one obtains:

(h) $$\delta D_{CN} = 11.6\ \delta^{18}O_{C} - 320$$

Since (a) and (b) are for aquatic plants (h) represents the δD_{CN}- $\delta^{18}O_{C}$ relationship for AP # 2 only if the tree had not undergone any evaporative enrichment of leaf water during its growth (i.e. assuming 100% relative humidity). However, since the mean growing season relative humidity for AP # 2 for the period 1903-32 A.D. varied from 67 to 83%, it is most likely that the tree did undergo evapotranspiration and consequent enrichment in δD and $\delta^{18}O$ of leaf water (the source water for cellulose synthesis) during its growth. In such a case the slope of equation (h) is expected to be less than the empirically calculated value of 11.6. Since the δD, $\delta^{18}O$ values of precipitation and atmospheric water vapour are not available, it is not possible to calculate the value of the expected slope for the mean humidity of 75% experienced by the tree.

The experimentally obtained slopes of 7.0 ± 0.3 and 8.6 ± 0.4 with a mean of 7.8 ± 0.8 is much less than 11.6 indicating the effect of humidity on leaf water and consequently on the tree ring cellulose.

One inherent assumption made in the derivation of equation (h) is that the biochemical fractionation factor is independent of temperature. This appears to be true for oxygen isotopes in the range of 15-25°C (DeNiro and Epstein, 1981). However, there appears to be a temperature dependence of the biochemical fractionation factor for hydrogen isotopes. (DeNiro and Epstein, 1981). For example, White (1983) has estimated a value of 1.6 °/oo per °C for white pines from New York. If this is the case, then some scatter can be expected in the δD_{CN} - $\delta^{18}O_C$ relationship, as observed in the present case.

Another point of interest is that the slopes for the two radii R_1 and R_2 are different. Since the microclimate experienced by the tree cannot be very different for the two sides of the tree in question, it appears that this difference is brought about by some fractionation mechanism taking place during the translocation of photosynthates to the trunk. It is also possible that the harmone control on cambial growth along different directions of the trunk might be responsible for causing the observed difference in the slopes and the scatter.

Though the data are limited, it must be noted that existence of a linear relationship between δD_{CN} and $\delta^{18}O_C$ has been demonstrated for the first time.

4.2.4 Correlation between climatic parameters and isotopic records

As discussed in Chapter 2, most of the earlier work in isotope dendroclimatology has been on spatial correlations between δ values and climatic parameters, mainly temperature. This is because the relationship between δD and $\delta^{18}O$ of meteoric water is better known on a spatial scale rather than on a temporal scale in a given region. For the Kashmir Valley δD and $\delta^{18}O$ values of precipitation are not available even for the recent years. Therefore, it was decided to compare the δD, $\delta^{13}C$ and $\delta^{18}O$ values from tree ring cellulose with the available climatic parameters for this region.

Because of the intra ring variations, tree to tree and species to species variations in δ values, mean δ values for the site have to be calculated for each year to compare with the instrumental weather records. Annual rings (for the period 1903-32) from 3 radial sections of AP # 2 and 1 radial section of AP # 3 have been analysed for δD and $\delta^{13}C$. Two radial sections of AP # 2 have been analysed for $\delta^{18}O$. Though the number of trees analysed is limited, as a first approximation the yearwise means of these measurements (for each isotope) have been taken to represent the mean isotopic record from this site.

The instrumental weather records were available from the Monthly Weather Review published by the India

Meteorological Department, New Delhi. The Gulmarg weather station is only 8 kms. away from the tree site. Hence it can be assumed that the climatic conditions experienced by the trees was reasonably close to those recorded by the weather station. Monthly values of temperature, humidity, rainfall, wind velocity and cloud amount were available from this station for April to September of each year from 1903 to 1932 A.D. The mean growing season value for each year was calculated for all the climatic parameters except rainfall, where the total growing season rainfall was calculated.

The aim of the present exercise is to obtain an estimate of the relative degrees of influence of each of the climatic parameters on δ values rather than a formula linking them for the purpose of palaeoclimatic reconstruction.

While temperature influences the values of δD and $\delta^{18}O$ of the meteoric water sampled by the tree and of the leaf water through evapotranspiration and perhaps the biochemical fractionation factor, humidity and wind velocity determine the equilibrium and kinetic enrichment of the leaf water δD and $\delta^{18}O$ values. These factors may also be affected by the amount of precipitation received by the tree (Epstein, et al., 1977). Apart from these the cloud amount may also influence the δ values of tree ring cellulose. Schiegl (1974) notes: "On overcast days there is hardly any assimilation because of insufficient solar radiation". Because of the

above considerations, the δD, $\delta^{13}C$ and $\delta^{18}O$ records of tree ring cellulose were individually regressed with temperature (T), humidity (H), precipitation (P), wind velocity (V) and cloud amount (C). Since the ring widths are generally sensitive to the mean maximum summer temperature (T_{max}) (Hughes, M.K., personal communication, 1982), this was also included in the multiple regression. There was a significant correlation between T and T_{max} (0.57). Deletion of either of these from the multiple regression did not alter the multiple correlation coefficient significantly.

It is to be noted here that such multiple regression studies, which could yield valuable information on the relative influences of the various climatic parameters on the tree ring isotopic record, have not been attempted so far.

