A PHYSIOLOGICAL INVESTIGATION

OF THE

ADAPTIVE SIGNIFICANCE OF JUVENILITY

IN

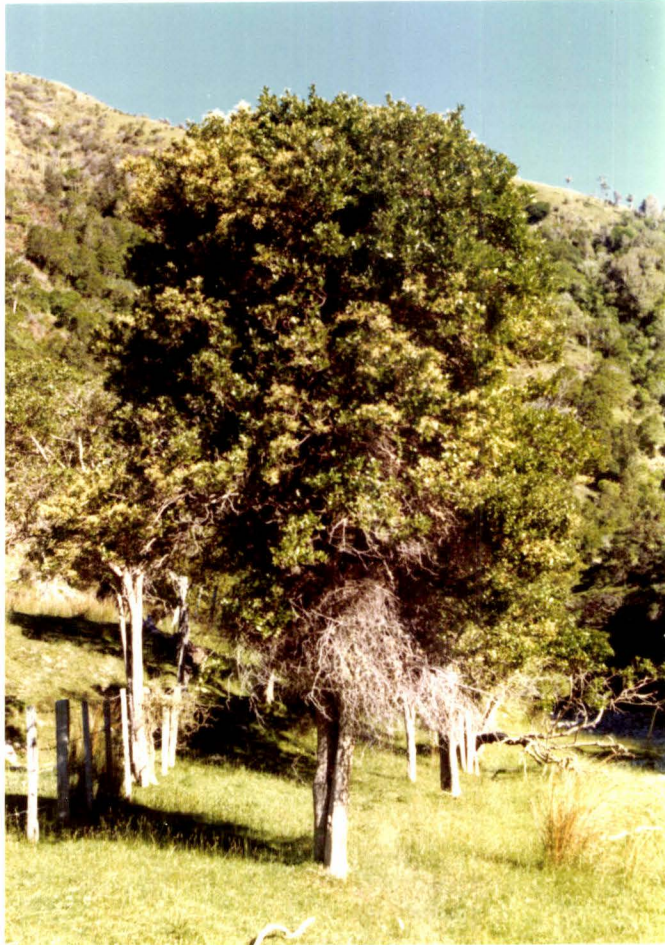<u>Pennantia</u> <u>corymbosa</u> Forst.

A thesis presented in partial fulfilment
of the requirements for the degree of

Master of Science in Botany

at Massey University

David Grant Hollows
1978

Mature Specimen of _Pennantia corymbosa_ Forst.

ABSTRACT

The responses of the juvenile and adult growth forms
of Pennantia corymbosa Forst. to a range of light intensities,
leaf temperatures, shoot water potentials and wind velocities
were investigated.

Results tend to indicate that the small-leafed
divaricating juvenile is better adapted to open habitats than
the adult. Responses to light intensity were similar for both
growth forms. Measurements of photosynthetic rates at various
light intensities after pretreatment at low and high irradi-
ances revealed little difference in response between juvenile
and adult, with both showing a similar increase in photosyn-
thetic rates and light saturation points after the pretreat-
ment light intensity was increased. Granal stacking in
chloroplasts from juvenile and adult leaf palisade was reduced
after growth at the higher pretreatment light intensity to the
same extent in juveniles and adults. Solarization, despite
the presence of a hypodermis, was greater in the adult, while
the activity of Ribulose -1,5- diphosphate carboxylase was
greater in the juvenile.

The indication that the juvenile is better adapted
to open habitats is also supported by the results of experi-
ments into the response of photosynthetic rates to a range
of temperatures. The data revealed a higher mean temperature
optimum for the juvenile than for the adult leaves (21°C c.f.
18°C).

The hypothesis that the juvenile might be better
adapted to edaphic water stress was tested by withholding
water for 14 days and measuring the rates of photosynthesis
and transpiration as shoot water potential decreased. Rates
of photosynthesis and transpiration declined in both juvenile
and adult leaves as shoot water potential decreased. However,

the juvenile was able to maintain a higher rate of photosynthesis at comparable low water potentials than the adult which indicates that the juvenile is the more drought tolerant of the two.

