

WATER RELATIONS OF *MYRICA ESCULENTA* BUCH.-HAM.  
EX D.DON AND *LYONIA OVALIFOLIA* (WALL.) DRUDE  
IN KATHMANDU

A DISSERTATION SUBMITTED FOR THE PARTIAL FULFILLMENT OF  
THE REQUIREMENTS FOR THE MASTER OF SCIENCE IN BOTANY

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## RECOMMENDATION

This is to certify that the dissertation work entitled **Water Relations of *Myrica esculenta* Buch.-Ham. Ex D.Don and *Lyonia ovalifolia* (Wall.) Drude in Kathmandu** submitted by Mr. Dol Raj Luitel for the partial fulfillment of M.Sc. degree in Botany, has been carried out under my supervision. The entire work is based on the results of his own work and has not been submitted for any other degree to the best of my knowledge.

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## LETTER OF APPROVAL

This dissertation paper submitted by Mr. Dol Raj Luitel entitled "**Water relations of *Myrica esculenta* Buch.-Ham. ex D.Don and *Lyonia ovalifolia* (Wall.) Drude in Kathmandu**" has been accepted as a partial fulfillment of Master's Degree in Botany.

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## ABSTRACT

Variation in water relation parameters and phenomorphological processes were studied in *Myrica esculenta* Buch.-Ham. ex D.Don., an evergreen and *Lyonia ovalifolia* (Wall.) Drude., a deciduous species on their natural habitat at Hattiban forest of Kathmandu, Nepal. Phenology, specific leaf mass and water relation parameters such as Pressure-Volume (P-V) curve analysis, xylem conductance, leaf relative water content were studied from August 2002 to June 2003 at an interval of one month.

During the dry months, plants maintained high turgor pressure, low osmotic potential and low bulk modulus of elasticity. At the same time plant entered phenologically more active period i.e. new leafing. Lower water potential facilitated water extraction from dry soil and help to maintain proper turgidity of cell (osmotic adjustment). The lower value of both LRWC<sub>pd</sub> and LRWC<sub>md</sub> at dry months of the year in both species showed lower water content in the plant that induced the senescence of leaves. Plants never reached to its turgor loss point during observation. SLM of *Myrica esculenta* an evergreen species was higher than *Lyonia ovalifolia* a deciduous species.



## ACRONYMS AND ABBREVIATIONS

$\bar{X}$	Arithmetic Mean
Al	Leaf Surface Area
$A_{we}$	Effective Xylem Area
$A_{xyl}$	Cross Sectional Area of Xylem
BP	Balance Pressure
$g_s$	Stomatal Conductance
HMG/N	His Majesty's Government of Nepal
HV	Huber Value
$K_1$	Leaf Specific Conductivity
$K_h$	Hydraulic Conductivity
$K_s$	Specific Conductivity
LRWC	Leaf Relative Water Content
$LRWC_{md}$	Midday Leaf Relative Water Content
MPa	Mega Pascal
$LRWC_{pd}$	Predawn Leaf Relative Water Content
PG	Pressure Gradient
PLC	Percent Loss of Hydraulic Conductivity
P-V Curve	Pressure Volume Curve
r	Karl Pearson Coefficient of Correlation
RWC	Relative Water Content
$RWC_z$	Relative Water Content at Zero Turgor
SLA	Specific Leaf Area
SLM	Specific Leaf Mass
X	Coefficient Factor of Leaf Area
$\epsilon$	Bulk Modulus of Elasticity
$\sigma$	Standard Deviation
$\psi_p$	Pressure Potential
$\psi_{pf}$	Pressure Potential at Full Turgor
$\psi_s$	Osmotic Potential
$\psi_{sf}$	Osmotic Potential at Full Turgor
$\psi_{sz}$	Osmotic Potential at Zero Turgor
$\psi_w$	Water potential



# CONTENTS

ACKNOWLEDGEMENT

ACRONYMS AND ABBREVIATIONS

LIST OF TABLES, FIGURES AND PLATES

ABSTRACT

<b>1.</b>	<b>INTRODUCTION</b>	<b>1-8</b>
1.1	Background	1
1.2	Plant Water Status	3
1.3	P-V Curve Analysis	4
1.4	Xylem Conductance	5
1.5	Specific Leaf Mass (SLM)	6
1.6	Phenology	6
1.7	Justification	7
1.8	Objectives	8
1.9	Limitation	8
<b>2.</b>	<b>LITERATURE REVIEW</b>	<b>9-23</b>
2.1	Historical Background	9
2.2	Major Publications	10
2.3	Recent Works	10
	2.3.1 Plant Water Status	11
	2.3.2 P-V curve analysis	13
	2.3.3 Xylem conductance	16
	2.3.4 Specific Leaf Mass (SLM)	19
	2.3.5 Phenology	21
<b>3.</b>	<b>SITE DESCRIPTION</b>	<b>24-26</b>
3.1	Location	24
3.2	Climate	24
3.3	Vegetation	24
3.4	Species Characteristics	25



<b>4.</b>	<b>MATERIALS AND METHODS</b>	<b>27-36</b>
4.1	Pressure Volume (P-V) Curve Analysis	27
4.1.1	Sample collection and rehydration	28
4.1.2	Bench drying and B.P. Measurement	28
4.1.3	Components of plant water potential	29
4.1.4	Osmotic Potential ( $\psi_s$ )	29
4.1.5	Turgor potential (pressure potential)	30
4.1.6	Relative Water Content (RWC) at zero turgor	30
4.1.7	Bulk modulus of elasticity ( $\epsilon$ )	30
4.2	Xylem Conductance	31
4.2.1	Pressure Gradient (PG)	32
4.2.2	Effective xylem area (Awe)	32
4.2.3	Huber value (HV)	33
4.2.4	Hydraulic conductivity (Kh)	34
4.2.5	Leaf specific conductivity (Kl)	34
4.2.6	Specific conductivity (Ks)	34
4.3	Relative Water Content (RWC) of leaves	34
4.4	Specific Leaf Mass (SLM)	35
4.5	Phenology	35
4.6	Statistical Analysis	35
4.6.1	Mean ( $\bar{X}$ )	35
4.6.2	Standard Deviation ( $\sigma$ )	36
4.6.3	Coefficient of Correlation	36
<b>5.</b>	<b>RESULTS</b>	<b>37-48</b>
5.1	Pressure Volume (P-V) Curve Analysis	37
5.2	Xylem Conductance	39
5.3	Leaf Relative Water Content (LRWC)	41
5.4	Specific Leaf Mass (SLM)	43
5.5	Phenology	44
<b>6.</b>	<b>DISCUSSION</b>	<b>49-56</b>
<b>7.</b>	<b>CONCLUSION</b>	<b>57</b>
<b>8.</b>	<b>REFERENCES</b>	<b>58-73</b>
<b>9.</b>	<b>APPENDICES</b>	<b>74-77</b>



## LIST OF TABLES

Table 1	Karl Pearson coefficient of correlation between various parameters for <i>Myrica esculenta</i>	47
Table 2	Karl Pearson coefficient of correlation between various parameters for <i>Lyonia ovalifolia</i>	48

## LIST OF FIGURES

Fig. 1	Climatic data of study site from August 2002 to June 2003	26
Fig. 2	Parameters obtained from P-V curve analysis in <i>Myrica esculenta</i>	38
Fig. 3	Parameters obtained from P-V curve analysis in <i>Lyonia ovalifolia</i>	39
Fig. 4	Seasonal variation in various parameters of hydraulic conductivity in <i>Myrica esculenta</i>	40
Fig. 5	Seasonal variation in various parameters of hydraulic conductivity in <i>Lyonia ovalifolia</i>	41
Fig. 6	Variation in Leaf Relative Water Content (LWRC) in <i>Myrica esculenta</i>	42
Fig. 7	Variation in Leaf Relative Water Content (LWRC) in <i>Lyonia ovalifolia</i>	42
Fig. 8	Specific leaf mass (gm.cm <sup>-2</sup> ) of <i>Myrica esculenta</i>	43
Fig. 9	Specific leaf mass (gm.cm <sup>-2</sup> ) of <i>Lyonia ovalifolia</i>	44
Fig. 10	Phenological activities in <i>Myrica esculenta</i>	44
Fig. 11	Phenological activities in <i>Lyonia ovalifolia</i>	45

## LIST OF PLATES

Plate 1	Pressure chamber to measure balance pressure	29
Plate 2	Twig segment fitted with vertical column of oxalic acid solution	31
Plate 3	Twig segment fitted with vertical column of saffranin solution to stain effective xylem area	31
Plate 4	A twig with flower of <i>Myrica esculenta</i>	45
Plate 5	A twig with mature leaves of <i>Lyonia ovalifolia</i>	46



# 1. INTRODUCTION

## 1.1 Background

Growth and development of plants are influenced by many environmental factors, of which water is the most important in terrestrial ecosystem. Plant water status strongly influences plant growth and biomass production, through its effect on leaf and root expansion and on photosynthesis (Ludlow *et al.*, 1983). Although water is the most abundant molecule on earth's surface, the availability of water is the factor that most strongly restricts terrestrial plant production on a global scale. Terrestrial plants are exposed to water regimes that change seasonally and diurnally. If they are to survive, they must adjust physiologically and/or morphologically, have a broad range of tolerance, or avoid the drought period. Low water availability limits the productivity of many natural ecosystems, particularly dry climates. Loss in crop yield due to water stress exceeds losses due to all other biotic and environmental factors combined (Lambers *et al.*, 1998). Higher plants are more sensitive to their water supply and respond more quickly to soil water deficit than to any other environmental factors. Availability of soil water to the roots is the most important ecological factors governing the distribution of higher plants (Noggle and Fretz, 1992).

Trees in Himalayas are subjected to drought for several months because of rain pattern. Study of their response to drought can contribute to the understanding of patterns of distribution, performance and plant water relations in general (Zobel and Singh, 1995). The essential factor in plant water relations is maintenance of sufficiently high water content or turgidity to permit normal functioning of physiological process and growth. All other processes considered in water relations are important chiefly because they affect the internal water relations of trees and thereby modify the



physiological processes and conditions which affect growth (Kramer and Kozlowski, 1960).

The Himalayan climate is characterized by the concentrated rainfall of the monsoon generally three to four months (June-September). Such warm season with concentrated rainfall and complementary eight to ninth dry months are likely to have profound effects on adaptation and ecosystem processes (Zobel and Singh, 1997). So study of the water relations in plants of such climate helps to understand their adaptive mechanism and phenomorphological state in dry months of the year. Study of plant water relations in general provide a cornerstone for interpreting vegetation and plant distributions across environmental gradients (Zobel and Singh, 1995).

The effects of water deficit have been investigated over the past decades at different levels of plant organization and functioning, covering areas ranging from eco-physiology to cellular metabolism (Turner, 1986). Mostly water relations of temperate trees have been done where precipitation is concentrated in winter (Hinckely *et al.*, 1978; Lassoie *et al.*, 1985; Bannister and Kissel, 1986; Young, 1987; Medina and Francisco, 1994; Lowenstein and Pallardy, 1998; Munne and Alegre, 1999; Kolb and Stone, 2000). However, very few works have been conducted on water relations of trees where rainfall is concentrated in warm season.

More detailed study of water relation parameters in Himalayas is necessary to draw the conclusion about adaptive mechanism of species in response to prolonged drought period in particular and to understand the response of ecosystem in general. The present study deals with seasonal variation in water status of plants taking relative water contents of leaves, xylem conductance, specific leaf mass and analysis of pressure volume curve. Effects of water status on phenology of *Myrica esculenta*, an evergreen species and *Lyonia ovalifolia*, a deciduous species are studied in subtropical forest.



## 1.2 Plant Water Status

Plant water status is used in relative sense to refer any measure of general state of a plant in relation to water. It may refer to the plant water potential, its relative water content or its turgor. In general it is perhaps best used to qualitatively describe turgor or relative water content rather than water potential (Passioura, 1982).

The water status is based on physically defined reference rather than a biological one (Kramer and Boyer, 1995). The water potential is the expression of thermodynamic state of water, rather than its total quantity, that influence the biochemical activity of protoplasm. The minimal water potential necessary for normal metabolic activity is species-specific and determines the range of distribution of the various species (Larcher, 1995).

The plant water status can be expressed in term of Relative Water Content (RWC). The metabolic activities like photosynthesis, protein synthesis, nitrate reduction, leaf senescence are better correlated with change in cell volume and RWC (Kozlowski, *et al.*, 1991). RWC is not applicable to soil water status, while water potential units are applicable for description of both plant and soil water status. The leaf relative water content technique has been used to quantify the moisture status of plants (Naidu and Swamy, 1995).

Determining tree water potential during drought has become a corner stone in interpreting vegetation and species distribution and ecosystem characteristics across environmental gradient (Zobel *et al.*, 1976). Although, water potential is the most important parameter of plant water relations (Larcher, 1995) to describe plants and soil water status but no single measure of plant water status can be expected to be correlated with numerous effects of plant water stress (Kramer, 1988). So other variables like leaf Relative Water Content (Naidu and Swamy, 1995), Xylem conductance (Tyree and Ewers, 1996), P-V curve analysis (Tyree and Hammel, 1972) are other important indicators of plant water status.



### 1.3 P-V Curve Analysis

The pressure-volume (P-V) curve is widely used to analyze plant water relations (Koide *et al.*, 1989; Pallardy *et al.*, 1991). P-V curves indicate how components of water potential change as plant tissue loses water and measure the elasticity of tissue.

The relationship between water potential and relative water content is not constant. It varies among the species and with the immediate environment of the plant. The shape of relationship is determined by the osmotic potential of the plant, the proportion of apoplastic water in the plant (i.e., outside the living cells) and elasticity of the cell wall. P-V curve analysis is routinely used for estimating various tissue water relations parameters (Tyree and Hammel, 1972). Most studies of osmotic adjustment, turgor loss and role of elastic modulus in sensing water deficits deal with leaves and rely on use of P-V curve (Pallardy *et al.*, 1991).

Osmotic adjustment and elastic adjustments are the important mechanism of drought resistance and to maintain turgor pressure in plant cells. Both of these mechanisms help to maintain turgor at lower tissue water potential and prevent mechanical damages to plasma membrane (Fan *et al.*, 1994). The active accumulation of solutes in cell, is osmotic adjustment which promotes water absorption at lower water potential. Elastic adjustment results from modification in the cell walls which make them more elastic, thereby facilitating tissue shrinkage during dehydration (Blake and Tshaplinski, 1992). The elasticity of cell wall determines the rate of change of turgor pressure of a cell with respect to change in water content (Cheung *et al.*, 1975). P-V curve allows to determine all these factors.



## 1.4 Xylem Conductance

The balance of the water within plant is determined by the relative rate of water loss and uptake (Herzog *et al.*, 1995). It is now generally agreed that water is pulled up through the xylem by the decrease in water potential developed in leaf cells by loss of water during transpiration (Kozlowski *et al.*, 1991).

The hydraulic architecture is affected by several factors such as cell diameter, cell shape and cell number as well as the number of pits and characteristics of pit membrane (Schulze *et al.*, 1987). Thus, hydraulic architecture can potentially limit the flow of water to leaves controlling leaf water potential, stomatal conductance and gas exchange (Tyree and Ewers, 1996).

The amount of water moved through the vascular system in unit time is dependent on the specific properties of xylem, such as the conducting area (Cross sectional area of vessels) and flow resistances, on the physiological state of the plant (e.g. degree of stomatal opening) and an environmental conditions. The larger the conducting area, the higher the flow velocity (Larcher, 1995).

For xylem conductance the important parameters considered are Huber Value (HV), hydraulic conductivity ( $K_h$ ), specific conductivity ( $K_s$ ) and leaf specific conductivity ( $K_l$ ).

To consider the water relations of whole plant, huber value (i.e. relative xylem conducting area which is the ratio of xylem conducting area and leaf surface area beyond the segment) is more indicative than total cross sectional area (Larcher, 1995).

Hydraulic conductivity ( $K_h$ ) is the ratio of water flux through the existed stem segment and the pressure gradient causing to flow. The specific conductivity ( $K_s$ ) is the hydraulic conductivity per unit cross sectional area of xylem and depends upon the diameter of conducting elements and its types of perforation.



Leaf specific conductivity ( $K_l$ ) is the measure of hydraulic sufficiency of the segment to supply water to leaves distal to that segment (Tyree and Ewers, 1996).

### **1.5 Specific Leaf Mass (SLM)**

The specific leaf mass (SLM) gives the information about productivity of any plant. Various studies have indicated the close relation of nutrient accumulation in leaves with water relations of trees. Himalayan trees have relatively high leaf nitrogen concentration for a given specific leaf area (SLA) and are less efficient at recovering nitrogen from senescing leaves than elsewhere (Zobel and Singh, 1997). The leaves of evergreen species have higher SLM ( $\text{g}/\text{cm}^2$ ) and lower nitrogen and phosphorus content on a mass basis than deciduous species (Medine, 1984; Mooney *et al.*, 1984; Castellanos *et al.*, 1989; Sobrado, 1991).

### **1.6 Phenology**

Phenological observations provide background information on functional rhythms of organisms and communities. It examines how species populations and communities have developed seasonal patterns of various functions under the influence of weather patterns and climate (Singh and Singh, 1992).

Phenology has been defined as the timing of biological events and their relationship to seasonal climatic change (Lieth, 1974). The study of phenomorphological events provides an indication of ecosystem function i.e., they show how species exploit environmental resources for growth and reproductive activities that underlies the structure and evolution of each community (Lieth, 1974; Orshan *et al.*, 1988).

Trees are the largest structural component and the dominant producers in tropical forest (Borchert, 1995). Seasonal patterns of tree development have therefore a major influence on ecosystem properties. The seasonality of tropical tree phenology is mainly determinal by the duration and intensity of



seasonal drought (Mooney *et al.*, 1994; Murphy and Lugo, 1986). Seasonal water stress is likely to determine the timing of phenological events.

Flowering, fruiting, seed germination and seedling establishment are important for ecosystem structure while leaf life span is critical for ecosystem dynamics. Leaf life span not only control nutrient dynamics (Eckstein *et al.*, 1999) but also it has phylogenetic significance (Rogers and Clifford, 1993). Thus foliar phenology is important because of its relations to processes and factors such as tree growth periodicity, flowering, fruiting, plant water stress, leaf gas exchange and longevity, plant-herbivore interaction and ecosystem properties (Reich *et al.*, 1991; Wright, 1991).