4.2.4.1. δD record in tree rings and climatic parameters:

When the mean δD record for the 30 year period (1903-32 A.D.) was regressed with the climatic parameters, the following relationship was obtained:

$$(1) \qquad \delta D = -171.79 + 1.01\ T - 0.01\ P - .72\ H - 0.69\ W - 4.14\ C + 2.32\ T_{max}$$

where T is the mean growing season temperature (^{o}F), T_{max} is the mean maximum growing season temperature (^{o}F), P is the precipitation (cents), H is the relative humidity (%),

and C is the cloud amount (octas). Since the dimensions of these climatic parameters are different, it is not possible to compare the coefficients of equation (1). For doing this, each variable has to be standardised using the Z transform

$$Z_i = \frac{x_i - \mu}{\sigma}$$

where x_i is the variable, μ is the mean and σ, the standard deviation of x_i (i runs from 1 to 30). Thus all the standardised variables (dimensionless) have a mean of zero and standard deviation of unity.

For the standardised variables, the equation (1) becomes

$$(2) \qquad \delta D = 0.16\ T - 0.40\ P - 0.21\ H - 0.05\ W - 0.19\ C + 0.25\ T_{max}$$

The multiple correlation coefficient (R) is 0.79, which is significant at 0.01 level. (F value = 6.45, degrees of freedom for regression = 6, degrees of freedom for deviations = 23). Thus 63% (R^2) of the variance in the δD record is accounted for by these climatic variables jointly. The individual correlation coefficients for each of the climatic parameters are listed in table 4.10. Individually, mean temperature, maximum temperature, precipitation, humidity, wind velocity and cloud amount account respectively for 18%, 29%, 39%, 37%, 2% and 4% of the variance in δD.

Table 4.10

Linear correlation coefficients between the isotopic ratios, ring width and mean growing season climatic parameters

Variable	Temperature T	Mean maximum temperature T_{max}	Amount of Precipiration P	Relative Humidity H	Wind velocity W	Cloudiness C
δD	0.43	0.54	-0.63	-0.61	-0.15*	-0.20*
$\delta^{13}C$	0.17*	0.01*	-0.35	-0.40	0.35	-0.66
$\delta^{18}O$	0.11*	0.25*	-0.35	-0.50	0.03*	-0.31
Ring width	-0.24*	-0.10*	0.42	0.33	-0.09*	0.52

* Not significant at 0.1 level (two tailed t test)

It is seen from equation (2) that the amount of precipitation has the maximum effect on δD of tree ring cellulose. There is an anticorrelation between the two (-0.63) which is significant at 0.05 level. Dansgaard (1964) has noted such an "amount effect" for precipitation samples collected and analysed by the International Atomic Energy Agency. He observed that the isotopic ratios were "low in rainy months and high in months with sparse rain". He stated: "this 'amount effect' is found all the year round at most tropical stations and in summer at mid latitudes". If such an amount effect exists for the precipitation in Kashmir region, then this effect could lead to an anticorrelation between the amount of precipitation and δD of tree ring cellulose utilizing this precipitation. This effect has recently been detected in tree ring cellulose by Lawrence and White (1984) and Gray and Song (1984) as well.

While wind velocity and cloud amount do not show a significant correlation even at 0.1 level, humidity and temperature show correlations significant at 0.05 level. The negative correlation (-0.61) with humidity is expected because when the humidity is more, the evapotranspirative enrichment in δD of leaf water is less and consequently the δD of the cellulose nitrate from the tree ring is less. On the contrary when the humidity is low, δD of the cellulose nitrate is expected to be higher.

There is not much difference between the correlations between δD and T and δD and T_{max}. However, in both cases the correlation is positive. This is also expected since the δD of the precipitation sampled by the tree is directly related to the temperature at the site of precipitation and the evaporative enrichment of deuterium in the leaf water is also directly related to temperature. During the subsequent biosynthesis of cellulose deuterium gets depleted. The temperature dependence of this process is not yet understood. Since this effect is in a direction opposite to that of the other two mentioned above, the net correlation between δD of cellulose nitrate and temperature is not as high as one can expect.

4,2.4.2. $\delta^{13}C$ record in tree rings and climatic parameters:

When the mean $\delta^{13}C$ record from tree rings was regressed with the climatic parameters, the following relationship was obtained:

(3) $$\delta^{13}C = -17.00 + 0.07\ T - 0.00\ P - 0.06\ H + 0.16\ W - 0.36\ C - 0.08\ T_{max}$$

(symbols and dimensions are explained in section (4.2.4.1).

For the standardised (see sec. 4.2.4.1) variables the relation is:

(4) $$\delta^{13}C = 0.27\ T - 0.12\ P - 0.40\ H + 0.26\ W - 0.39\ C - 0.21\ T_{max}$$

The multiple correlation coefficient (R) is 0.77, which is significant at 0.01 level (F value = 5.70, degrees of freedom for regression = 6, degrees of freedom for deviations = 23). Thus 60% (R^2) of the variance in $\delta^{13}C$ record is accounted for by the climatic variables jointly. The individual correlation coefficients for each of the climatic parameters are listed in table 4.10.

Individually, the mean temperature, mean maximum temperature, precipitation, humidity, wind velocity and cloud amount account respectively for 3%, 0.01%, 12.5%, 16%, 12.5% and 43% of the variance in $\delta^{13}C$.