Leaves of the juvenile also retain water better than those of the adult under moderately windy conditions. When plants were grown in a wind tunnel at wind speeds of up to 12 m sec^{-1} stomatal closure (as measured using a leaf diffusion resistance meter) occurred at lower wind speeds in the juvenile than the adult leaves.

The results obtained during this investigation thus support the hypothesis that the small-leafed divaricating juvenile of _Pennantia_ is better adapted to a dry, exposed habitat than is the large-leafed orthotropic adult.

ACKNOWLEDGEMENTS

"Wind is a most important factor in New Zealand"

Leonard Cockayne (1911)

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CHAPTER I

INTRODUCTION

A feature of the New Zealand flora is the prevalence of small-leafed (leptophyllous* and nanophyllous*) species with semi-divaricating and divaricating* habits. According to Greenwood and Atkinson (1977) there are 54 species from sixteen families that have a more or less divaricating growth habit, which constitutes approximately ten percent of the indigenous woody flora.

That the proportion of divaricating nanophylls in the New Zealand flora is atypically high is suggested by the paucity of recordings of like plants in other floras. However, the growth habit is not unique to New Zealand as divaricating plants do occur elsewhere, particularly in xeric environments.

Carlquist (1965) has noted the presence in Madagascar of a plant (Decaryia madagascariensis) with divaricating, thorny branches, while Bartlett and Bartlett (1976) have recorded a thorny divaricating shrub (Candalia microphylla) from Patagonia. Tucker (1974) lists 53 divaricating species from the species from the desert and chaparral communities of California and Arizona, though in these cases leaf size is much larger than that of most New Zealand divaricating species. Thus it is apparent that nowhere else are small-leafed divaricating plants such a large proportion of the woody flora.

The large number of distantly related plant families that contain divaricating species indicate the existance of strong selection pressures in favour of divarification at some stage during the evolutionary history of the New Zealand flora. The New Zealand flora has been geographically isolated since the Cretaceous (Fleming, 1962) and possibly the Permian (Darlington, 1965) thus giving ample time for selection pressures to operate.

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*See Glossary for definition

The occurrance of a large number of woody plants which exhibit heteroblastic development* is another feature of the indigenous flora, the life cycles of which have long been familiar to botanists (Hooker 1853, Kirk 1878). Eleven of the heteroblastic species have divaricating juveniles. These belong to nine genera in eight unrelated families (Philipson 1963).

Rumball (1961) conducted a survey of the distribution of heteroblastism by country from which it appears that, although the survey was not complete, the incidence of heteroblastism, like divarication, is atypically high in the New Zealand flora. Rumball (1961) reported two interesting Australian species, Flindersia maculosa and Eremocitrus glauca both from the Rutaceae, that are thorny xerophytic shrubs while juvenile and develop into the tree form later. The habit of Flindersia maculosa, leafy and slender at the top but bearing a mass of tangled branches and very few leaves at the base, is very similar to Eleocarpus hookerianus, an indigenous species with a divaricating juvenile. The presence of heteroblastic species with microphyllous+ non-divaricating juveniles in the Rèunion, Maurice and Rodriques group of islands has been reported by Friedmann and Cadet (1976). Unfortunately the proportion of species with microphyllous juveniles is not indicated.

A characteristic of heteroblastic development in indigenous species is the length of duration of the juvenile stage, which may last up to 60 years as in the case of the divaricating juvenile of Eleocarpus hookerianus (Bulmer 1958). It appears that there has been strong selection for an attenuation of the developmental processes that result in the adult plant. Thus it is again evident that there has been strong selection pressure in favour of the divaricating growth habit. The question then arises as to the nature of the selection pressure and therefore the type of environment to which divarication and juvenility is an adaptation.