## **1.7 Justification**

Although growth and development of plant is influenced by many environmental factors, water, the soul of nature, is the most important factor. Since Himalayan trees are exposed to eight to nine months of dry period water becomes a major limiting factor for many sites. Thence, the study of trees response to drought can contribute to understanding the local pattern of species distribution and performance (Zobel and Singh, 1995). An understanding of water relation is one of the necessary pre-requisites for the development of varieties of plants which will survive in the areas of longer drought and this can only be achieved by an extensive study of the regulatory and adaptive process which lead to change in water relations in a plant in response to environmental changes (Zimmermann and Steudle, 1978). Phenological phases of plants such as flower expansion, leaf expansion, shoot growth etc are known to be inhibited by even moderate water deficit. Thus, the leaf production, flowering, fruiting as well as senescence of the tree species are directly related to seasonal rainfall, soil moisture and the water stored in stem.

Extensive works on water relations of temperate trees have been conducted where precipitation is evenly distributed or concentrated in winter. However, very few works have been conducted in water relations of trees where rainfall



is concentrated in the warm season. Only few M.Sc. dissertations have been done in this field in Nepal Himalaya (Adhikari, 2000; Mainali, 2000; Tripathee, 2001; Shrestha, 2001; Bhattarai, 2001, Pokhrel, 2001; Nepal 2002 and Uprety, 2002). This study focuses on water relations of economically and taxonomically important tree species of Nepal i.e. evergreen species *Myrica esculenta* Buch.-Ham, ex D.Don. (Myricaceae) and deciduous *Lyonia ovalifolia* (Wall.) Dride (Ericaceae) in their natural habitat at Hattiban, Kathmandu.

## **1.8 Objectives**

The broad objective of this work is to determine the adaptive mechanism of *Myrica esculenta* and *Lyonia ovalifolia* in their natural habitat and to correlate the phenomorphological events in different seasons. This water relations work was performed with following specific objectives.

- 1) To document variation in water relations parameters i.e. osmotic potential, turgor potential, relative water content at zero turgor, leaf relative water content, xylem conductance and establish correlation among them (if any).
- 2) To know the timing of drought stress and understand possible mechanism of drought resistance.
- 3) To estimate variation in Specific Leaf Mass (SLM) and correlate with water relations parameters.
- 4) To understand the control of water relations on phenology.

## **1.9 Limitation**

Water relation study requires long term research to conclude the exact mechanism of plants to adopt with the scarcity and availability of water. As the present investigation is a part of M.Sc. study, its duration is only one year.



## 2. LITERATURE REVIEW

### 2.1 Historical Background

The first quantitative experiment was done by Stephen Hales on plant water relations in 1727 which was published in title of 'vegetable static (Kramer and Boyer, 1995). His important finding was that the rate of transpiration varied with species, temperature, time of day and intensity of light.

In the beginning of 19<sup>th</sup> century de Saussure (1804) found that roots differ in permeability to various solutes. The absorption of minerals by plants was not proportional to the absorption of water. In the year 1837, Dutrochet, on the basis of his osmotic theory explained phenomena like water uptake by plants exhibiting root pressure and release of spores from Sporangia. Osmotic pressure was first measured by Pfeffer in 1877. For the development of concept of water relations the work of Sachs (1882 a, b) and his contemporaries in the middle of 19<sup>th</sup> century can be considered a corner stone. Their study mainly concentrated on the effect of soil moisture, soil aeration and soil temperature on water absorption and root growth.

At the beginning of 20<sup>th</sup> century, Brown and Escombe (1900) described diffusion of water through stomata to be a purely physical process. Dixon (1914) published the "Transpiration and Ascent of Sap in Plants", which was a land mark in the study of water relations. Experiment of Stalfelt (1932) and Bange (1953) summarized by Slatyer (1967) indicated that stomatal closure has a large effect on transpiration in moving air where boundary resistance is low.

The development of thermocouple psychrometers (Spanner, 1951; Monteith and Woen, 1958; Richards and Ogata, 1958) and pressure equilibration (Scholander *et al.*, 1964) have made the measurement of water potential easy and reliable.



## 2.2 Major Publications

- a) "Transpiration and Ascent of Sap in Plants" by Dixon (1914).
- b) "Water Deficit and Plant Growth" vol. 1-7 by Kozlowski (1968-83).
- c) "The Physiological Ecology of Woody Plants" by Kozlowski (1972).
- d) "The Physiology and Biochemistry of Drought Resistance in Plants" by Paleg and Aspinall (1981).
- e) "Water Relations of Plants" by Kramer (1983).
- f) "Xylem Structure and Ascent of Sap" by Zimmermann (1983).
- g) "The Physiological Ecology of Woody Plants" by Kozlowski *et al.* (1991)
- h) "Forest Tree Ecophysiology" by Lassoie and Hinckley (1991).
- i) "Water Relations of Plants and Soils" by Kramer and Boyer (1995).
- j) "Physiological Plant Ecology" by Larcher (1995).
- k) "Plant Physiological Ecology" by Lambers *et al.* (1998).
- l) "Environmental biophysics" by Campbell and Norman (1998).

## 2.3 Recent Works

In recent time, some of the prominent scientists working on water relations and phenology are: R. Borchert (University of Kansas, Lawrence, USA); J.S. Sperry (University of Vermont, Vermont, USA); M.T. Tyree (USDA, Forest service, Northeastern Forest Experiment Station, Vermont, USA); D.B. Zobel (Oregon State University, Corvallis, USA). K. Kikuzawa (Hokkaido Forestry Research Institute, Hokkaido, Japan); A.G. Netting (University of New South Wales, Sydney, Australia); S.P. Singh (Kumaon University, Nainital, India).

The work of water relations in Nepal has started since 1998 through a joint project of Prof. Dr. D.B. Zobel (Oregon State University, Oregon, USA) and Prof. Dr. P.K. Jha (Central Department of Botany, T.U., Kathmandu, Nepal). Since, then following dissertation works on water relations in Master's Degree



in Botany was carried out under the supervision of Prof. Dr. P.K. Jha. These are water relations of *Castanopsis tribuloides* (Adhikari, 2000), *Shorea robusta* (Mainali, 2000; Tripathee, 2001), *Pinus roxburghii* (Shrestha, 2001), *Schima wallichii* (Bhattarai, 2001 and Pokharel, 2001), *Alnus nepalensis* and *Cotoneaster bacillaris* (Uprety, 2002), *Quercus lanata* and *Cinnamomum camphora* (Nepal, 2002).

### **2.3.1 Plant Water Status**

Hinckley *et al.* (1979) discussed on various drought avoidance mechanisms in eight species found in central Missouri. All the studied species showed well adapted to survive and to continue functioning during the severe drought of 1976 summer. They observed reduced growth, increased dieback and net photosynthetic rate was depressed to near the compensation point, when the condition of drought increased.

Calkin and Percy (1984) studied the seasonal change in plant water potential on three different species and they found greatest decrease in plant water potential on evergreen species (*Heteromeles arbutifolia*), intermediate on winter deciduous species (*Arcis occidentalis*) and least on drought deciduous species (*Aesculus californica*).

Pena and Grace (1986), using potted plants in green house, investigated the relationship between ultrasound emission rate and water potential before, during and after a period of simulated drought. On re-watering the plant, the density of the xylem recovered and after recovery, the plant that had been growing in drought failed to produce ultrasound emission when water potential fall. Mc Burney (1992) by measuring the water potential using a psychrometer attached to the leaf petiole and pressure chamber found that thickness of leaf was sensitive indicator of plant water status. The relationship between leaf thickness changes and water potential measured in detached leaves, was usually curvilinear and was strongly influenced by leaf age, stress history and leaf growth.



Plant-soil water relations was studied by Calson *et al.* (1994) on forestry and silvopastoral system in Oregon, USA and found that grazing of under story vegetation may reduce water stress of trees during dry periods by reducing transpirational water loss by the forage plants.

Naidu and Swamy (1995) studied leaf relative water content (LRWC) and biomass production in seven tree species. They reported significantly higher LRWC during winter than summer. Their results indicate that measurement of LRWC could be used as simple screening method for selecting the right type of tree species for energy plantations. Dawson (1996) concluded that small trees had lower overall rates of water loss at leaf and canopy levels than large trees.

Thomas and Hortman (1996) studied the plant water relations in mature Oak stands of Northern Germany. They observed that impact of drought on water relations was much lower in sessile oak and LRWC of damaged oak was not lower than those from leaves of healthy trees, even in extremely dry period.

Epron and Dreyer (1996) conducted an experiment on four years old potted saplings of *Quercus petraea* which were exposed to water shortage by withholding irrigation. They observed that after 10 days, predawn leaf water potential was decreased to - 2.0 MPa, leaf photosynthesis reduced by 55%, starch and sucrose concentrations decreased to 47% and 48% respectively, but the glucose and fructose concentration was increased by five fold in water stressed saplings compared with well watered plants. This indicated that drought involves changes in sugar composition which contribute to osmotic adjustment. Chone *et al.* (2001) determined the predawn ( $\psi_{\text{dawn}}$ ) leaf water potential ( $\psi_{\text{leaf}}$ ) and stem water potential ( $\psi_{\text{stem}}$ ) of mature leaves to know the water status of non-irrigated vine in vineyards during the growing season.  $\psi_{\text{stem}}$  was the most discriminating indicator for both moderate and severe water deficits. The difference between stem  $\psi$  and leaf  $\psi$  provided an indirect measurement for mean leaf transpiration, which varied with soil moisture conditions and vapour pressure deficit in the atmosphere.



### 2.3.2 P-V curve analysis

There is intricate relationship between water transport, turgor pressure, active and passive transports of solutes and elastic properties of cell wall which themselves control growth in plant cells (Zimmermann and Steudle, 1978). The study of pressure-volume method by analysing the leaf water relationship in four widespread forest trees (*Ilex opaca* Ait; *Cornus florida* L., *Acer rubrum* L. and *Liriodendron tulipifera* L.) Robert *et al* (1980) recorded the highest (least negative) initial osmotic potentials (the values of osmotic component at full turgidity) at the start of growing seasons. These values decreased (becoming progressively more negative) as the season progressed through a drought period. Following a period of precipitation at the end of drought period, initial osmotic potentials increased towards the values measured earlier in the season.

The rate of water exchange during shrinkage and swelling of a plant cell in response to external osmotic stress is not only controlled by the properties of the membrane but also by the elasticity of the cell wall. Bulk modulus of elasticity is expected to vary widely between species and to change as a result of ontogeny and environmental stress (Tyree and Jarvis, 1982).

From the comparative study of tissue water relations and seasonal change in  $\psi_{\text{leaf}}$  component of an evergreen tree (*Morisonia americana*) and two deciduous shrubs (*Cappairs verucosa* and *C. aristiquetae*) with two deciduous trees (*Humboltiella arborea* and *Lonchocarpus dipteroneurus*) and deciduous vine (*Mansoa verrucifera*) by analyzing the P-V Curve Sobardo (1986) showed that evergreen species could develop a higher leaf turgor and lose turgor at lower  $\psi_{\text{leaf}}$  than deciduous. The osmotic potential at full turgor in evergreen species ( $\cong -3$  MPa) is lower than in deciduous species (-2 MPa) rather than the elastic properties of leaf tissue.

Kubiske and Abrams (1990) indicated that artificial dehydration may significantly alter P-V relationships such as the plateau effect, resulting in enormous measurements of tissue elasticity and osmotic potentials. They also



suggested that linear regression of P-V curve at and above, the turgor loss point may be used to extrapolate saturated weight from non-rehydrated samples at various moisture deficits thus eliminating the plateau effect and other potential rehydration problems.

Fan *et al.* (1994) determined variation in tissue water relations and its contribution to turgor maintenance in species from contrasting ecological zones on seedlings of *Pinus baksiana* Lamb. *Picea mariana* (miu) B.S.P. and *Eucalyptus grandis* W. Hill ex. Maiden. They concluded that elastic adjustment ( $<-3.7$  MPa) was more important for turgor maintenance than osmotic adjustment ( $<-0.13$  MPa). Maximum bulk modulus of elasticity, but not osmotic potential at full turgor was significantly correlated with the RWC and water potential at zero turgor in drought seedlings. Both the inherent capacity for turgor maintenance of a species under drought and its ability to adjust to repeated drought should be considered in genetic relations for drought tolerance.

Zobel (1995) analyzed pressure-volume curve of three deciduous, three evergreen broadleaf, four pine and two other conifer species in the southern Appalachian mountain USA. He observed that sample rehydrated overnight did not differ significantly from results of sample rehydrated overnight plus 24 hours. He also found that water relations parameters usually varied with leaf form, patterns of variation among leaf forms differed for each combination parameters and season. The strongest generality was that leafy deciduous twigs and pines had high tissue elasticity. Whereas evergreen broad leaf species had low elasticity. Evergreen broadleaf species had a consistently high RWC at zero turgor.

The effect of water stress cycles on turgor maintenance processes in *Pyrus communis* was studied by Marsal and Girona (1997) and concluded that the  $\psi_{\text{leaf}}$  and turgor loss point of control leaves tended to decrease from spring to summer. Changes in leaf osmotic water potential at full turgor and in symplast water function did not explain the seasonal decrease in  $\psi_{\text{leaf}}$ .



From the study on *Fraxinus excelsor* by Peltier and Marigo (1998) indicated that drought tolerance is not only due to decrease in osmotic potential (osmotic adjustment) but also due to increase in elastic modulus (elastic adjustment). Osmotic adjustment is involved in the maintenance of turgor pressure and elastic adjustment in the facilitation of continued water uptake from drying soil.

Both elasticity and extent of osmotic adjustment depends upon the structure of tissue. Leaves have substantial and rapid capacity for osmotic adjustment and this capacity is more rapid in upper canopy leaves (Niinemets *et al.* 1999). Grammatikopoulos (1999) from the analysis of p-v curve reported less pronounced summer reduction of water potential at turgor loss point, in two Mediterranean species (*Pholmis fructicosa* L. and *Cistus creticus* L.). He observed that during summer there was gradual decrease in osmotic potential but increase in tissue elasticity. Both of these species used combination of osmotic and elastic adjustment in order to maintain their turgidity during dry period. In the summer, leaf of *P. fructicosa* tolerate dehydration quite successfully surviving at RWC value as low as 17%. This improved dehydration tolerance of summer leaves was attributed to possession of physiological and biophysical adaptation to water stress, reflected in lower osmotic potential at full turgor and at turgor loss point.

Tognetti *et al.* (2000) compared water relations of Mediterranean shrub *Erica arborea* L. *Myrtus communis* L. and *Juniperus communis* L. in elevated and ambient CO<sub>2</sub> and showed that during drought higher turgor potential was maintained under elevated CO<sub>2</sub> so that growth should be affected less than for plants exposed to drought in ambient CO<sub>2</sub>. Elevated CO<sub>2</sub> had the potential to alter the competitive relationship in terms of tissue water relations, by differentially altering the mechanism to withstand stress period. Thus it might represent an important factor in controlling species composition and diversity.



Adhikari (2000) from the study on *Castanopsis tribuloides* in Phulchoki, Lalitpur, Nepal found that osmotic adjustment is the most effective strategy of drought tolerance but elastic adjustment has less effective role. Bhattra (2001) showed elastic adjustment in *Schima wallichii* as the most effective in phenologically active period i.e. during summer drought from the study.

Zobel *et al.* (2001) calculated  $\psi_{st}$ ,  $\psi_{sz}$ , RWC<sub>Z</sub> and index of tissue elasticity by developing p-v curve for the seedlings of *Chamaecyparis lawsoniana* A. murr. From the study of some desiccation tolerant angiosperms Willigen *et al.* (2001) showed that wall folding induced the reduction in cell volume. The wall folding prevented the development of negative turgor and physical stress in the cell and contributed to desiccation tolerance. Arndt *et al.* (2001) determined the physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. They found that as drought developed osmotic potential at full turgor decreased and total solute concentrations increased indicating osmotic adjustment.

Uprety (2002) studied *Alnus nepalensis* and *Cotoneaster bacillaris* and observed that osmotic adjustment was more pronounced in *Alnus nepalensis* but both osmotic and elastic adjustment were significant in *Cotoneaster bacillaris* during drought. He also mentioned higher amount of starch accumulation at the time of leaf maturation. However at the time of bud break, twig growth, leafing and flowering the starch level decreased but sucrose level increased.

### **2.3.3 Xylem conductance**

The hydraulic conductivity of tree depends on sap wood water content and dry wood density (Waring and Running, 1978). Zimmermann (1978) suggested that very large  $K_1$  values in the trunks of dicotyledons trees are the result of large vessels which have low resistance to water flow.

Ewers (1985) compared  $K_1$ , Huber value, specific conductivity and vessel diameters for conifer trees, dicotyledons trees and lianas. He observed that



lianas were similar to dicot trees and conifers in having hydraulic constriction (low  $K_I$ ) at branch junctions. But, lianas generally had greater  $K_I$  and specific conductivities but lower Huber values than did conifers. Dicot trees were intermediate in these values.

Gartner *et al.* (1990) recorded that excised stem segments of vines had higher specific hydraulic conductivities than did trees during the dry season in the deciduous forest in Jalisco, Mexico. Ishida *et al.* (1992) studied the diurnal changes of leaf water potential and stomatal conductance for 12 deciduous shrubs and the saplings in the understory of a temperate forest. They observed three different responses to an increase in transpiration rate: (a) plant had low leaf water potential and slightly increased soil to leaf hydraulic conductance, (b) plants decreased leaf water potential and increased the hydraulic conductance; and (c) plant had high leaf water potential and largely increased the hydraulic conductance.

Joyce and Steiner (1995) studied the hydraulic parameters on *Fraxinus americana* and recorded the highest ( $K_I$ ) in main stem and lowest in leaves bearing lateral shoot. Terminal shoot bearing leaf were larger and had significantly greater mean  $K_I$  than subjacent lateral shoots. They also pointed out that  $K_I$  of shoots bearing leaf from south facing branches was greater than that of north facing branches.