It is seen that $\delta^{13}C$ is insensitive to T and T_{max}. Humidity and cloud amount have the maximum influence on $\delta^{13}C$ and both are negatively correlated with $\delta^{13}C$. Also, there is a significant anticorrelation between the amount of precipitation and $\delta^{13}C$. These anticorrelationsare in line with expectations as described below:

Low humidity causes a reduction in g, the stomatal conductance of the CO_2. This perhaps is the result of the trees opposing effect to minimise the increasing evapotranspiration due to low humidity. Consequently, the partial pressure of CO_2 inside the leaf (C_i) goes down. Similar

reduction in C_i is caused by low precipitation, when the assimilation rate of CO_2 (A) is reduced. According to Francey and Farquhar's (1982) model, such a reduction in C_i causes an increase in $\delta^{13}C$ of cellulose (see section 2.5). Also, when the cloud amount is high, the light levels are low. A low irradiances, A and to a lesser extent, g are reduced. The net result is that C_i increases and consequently $\delta^{13}C$ decreases. Evidences for the above effects have also been found by other workers recently (Francey and Farquhar, 1982).

The significant positive correlation between wind velocity and $\delta^{13}C$ can also be explained as follows: When the wind velocity is more, the evapotranspiration is more as in the case of low humidity. Thus a reduction in g and C_i will result and therefore $\delta^{13}C$ will be more. Thus the results of the present work are consistent with Francey and Farquhar's (1982) model.

From fig. 4.6, a declining trend (~ 1.5 $^{o}/_{oo}$) in the mean $\delta^{13}C$, especially from 1911, can be seen. This has also been observed in the case of Dutch trees by Tans and Mook (1980). This decline in $\delta^{13}C$ is expected because of the fossil fuel contribution to the atmospheric CO_2 and also the forest soil CO_2 contribution due to land use (see section 2.5 for details).

Another point of interest is that there is an auto-correlation of 0.55 in the $\delta^{13}C$ record, with one year lag, which is significant at 0.05 level. The autocorrelation, which is a measure of "persistence", is however absent in δD, $\delta^{18}O$ and ring width records. Also the autocorrelation coefficients with more than a year lag (upto 5 years) are not significant. Storage and reuse of photosynthates of the previous year could not be responsible for the autocorrelation with one year lag seen in the mean $\delta^{13}C$ record, because this is absent in δD, $\delta^{18}O$ and ring width records.

Such an autocorrelation has been found in the case of ring width by Tans and Mook (1980). However, because of the "lack of a sufficient number of data points and inhomogeneous character of the time series" (data points are not spaced at regular intervals of one year), the autocorrelation for the $\delta^{13}C$ record was not calculated by them. However, they expected an autocorrelation in $\delta^{13}C$ as well based on the following observations:

The potential for tree growth represents the integrating effect that prior seasons have on the physicall well-being of the tree. (The influence on tree growth of climatic factors prior to the growing season has been described in detail by Fritts (1976)). This general physical state of the tree can have an influence on $\delta^{13}C$, if, for instance, growth starts earlier & is more vigorous in spring, or through

different concentrations of growth regulating substances, which may shift the rate limiting step in photosynthesis and hence the fractionation. This effect on fractionation, however, will be of a local and transitory nature. Thus the climate of the previous season can have an indirect effect on the $\delta^{13}C$ of the current season, which might lead to the observed autocorrelation.

Tans and Mook (1980) have also observed that this effect might be related to ring width. However, the present data do not show this effect in ring width, δ D or $\delta^{18}O$. Therefore it is unlikely that Tans and Mook's explanation is valid in the present case. The autocorrelation seen only in $\delta^{13}C$ appears to favour a causal mechanism extraneous to the tree and a likely reason for this effect might be that there is an autocorrelation in the $\delta^{13}C$ of atmospheric CO_2, which has been increasing steadily, (and has seasonal cycles as well), as observed by Keeling et al (1979).

4.2.4.3. $\delta^{18}O$ record in tree rings and climatic parameters:

When the mean $\delta^{18}O$ record from tree rings was regressed with the climatic parameters, the following relationship was obtained:

(5) $$\delta^{18}O = 35.61 - 0.04\ T - 0.00\ P - 0.14\ H + 0.05\ W - 0.62\ C + 0.14\ T$$

(symbols and dimensions are explained in section 4.2.4.1).

For the standardised (see section 4.2.4.1) variables, the relation is:

$$(6) \qquad \delta^{18}O - 0.06\ T - 0.12\ P - 0.36\ H + 0.03\ W - 0.25\ C + 0.13\ T_{max}$$

The multiple correlation coefficient (R) is 0.57, which is not significant even at 0.05 level. (F value = 1.85, degrees of freedom for regression = 6, degrees of freedom for deviations = 23). Thus, only 33% (R^2) of the variance in the $\delta^{18}O$ record is accounted for by the climatic variables jointly. The individual correlation coefficients for each of the climatic parameters are listed in table 4.10.

Individually, the mean temperature mean maximum temperature, precipitation, humidity, wind velocity and cloud amount account respectively for 1%, 6%, 13%, 25%, 0.1% and 10% of the variance in $\delta^{18}O$.

It is seen that $\delta^{18}O$ in tree ring cellulose in insensitive to T, T_{max} and the wind velocity (W). Amount of precipitation, humidity and cloudiness are significantly, but negatively correlated with $\delta^{18}O$. Thus the "amount effect" found in the case of δD (see section 4.2.4.1) is also detectable in $\delta^{18}O$ of tree ring cellulose. Also, the anticorrelation of $\delta^{18}O$ with humidity is similar to that obtained for δD. These anticorrelations are as expected (see section 4.2.4.1).