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+ Technically nanophyllous

* See Glossary for definition

Juvenility in the New Zealand flora (and as a consequence divarication) was first studied experimentally by Leonard Cockayne (1899, 1900, 1901) who raised seedlings of many indigenous plants. Cockayne (1929) in FLORA AND VEGETATION OF NEW ZEALAND states that in 106 cases considered the juvenile is the more mesophytic* and in seventeen cases the more xerophytic* plant when compared to the adult. Cockayne included the eleven species of divaricating juveniles in the xerophytic category. Cockayne (1911) deduced that divaricating shrubs are xerophytes because:

> "The ecological factors governing this
> growth-form appear to be wind, in the
> first place, and then various other
> xerophytic stimuli, of which soil must
> play an important part."

Leonard Cockayne (1911) did not consider the present day New Zealand climate to differ from others to a degree that would allow for the evolution of the divaricating growth habit to such a unique extent. Cockayne considered that the divaricating habit is a xeromorphic growth form resulting from adaptation to "steppe climates" that he hypothesized occurred during the Pleistocene when conditions were cooler and drier than at present.

Rattenbury (1962) supported Cockayne's hypothesis of divarication as an adaptation to a harsh Pleistocene climate. However, he considered that cold climatic conditions resulting in low soil temperature which would retard root absorption combined with strong winds, rather than low soil moisture, could have led to the evolution of divarication during the Pleistocene.

Wardle (1963) considers that the concentration of divaricating, small-leafed, species in forest and mesic scrub communities does not support Cockayne's hypothesis. Instead he suggests that divaricating jubenile forms are adapted to present day fairly dry forest environments, and that their xeromorphy enables them to survive on drier sites while the development of adult foliage is related to the development of larger root systems.

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* See Glossary   for definition

Dansereau (1964), while discussing the prevalence of leptohyllous species in the indigenous flora, found it difficult to see the ecological significance of having small leaves with regard to the present climate, and considered such forms to be non-disadvantageous at the present but advantageous at some time during the past.

Denny (1964) from her studies on the habit heteroblastism of _Sophora microphylla_ found that dryness and long days bring about a phenotypic response which results in more divarication, that genotypic populations have developed in areas where the environment is dry. Furthermore where the plants are from a shaded or damp habitat there is little divarication. The presence of two species of _Sophora_ that show marked divarication (along with many other divaricating species) in Canterbury and Central Otago which has the driest climate in New Zealand combined with dry "Northwesters" suggested to Denny that divaricating plants are adapted to fairly dry continental-type climates which still exist.

Went (1971) suggested a non-adaptative explanation. He proposed that a particular chromosome segment carrying the genes controlling divarication was transferred asexually between families, perhaps by an insect vector.

Evidence that small-leafed juveniles (although not associated with divarication in this instance) are an adaptation to edaphic water stress is presented by Friedmann and Cadet (1976) who conducted a survey of the geographical distribution of heterophyllous species in the Réunion, Maurice and Rodriques Islands. There was found to be a significant relation between the occurrence of heterophylly and the xeric condition of the enviornment to which it was concluded microphylly is an adaptation.

Several workers have suggested that divarication could possibly be an adaptation against browsing by moas. This theory has been recently discussed by Greenwood and Atkinson (1977) in some depth. However, the theory must remain speculative due to the difficulty of investigating such an hypothesis experimentally.

The anatomy and physiology of divaricating plants, and in the case of divaricating juveniles the corresponding adults, has been investigated over the last 50 years or so, with the aim of determining the habitat to which the divaricating growth habit is an adaptation.

Fitzgerald (1923 quoted by Rumball 1961) claimed that the juvenile leaves of Paratrophis microphylla, Plagianthus betulinus and Pennantia corymbosa are more xeromorphic both internally and externally than those of the adult. The criteria for xeromorphy used by Fitzgerald are not known as they were not mentioned by Rumball and the original thesis is not available.