Lovisollo and Schubert (1998) showed that shoot  $K_h$ ,  $K_s$  and  $K_I$  were lower in water stressed plants. At the higher stress level,  $K_h$  was further reduced by vessel embolisms. Stomatal conductance ( $g_s$ ) was linearly correlated with  $K_I$  at low stress levels, suggesting a concerted regulation of water flow, while at higher stress levels  $g_s$  decreased without change in  $K_I$ .

Irvine *et al.* (1998) studied the impact of drought on physiology of 41 years old *Pinus sylvestris* L. Their result suggested that in response to soil water deficit mature *Pinus sylvestris* close its stomata sufficiently to prevent the development of substantial system embolism. As the soil water deficit



developed the hydraulic resistance between soil and needles increased due to decrease in pre-dawn needle water potential.

Bond *et al.* (1999), from their study on stomatal behaviour in four woody trees concluded that much of the variation among the species in stomatal response to soil and atmospheric water deficit could be explained by  $K_i$  and  $\Psi_{\text{threshold}}$ . The relationship between  $\Psi_{\text{threshold}}$  and xylem vulnerability to cavitation differed among these species.

Water supply regulates the development of wood. For example, plants with increased water supply produces wider and longer vessels. However, wood anatomical characters appear stable despite changing environment but functional capacity of wood, measured as specific conductivity, changes significantly (Arnold and Mouseth, 1999).

Brodribb and Hill (2000) suggested that decrease in whole plant conductivity in *Toona australis* resulted from a decrease in xylem conductivity due to stress induced cavitation. Hydraulic properties in relation to soil moisture, leaf habit and phylogenetic lineage of 17 species of *Quercus* that occur sympatrically in northern central Florida (USA), were investigated by Banes and Holbrook (2001). They recorded increased leaf area per shoot and decreased Huber values with increasing soil moisture. As a result, maximum hydraulic conductance and maximum transpiration were found positively correlated with mean soil moisture when calculated on a sapwood area basis, but not on a leaf areas basis. The significant difference in specific conductivity were observed between species, but these differences were not explained by leaf habit or by evolutionary lineage.

Pressure gradients, pressure potential, ion concentrations, volume flow as well as turgor pressure gradient are the major driving forces for water lifting in the xylem conduit (Zimmermann *et al.* 2002).



Cordero and Nilsen (2002) studied the limits to maximum water transport in three *Rhododendron* species that were exposed to frequent winter freeze thaw events (*R. maximum* and *R. catawbiense*) and to a severe summer drought (*R. macrophyllum*). From two years study, they observed that loss of hydraulic conductivity ( $K_h$ ) was significantly higher in winter than summer for *R. macrophyllum* but seasonal changes in  $K_h$  were variable in two other species. They also indicated that a single freeze-thaw event significantly increased  $K_h$ , and *R. catawbiense* had the highest  $K_h$  in response to freeze treatment but had small stem diameter and suffered high embolism in response to a single freeze thaw event. The winter induced and drought induced embolism cause a significant reduction in hydraulic conductivity in all species during the periods when drought or freeze-thaw events occurred in their native habitat.

Stout and Sala (2003) suggested that roots and shoots of *Pinus ponderosa* were significantly more vulnerable to water stress induced cavitation than those of *Pseudotsuga menziesii*. But, shoot specific conductivity did not differ between species in the field. They also indicated that roots were more vulnerable to cavitations than shoots in both the species.

#### **2.3.4 Specific Leaf Mass (SLM)**

According to Singh and Singh (1992), the leaf life span was not strongly correlated with leaf mass, specific leaf mass or leaf production efficiency (net primary productivity per unit area). The leaf mass and productivity depends upon forest types, being highest (10.0-28.2 kg/ha) for evergreen broad leaved species while least in deciduous species (3.7-8.6 kg/ha). The leaf mass duration (leaf mass x month of year with leaf) was related directly to Net Primary Productivity (NPP) and inversely to leaf production efficiency (NPP/Leaf Mass).

The ratio of dry weight to the leaf area of fully mature leaves were about 75 and 17 g cm<sup>-2</sup>, in evergreen and deciduous species, respectively (Sobrado, 1986). This result indicated that SLM of evergreen species is higher than



deciduous species. Prior *et al.* (1997) found that specific leaf area was higher in the deciduous species than evergreen species. Their finding was concluded by studying *Terminalia ferdinandiana* a deciduous tree of northern Australia Savanas and *Eucalyptus tetrodonta*, a co-occurring evergreen tree.

Wilson *et al.* (1999) concluded that high SLM leaves worked better in resources poor environment where retention of captured resource was a higher priority, while low SLM leaves worked best in resource rich environment and short lived and vulnerable to herbivore.

Awada *et al.* (2003) came to conclusion from the study of seasonal dynamics of net photosynthesis in two year old seedlings of *Pinus brutia*, *Pinus pinea* and *Pinus pinaster* that total chlorophyll concentration per unit leaf area, specific leaf area (SLA) and height, significantly increased in *P. pinea* in response to shade, but not in other. In response to shade, *P. brutia* showed a significant increase in total chlorophyll concentration but not SLA. Their study showed *P. pinaster* and *P. brutia* were more light demanding than *P. pinea*.



### 2.3.5 Phenology

Most trees in seasonal dry tropics shed their leaves during the dry season and many species flower or flush soon after leaf shedding. Flower expansion and shoot growth involve cell expansion known to be inhibited by even moderate water deficit (Bradford and Hsiao, 1982).

Study on tree layer phenology of Indian central Himalayan forest between 300–2200m elevation indicated that all the dominant tree species are evergreen with concentrated summer leaf drop (around April) and simultaneous leafing (Ralhan *et al.* 1985). The degree of evergreenness increased with increasing elevation and decreasing summer dryness. He also pointed out that *Myrica esculenta* an evergreen species flower during summer.

A phenomorphological survey was carried out in central Italy by de Lillis and Fontanella (1992) to study the effects of increasing water stress on some characteristic species of the Mediterranean marquis. Nutrient content and leaf water potential were examined. The result showed that three different groups existed, which diverged in the modulation of growth activity: i) Evergreen sclerophyllous species, ii) Drought deciduous species, and iii) semi-deciduous species. Wang (1992) investigated the relationship between the foliar phenology of forty-three north temperate tree species and the winter impairment of hydraulic conductivity. He observed that eight were non-porous conifers of which all but one was evergreen.

The development of tree during the dry season varied considerably between species and between sites of different moisture availability (Borchert, 1994a). He observed the strong correlation of leaf shedding, flowering and shoot growth with seasonal changes in tree water status in the tropical dry lowland forest of Guanacaste, Costa Rica.

Borchert (1994 b) monitored phenology and seasonal changes in tree water status during two consecutive dry seasons in more than 150 trees of 37 species



growing at different sites in the tropical dry lowland forest of Costa Rica where many trees flower or form new shoots soon after leaf shedding during dry season i.e. during the period when trees are likely to be severally water stressed. From this work, he concluded that the elimination of transpiration water loss during leaf shedding in conjugation with utilization of residual water enabled rehydration of stem tissue and subsequent flowering or flushing during the dry season. Rate and degree of rehydration varied strongly with the availability of water stored in tree trunks grown in the sub soil. Stored water buffered the impact of seasonal drought and enabled flowering and flushing during the dry season.

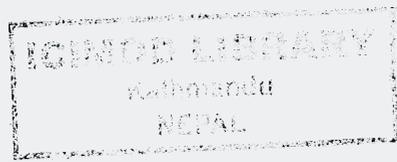
Borchert (1995) studied herbarium material for the analysis of tropical tree phenology. Flowering time and geographical distribution were graphed for 1673 flowering collection from 18 species native to Neo-tropical dry forest. From this work, he came to conclusion that inter-specific differences in the timing of phenology relative to the dry season indicate difference in control of phenology by seasonal drought.

According to Kikuzawa (1995) to avoid leaf shedding plants adopt two alternative modes of leaf emergence. One is successive leaf emergence, in which plants expand one leaf at a time, the leaf utilize full sunlight and is replaced by a second leaf only when its photosynthetic activity decline from maximum. He further pointed out that leaf longevity was short when the maximum photosynthetic rate of leaf was high.

Water shortage during summer and low temperature during winter determine the phenology and limit the growth of leaves (Kyparissis and Manetas, 1997). Volkenburgh (1999) indicated that the rate of leaf expansion and the extent to which they expand is determined partly by genetic constrains and partly by environmental signaling to the plant.

Shrestha (2001) studied water relations and phenology of *Pinus roxburghii* and observed that higher wood water storage capacity and leaf senescence at the





time of bud break which were the important strategies developed by the plant to maintain the favorable water status during dry but phenologically active period. The role of water in the development and yield of coffee crop (*Coffea arabica* L.) was studied by Carr (2001). He mentioned that water must be freely available during the period of fruit expansion to ensure large and high quality seed yields.

The bud break, new leafing, flowering and fruiting occurred during drought period (around April-May) due to supply of water from stem, that also supported by tree water potential, wood water storage and climatic condition indicated by Nepal (2002) from the study on *Quercus lanata* and *Cinnamomum camphora*.



## 3. SITE DESCRIPTION

### 3.1 Location

The study site is the Hattiban Community Forest, at Chalnakhel Village Development Committee, Ward No. 9, Kathmandu. It is located at about 14 km south from the center (Ratna Park) of Kathmandu towards Chovar in between 27° 38' 12.3" N to 27° 38' 13.1" N latitude and 85° 16' 47.2" E to 85° 16' 44.2" E longitude. The altitude ranges from 1377 m to 1405 m. The study site lies on north-east face of the, Hattiban hill with 30° to 45° slope.

### 3.2 Climate

The study area lies on the subtropical zone (Chaudhary, 1998) having cool and dry winter (October-February), hot and dry summer (March-May) and hot and humid monsoon (June-September) The climatic data recorded at Tribhuvan International Airport Station, Kathmandu, about 15 km north from study site is presented here. The maximum rainfall was recorded in August with 499.9 mm, followed by June with 227.8 mm. The average maximum and minimum temperatures were recorded in May (26.6° C) and January (2° C).

### 3.3 Vegetation

The vegetation of the study area is typically subtropical. The upper canopy of the forest is dominated by *Engelhardtia spicata* and *Castanopsis indica* while lower canopy is dominated by *Myrica esculenta* and *Lyonia ovalifolia* in the moist places. However, *Pinus* sp. are more frequent on the uppermost part of hill. The important associate species are *Fraxinus floribunda*, *Schima wallichii*, *Toona ciliata*, *Bauhinia variegata*, *Albizia procera*, *Ficus semicordata*, *Michelia* sp., *Erythriana arborescens*, *Maesa chisia*, *Woodfordia fruticosa*, *Quercus lanata* etc.



### 3.4 Species Characteristics

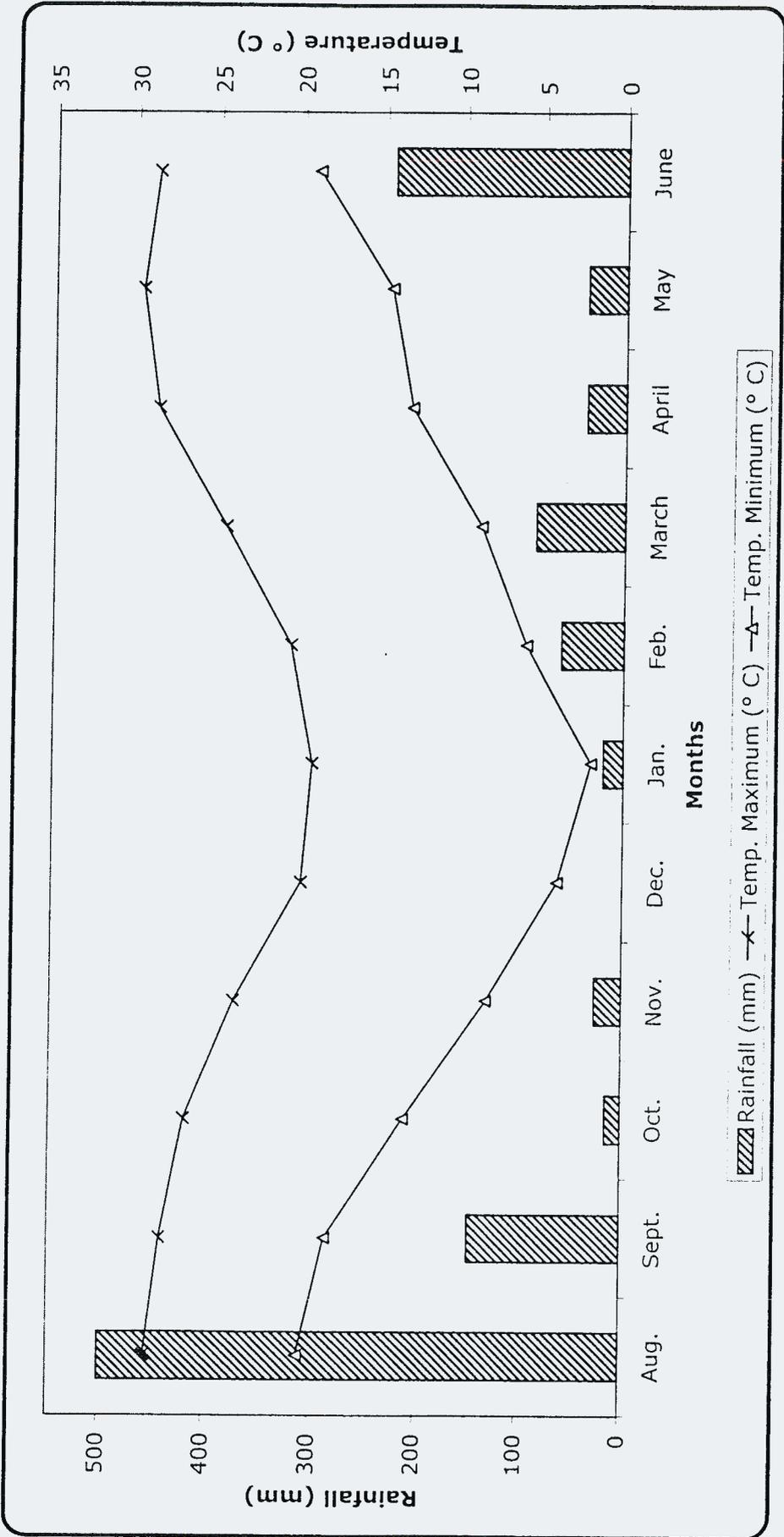
*Myrica esculenta* Buch.-Ham. ex D.Don belong to family Myricaceae.

A small evergreen tree with narrow oblong to lanceolate entire leathery dark green leaves, dotted beneath with minute resinous glands. Being dioecious plant, male flower spikes reddish, in branched axillary clusters; female flowers in slender spikes, or occasionally at end of male spikes. Leaves short-stalked, pale or rust coloured beneath, mostly 8-12 cm; bark greyish-brown, rough, vertically fissured, shoots greyish, fruit reddish with red flash and a rough stone to 1.5 cm. (Polunin and Stainton, 1999). A multipurpose tree species, *Myrica* is one of the non-leguminous angiosperms nodulated by *Frankia* spp. with negatively geotropic roots growing from the apex of nodule lobes. The fruits of *Myrica* are edible. The bark of *Myrica esculenta* have medicinal value, though flowers, fruits, seeds and arillus are also used for medicine (Yadav, 2000).

*Lyonia ovalifolia* (Wall.) Drude belong to family Ericaceae.

A small deciduous tree up to 10m; flowers in long nearly horizontal axillary clusters of 5-15 cm length, white, numerous, 6-12 mm long flask-shaped, constricted at the mouth and with 5 short recurved lobes and finely hairy; calyx-lobes triangular-lanceolate, 2.3 mm long; leaves 8-15 cm, leathery, ovate, apex acute or long pointed, hairless above, often shaggy-haired beneath when young, short-stalked; capsule globular 4 mm long, 5 valve, bark brown with peelings in narrow steps (Polunin and Stainton, 1999).





Source: Department of Hydrology and Meteorology, HMG/N.

Fig. 1 Climatic data of study site from August 2002'to June 2003



## 4. MATERIALS AND METHODS

*Myrica esculenta* Buch.-Ham. ex D.Don. and *Lyonia ovalifolia* (Wall.) Drude. were taken as experimental plants. Three plants each of *Myrica esculenta* and *Lyonia ovalifolia* were marked in the study site and the readings were taken from these plants from August 2002 to June 2003 at monthly interval. The phenological observations of the marked plants were taken in every field visit.

Xylem conductance, Relative Water Content (RWC) of leaves, and Specific Leaf Mass (SLM) were measured in the Laboratory of Ecology, Central Department of Botany, Tribhuvan University and osmotic potential of tissues, its elasticity and relative water content at zero turgor were determined by analyzing the pressure-volume (P-V) curves developed through a series of laboratory works for which the materials were collected from the field with essential precautions.

### 4.1 Pressure Volume (P-V) Curve Analysis

The pressure volume (P-V) analysis was performed using pressure chamber by free transpiration technique. The technique was developed by Scholander *et al.* (1964), refined by Tyree and Hammel (1972), Tyree (1981) and Tyree and Richter (1981). However, theoretical aspect of pressure volume technique was discussed in detail by Tyree and Jarvis (1982).

P-V curves were developed and used to illustrate the relationship between water potential and relative water content (RWC) which help to determine osmotic potential at both full turgor and zero turgor, RWC at zero turgor, pressure potential at full turgor and an index of elasticity.



#### **4.1.1 Sample collection and rehydration**

Three twigs of each plant having at least 3-4 leaves were collected in the field from marked trees, mostly before sunrise in the morning from the secondary branches. The cut end of the twigs were immediately immersed in water containing bottles which was covered by black polythene bag without any leakage to ensure the full saturation of sample twig. Then, the sample were brought to the laboratory and allowed for rehydration for 24 h. It allowed to absorb water under the condition of minimum transpiration, and water potential become less than -0.3 MPa (Zobel, 1996 b).