While cloudiness has no effect on δD, it has a significant anticorrelation with $\delta^{18}O$ as in the case of $\delta^{13}C$, though the magnitude is lower. This is because, as explained in section 4.2.4.2, cloudiness affects the light intensity available for photosynthesis, which in turn has as influence on the CO_2 assimilation rate (A) and the stomatal conductance (g) of CO_2 in the leaf. Though the cellulose oxygen has been shown to represent the leaf water isotopically, the pathway of oxygen to cellulose is through the CO_2, which gets equilibrated with leaf water. (see section 2.4). Hence, the cloudiness is expected to affect $\delta^{18}O$ (and $\delta^{13}C$) and not δD.

At present the oxygen isotopic fractionation in the various steps of biosynthesis of cellulose is poorly understood. Ferhi et al (1983) have suggested that even the atmospheric oxygen may contribute to the cellulose oxygen. In view of these complications it is not very clear as to how the anticorrelation between cloudiness and $\delta^{18}O$ comes about.

4.2.4.4. Ring width record and climatic parameters:

Because of the large variations in absolute values and trends in ring width measurements, it is not possible to do multiple regression analysis on the limited number of measurements available. The linear correlation coefficients with the various climatic parameters have been calculated individually and listed in table 4.10. It is seen

that ring width is insensitive to mean temperature, mean maximum temperature and wind velocity. However, significant positive correlations are found with humidity, amount of precipitation and cloudiness. This is expected because low precipitation and low humidity signify lower water-availability to the tree and consequently ring width will be low. Higher cloudiness, perhaps signifies higher rainfall and consequently the ring width may be broader.

It is seen that the effect of humidity, amount of precipitation and cloudiness are opposite in the case of isotopic ratios as has been explained in sections 4.2.4.2 - 3. Thus a parametric negative correlation between isotope ratios and ring width is expected and has been observed in section 4.2.3. The insensitivity of ring width to temperature is also expected because temperature is not the growth limiting factor in the Gulmarg tree site.

Summarising, though significant correlations have been found between climatic parameters and isotopic records in trees, extraclimatic factors also seem to have some influence on them. Therefore, it is necessary to choose proper sites where the trees are climatically stressed (by lack of rain or low temperatures for instance) in order to maximise the climatic signal in the isotopic record and minimise the extraclimatic "noise" in the signal.

CHAPTER 5

SUMMARY AND RECOMMENDATIONS

5.1 Summary of the Results

Intra ring isotopic variations have been studied in detail in the case of Abies pindrow. The common signal obtained from the three radii of this tree which had a lobate growth was 95% for δD and $\delta^{18}O$ and 89% for $\delta^{13}C$. Thus the maximum "noise" due to intra ring variations in Abies pindrow has been estimated to be 5% for δD and $\delta^{18}O$ and 11% for $\delta^{13}C$. This "noise" is expected to be lower in the case of normal trees with less asymmetric growth. Though the absolute values of δD, $\delta^{13}C$ and $\delta^{18}O$ differ along the three radii, the patterns are similar in each case. Thus for

the purpose of analysis of trend, it seems that measurement along a single radial strip of Abies pindrow would suffice. This is substantiated by the fact that the trend in the D and $\delta^{13}C$ record from this tree parallels those obtained from another tree of the same species growing nearby. Such a detailed study of the intraring isotopic variations are reported for the first time.

Secondly, the coherence of the climatic signal from several trees in a given climatic regime has been ascertained. The coherence in δD and $\delta^{13}C$ records are 92% and 83% respectively for 2 trees (Abies pindrow) of the same species and 79% and 84% respectively for 4 trees of different species (Abies pindrow, Pinus wallichiana and Cedrus deodara), all growing near one another. The former result is for whole ring (cellulose nitrate) measurements whereas the latter are for early wood measurements. It appears that there is some "noise" due to species effect in the δD signal.

The results for $\delta^{13}C$ record are consistent with Francey and Farquhar's (1982) model for carbon isotopic fractionation in C_3 plants. The mean difference of 1.6 $^{o}/_{oo}$ between the $\delta^{13}C$ records obtained from 2 trees agrees well with the theoretically calculated value using their model.

There are significant anticorrelation between δD and $\delta^{13}C$ and ring width variations. This is perhaps

because of the opposing effects of climatic parameters like humidity and precipitation on the isotopic ratios and ring widths. Wigley et al's (1978) model fails to explain the observed results.

δD of cellulose nitrate and $\delta^{18}O$ of cellulose from Abies pindrow along two radii are seen to be linearly related with a mean slope of 7.8 ± 0.8. This is the first result of its kind. This seems to agree with the empirically calculated slope using recent results on δD and $\delta^{18}O$ of plant cellulose and water.

Multiple linear regression of the isotopic records (δD, $\delta^{13}C$ and $\delta^{18}O$) with mean growing season climatic parameters like temperature, maximum temperature, humidity, precipitation, wind velocity and cloud amount yielded significant multiple correlations in the case of δD and $\delta^{13}C$.

Individually, δD is found to be significantly influenced by temperature, humidity and rainfall; $\delta^{13}C$ is influenced by precipitation, humidity, wind velocity and cloud amount; $\delta^{18}O$ is sensitive only to precipitation, humidity and cloud amount.