Johnston (1948) compared the anatomy of juvenile and adult leaves of Carpodetus serratus taken from shaded and open habitats. Using the criteria for xeromorphy described by Maximov (1928) (i.e., smaller and thicker leaves, closer venation, increased stomatal density and strongly developed palisade mesophyll) he found that the adult "shade" leaves were more xeromorphic than the juvenile "shade" leaves, while the reverse was found for the juvenile and adult "sun" leaves. However, adult "shade" leaves were sampled from the forest margin and juvenile "shade" leaves from the forest floor, thus it is possible that the degree of xeromorphy in adult "shade" leaves was due to differences in humidity. Therefore it is only valid to compare "sun" leaves in which the juvenile is more xeromorphic. Johnston also found that the transpiration rate in "sun" leaves was greater for the juvenile than the adult, which Maximov (1928) describes as a xeromorphic characteristic. Johnston noted that the differences in the anatomy and physiology of "sun" and "shade" leaves of Carpodetus were similar to the differences in juvenile and adult leaves. Furthermore, he concluded that the juvenile leaves were more plastic, reacting more completely when exposed to similar environmental conditions as the adult.

Keen (1970) investigated the anatomy and physiology of small and large-leafed plants in the genera Coprosma, Melicope and Plagianthus. Leaf anatomy studies showed that all the small-leafed plants are xeromorphic, while the large-leafed plants are typical mesomorphs. From elementary physiological

studies Keen concluded that the small-leafed species are not
necessarily more drought resistant than the large, but are
better adapted to grow under conditions of physiological
drought, mainly because of a reduced internal resistance to
water movement, more efficient heat exchange processes and
a greater resistance to wilting.

It is evident that further research on heteroblastic
species is needed to determine the adaptive significance of
divarification and juvenility in the indigenous flora. By
selecting a divaricating juvenile it is possible to make
a comparative investigation between divaricating and non-
divaricating plants of the same species, thereby minimizing
genetic differences and concentrating on developmental
changes.

To gain information on the habitat to which a plant
species or ecotype is adapted it is possible to monitor the
response to a range of combinations of environmental parameters.
Physiological changes are an accurate indication of the effect
that a given set of parameters is having on the plant because
of the rapidity of functional responses to changes in
environment. Therefore the emphasis in this investigation is
physiological, with some anatomical and biochemical work where
considered appropriate.

The responses of rates of photosynthesis and transpiration
to changes in factors of the environment are easier to interpret
than the responses of other plant characteristics. Such responses
have a definite application to describing the adaptation of
plants to their environment, (Jarvis 1969). Thus investigations
into the effects of a range of defined environments on the rates
of photosynthesis and/or transpiration have been under taken
to test for physiological differences between divaricating
juvenile and non-divaricating adult plants.

CHAPTER   2

MATERIALS AND METHODS

2:1   Choice and Description of Experimental Material

To investigate the adaptive significance of
divarication a plant species with a small-leafed divaricating
juvenile was chosen, thereby making it possible to conduct a
comparative study of a small-leafed divaricating and a larger
leafed orthotropic* plant while minimizing differences in
genetic constitution.

The species used was Pennantia corymbosa Forst.
(see Frontispiece) which is commonly found in lowland forest
from latitude 35° southwards (Allen, 1961). The juvenile
plant, which can grow to a height of up to two metres, is
shrubby with slender, flexible more or less interlacing
branchlets (Fig. 2 ,p. 9). Technically the growth habit of
the juvenile is semi-divaricating as not all of the branchlets
are at an angle of 90° or greater from the main axis.   The
juvenile leaf is obovate with a lamina 7 - 15 mm long and
5 - 10 mm broad. The adult leaf is obovate-oblong with a
lamina 5 - 10 cm long and 2 - 4 cm broad (Fig. 3 ,p. 9). The
adult tree can grow to a height of eight to ten metres.

Juvenile Pennantia can be observed growing in a wide
range of habitats, from lowland forest floor to open river
flats and exposed montane outcrops. This apparent broad eco-
logical tolerance has led to speculation as to the adaptive
significance of the juvenile phase, and therefore the divar-
icating growth habit.

It was hoped in this investigation to determine
whether the juvenile growth form is physiologically adapted
to a shaded or exposed habitat when compared to the adult.