#### **4.1.2 Bench drying and B.P. Measurement**

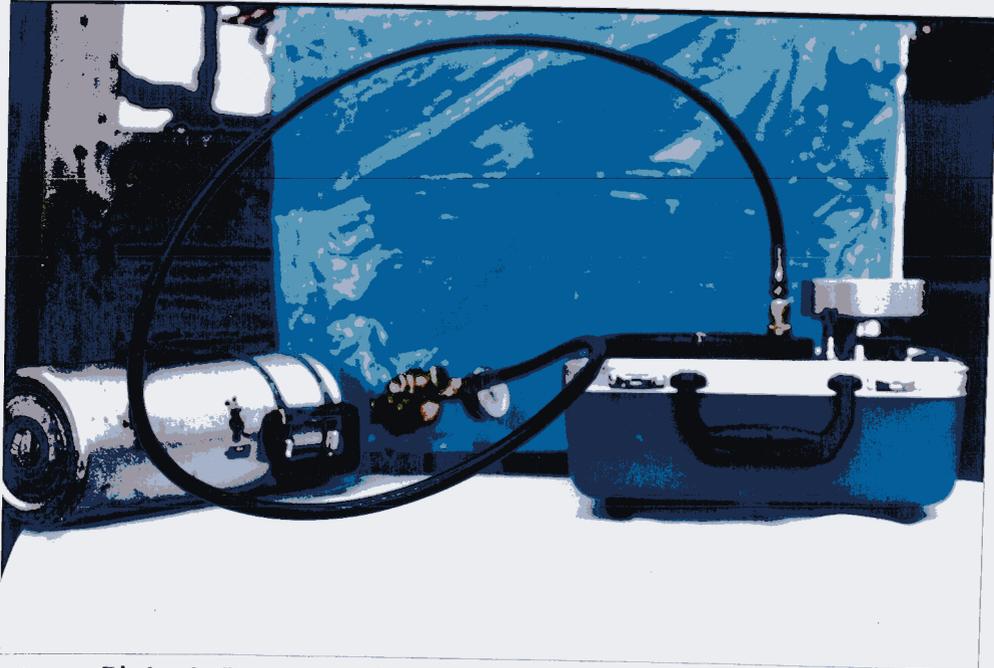
The twig was re-cut obliquely to a size appropriate to pressure chamber and fitted into the stopper. Leaf surface was soaked with tissue paper to remove surface water and dead parts were removed. The weight of twig with stopper was taken and it was fitted to chamber lid and BP was measured. The time, weight and balance pressure (BP) were measured as adopted by Zobel (1996 a). The twig was then hanged in an open area so as to allow free transpiration. After a interval of time (usually 5-10 minutes in first few readings and upto one hour in later readings) measurements of mass and BP were repeated. The process was continued till the leaf completely wilted or reading of BP crossed 30 bars. After last measurement the sample was separated from stopper and the later was weighed. The twig was placed in hot air oven at 80°C for 24 hours and dry weight of twig was recorded. Then, RWC was calculated for each measurement, using following relation.

$$\text{RWC (\%)} = \frac{F - D}{S - D} \times 100$$

Where,

- F = Fresh weight of sample twig
- S = Weight of sample twig at full saturation
- D = Dry weight of sample twig





**Plate 1 Pressure chamber to measure balance pressure**

#### **4.1.3 Components of plant water potential**

The curves were analyzed by plotting the RWC against the inverse of balance pressure (1/BP) to find different components of plant water potentials.

#### **4.1.4 Osmotic Potential ( $\psi_s$ )**

According to Kramer and Boyer (1995),

$$\psi_s = \frac{nRT}{V}$$

Where,

R = Gas constant

T = Temperature

n/v = molar concentration of the solute

Thus,

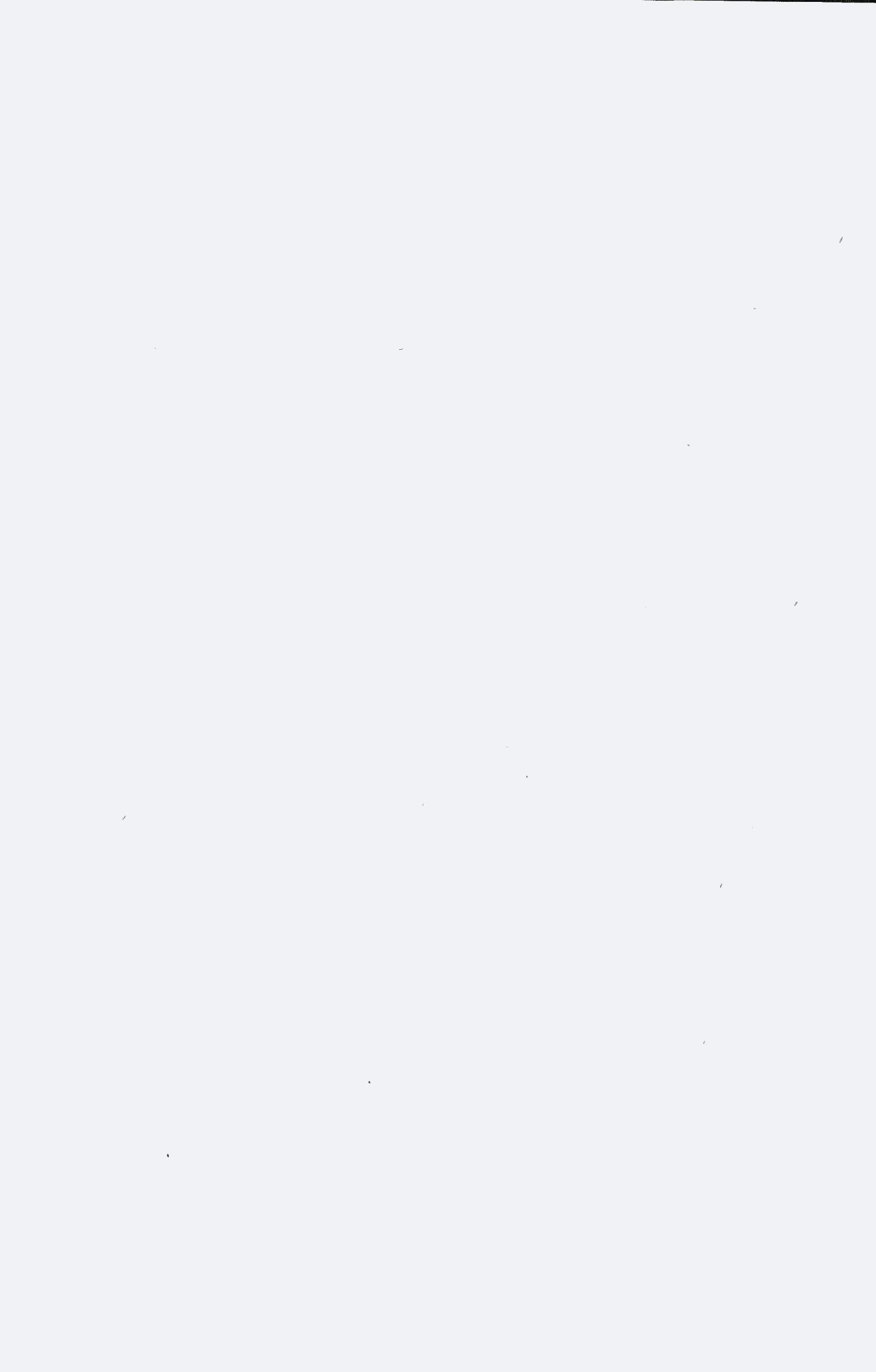
$$\frac{1}{\psi_s} = -\frac{V}{nRT}$$

Where, n represents number of solutes and V is volume of cell.

In turgor loss tissue

$$\frac{1}{-BP} = -\frac{V}{nRT}$$

or, 
$$\frac{1}{BP} = \frac{V}{nRT}$$



When pressure is applied the water removed from the cell thus affecting  $V$  but the number of moles of solute ( $n$ ) remain the same. As  $T$  and  $n$  are constant,  $nRT$  is also constant. So the graph of  $1/BP$  against  $V$  becomes linear. The linear portion of  $P$ - $V$  curve represents changes in  $\psi_s$  along with cell volume.

The linear portion of  $P$ - $V$  curve and its regression line was used to calculate  $\psi_s$  at full turgor (at a point where the extrapolation of linear portion meets the abscissa) and at zero turgor (at a point where the curve meets linear).

#### **4.1.5 Turgor potential (pressure potential)**

The  $P$ - $V$  curve changes in its nature at zero turgor point. At any positive turgor, the  $P$ - $V$  curve is smooth which measures  $\psi_s$  and  $\psi_p$ . The calculated  $\psi_s$  was subtracted from  $\psi_w$  ( $\psi_w = -BP$ ) to determine  $\psi_p$  at any RWC.

'Plateaus' occurred in few samples. The plateaus are apparently associated with apoplastic water that is accumulated in intercellular spaces of the leaf near full turgidity and act to buffer change in the leaf xylem pressure potential as tissue dehydrates (Parker and Pallardy, 1987). A plateau from either natural or artificial rehydration violates the requirements of constant apoplastic water content causes an abnormally rapid decrease in RWC relative to that in  $\psi_w$  (Tyree and Hammel, 1972; Parker and Pallardy, 1987). It is reported that plateau is formed when tissue has high  $\psi_p$  and  $\psi_w$  remain stable or decline only slightly with tissue dehydration (Bahari *et al.*, 1985). Data sets with plateaus were corrected by assuming that the beginning of steep decline in water potential represented full turgor.

#### **4.1.6 Relative Water Content (RWC) at zero turgor**

RWC at point where the  $P$ - $V$  curve becomes linear (turgorloss point) was determined as RWC at zero turgor.

#### **4.1.7 Bulk modulus of elasticity ( $\epsilon$ )**

The elasticity of tissues is expressed as the bulk modulus of elasticity ( $\epsilon$ ). Volumetric modulus of elasticity is the proportionality parameter between the observed change in pressure ( $\Delta P$ ) and the change in relative cell volume ( $\Delta V/V$ ), (Zimmermann, 1978).



$$\varepsilon = \frac{\Delta P}{\frac{\Delta V}{V}}$$

Where,

- $\Delta P$  = Change in pressure potential between full turgor & zero turgor
- $\Delta V$  = Change in RWC between full turgor and zero turgor
- $V$  = RWC at full turgor

## 4.2 Xylem Conductance

At least one year old twig was collected in the field and cut end immediately dipped in water and carried to lab. A straight portion of twig approximately pencil size (not less than 14 cm) with no leaves in the portion was cut under water. Bark of the lower end was removed and it was fitted with continuous vertical column of oxalic acid solution (0.10 M, 1.26 g/lit) and allowed to pass. The twig was allowed to remain vertical till the solution started to drop from the free (upper) end. Paraffin tape was used to check leakage, if any.

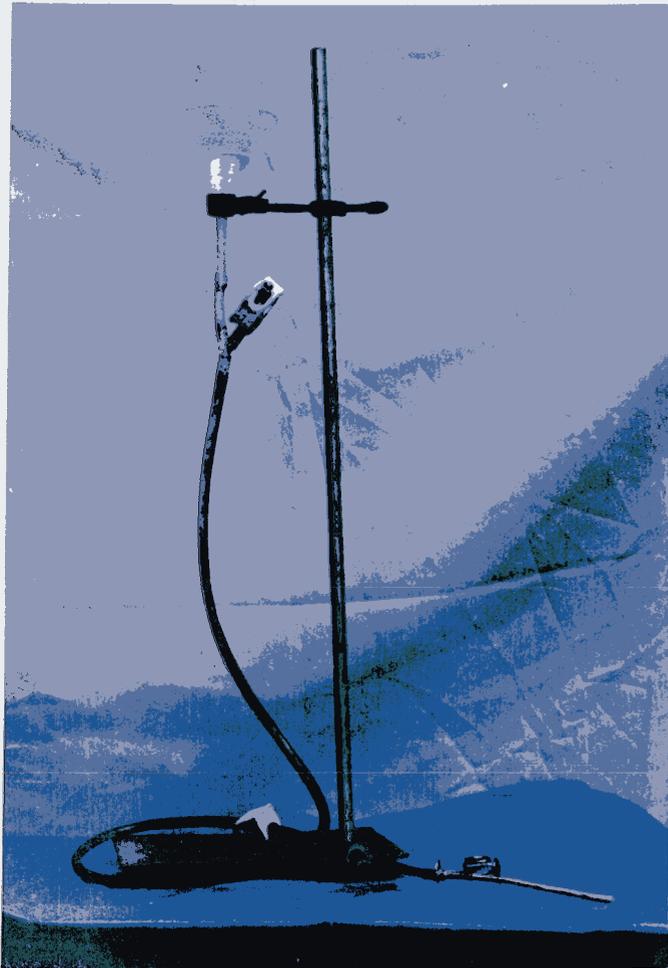


Plate 2 Twig segment fitted with vertical column of oxalic acid solution

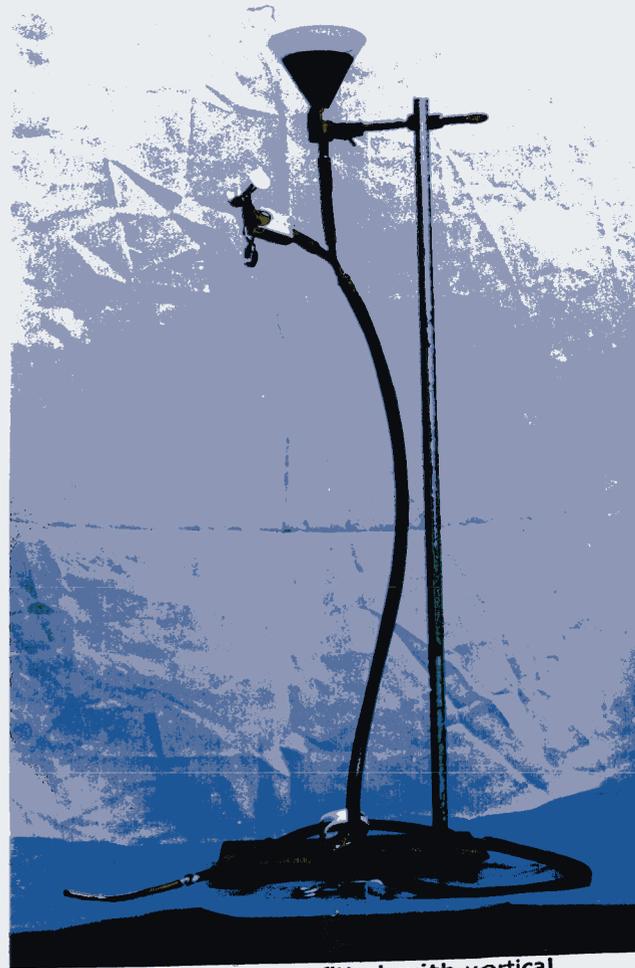
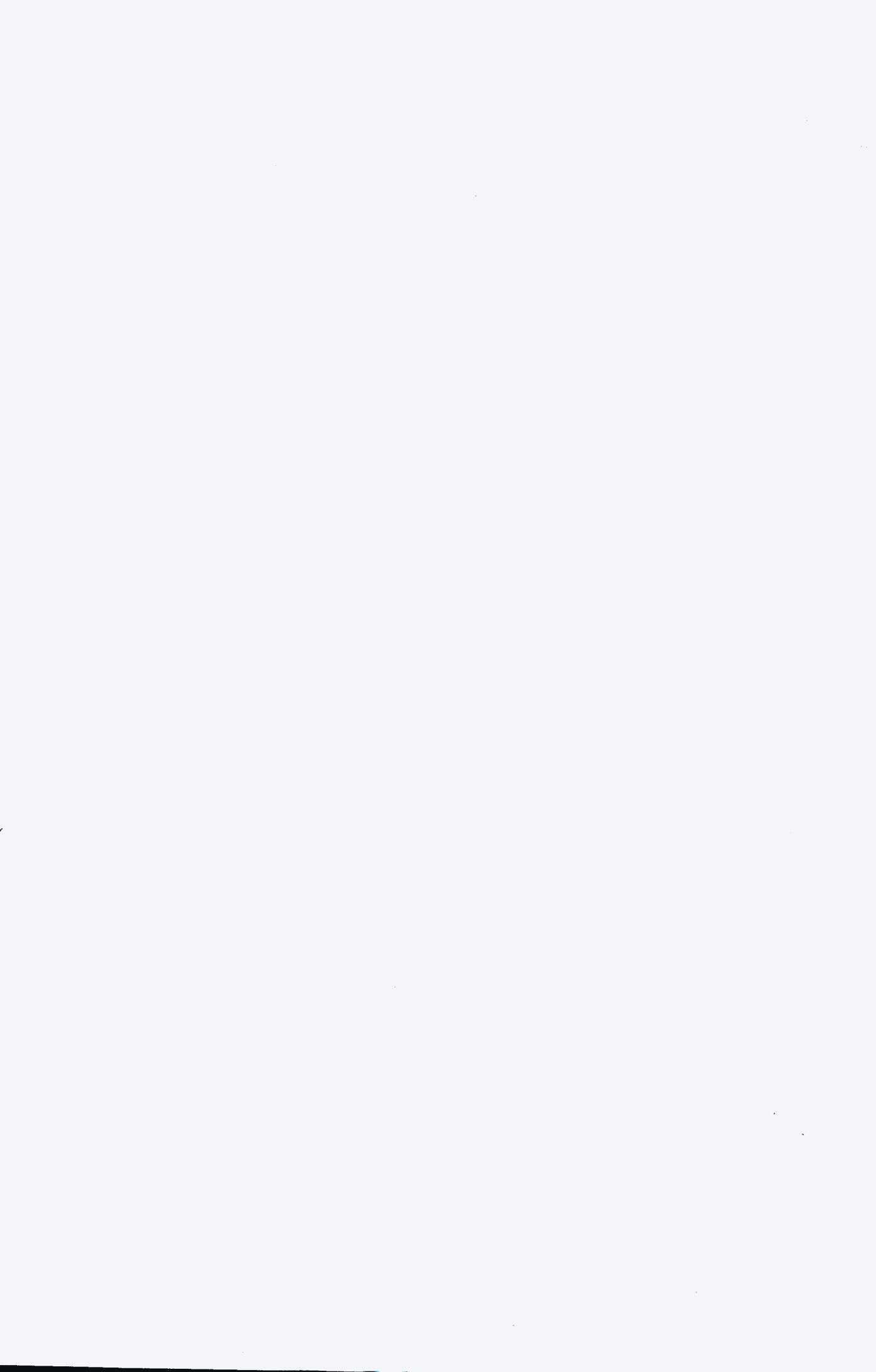


Plate 3 Twig segment fitted with vertical column of safranin solution



During measurement the twig segment was placed horizontally at fixed level from the oxalic acid reservoir level. A small plastic tube (vial) with one end closed by paraffin tape and loosely packed with tissue paper was weighed. When flow from the twig started, the flow of solution per minute collected in tube was weighed. The difference in the weight of plastic tube before and after flow was calculated, that give the flow rate. The process was repeated until nearly constant flow rate was recorded. The sample was then transferred to another tube containing 0.1% saffranin solution (double filtered) attached to the reservoir. The twig was allowed to remain vertical; once the saffranin started flowing from the free end the twig was removed. Then length of twig, upper and lower diameter of xylem of twig as well as upper and lower diameter of pith was measured. The stained section of the twig was observed and proportion of stained xylem was considered as active xylem. Then, all parameters of xylem conductance i.e. Huber Value (HV), hydraulic conductivity ( $K_h$ ), specific conductivity ( $K_s$ ) and leaf specific conductivity ( $K_l$ ) were calculated.