The "amount effect" for δD and $\delta^{18}O$ which perhaps exists for precipitation in Kashmir, has been detected in tree-ring cellulose. The sign of the various correlation coefficients are as expected from empirical considerations.

Since an accurate evaluation of the dependence of δ values on climatic parameters needs more extensive measurements, the above results are to be considered as first approximation in indicating the exact relative influences of the different climatic parameters on the isotopic records in tree ring cellulose.

5.2 Recommendations

Though significant correlations have been obtained between isotopic records and climatic parameters, there is considerable "noise" due to apparent extraclimatic factors like tree physiology, species effects, etc. It is, therefore, important to choose proper sites where the tree growth is climatically stressed, either due to low temperature or low precipitation, and measure the isotopic ratios. In such places the extraclimatic "noise" is perhaps, minimised significantly and hence the climatic signal can be optimised.

Teak trees (*Tectona grandis*) in the west coast of India offer such a possibility, because their growth is controlled essentially by the Indian summer monsoon. If one is able to demonstrate a definite relationship between δ values of cellulose from teak trees and the intensity of summer monsoons, this could be of great use in reconstructing the monsoon history over several hundred years. This, of course, depends on the availability of long tree ring sequences of teak from

the west coast. Efforts must be made to build such chronologies using wood from old buildings and buried logs. Such tree ring based monsoon reconstructions would be of great use to monsoon-dependent Indian agriculture.

More trees from the Gulmarg area have to be analysed to substantiate the conclusions made in this work. Also fast grown species like Chinar (Platanus orientalis) can be studied for high resolution climatic reconstruction.

Measurement of δD and $\delta^{18}O$ of precipitation samples must be undertaken all over India. This will not only help to interpret the tree ring data, but would also serve as a data base for other monsoon studies, palaeoclimatic reconstructions and climatic models.

More controlled growth chamber experiments must be done to characterise the isotopic fractionations taking place during the biosynthesis of cellulose and other components of wood. Models can be developed for the isotopic enrichment in leaves, which will help a great deal in palaeoclimatic reconstruction using the tree ring isotopic record.

Summarising, one can say that there is a lot of scope for isotope dendroclimatology in India in the near future.

REFERENCES

Arnold, L.D. (1979) The Climatic response in the partitioning of the stable isotopes of carbon in juniper trees from Arizona. Ph.D. Thesis, submitted to the University of Arizona, U.S.A.

Bender, M.M. and Berge, A.J. (1982) Carbon isotope records in Wisconsin trees, Tellus, 34, p.500-504.

Bhattacharya, S.K. and Gopalan, K. (1981) A bakeable glass pirani gauge for monitoring low vacuum. P.R.L. Technical Note, 81-06.

Bottinga, Y. and Craig, H. (1969) Oxygen isotope fractionation between CO_2 and water and the isotopic composition of marine atmospheric CO_2. EPSL, 5, p.285-295.

Brenninkmeijer, C.A. and Mook, W.G. (1981) A batch process for direct conversion of organic oxygen and water to CO_2 for $^{18}O/^{16}O$ analysis. Int. J. Appl. Radiat. Isot., 32, p.137-141.

Browning, B.L., (1975) The chemistry of wood (Ed.) R.E. Krieger, Huntington, New York.

Burk, R.L. (1979) Factors affecting oxygen-18/oxygen-16 ratios in cellulose. Ph.D. Thesis, submitted to the University of Washington, U.S.A.

Burk, R.L. and Stiver, M. (1981) Oxygen isotope ratios in trees reflect mean annual temperature and humidity. Science, 211, p.1417-1419.

Craig, H. (1953) The geochemistry of the stable carbon isotopes, Geochim. Cosmochim. Acta. 3, p.53.

Craig, H. (1954) Carbon-13 variations in Sequoia rings and the atmosphere. Science, 119, p.141-143.

Craig, H. (1961) Standard for reporting concentrations of deuterium and oxygen-18 in natural waters, Science 133, p.1833.

Craig, H. and Gordon, L.I. (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere, In: Stable Isotopes in Oceanographic Studies and Palaeotemperatures, Spoleto, July 26-30, 1965.

Dansgaard, W. (1964) Stable isotopes in precipitation, Tellus 16 p.436-468.

Dansgaard, W., Johnson, S.J., Clausen, H.B. and Langway, C.C. (Jr.), (1971) Climatic record revealed by Camp Century ice core. In: Late Cenezoic Glacial Ages (Ed). Turekian, K.K., Yale University.

DeNiro, M.J. and Epstein, S. (1979) Relationship between the oxygen isotope ratios of terrestrial plant cellulose, carbon dioxide and water. Science, 204, p.51-53.

DeNiro, M.J. (1981) The effects of different methods of preparing cellulose nitrate on the determination of the D/H ratios of non-exchangeable hydrogen of cellulose. EPSL, 54, p.177-185.

DeNiro, M.J. and Epstein, S. (1981) Isotopic composition of cellulose from aquatic organisms. Geochim. Cosmochim. Acta, 45, p.1885-1894.

Emiliani, C. (1955) Pleistocene temperatures J. Geol. 63, p.538-578.

Epstein, S., Buchsbaum, R., Lowenstam, H.A. and Urey, H.C. (1953) Revised carbonate water isotopic temperature scale, Geol. Soc. Am. Bull., 64, p.1315-1326.