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*See Glossary for definition

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Figure 2   Growth Habits of Juvenile (J) and Adult (A)
            Pennantia corymbosa Forst.

Figure 3   Comparison of Leaf Sizes of Juvenile (J) and
            Adult (A) Pennantia corymbosa Forst.

2:2   Growing Conditions and Experimental Design

To determine whether the juvenile and adult growth forms of Pennantia are adapted to different environments two groups of experiments were conducted.

The first group of experiments (Group A see Table I, p. 11) investigated the responses of juvenile and adult plants to variations in light intensity, temperature and water stress, the physiological responses being monitored using a leaf chamber.

The second group of experiments (Group B see Table II, p. 12) investigated the effect of wind velocity on day and night transpiration rates and leaf diffusion resistance on plants of juvenile and adult Pennantia.

For the Group A experiments the plants used had been struck from cuttings taken at Tokomaru, 20 Km south of Palmerston North, about four years previously and had been pruned to a height of approximately 30 cm. The growing medium consisted of a 50:50 pumice and peat mixture with the pH adjusted to 7 with the addition of lime. The plants were grown in plastic containers 17 cm x 17 cm x 20 cm in size and watered to saturation point on alternate days. Nutrients in the form of 200 ml of Hoagland's solution modified by the addition of iron chelate were supplied weekly.

Two juvenile and two adult plants were grown outside under shade cloth at a low light intensity for a period of four months and further pretreated for eight weeks in a defined environment in a Temperzone growth cabinet. The growth cabinet light intensity of 4 n Einsteins $cm^{-2}sec^{-1}$ photosynthetically active radiation (PHAR) was equivalent to approximately two percent of full sunlight.

After the responses to growth at low light intensity had been investigated (see Chapter 3) pretreatment irradiance was increased for a further eight weeks. For two weeks a light intensity of 80 n Einsteins $cm^{-2}sec^{-1}$ was employed but because of solarization made evident by incipient chlorosis (particularly in the adult leaves) the light intensity was reduced to 40 n Einsteins $cm^{-2}sec^{-1}$ PHAR (approximately 20 percent of full sunlight) for the remaining six weeks. Illumination was provided by six mercury iodide HPIT and two tungsten halogen lamps.

TABLE I

EXPERIMENTS IN GROUP A

PHYSIOLOGICAL INVESTIGATIONS USING LEAF CHAMBER

1. Effect of Low and High Irradiance Pretreatments
   on Photosynthesis

2. Effect of Temperature on Photosynthesis

3. Effect of Water Stress on Photosynthesis and
   Transpiration

BIOCHEMICAL INVESTIGATIONS

1. Effect of Light Intensity on Total Chlorophyll Content

2. Ribulose-1,5-diphosphate Carboxylase Activities

ANATOMICAL INVESTIGATIONS

1. Effect of Light Intensity on Chloroplast Ultrastructure

2. Leaf Anatomy

TABLE II

EXPERIMENTS IN GROUP B

PHYSIOLOGICAL INVESTIGATIONS USING WIND TUNNEL

1. Effect of Wind Velocity on Transpiration Rate

2. Effect of Wind Velocity on Night Transpiration Rate

3. Effect of Wind Velocity on Leaf Diffusion Resistance

ANATOMICAL INVESTIGATIONS

1. Examination of Stomatal Structure using Scanning
   Electron Microscopy

2. Estimation of Stomatal Frequency

From field observations the growth rate of <u>Pennantia</u> appears to be greatest during spring and autumn conditions. Although the lack of summer growth is probably a passive reponse to water stress, to safeguard against the possibility of a summer dormancy a spring, rather than a summer, environment was simulated in the growth cabinet. Thus a thirteen hour photoperiod and a $18^{\circ}C/13^{\circ}C$ day/night temperature regime were decided upon. Relative humidity was maintained at 70%.

Under growth cabinet conditions at the higher light intensities a plastochron* of five to six days for both the juvenile and adult plants was measured, resulting in nine to ten leaves per shoot being produced during the pretreatment interval. Thus adequate leaf material for the leaf chamber investigations was grown at the high light intensity.