Now the various parameters of xylem conductance were calculated as follow:

#### 4.2.1 Pressure Gradient (PG)

The pressure gradient was calculated by using following formula.

$$PG \text{ (MPa/m)} = \frac{\text{Pressure Head (cm)}}{10.2} \times \frac{1}{\text{Length of segment (m)}} \times \frac{1}{1000}$$

#### 4.2.2 Effective xylem area (Awe)

Effective xylem area ( $A_{we}$ ,  $m^2$ ) = Average xylem area  $\times$  proportion of strained xylem.

$$\text{Average xylem area} = \frac{\pi}{8} \{ (dlw)^2 - (dlp)^2 + (dsw)^2 - (dsp)^2 \}$$

where,

dlw = diameter of wood and pith at larger end.

dlp = diameter of pith at larger end.

dsw = diameter of wood and pith at smaller end.

dsp = diameter of pith at smaller end.



### 4.2.3 Huber value (HV)

The ratio of xylem conducting area ( $A_{xyl}$ ) and the leaf surface area or the leaf mass of transpiring parts of the plant that are supplied with water by this conducting tissue is called Huber Value (Huber, 1928; Zimmermann and Brown, 1974).

Thus,

$$HV = \frac{A_{xyl}}{A_l}$$

Where,

HV = Huber value

$A_{xyl}$  = Xylem conducting area

$A_l$  = Leaf surface area

Vernier Caliper and scale were used to measure  $A_{xyl}$ . To determine  $A_l$  the linear dimension of leaf i.e. length (l) and breadth (b) were multiplied by coefficient factor (x).

The x for *Myrica esculenta* and *Lyonia ovalifolia* was calculated as 0.65 and 0.71 respectively. The length was measured along the mid rib and breadth at the widest part of leaf. X can be determined by following Goodall (1947) and Winter *et al.* (1956) by examining 20 leaves of all size classes. But, to determine the coefficient factors for *Myrica esculenta* and *Lyonia ovalifolia*, 40 healthy and mature leaves from each plant species were collected from the field. The leaves were placed on a graph paper and their margins were outlined. Then, actual area of respective leaves were obtained by counting the squares within the outline.

$$x = \frac{A_l}{l \times b}$$

Where,

x = coefficient factor

$A_l$  = Actual area of leaf

l = Length of leaf along mid rib

b = Breadth of leaf from widest part



#### 4.2.4 Hydraulic conductivity (K<sub>h</sub>)

Hydraulic conductivity is expressed as:

$$\text{Hydraulic conductivity (kg. m. sec}^{-1} \text{ MPa}^{-1}) = \frac{\text{Flowrate}}{\text{Pressure gradient}}$$

#### 4.2.5 Leaf specific conductivity (K<sub>l</sub>)

Leaf specific conductivity (K<sub>l</sub>) is equal to hydraulic conductivity (K<sub>h</sub>) divided by leaf area distal to the segment (A<sub>l</sub>).

$$K_l (\text{Kg. m}^{-1} \text{ Sec.}^{-1} \text{ MPa}^{-1}) = \frac{\text{Hydraulic conductivity}}{\text{Leaf area}}$$

#### 4.2.6 Specific conductivity (K<sub>s</sub>)

Specific conductivity (K<sub>s</sub>) is a measure of the porosity of wood which is equal to hydraulic conductivity (K<sub>h</sub>) divided by effective xylem area (A<sub>we</sub>).

$$\begin{aligned} K_s (\text{Kg. m}^{-1} \text{ Sec.}^{-1} \text{ MPa}^{-1}) &= \frac{\text{Hydraulic conductivity}}{\text{Effective xylem area}} \\ &= \frac{K_h}{A_{we}} \end{aligned}$$

### 4.3 Relative Water Content (RWC) of leaves

Relative water content (RWC) is the amount of water present in the plant tissue as a fraction of the total water present in fully hydrated tissues (Pallardy *et al.*, 1991).

For the RWC of leaves, the sample were collected twice in a day at the interval of one month; predawn, when the leaf is most saturated and midday around 1 PM when the leaf has minimum amount of water in diurnal cycle. To determine the RWC of leaves, five mature, healthy and uniform size leaves were collected from the sample trees and immediately kept in plastic bags having zipper to check the loss of water from leaves. These samples were brought to the lab and fresh weight was measured by electric balance. Then, the leaves were wrapped by filter paper soaked in water and kept at room



temperature at least 24 h. to determine the saturated weight. After that leaves were dried in oven at 80° C for at least 24 h. dry weight of leaves were measured. Then, RWC of leaves was calculated by using following formula.

$$\text{RWC (\%)} = \frac{F - D}{S - D} \times 100$$

Where, F = Fresh weight of leaves  
 S = Saturated weight of leaves  
 D = Dry weight of leaves

#### 4.4 Specific Leaf Mass (SLM)

Five healthy, mature and uniform size leaves from each sample plant were collected from healthy twig. The area of leaves were calculated by multiplying length, breadth and coefficient factor. The leaves were oven dried at 80°C for at least 24 h. and dry mass was measured. The SLM was calculated by using following formula.

$$\text{SLM} = \frac{\text{Dry mass of leaves (g)}}{\text{Surface area of leaves (cm}^2\text{)}}$$

#### 4.5 Phenology

The phenological observations were made in marked (sample) plants as well as adjoining trees of the same species in every field visit. In the present study, observation was focused on bud break, leaf flushing, shoot elongation, flowering, fruiting and leaf fall.

#### 4.6 Statistical Analysis

##### 4.6.1 Mean ( $\bar{X}$ )

The single value representing many replicates in experiments is arithmetic mean ( $\bar{x}$ ) which is calculated as follows:

$$\bar{X} = \frac{\sum x}{n}$$

Where, X = Measured value of an item  
 n = Size of a sample



### 4.6.2 Standard Deviation ( $\sigma$ )

Standard deviation measures the variability that indicates, by how much all the values in a distribution typically deviates form the arithmetic mean.

Standard deviation ( $\sigma$ ) is calculated as follows:

$$\sigma = \sqrt{\frac{\sum(X - \bar{X})^2}{n - 1}}$$

### 4.6.3 Coefficient of Correlation

The strength of the linear relationship between two variables  $x$  and  $y$  can be measured by coefficient of correlation ( $r$ ). The Karl Pearson sample correlation coefficient as described by Hosmand (1988).

$$r = \frac{\sum xy - n\bar{x}\bar{y}}{\sqrt{(\sum x^2 - n\bar{x}^2)(\sum y^2 - n\bar{y}^2)}}$$

The correlation coefficient can assume values between +1 and -1, values of  $r$  refer to perfect positive and negative linear correlation respectively between variables of interest. When  $r$  equals to zero, there is no linear correlation.



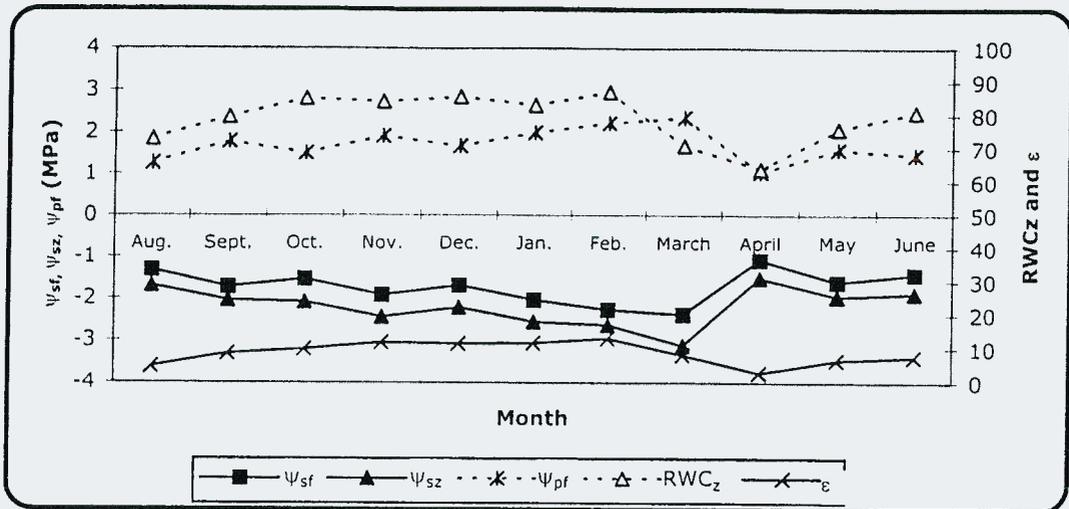
## 5. RESULTS

### 5.1 Pressure Volume (P-V) Curve Analysis

The parameters obtained from pressure-volume curve analysis for *Myrica esculenta* (Fig. 2) showed seasonal variation. The lowest value of  $RWC_z$  was observed in March (71%) and highest in February (87%). Throughout the year  $\psi_{sf}$  and  $\psi_{sz}$  showed similar pattern of seasonal variation.  $\psi_{sf}$  and  $\psi_{sz}$  were recorded lowest during March (-2.38 MPa and -3.12 MPa) and highest during April (-1.07 MPa and -1.51 MPa respectively).  $\psi_{sf}$  showed positive correlation with  $\psi_{sz}$  ( $r = 0.758$ ) and negative correlation with  $\psi_{pf}$  ( $r = -0.817$ ),  $RWC_z$  ( $r = -0.661$ ),  $\epsilon$  ( $r = -0.735$ ), SLM ( $r = -0.559$ ) and  $LRWC_{pd}$  ( $r = -0.502$ ).

The highest and lowest values of  $\psi_{pf}$  was recorded in March (2.35 MPa) and April (1.05 MPa) respectively. The  $\psi_{pf}$  showed strong negative correlation with  $\psi_{sz}$  ( $r = -0.963$ ) and  $\psi_{sf}$  ( $r = -0.817$ ). The  $\psi_{pf}$  value smoothly increased from August-March but decreased from April. The highest and lowest values of bulk modulus of elasticity ( $\epsilon$ ) were recorded in February (13.07 MPa) and April (2.91 MPa) respectively. The  $\epsilon$  showed negative correlation with  $\psi_{sf}$  ( $r = -0.735$ ) and  $\psi_{sz}$  ( $r = -0.669$ ) but high positive correlation with  $\psi_{pf}$  ( $r = 0.715$ ) and  $RWC_z$  ( $r = 0.898$ ) (Table 1).



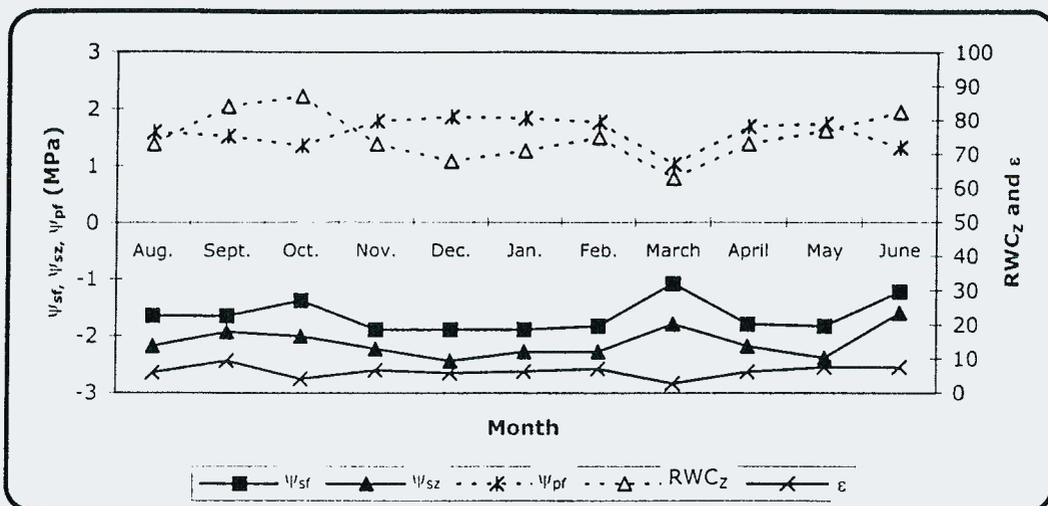


**Fig. 2** Parameters obtained from P-V curve analysis. Osmotic potential at zero turgor ( $\psi_{sz}$ ), osmotic potential at full turgor ( $\psi_{sf}$ ), pressure potential at full turgor ( $\psi_{pf}$ ), relative water content at zero turgor (RWC<sub>z</sub>) and bulk modulus of elasticity ( $\epsilon$ ) for *Myrica esculenta* form August 2002 to June 2003.

*Lyonia ovalifolia* also showed similar result as *Myrica esculenta* in  $\psi_{sf}$ ,  $\psi_{sz}$ , RWC<sub>z</sub> and  $\epsilon$  parameters (Fig. 3). The lowest value of RWC<sub>z</sub> was observed in March (63%) and highest in October (87%). RWC<sub>z</sub> did not show significant correlation with any other parameters. Throughout the year  $\psi_{sf}$  showed similar pattern of seasonal variation.  $\psi_{sf}$  and  $\psi_{sz}$  were recorded highest in March (-1.07 MPa, -1.78 MPa) and lowest in December (-1.88 MPa, -2.43 MPa) respectively.  $\psi_{sf}$  showed high positive correlation with  $\psi_{sz}$  ( $r = 0.88$ ) but strong negative correlation with  $\psi_{pf}$  ( $r = -0.981$ ).

The highest and lowest values of  $\psi_{pf}$  was recorded in December (1.86 MPa) and March (1.02 MPa) respectively. The  $\psi_{pf}$  showed strong negative correlation with  $\psi_{sf}$  ( $r = -0.98$ ) and  $\psi_{sz}$  ( $r = -0.863$ ) and weak negative correlation with  $K_h$  ( $r = -0.529$ ). The lowest value of  $\epsilon$  was observed in March (2.770 MPa) and highest in September (9.462 MPa) (Table 2).





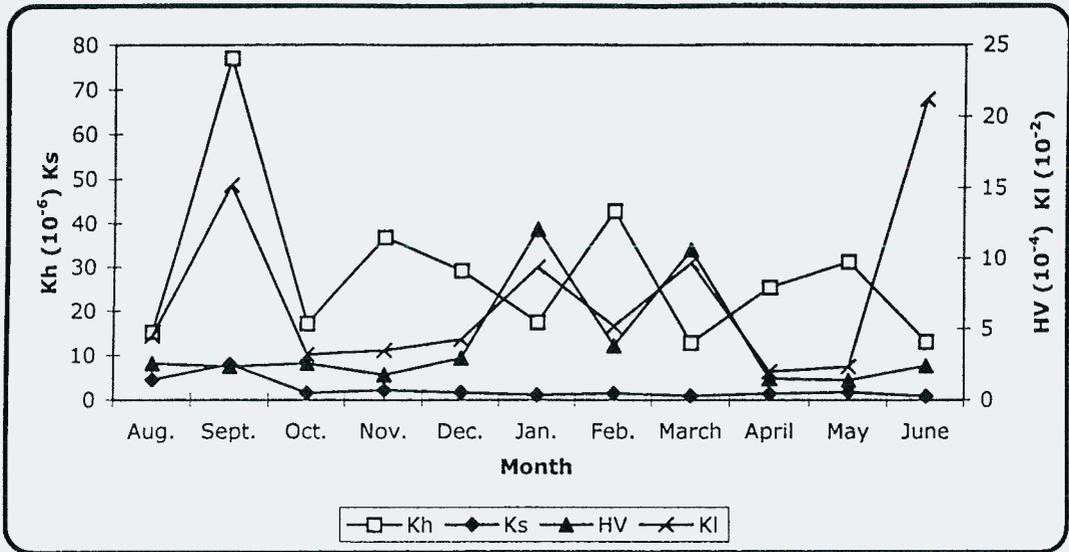
**Fig. 3** Parameters obtained from P-V curve analysis. Osmotic potential at zero turgor ( $\psi_{sz}$ ), osmotic potential at full turgor ( $\psi_{sf}$ ), pressure potential at full turgor ( $\psi_{pf}$ ), relative water content at zero turgor (RWC<sub>z</sub>) and bulk modulus of elasticity ( $\epsilon$ ) for *Lyonia ovalifolia* form August 2002 to June 2003.

## 5.2 Xylem Conductance

The hydraulic conductivity ( $K_h$ ) of *Myrica esculenta* was found highest during September ( $77.04 \times 10^{-6} \text{ kg.m. sec}^{-1} \text{ MPa}^{-1}$ ) and followed by February ( $42.78 \times 10^{-6} \text{ kg.m. sec}^{-1} \text{ MPa}^{-1}$ ) and became minimum during March ( $12.82 \times 10^{-6} \text{ kg.m. sec}^{-1} \text{ MPa}^{-1}$ ). The  $K_h$  did not show any significant correlation with any other parameters except  $K_s$ . It is positively correlated with  $K_s$  ( $r = 792$ ).