Epstein, S., Yapp, C.J. and Hall, J.H. (1976) The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. EPSL 30, p.241-251.

Epstein, S. and Yapp, C.J. (1976) Climatic implications of the D/H ratio of hydrogen in C-H groups in tree cellulose EPSL 30, p.252-261.

Epstein, S., Thompson, P. and Yapp, C.J. (1977) Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198, p.1209-1215.

Epstein, S. (1978) The D/H ratio of cellulose in a New Zealand Pinus radiata; A reply to the criticism of A.T. Wilson and M.J. Grinsted, EPSL 39, p.303-307.

Farmer, J.G. and Baxter, M.S. (1974) Atmospheric carbon dioxide levels as indicated by the stable isotope record in wood, Nature, 247, p.273-275.

Farquhar, G.D. (1980) Carbon isotope discrimination by plants : effects of carbon dioxide concentration and temperature via the ratio of intercellular and atmospheric CO_2 concentrations. In: Carbon dioxide and climate: Australian Research, Ed: G.I. Pearman, Australian Academy of Science, Canberra, p.105-110.

Ferhi, A., Letolle, R., Long, A. and Lerman, J.C. (1979) Factors controlling the variations of oxygen-18 in plant cellulose. In: Carbon dioxide effects Research and assessment program., Proc. Int. Meet. Stable Isotopes in Tree ring research, New York, Ed. G.C. Jacoby, p.71-82.

Ferhi, A., Bariac, T. and Letolle, R. (1983) $^{18}O/^{16}O$ ratios in leaf water and cellulose of aquatic and terrestrial plants. In: Palaeoclimates and Palaeowaters : A collection of environmental isotope studies, IAEA, p.85-99.

Fotidar, A.N. (1970) Some observations on two storied kail deodar forests with suggestions for their treatment. Proc. of the State Forestry Conference, Forest Department, Jammu and Kashmir, Srinagar, p.70-74.

Francey 1979

Francey, R.J. (1981) Tasmanian tree rings belie suggested anthropogenic $^{13}C/^{12}C$ trends. Nature, 290, p.232-235.

Francey, R.J. and Farquhar, G.D. (1982) An explanation of $^{13}C/^{12}C$ variations in tree rings. Nature, 297, p.28-31.

Freyer, H.D. and Weisberg, L. (1973) ^{13}C decrease in modern wood due to the large scale combustion of fossil fuels. Die Naturwissenchaften, 60, p.517.

Freyer, H.A. and Weisberg, L. (1974) Dendrochronology and ^{13}C content in atmospheric CO_2. Nature, 252, p.757.

Freyer, H.D. and Weisberg, L. (1975) Anthropogenic carbon-13 decrease in atmospheric CO_2 as recorded in modern wood. In: Isotope Ratios as Pollutant Source and Behavioural Indicators, IAEA, Vienna, Unipub, New York, p.49-62.

Freyer, H.A. (1979) On the ^{13}C record in the tree rings. Part I. ^{13}C variations in northern hemispheric trees during the last 150 years, Tellus, 31, p.124-137.

Freyer, H.D. (1979a) On the ^{13}C record in tree rings. Part II. Registration of microenvironmental CO_2 and anamolous pollution effect. Tellus, 31, p.308-312.

Freyer, H.D. and Belacy, N. (1983) $^{13}C/^{12}C$ records in Northern Hemispheric trees during the past 500 years - anthropogenic impact and climatic superpositions. JGR, 88, C11, p. 6844-6852.

Fritts, H.C. (1976) Tree rings and Climate, Academic Press, New York.

Gat, J.R. (1981) Properties of the isotopic species of water: The 'isotope effect'. In: Stable Isotope Hydrology. Deuterium and Oxygen-18 in the water cycle, IAEA, Chp.2, p.8.

Gat, J.R. (1981) Isotopic Fractionation. In: Stable Isotope Hydrology, Deuterium and Oxygen-18 in the Water Cycle. IAEA, Chp.3, p.29.

Gonfiantini, R. (1984) Advisory group meeting on stable isotope reference samples for geochemical and hydrological investigations. International Atomic Energy Agency, Vienna, 19-21 September 1983, Report to the Director General, p.2.

Gray, J. and Thompson, P. (1976) Climatic information from $^{18}O/^{16}O$ ratios of cellulose from tree rings. Nature, 262, p.481-482.

Gray, J. and Thompson, P. (1977) Climatic information from $^{18}O/^{16}O$ analysis of cellulose, lignin and whole wood from tree rings. Nature, 270, p.708-709.

Gray, J. and Thompson, P. (1979) Natural variations in the ^{18}O content of cellulose. In: Carbon dioxide effects research and assessment program. Proc. Int. Meet. on Stable Isotopes in tree ring research, New York, Ed. G.C. Jacoby, p.84-92.

Gray, J. (1981) The use of stable isotope data in climate reconstruction, In: Climate and History: Studies on past climate and their impact on man (Ed) T.M.L. Wigley, M.J. Ingrams and G. Farmer, Cambridge University Press, p.53-81.

Gray, J. and Song, S.J. (1984) Climatic implications of the natural variations of D/H ratios in tree ring cellulose. EPSL, 70 p.129-138.

Gray, J., Yonge, C. and Thompson, P. (1984) Simultaneous determination of $^{18}O/^{16}O$ and D/H in compounds containing C, H and O, Int. J. Appl. Radiat. Isot. 35, 6, p.525-530.

Grinsted, M.J. and Wilson, A.T. (1979a) Hydrogen isotopic chemistry of cellulose and other organic material of geochemical interest. New Zealand Journal of Science, 22, p.281-287.

Grinsted, M.J., Wilson, A.T. and Ferguson, C.W. (1979) $^{13}C/^{12}C$ variations in Pinus longaeva (Bristlecone pine) cellulose during the last millenium. EPSL, 42, p.251-253.

Grinsted, M.J. and Wilson, A.T. (1979b) Variations of $^{13}C/^{12}C$ ratio in cellulose of Agathis australis (kauri) and climatic change in New Zealand during the last millenium. New Zealand Journal of Science, 22, p.55-61.

Green, J.W. (1963a) Wood cellulose. In: Methods in carbohydrate chemistry III (ed.) R.L. Whistler, Academic Press, New York, p.9.

Green, J.W. (1963b) Nitration with a mixture of nitric and phosphoric acids, In: Methods in carbohydrate chemistry III (ed.) R.L. Whistler, Academic Press, New York, p.222.

Hardcastle, K.G. and Friedman, I. (1974) A method for oxygen isotope analyses of organic material. Geophy. Res. Lett. 1, 4, p.165-167.

Harkness, D.D. and Miller, B.F. (1980) Possibility of climatically induced variations in the ^{14}C and ^{13}C enrichment patterns as recorded by a 300 year old Norwegian pine. Radiocarbon, 22, 2, p.291-298.

Hendy, C.H. (1971) The isotopic geochemistry of speleotherms I. The calculation of the effects of different modes of formation on the isotopic composition of speleotherms and their applicability as palaeoclimatic indicators. Geochim. Cosmochim. Acta., 35, p.801.

Hughes, M.K., Kelly, P.M., Pilcher, J.R. and LaMarche, V.C. (Jr.) (1982) Climate from tree rings, Cambridge University Press, Cambridge.

Jansen, H.S. (1962) Comparison between ring dates and ^{14}C dates in a New Zealand kauri tree. New Zealand Journal of Science, 5, p.74-84.

Keeling, C.D., Mook, W.G. and Tans P.P. (1979) Recent trends in the $^{13}C/^{12}C$ ratio of atmospheric CO_2. Nature, 277, p.121-123.

Kramer, P.J. and Kozlowski, T.T. (1960) Physiology of trees, McGraw Hill Book Company, New York, p.42.

Kramer, P.J. and Kozlowski, T.T. (1979) Physiology and Woody Plants, Academic Press, New York.

Krishnamurthy, R.V. and DeNiro, M.J. (1981) Sulfur interference in the determination of hydrogen concentration and stable isotope composition in organic matter Annl. Chem. 54, p.153-54.

Krishnamurthy, R.V. (1984) Stable isotope studies on sedimentary deposits and ground waters and their climatic implications, Ph.D. Thesis, submitted to the Gujarat University, Ahmedabad 380 009, India.

Lawrence, J.R. and White, J.W.C. (1984) Growing season precipitation from D/H ratios of Eastern White Pine. Nature, 311, p.558-60.

Leavitt, S.W. and Long, A. (1983a) An atmospheric $^{13}C/^{12}C$ reconstruction generated through removal of climatic effects from tree ring $^{13}C/^{12}C$ measurements, Tellus, 35B, p.92-102.

Leavitt, S.W. and Long, A. (1983b) Possible climatic response of ^{13}C in leaf cellulose of pinyon pine in Arizona, U.S.A. Isotope Geoscience, 1, p.169-180.

Leavitt, S.W. and Long, A. (1984) Sampling strategy for stable carbon isotope analysis of tree rings in pine. Nature, 311, p.145-147.

Libby, L.M., (1972) Multiple thermometry in palaeoclimate and historic climate. J.G.R. 77, p.4310-4317.

Libby, L.M. and Pandolfi, L.J. (1974) Temperature dependence of isotope ratios in tree rings, Proc. Nat. Acad. Sci. USA, 71, p.2482-2486.

Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshall III, J., Becker, B. and Giertz-Stenbenlist, V. (1976) Isotopic tree thermometers, Nature, 261, p.284-288.

Long, A. (1982) Stable isotopes in tree rings. In: Climate from tree rings (Ed.) Hughes, M.K., Kelly, P.M., Pilcher, J.R. and LaMarche, V.C. (Jr.), Cambridge University Press, Cambridge, p.13-18.

MacIntyre, F. (1979) Carbon-13 in tree rings indicates no record of sea-surface temperature. Science, 205, p.1127-1129.

Mazany, T., Lerman, J.C. and Long, A. (1980) Carbon-13 in tree ring cellulose as an indicator of past climates. Nature, 287, p.432-435.

McKinney, C.R., McCrea, J.M., Epstein, S., Allen, H.A. and Urey, H.C. (1950) Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios, Rev. of Sci. Instr. 21, p.724-730.

Northfelt, D.W., DeNiro, M.J. and Epstein, S. (1981) Hydrogen and carbon isotopic ratios of the cellulose nitrate and saponifiable lipid fractions prepared from annual growth rings of a California red wood. Geochim. Cosmochim. Acta. 45, p.1895-1898.