Likewise specific conductivity ( $K_s$ ) showed similar pattern as  $K_h$ . It was maximum during September ( $8.043 \text{ kg.m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) followed by August ( $4.526 \text{ kg.m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) and became minimum during June ( $0.877 \text{ kg.m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ). The  $K_s$  showed significant correlation with  $K_h$  ( $r = 0.722$ ) in *Myrica esculenta*. Leaf specific conductivity ( $K_l$ ) showed variation during the different season. It was highest during September ( $0.152 \text{ kg.m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ) and lowest in April ( $0.019 \text{ kg.m}^{-1} \text{ sec}^{-1} \text{ MPa}^{-1}$ ). The  $K_l$  showed significant positive correlation with HV ( $r = 0.569$ ). The maximum HV was observed in January ( $1.2089 \times 10^{-3}$ ) and minimum in April ( $0.150 \times 10^{-3}$ ). HV showed positive correlation with  $\psi_{pf}$  ( $r = 0.633$ ) and significant negative correlation with  $\psi_{sz}$  ( $r = -0.721$ ) (Table 1).



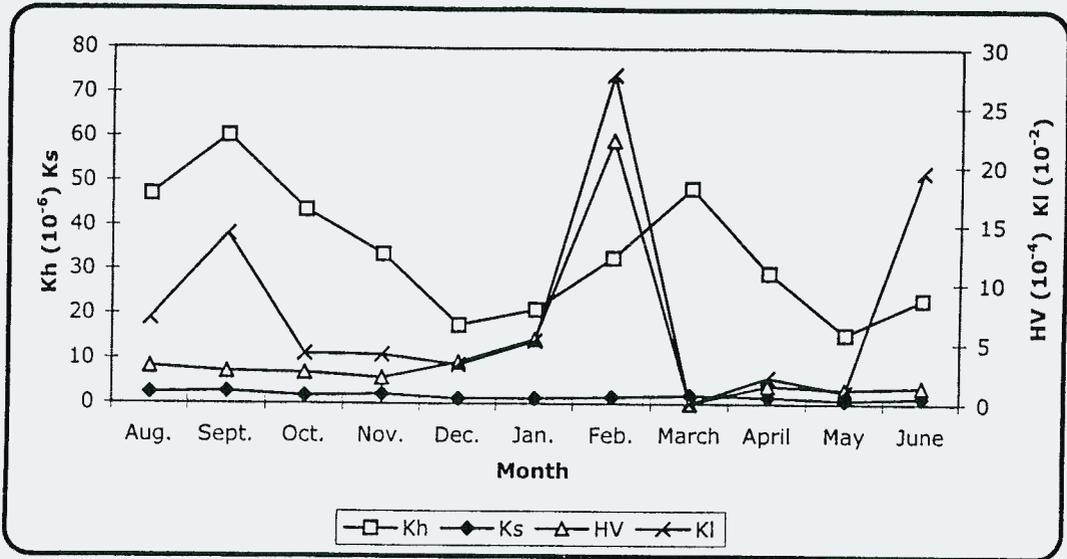


**Fig. 4 Seasonal variation in various parameters of hydraulic conductivity. Huber value (HV), hydraulic conductivity ( $K_h$ ,  $\text{kg.m.sec}^{-1} \cdot \text{MPa}^{-1}$ ), specific conductivity ( $K_s$ ,  $\text{kg.m}^{-1} \cdot \text{sec}^{-1} \cdot \text{MPa}^{-1}$ ) and leaf specific conductivity ( $K_l$ ,  $\text{kg.m}^{-1} \cdot \text{sec}^{-1} \cdot \text{MPa}^{-1}$ ) in *Myrica esculenta* from August 2002 to June 2003.**

In *Lyonia ovalifolia*, the highest  $K_h$  value observed during September ( $60.25 \times 10^{-6} \text{ kg.m. sec}^{-1} \cdot \text{MPa}^{-1}$ ) and lowest during May ( $15.48 \times 10^{-6} \text{ kg.m. sec}^{-1} \cdot \text{MPa}^{-1}$ ). Similarly highest and lowest value of  $K_s$  was observed during September ( $2.684, \text{ kg.m}^{-1} \text{ sec}^{-1} \cdot \text{MPa}^{-1}$ ) and May ( $0.801, \text{ kg.m}^{-1} \cdot \text{sec}^{-1} \cdot \text{MPa}^{-1}$ ). However,  $K_l$  value reached maximum during February ( $0.277, \text{ kg.m}^{-1} \cdot \text{sec}^{-1} \cdot \text{MPa}^{-1}$ ) and minimum to May ( $0.0098, \text{ kg.m}^{-1} \cdot \text{sec}^{-1} \cdot \text{MPa}^{-1}$ ). Similarly HV reached maximum during February ( $2.22 \times 10^{-3}$ ) and minimum in May ( $0.128 \times 10^{-3}$ ).

$K_h$  was positively correlated with  $K_s$  ( $r = 8.59$ ). But, HV showed higher negative correlation with pd-LRWC ( $r = -0.724$ ) and md-LRWC ( $r = -0.731$ ) and poor positive correlation with  $K_l$  ( $r = 0.569$ ) (Table 2).





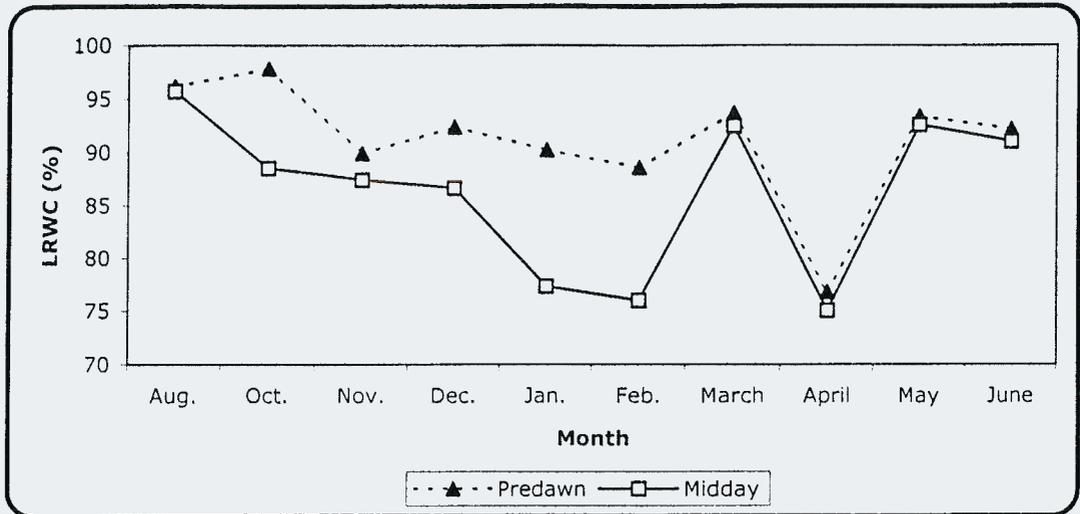
**Fig. 5 Seasonal variation in various parameters of hydraulic conductivity. Huber value (HV), hydraulic conductivity (Kh,  $\text{kg.m.sec}^{-1} \cdot \text{MPa}^{-1}$ ), specific conductivity (Ks,  $\text{kg.m}^{-1}.\text{sec}^{-1} \cdot \text{MPa}^{-1}$ ) and leaf specific conductivity (KI,  $\text{kg.m}^{-1}.\text{sec}^{-1} \cdot \text{MPa}^{-1}$ ) in *Lyonia ovalifolia* from August 2002 to June 2003.**

### 5.3 Leaf Relative Water Content (LRWC)

The LRWC of *Myrica esculenta* at predawn and midday showed seasonal variation. The highest value of predawn LRWC was observed in October (97.82%) and lowest in April (76.81%) whereas highest value of midday LRWC was observed in August (95.7%) and lowest in April (75.05%). The difference in  $\text{LRWC}_{\text{pd}}$  and  $\text{LRWC}_{\text{Md}}$  was narrowest in April (76.81% and 75.05%) and August (96.14% and 95.7%) but widest in October (97.82% and 88.49%) and December (92.39% and 86.62%) (Fig. 6).

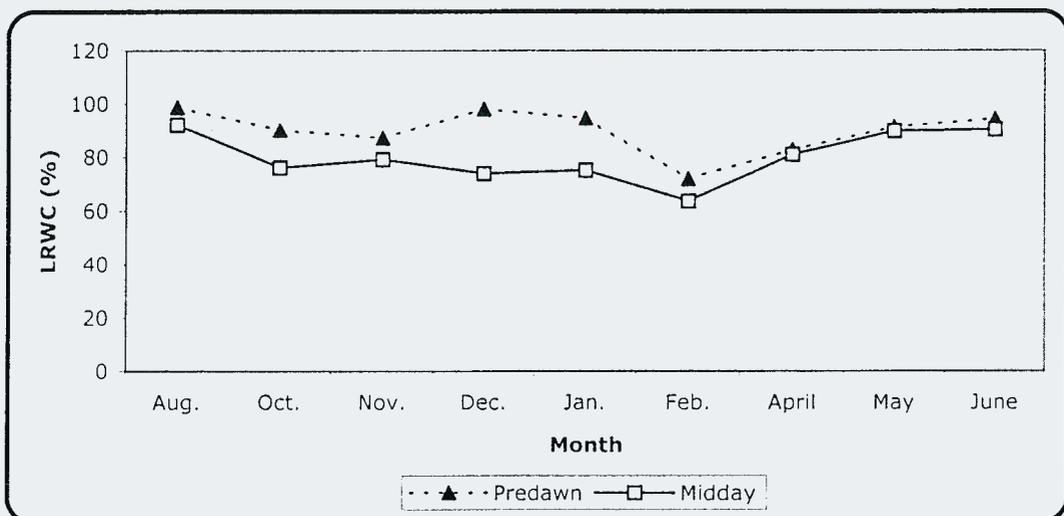
However, both  $\text{LRWC}_{\text{pd}}$  and  $\text{LRWC}_{\text{Md}}$  did not show significant correlation with any other parameters (Table 1).





**Fig. 6 Variation in Leaf Relative Water Content (LRWC) in *Myrica esculenta* from August 2002 to June 2003.**

In case of *Lyonia ovalifolia* maximum value of pd-LRWC was observed in August (98.70%) and lowest in February (72.02%) whereas highest value of midday LRWC was also observed in August (92.16%) and lowest in February (63.69%). The difference in LRWC<sub>pd</sub> and LRWC<sub>Md</sub> was narrowest in May (91.33% and 89.89%) and widest in December (98.08% and 73.95%) (Fig. 7). LRWC<sub>pd</sub> showed negative correlation with HV ( $r = -0.724$ ) and KI ( $r = -0.699$ ). Whereas LRWC<sub>Md</sub> showed negative correlation with HV ( $r = -0.731$ ) and positive correlation with LRWC<sub>pd</sub> ( $r = 0.606$ ) (Table 2).

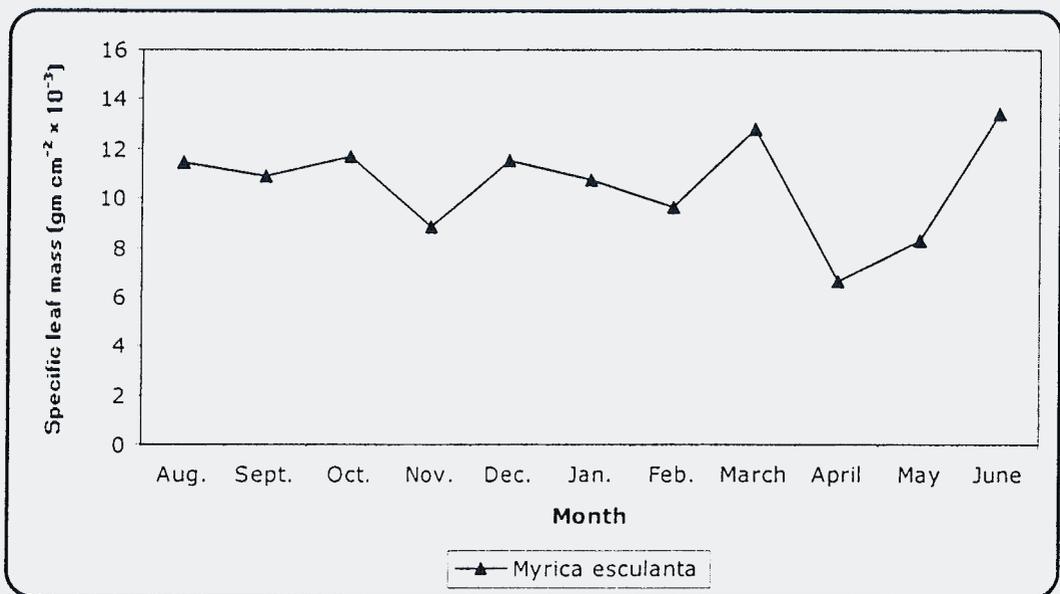


**Fig. 7 Variation in Leaf Relative Water Content (LRWC) in *Lyonia ovalifolia* from August 2002 to June 2003.**



## 5.4 Specific Leaf Mass (SLM)

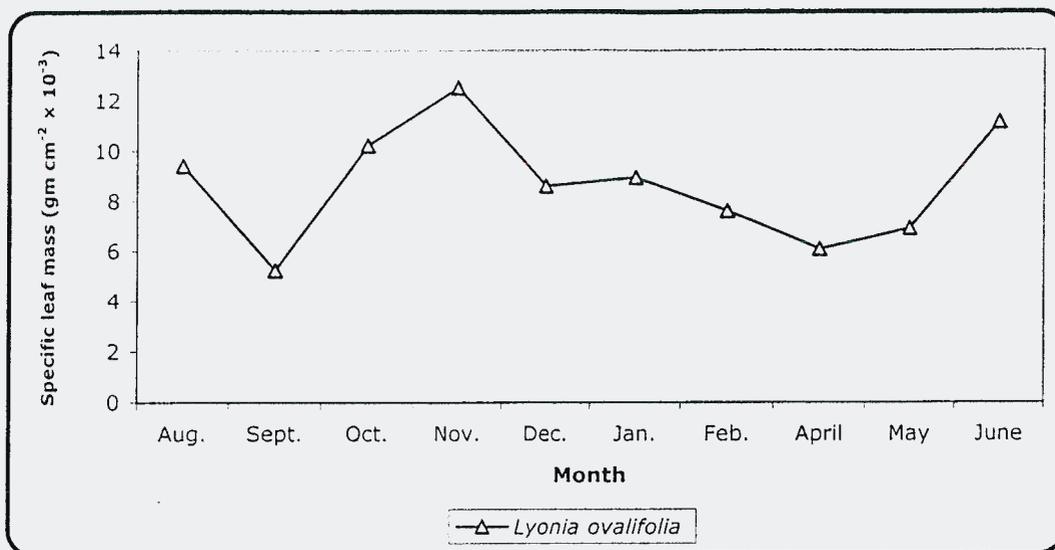
The maximum Specific Leaf Mass (SLM) was found in June ( $13.41 \times 10^{-3}$  gm.cm<sup>-2</sup>) and minimum in April ( $6.61 \times 10^{-3}$  gm.cm<sup>-2</sup>) in *Myrica esculenta* (Fig. 8). From April it continuously increased till June. The SLM showed positive correlation with  $K_l$  ( $r = 0.642$ ) and  $RWC_{pd}$  ( $r = 0.697$ ) and negative correlation with  $\psi_{sf}$  ( $r = -0.559$ ) (Table 1)



**Fig. 8 Specific leaf mass (gm.cm<sup>-2</sup>) of *Myrica esculenta* from August 2002 to June 2003.**

In case of *Lyonia ovalifolia*, the maximum SLM value was observed during November ( $12.52 \times 10^{-3}$  gm.cm<sup>-2</sup>) and minimum during September ( $5.245 \times 10^{-3}$  gm.cm<sup>-2</sup>). This value increased from September to November and again decreased (Fig. 9). SLM of *Lyonia ovalifolia* did not show any significant correlation with other parameters (Table 2).

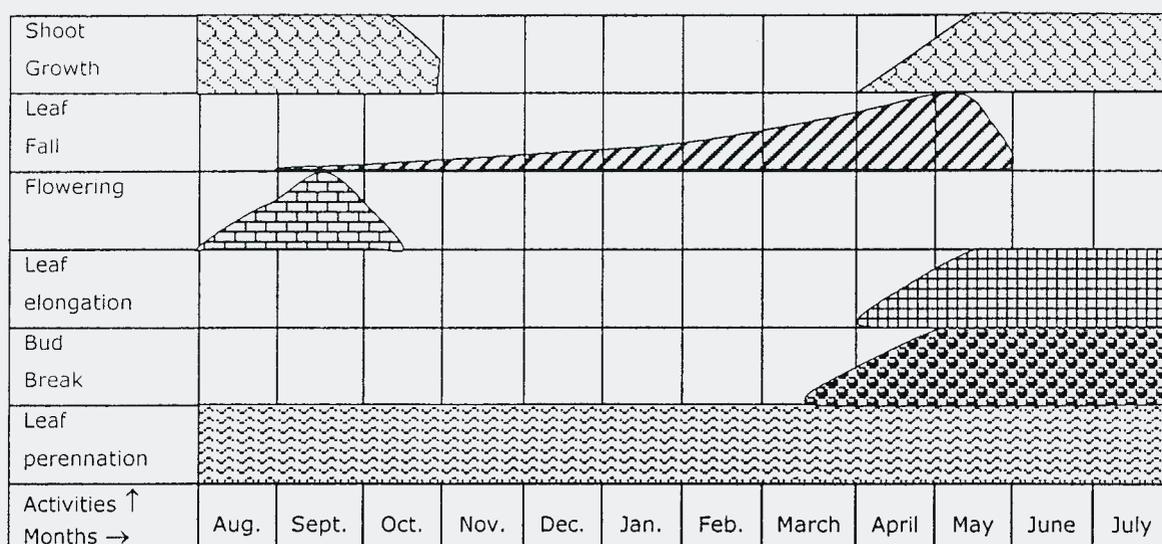




**Fig. 9** Specific leaf mass (gm.cm<sup>-2</sup>) of *Lyonia ovalifolia* from August 2002 to June 2003.

### 5.5 Phenology

In *Myrica esculenta* leaf fall started from September and completed at the middle of May. Simultaneously, new leafing ( $\cong 10\%$ ) started at the middle of March. So, leaf life span of *Myrica esculenta* is more than one year (about 13 months). The flowering started at the early August and completed in early October. After flowering, being male plant, flower completely fall within October. The bud break started at late March; and shoot growth continued till September (Fig. 10).



**Fig. 10** Phenological activities in *Myrica esculenta*.





Plate 4 A twig with flower of *Myrica esculenta*

In case of *Lyonia ovalifolia*, the leaf fall started from September December and continued to March. The complete leafless plant was found during March to April. In early February bud break started and completed after May. The leaf maturation and twig growth continued till early April to September. The flowering of the *Lyonia ovalifolia* started at the end of April and continued till June then mature fruits persisted till late June. Mature leaf persisted except in March. The average leaf life span of *Lyonia ovalifolia* is about 10 months (Fig. 11).

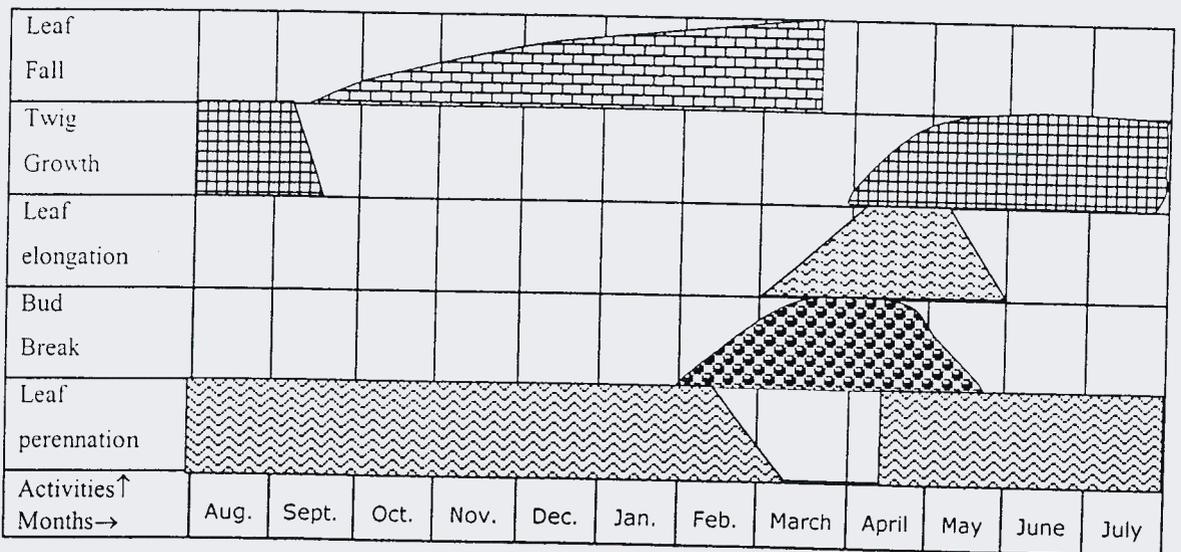


Fig. 11 Phenological activities in *Lyonia ovalifolia*.





Plate 5 A twig with mature leaves of *Lyonia ovalifolia*



**Table 1 Karl Pearson coefficient of correlation between various parameters for *Myrica esculenta*.**

Parameters	HV	K <sub>h</sub>	K <sub>s</sub>	K <sub>l</sub>	LRWC <sub>pd</sub>	LRWC <sub>md</sub>	ψ <sub>sf</sub>	ψ <sub>sz</sub>	ψ <sub>pf</sub>	RWC <sub>z</sub>	ε
K <sub>h</sub>	-0.355	-									
K <sub>s</sub>	-0.285	0.792	-								
K <sub>l</sub>	0.201	0.050	0.218	-							
LRWC <sub>pd</sub>	-0.024	0.023	0.343	0.272	-						
LRWC <sub>md</sub>	-0.224	-0.474	0.307	0.365	0.416	-					
ψ <sub>sf</sub>	-0.437	-0.017	-0.032	-0.241	-0.502	-0.287	-				
ψ <sub>sz</sub>	-0.721	0.120	0.282	-0.076	-0.001	0.098	0.758	-			
ψ <sub>pf</sub>	0.633	0.072	-0.127	0.176	0.024	-0.166	-0.817	-0.963	-		
RWC <sub>z</sub>	0.007	0.167	-0.047	0.119	0.612	-0.025	-0.661	-0.342	0.421	-	
ε	0.298	0.166	-0.152	0.038	0.361	-0.223	-0.735	-0.669	0.715	0.898	-
SLM	0.359	-0.336	0.026	0.642	0.697	0.551	-0.559	-0.325	0.289	0.315	0.226



Table 2 Karl Pearson coefficient of correlation between various parameters for *Lyonia ovalifolia*.

Parameters	HV	$K_h$	$K_s$	$K_l$	LRWC <sub>pd</sub>	LRWC <sub>md</sub>	$\psi_{sf}$	$\psi_{sz}$	$\psi_{pf}$	RWC <sub>z</sub>	$\epsilon$
$K_h$	-0.050	-									
$K_s$	-0.094	0.879	-								
$K_l$	0.569	0.090	0.069	-							
LRWC <sub>pd</sub>	-0.724	-0.013	0.038	-0.699	-						
LRWC <sub>md</sub>	-0.731	0.145	0.131	-0.293	0.606	-					
$\psi_{sf}$	-0.249	0.398	0.197	0.199	0.203	0.396	-				
$\psi_{sz}$	-0.237	0.385	0.335	0.384	0.092	0.375	0.886	-			
$\psi_{pf}$	0.291	-0.529	-0.301	-0.192	-0.149	-0.414	-0.981	0.863	-		
RWC <sub>z</sub>	-0.150	0.117	0.193	0.136	-0.091	0.198	0.134	0.319	-0.109	-	
$\epsilon$	0.057	-0.107	0.077	0.330	-0.208	0.232	-0.466	-0.102	0.476	0.470	-
SLM	-0.149	-0.460	-0.057	-0.312	0.312	0.141	-0.363	-0.193	0.470	0.428	0.290



## 6. DISCUSSION

The water relations parameters measured in *Myrica esculenta* an evergreen species and *Lyonia ovalifolia*, the deciduous species in Hattiban natural forest were P-V curve analysis, Leaf Relative Water Content (LRWC) and xylem conductance. Along with these SLM was determined and phenological events were recorded at monthly interval.

The parameters analyzed from the P-V curve included osmotic potential at full turgor ( $\psi_{sf}$ ), osmotic potential at zero turgor ( $\psi_{sz}$ ), pressure potential at full turgor ( $\psi_{sf}$ ), and relative water content at zero turgor (RWC<sub>Z</sub>). *Myrica esculenta* had the lowest values of osmotic potential  $\psi_{sf}$  and  $\psi_{sz}$  and highest value of pressure potential of full turgor in March, a dry month. Leaf relative water content was also higher, indicating that tissues were sufficiently saturated with water to allow new growth such as vegetative bud break which began in March. When soil water potential declines in dry months, live cells adjust their water status by accumulating osmotically active compounds, which reduces the osmotic potential (Lambers, 1998). Increasing dehydration due to higher evapo-transpiration also increases solute concentrations and decreases osmotic potential (Tyree and Jarvis, 1982). Maintenance of a positive turgor is critical for cell growth because cell expansion is highly sensitive to dehydration (Hsiao, 1973). Reduction in osmotic potential helped the plant to maintain turgor during dry month (Blake and Tschaplinski, 1992). This result indicates that *Myrica esculenta* appears to exhibit good osmoregulation (i.e. osmotic adjustment) during drought. Similar result was obtained by Saliendra and Meizer (1991), Ishida *et al.* (1992), Kumari and Veeranjaneulu (1996), Peltier and Marigo (1998), and Uprety (2002). Osmotic adjustment is one of the adaptational strategies of plants having ability to tolerate low water potential (Abrams, 1990).



However, in case of *Lyonia ovalifolia* seasonal variation in osmotic potential is slight different from *Myrica esculenta*. Throughout winter (November to February)  $\psi_{sf}$  remained uniform. Least  $\psi_{sz}$  observed in December indicated increase in solute concentrations in cells due to low water availability. The work of Tiwari (1998) in foot hill of Kumaun Himalaya on *Shorea robusta* showed  $\psi_{sf}$  and  $\psi_{sz}$  remained more or less constant from monsoon to winter and then declined during early summer. Phenologically, the dormant bud break at the end of February and initiation of new leaf and senescence was almost completed. Osmotic adjustment has been documented in many wood species (Abram, 1988). However its importance for turgor maintenance and growth has been questioned (Munns, 1988). Lack of significant positive correlation between  $\psi_{sf}$  and  $RWC_z$  suggests that osmotic adjustment contributed little to turgor maintenance. Same result was achieved by Zwiazek and Blake (1989, 1990).

Increased water stress during early dry season generally cause a decline in water potential and induce leaf shedding (Borchert, 1995). The considerable water stress was faced by plants in February which was indicated by lowest value of LRWC. It induced rapid leaf fall and plant became leaf less. It reduced water loss from evapo-transpiration which increased the osmotic potential in March to the highest value.

In *Myrica esculenta*, the values of  $\psi_{pf}$  were always greater than 1MPa and highest value was found in March (2.355 MPa) corresponding to lowest osmotic potential at full turgor ( $\psi_{sf} = -2.380$  MPa) and osmotic potential at zero turgor ( $\psi_{sz} = -3.125$  MPa). So, phenologically active period (new leafing) and most drought condition in March matched in *Myrica* with highest value of  $\psi_{pf}$ . This result indicates that turgidity in cells are essential during phenologically active period, that was maintained through osmotic adjustment. Higher value of  $\psi_{pf}$  in March made leafing process smooth. In contrast, when new leaves started to mature at April, the  $\psi_{pf}$  value dropped to



least (1.05 MPa).  $\psi_{\text{pf}}$  value showed almost same pattern in *Lyonia ovalifolia* with phenology too.

In *Myrica esculenta*, an evergreen species, RWC<sub>Z</sub> did not change significantly from September to February (79.5% to 87%). Among evergreen species seasonal changes in cell wall properties and osmotic potential are such that RWC<sub>Z</sub> remain constant throughout the year (Holbrook *et al.*, 1995). Present study showed some similarity with evergreen species of neotropical dry forest (Borchert, 1995). Mainali (2000) and Tripathee (2001) reported higher value of RWC<sub>Z</sub> (80%-87.6%) and (75%-80.9%) respectively in *Shorea robusta* in Makawanpur district. However, in *Myrica* the RWC<sub>Z</sub> varies from 71% to 87% indicating that this plant can maintain turgor to lower RWC than *Shorea robusta*. Increasing and decreasing of RWC<sub>Z</sub> is affected by soil water potential because it shows positive correlation with  $\psi_{\text{soil}}$  (Pokherel, 2001).

*Lyonia ovalifolia*, the deciduous species, showed lowest RWC<sub>Z</sub> (63%) in March a hot and dry month and highest (87%) in October, in the first month of winter season. This result indicates that *Lyonia ovalifolia* can maintain turgor to lower level of water content than *Myrica esculenta* (63% Vs 71%). At the same time *Lyonia* was phenologically active with rapid growth of new leaves. It also showed that plant maintain turgor at dry condition where the  $\psi_{\text{soil}}$  fall to lowest level.

Change in bulk modulus of elasticity i.e. cell wall elasticity is another important adaptational mechanism during the phenological process occurring mostly in dry season. During drought periods the cell wall becomes more elastic decreasing the bulk modulus of elasticity ( $\epsilon$ ) because value of  $\epsilon$  is inversely proportional to the elasticity of cell wall i.e. smaller  $\epsilon$  value indicates greater elasticity (Fan *et al.*, 1994). The general phenomenon observed by Patakas *et al.* (1997) is that the cell becomes more extensible during growth and development whereas once an organ is formed the cell become less and less extensible. In present study, the lowest value of  $\epsilon$  observed during April



(2.916 MPa) in drought condition but phenologically the period of leaf initiation as well as leaf maturation and thereafter value of  $\epsilon$  increased and became maximum in February (13.07 MPa) which was phenologically inactive period in *Myrica esculenta*. However, value of  $\epsilon$  was lowest during March (2.77 MPa) which was leaf initiation period and became maximum in September (9.462 MPa) during which leaf senescence began in *Lyonia ovalifolia*. In short, value of  $\epsilon$  decreased during phenologically active period and thereafter increased. Such result was also observed by Patakas *et al.* (1997), Saliendra *et al.* (1995) and Uprety (2002). A small  $\epsilon$  (low rigidity) contributes to turgor maintenance in much the same way as decrease in osmotic potential (Robichaux *et al.*, 1986).

Huber value (HV), hydraulic conductivity ( $K_h$ ), specific conductivity ( $K_s$ ), and leaf specific conductivity ( $K_l$ ) were the parameters measured to describe the xylem conductance of plants. These values in *Myrica esculenta* were found high at the end of rainy season (beginning of dry period) in September and lowest value observed during March and April (Fig. 6). Sobrado (1993) observed marked seasonal decline in hydraulic conductivity during draught. The decrease in values ( $K_h$ ,  $K_s$ ,  $K_l$ , HV) as the season becomes drier was due to drought induced embolism in the xylem conduit suggesting that xylem embolism increases with progressive drought and diminishes stem cross-sectional area available for water transport (Holbrook *et al.*, 1995). Embolism in xylem of woody plants, which disrupt water flow and reduce hydraulic conductivity, may be induced by drought, excessive transpiration or winter cycle of freezing and thawing of xylem water (Tyree and Sperry, 1989).

The value of  $K_h$  was lowest during April at the time when leaf senescence completed and new leaf began to emerge, suggesting that the xylem embolism increased with progressive drought and diminished the stem cross sectional area available for water transport in *Myrica*.  $K_h$  showed higher value during September which may be due to new wood formation (Bhattarai, 2001).



The highest HV was observed in January ( $1.2089 \times 10^{-3}$ ). The HV is the ratio of xylem cross sectional area to leaf surface area. The highest HV in January was due to decrease in leaf area. At the same time phenology showed continued leaf fall. The highest value of HV in January might be due to winter wood formation. Similar result was observed in *Shorea robusta* in Hetauda (Tripathee, 2001) and *Quercus lanata* in coronation garden, Kirtipur (Nepal, 2002). However, lowest value of HV observed during March ( $0.15 \times 10^{-3}$ ) may be due to drought induced cavitation in *Myrica*.

The specific conductivity ( $K_s$ ) depends upon the diameter of conducting elements (Larcher, 1995) and is the porosity of wood (Tyree and Ewers, 1996). It showed highest value during September (at the end of rainy season) and lowest in July (rainy season). This might be due to embolism and deposition of tyloses in conducting elements. Leaf specific conductivity ( $K_l$ ) showed similar trend as  $K_s$ .

In case of *Lyonia ovalifolia*, the HV and  $K_l$  were observed highest during February and  $K_h$  and  $K_s$  were found highest in September. But lowest value of HV,  $K_h$ ,  $K_s$  and  $K_l$  were observed in May (a dry month). All the values decreased to minimum during drought which may be due to xylem embolism. The correlation analysis showed higher correlation between  $K_h$  and  $K_s$  ( $r = 0.879$ ) which is higher than in *Myrica*. Phenomorphological observations showed that leaf senescence started in September and new leaf began to emerge from May when the xylem conductance values were lowest.

In April leaf fall was completed and new leafing was very fast in *Myrica esculenta* showing lowest SLM value ( $6.61 \times 10^{-3} \text{ gm.cm}^{-2}$ ). Lower values of SLM of expanding leaves were also reported in *Shorea robusta* (Tripathee, 2001) and *Cinnamomum camphora* (Nepal, 2002) of Central Nepal. The highest value of SLM was observed in June ( $13.41 \times 10^{-3} \text{ gm.cm}^{-2}$ ). As the monsoon begin in June, the leaves were fully expanded with sufficient water and photosynthetic rate at its peak. Hence greater mass was accumulated, giving



highest value of SLM at the end of rainy season i.e. September. An increase in SLM may be pronounced by an increase in leaf thickness, which may be due to leaf maturation and increase in density (Witkowski and Lamont, 1991).

In *Lyonia ovalifolia* higher SLM value was observed in November ( $12.52 \times 10^{-3}$  gm.cm<sup>-2</sup>). In November, leaf fall continued and intact lower leaves turned to yellow with axillary bud. There was rapid increase in SLM from September to November, due to accumulation of dry matter in leaf. With the beginning of leaf senescence and fall, SLM decreased continuously from September to April. From senescing leaves nutrients are retranslocated to stem and made available to next growth of new leaves and shoot. Higher SLM of mature leaf and lowest in senescencing leaf were also reported by Shiraishi *et al.* (1996), Lei and Lechowicz (1998), Mainali (2000), Nepal (2002) and Uprety (2002). Shiraishi *et al.* (1996) and Lei and Lechowicz (1998) showed strong positive correlation between SLM and intensity of light. But, present study did not show any significant correlation with any other parameters of water relations.

LRWC was measured to determine the moisture status of *Myrica esculenta* and *Lyonia ovalifolia*. LRWC<sub>pd</sub> were found higher than LRWC<sub>md</sub>. Before sunrise, there is established an equilibrium in soil-plant-atmospheric continuum (Ritchie and Hinckley, 1975). In *Myrica* both predawn and midday values of LRWC showed lowest in March (76.81% and 75.05%). In severe drought condition  $\psi_{\text{soil}}$  decline and plant water potential decreases to minimum. From April LRWC increased upto August due to availability of water during rainy season and become maximum in August (97.82% at predawn and 88.49% at midday). LRWC always remain greater than RWC<sub>z</sub> in *Myrica*. Naidu and Swamy (1995) observed almost similar result in *Dolichandrone atrovivens* in India.

For *Myrica esculenta* RWC<sub>z</sub> was lowest and tissue elasticity highest in April. In the same month LRWC<sub>pd</sub> and LRWC<sub>md</sub> were also lowest. These indicate the lowest level of water status. But LRWC did not exceed the RWC<sub>z</sub> indicating



that plant was not under water stress even during the driest months of year. Favourable water status for new growth was maintained by leaf fall which reduced water loss by transpiration and increasing tissue elasticity. Almost similar result showed by *Lyonia* too.