Pearman, G.I., Francey, R.J. and Fraser, P.J.B. (1976) Climatic implications of stable carbon isotopes in tree rings, Nature, 260, p.771-773.

Peng, T.H., Broecker, W.S., Freyer, H.D. and Trumbore, S. (1983) A deconvolution of the tree ring based $\delta^{13}C$ record. JGR, 88, p.3609-3620.

Raina, A.N. (1977) Geography of Jammu and Kashmir, National Book Trust, New Delhi, p.51-52.

Rao, K.R. and Juneja, K.B.S., (1971) A handbook of field identification of fifty important timbers of India, Forest Research Institute Press, Dehra Dun, p.68, 71, 80.

Raza, M., Ahmed, A., Mohammed, A. (1978) The valley of Kashmir. A geographical interpretation, Vol I. The Land, Vikas Publishing House Pvt. Ltd., New Delhi, p.109-110.

Rittenberg, D., and Ponticarvo, L. (1956) A method for determination of the ^{18}O concentration of the oxygen in organic compounds, Int. J. Appl. Rad. Iso., 1, p.208-214.

Schlegl, W.E. (1974) Climatic significance of deuterium abundance in growth rings of Picea., Nature, 251, p.582-584.

Smith, B.N. and Epstein, S. (1971) Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant. Physiol., 47, p.380-384.

Sternberg, L.S.L. and DeNiro, M.J. (1983) Biogeochemical implications of the isotopic equilibrium fractionation factor between the oxygen atoms of acetone and water, Geochim. Cosmochim. Acta, 47, p.2271-2274.

Stuiver, M. (1978) Atmospheric CO_2 increases related to carbon reservoir changes. Science, 199, p.253-258.

Stump, R.K. and Frazer, J.W. (1973) Simultaneous determination of carbon, hydrogen and nitrogen in organic compounds. Nucl. Sci. Abs., 28, p.7848.

Sufi, G.R. (1970) The regeneration of silver fir and spruce in eastern Himalayas with special reference to the State of Jammu and Kashmir, Proc. of the State Forestry Conference, Forest Department, Jammu and Kashmir, Srinagar, p. 20-24.

Tans, P.P. and Mook, W.G. (1980) Past atmospheric CO_2 levels and the $^{13}C/^{12}C$ ratios in tree rings. Tellus, 32, p.268-283.

Thompson, P. and Gray, J. (1977) Determination of $^{18}O/^{16}O$ ratios in compounds containing C, H and O. Int. J. Appl. Radiat. Isot., 28, p.411-415.

Timell, T.E. (1957) Nitration as a means of isolating alpha cellulose component of wood, Tappi, 40, p.30-33.

Troup, R.S. (1921) The Silviculture of Indian Trees Vol III, The Clarendon Press, Oxford, p.1015-1154.

Urey, H.C. (1947) The thermodynamic properties of isotopic substances, J. Chem. Soc., p.562-581.

van der Straatten, C.M. (1981) Deuterium in organic matter. Ph.D. Thesis, submitted to Rijksuniversiteit te Groningen, Netherlands.

van der Straatten, C.M. and Mook, W.G. (1983) Stable isotopic composition of precipitation and climatic variability. In: Palaeoclimates and Palaeowaters: A collection of environmental isotope studies, IAEA, p.53-64.

White, J.W.C. (1983) The climatic significance of D/H ratios in white pine in the north eastern United States. Ph.D. Thesis, submitted to the Columbia University, New York.

Wigley, T.M.L., Gray, B.M. and Kelly, P.M. (1978) Climatic interpretation of $\delta^{18}O$ and δD in tree rings, Nature, 271, p.92-94.

Wigley, T.M.L. (1982) Oxygen-18, carbon-13 and carbon-14 in tree rings. In: Climate from tree rings (Ed.) Hughes, M.K., Kelly, P.M., Pilcher, J.R. and LaMarche, V.C. (Jr.), Cambridge University Press, Cambridge, p.18-21.

Williamson, J.H. (1968) Least squares fitting of a straight line. Canadian Journal of Physics, 46, p.1845-47.

Wilson, A.T. and Grinsted, M.J. (1975) Paleotemperatures from tree rings and the D/H ratio of cellulose as a biochemical thermometer. Nature, 257, p.387-388.

Wilson, A.T. and Grinsted, M.J. (1976) The possibilities of deriving past climate information from stable isotope studies on tree rings. Proc. Int. Conf. on Stable Isotopes, August 1976, New Zealand.

Wilson, A.T. and Grinsted, M.J. (1977) $^{13}C/^{12}C$ in cellulose and lignin as palaeothermometers. Nature, 265, p.133-135.

Wilson, A.T. (1978) Pioneer agriculture explosion and CO_2 levels in the atmosphere, Nature, 273, p.40-41.

Yapp, C.J. (1980) The variations and climatic significance of D/H ratios in the carbon-bound hydrogen of cellulose in trees. Ph.D. Thesis, submitted to the California Institute of Technology, U.S.A.

Yapp, C.J. and Epstein, S. (1982a) A reexamination of cellulose carbon-bound hydrogen δD measurements and some factors affecting plant-water D/H relationships. Geochim. Cosmochim. Acta, 46, p.955-965.

Yapp, C.J. and Epstein, S. (1982b) Climatic significance of hydrogen isotope ratios in tree cellulose. Nature, 297, p.636-639.

Yapp & Epstein (1982) JGR.