In *Lyonia ovalifolia* LRWC<sub>pd</sub> was recorded highest in August (98.70%) lowest in February (72.02%), and similarly LRWC<sub>md</sub> highest in August (92.16%) and lowest in February (63.69%). Both predawn and midday values of LRWC were lowest in February. Water lost by evapo-transpiration from senescing leaf is higher which decrease the water content of leaf to minimum. This is followed by leaf drop. Low LRWC during winter season may be due to limited transpirational water loss associated with low temperature, low light intensity and shorter sunshine hours (Naidu and Swamy, 1995). LRWC in *Lyonia* also showed higher value than RWC<sub>Z</sub>. Correlation analysis showed significant negative correlation of LRWC<sub>pd</sub> with HV ( $r = -0.724$ ) and K<sub>1</sub> ( $r = -0.699$ ). LRWC<sub>md</sub> also showed negative correlation with HV ( $r = -0.731$ ) but showed positive correlation with LRWC<sub>pd</sub> ( $r = 0.606$ ).

The annual minimum value of LRWC<sub>pd</sub> was higher in *M. esculenta*, an evergreen species than in *L. ovalifolia*, a deciduous species. SLM is also closely related with leaf life span. Short lived leaf has lower SLM and vice versa. Except in November *M. estulenta* had higher SLM than *L. ovalifolia*.

In *Myrica*, phenological processes like leaf initiation, leaf senescence and bud break were concentrated in the drier months (March-April) of the year. The seasonality of tree phenology is mainly determined by the duration and intensity of seasonal drought (Mooney *et al.*, 1994). However, flowering and leaf maturation occurred during summer. It is an established fact that high turgor is required for these processes. The leaf senescence of *Myrica* started from the August and completed at April. Evergreen species shed their leaves throughout the year but relatively more during the summer (Ralhan *et al.*,



1985). *M. esculenta* did not show multiple leafing. *M. esculenta* maintained its wet season flowering character on all sites wherever it occurred

In *L. ovalifolia* leaf senescence started from September and completed in March. More than 50% leaves dropped during February. New leafing started in March–April, which were dry months. The shoot expansion started from late March. Shoot expansion appears to require both the release of xylem tension by leaf shedding and full hydration of the terminal branches (Borchert, 1994 a, b).



## 7. CONCLUSION

Water relation parameters showed variation in different seasons. Both *Myrica esculenta* and *Lyonia ovalifolia* indicated that seasonality and climatic conditions had strong control over water status of plants. In *Myrica* osmotic potential was minimum during March when plant enter phenologically more active period i.e. active new leafing conditions. So, lower osmotic potential facilitated water extraction from dry soil and help to maintain proper turgidity of cells. Dehydration was postponed in *Myrica esculenta* by shedding old leaves during dry months.

*Lyonia ovalifolia* was also phenologically more active during dry months of the year. During cold and dry months plant maintained high turgor pressure, low osmotic potential, and low bulk modulus of elasticity. The lower value of both  $LRWC_{pd}$  and  $LRWC_{md}$  during dry months of the year in both species showed lower water content in plant that induced the senescence of leaves. However,  $LRWC_{pd}$  and  $LRWC_{md}$  never reached the turgor loss point both in *M. esculenta* and *L. ovalifolia*. So the plants were not under severe water stress even during hot and dry months of year.

Higher value of xylem conductance was observed during September in *Myrica* and during January and February in *Lyonia*. Thereafter the values decreased and reached to minimum in March to May probably due to xylem embolism in drought conditions.

Flowering and fruiting occurred in monsoon season in *Myrica* which require higher cell turgidity. SLM was highest for mature leaf and lowest for senescing leaf. SLM of *Myrica esculenta* an evergreen species was higher than *Lyonia ovalifolia*, a deciduous species.

It is concluded that studied species showed both drought resistance and avoidance mechanism. So growth and development remain unaffected in this environment even in severe drought condition.



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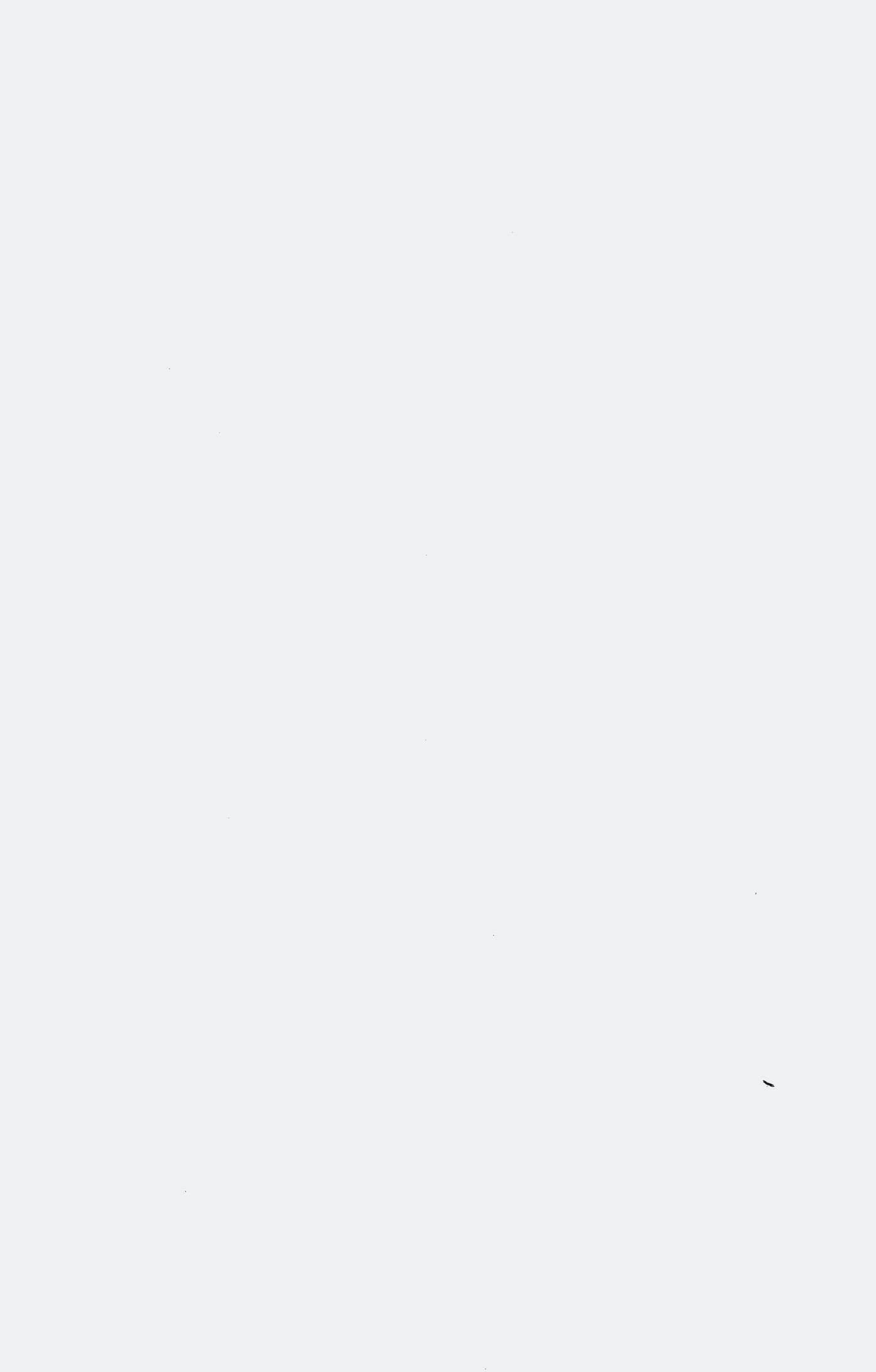
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## 9. APPENDICES

**Appendix I : Various parameters of xylem conductance seasonal variations.**

Huber value (HV), Hydraulic conductivity ( $K_h$ , Kg. m. sec.<sup>-1</sup> MPa<sup>-1</sup>), specific conductivity ( $K_s$ , Kg. m.<sup>-1</sup> sec.<sup>-1</sup> MPa<sup>-1</sup>), leaf specific conductivity ( $K_l$ , kg. m.<sup>-1</sup> sec.<sup>-1</sup> MPa<sup>-1</sup>). Sample Size = 1 for *Myrica esculanta*

Parameters Month	HV	$K_h$	$K_s$	$K_l$
August	$2.57 \times 10^{-4}$	$15.25 \times 10^{-6}$	4.526	$4.50 \times 10^{-2}$
Sept.	$2.36 \times 10^{-4}$	$77.04 \times 10^{-6}$	8.043	$15.21 \times 10^{-2}$
Oct.	$2.59 \times 10^{-4}$	$17.24 \times 10^{-6}$	1.539	$3.19 \times 10^{-2}$
Nov.	$1.778 \times 10^{-4}$	$36.77 \times 10^{-6}$	2.186	$3.49 \times 10^{-2}$
Dec.	$2.965 \times 10^{-4}$	$29.21 \times 10^{-6}$	1.693	$4.26 \times 10^{-2}$
Jan.	$12.089 \times 10^{-4}$	$17.53 \times 10^{-6}$	1.159	$9.38 \times 10^{-2}$
Feb.	$3.81 \times 10^{-4}$	$42.78 \times 10^{-6}$	1.514	$5.19 \times 10^{-2}$
March	$10.60 \times 10^{-4}$	$12.82 \times 10^{-6}$	0.952	$9.66 \times 10^{-2}$
April	$1.50 \times 10^{-4}$	$25.45 \times 10^{-6}$	1.394	$1.99 \times 10^{-2}$
May	$1.39 \times 10^{-4}$	$31.19 \times 10^{-6}$	1.758	$2.33 \times 10^{-2}$
June	$2.415 \times 10^{-4}$	$13.03 \times 10^{-6}$	0.877	$21.19 \times 10^{-2}$

**Appendix II : Various parameters of xylem conductance seasonal variations.** Huber value (HV), Hydraulic conductivity ( $K_h$ , Kg. m.sec.<sup>-1</sup> MPa<sup>-1</sup>), specific conductivity ( $K_s$ , Kg. m.<sup>-1</sup> sec.<sup>-1</sup>, MPa<sup>-1</sup>), leaf specific conductivity ( $K_l$ , kg. m.<sup>-1</sup> sec.<sup>-1</sup> MPa<sup>-1</sup>). Sample Size = 1 for *Lyonia ovalifolia*

Parameters Month	HV	$K_h$	$K_s$	$K_l$
August	$3.095 \times 10^{-4}$	$47.11 \times 10^{-6}$	2.427	$7.13 \times 10^{-2}$
Sept.	$2.704 \times 10^{-4}$	$60.25 \times 10^{-6}$	2.684	$14.27 \times 10^{-2}$
Oct.	$2.601 \times 10^{-4}$	$43.50 \times 10^{-6}$	1.789	$4.19 \times 10^{-2}$
Nov.	$2.162 \times 10^{-4}$	$33.49 \times 10^{-6}$	2.116	$4.11 \times 10^{-2}$
Dec.	$3.502 \times 10^{-4}$	$17.53 \times 10^{-6}$	1.156	$3.25 \times 10^{-2}$
Jan.	$5.408 \times 10^{-4}$	$20.98 \times 10^{-6}$	1.209	$5.23 \times 10^{-2}$
Feb.	$22.20 \times 10^{-4}$	$32.61 \times 10^{-6}$	1.470	$27.74 \times 10^{-2}$
March	—	$48.35 \times 10^{-6}$	1.823	—
April	$1.54 \times 10^{-4}$	$29.26 \times 10^{-6}$	1.480	$2.19 \times 10^{-2}$
May	$1.28 \times 10^{-4}$	$15.48 \times 10^{-6}$	0.801	$0.98 \times 10^{-2}$
June	$1.468 \times 10^{-4}$	$23.25 \times 10^{-6}$	1.329	$19.52 \times 10^{-2}$



Appendix III : Leaf Relative Water Content (LRWC, %), seasonal variation, values are mean with  $\pm$  standard deviation. LRWC calculated at two times of day; predawn collected leaves (before sunrise) and mid day (12-1 PM). Sample size = 5 for *Myrica esculanta*.

Month	LRWC	
	Predawn	Midday
August	96.14 $\pm$ 1.01	95.7 $\pm$ 0.167
Sept.	93.10 $\pm$ 0.46	—
Oct.	97.82 $\pm$ 1.52	88.49 $\pm$ 2.35
Nov.	89.87 $\pm$ 3.07	87.37 $\pm$ 1.37
Dec.	92.39 $\pm$ 6.26	86.62 $\pm$ 1.79
Jan.	90.22 $\pm$ 1.015	77.34 $\pm$ 11.20
Feb.	88.56 $\pm$ 1.57	75.99 $\pm$ 3.45
March	93.67 $\pm$ 10.78	92.45 $\pm$ 4.41
April	76.81 $\pm$ 2.53	75.05 $\pm$ 2.28
May	93.36 $\pm$ 16.40	92.57 $\pm$ 1.043
June	92.15 $\pm$ 3.27	90.98 $\pm$ 2.005

Appendix IV : Leaf Relative Water Content (LRWC, %), seasonal variation, values are mean with  $\pm$  standard deviation. LRWC calculated at two times of day; predawn collected leaves (before sunrise) and mid day (12-1 PM). Sample size = 5 for *Lyonia ovalifolia*.

Month	LRWC	
	Predawn	Midday
August	98.70 $\pm$ 0.404	92.16 $\pm$ 1.33
Sept.	—	—
Oct.	90.16 $\pm$ 3.85	76.23 $\pm$ 7.77
Nov.	87.23 $\pm$ 1.88	79.25 $\pm$ 2.17
Dec.	98.08 $\pm$ 0.82	73.95 $\pm$ 6.45
Jan.	94.70 $\pm$ 3.61	75.29 $\pm$ 11.34
Feb.	72.02 $\pm$ 3.27	63.69 $\pm$ 8.98
March	—	—
April	82.84 $\pm$ 10.18	81.06 $\pm$ 3.19
May	91.33 $\pm$ 6.12	89.89 $\pm$ 3.816
June	94.27 $\pm$ 6.76	90.27 $\pm$ 6.27



Appendix V : Parameters obtained from P-V curve analysis seasonal variation. Osmotic potential at zero turgor ( $\psi_{sz}$ , Mpa), relative water content at zero turgor (RWC<sub>Z</sub>, %) and bulk modulus of elasticity ( $\epsilon$ , MPa) for *Myrica esculanta*.

Month	$\psi_{sf}$	$\psi_{sz}$	$\psi_{pf}$	RWC <sub>Z</sub>	$\epsilon$
August	-1.315	-1.694	1.265	73	4.685
Sept.	-1.724	-2.0408	1.774	79.5	8.654
Oct.	-1.538	-2.083	1.488	85	9.920
Nov.	-1.923	-2.439	1.898	84	11.862
Dec.	-1.694	-2.222	1.669	85.5	11.510
Jan.	-2.040	-2.564	1.990	83	11.705
Feb.	-2.272	-2.631	2.222	87	13.070
March	-2.380	-3.125	2.355	71	8.120
April	-1.075	-1.515	1.050	64	2.916
May	-1.612	-1.960	1.587	76	6.612
June	-1.428	-1.886	1.453	81	7.65

Appendix VI : Parameters obtained from P-V curve analysis seasonal variation. Osmotic potential at zero turgor ( $\psi_{sz}$ , Mpa), relative water content at zero turgor (RWC<sub>Z</sub>, %) and bulk modulus of elasticity ( $\epsilon$ , MPa) for *Lyonia ovalifolia*

Month	$\psi_{sf}$	$\psi_{sz}$	$\psi_{pf}$	RWC <sub>Z</sub>	$\epsilon$
August	-1.639	-2.173	1.589	73	5.885
Sept.	-1.639	-1.923	1.514	84	9.462
Oct.	-1.369	-2.000	1.344	87	4.072
Nov.	-1.886	-2.222	1.786	73	6.614
Dec.	-1.886	-2.439	1.861	68	5.815
Jan.	-1.886	-2.272	1.836	71	6.331
Feb.	-1.818	-2.272	1.768	75	7.072
March	-1.075	-1.785	1.025	63	2.770
April	-1.785	-2.173	1.685	73	6.240
May	-1.818	-2.38	1.743	77	7.578
June	-1.2195	-1.587	1.3195	82.5	7.540



Appendix VII : Specific Leaf Mass (SLM, gm cm<sup>-2</sup>) seasonal variation.  
 Sample size = 5 for *Myrica esculanta* and *Lyonia ovalifolia*.

Month	<i>Myrica esculanta</i>	<i>Lyonia ovalifolia</i>
	SLM	SLM
August	11.45 × 10 <sup>-3</sup>	9.415 × 10 <sup>-3</sup>
Sept.	10.91 × 10 <sup>-3</sup>	5.245 × 10 <sup>-3</sup>
Oct.	11.63 × 10 <sup>-3</sup>	10.21 × 10 <sup>-3</sup>
Nov.	8.83 × 10 <sup>-3</sup>	12.52 × 10 <sup>-3</sup>
Dec.	11.53 × 10 <sup>-3</sup>	8.605 × 10 <sup>-3</sup>
Jan.	10.71 × 10 <sup>-3</sup>	8.95 × 10 <sup>-3</sup>
Feb.	9.62 × 10 <sup>-3</sup>	7.61 × 10 <sup>-3</sup>
March	12.79 × 10 <sup>-3</sup>	—
April	6.61 × 10 <sup>-3</sup>	6.09 × 10 <sup>-3</sup>
May	8.305 × 10 <sup>-3</sup>	6.92 × 10 <sup>-3</sup>
June	13.41 × 10 <sup>-3</sup>	11.14 × 10 <sup>-3</sup>

Appendix VIII : Climatic Data of Study Site from August 2002 to June 2003.

Months	Rainfall (mm)	Temp. Maximum (° C)	Temp. Minimum (° C)
Aug.	499.9	29	19.8
Sept.	148.0	28.1	18.2
Oct.	15.0	26.7	13.5
Nov.	26.5	23.8	8.4
Dec.	0.0	19.8	4.0
Jan.	19.5	19.2	2
Feb.	60.3	20.5	6
March	85.9	24.5	8.9
April	38.0	28.6	13.3
May	37.7	29.6	14.6
June	227.3	28.7	19.1

Source: Department of Hydrology and Metrology, HMG/N