At the low light intensity the plastochron indices were considerably longer (approximately two to three weeks). As the plants had been grown outside under shade cloth for four months at a light intensity approximating that of the growth cabinet, it was not considered crucial that some of the leaf material inserted into the leaf chamber had not grown in the defined environment. Furthermore, Gauhl (1970) has reported that the photosynthetic apparatus of mature leaves can adapt to changes in light intensity.

Two shoots from each plant were used for the physiological investigations thus giving four replicates per growth form. The small number of replicates was necessitated by the difficult and time consuming process of inserting the branchlets into the leaf chamber while ensuring that mutual shading was minimized and leaf orientation was approximately horizontal. To compensate, each datum is the mean of a minimum of ten readings for each defined set of environmental parameters, thus conferring a high degree of accuracy for each graphed point.

For the Group B experiments twelve-month-old rooted cuttings were used because their smaller size (15-20 cm) was more practical for insertion into the cylinder of the wind

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*See Glossary for definition

tunnel. The cuttings were taken in October 1975 from the same
population as the plants used in the Group A experiments and
grown in 10 cm plastic pots in the Botany/Zoology Department
glass house until the following spring when the wind tunnel
investigations were conducted. Plants were held in the glass
house when not in use. The growing medium and watering regime
were the same as those for the plants used in the Group A
experiments.

2:3   Description of Leaf Chamber and Method Used To Estimate Photosynthetic and Transpiration Rates

The standard methods for determining photosynthetic
activity involve measurement of either the rate of oxygen
efflux or CO_2 influx by analysing changes in the atmosphere
surrounding the assimilating tissue. Gasometric techniques,
those using infra-red gas analysers, can give very accurate
estimates of photosynthetic activity (Sestak et al, 1971).
The most commonly used gasometric techniques measure the
instantaneous rate of photosynthesis from changes in the
oxygen or CO_2 concentration in the air passing around the
assimilating tissue which is usually enclosed in a chamber.

Gas exchange systems have been classified by Larcher
(1969) into three types: closed, semi-closed and open.

In a closed system both the air input and output
from the assimilation chamber are connected to the gas analyser
with a pump in the interconnecting air line. The enclosed air
circulates between analyser and chamber where the CO_2 concen-
tration is changed gradually by the plant material. The rate
of CO_2 exchange is obtained from a curve of CO_2 concentration
against time at selected ambient CO_2 concentrations.

In a semi-closed system the gas analyser operates as
a null-point sensor regulating the supply of CO_2 or CO_2-free
air to the system, and the rate at which the gas is supplied
is recorded (Koller and Samish, 1964). The rate of CO_2 exchange
is calculated from the amount of CO_2 added to or removed from
the system during a certain period. The semi-closed system
integrates the CO_2 flux over a period of time. Consequently
the system is not as sensitive to transient, short-term

fluctuations in the rate of CO_2 exchange as the open system but it does provide easier regulation of the ambient CO_2 concentration.

In an open system (Sestak et al., 1971) an air stream of known, and in many cases conditioned, properties is passed through the assimilation chamber at a measured, constant flow rate. All or part of it is then passed through the gas analyzer before it leaves the system. In a completely open system there is no recirculation. The open system is particularly useful for investigation of single leaves, parts of plants or small plants. It allows the continuous recording of small rapid fluctuations and changes in photosynthetic rate with considerable accuracy.

In this investigation net photosynthetic and transpiration rates were measured in an open system leaf chamber (Fig. 4 ,p. 17) developed by H. G. McPherson (in publication) of the Plant Physiology Division D.S.I.R., Palmerston North. The leaf chamber proper is of perspex and has dimensions of 30 cm x 15 cm x 15 cm (Fig. 5 ,p. 19). It is built in two detachable halves and hinged to facilitate the entry of samples. Flexible rubber seals at each end allow larger leaves and shoots to project undamaged from the chamber.

Irradiance is provided by six mercury iodide HPIT and two tungsten halogen lamps, with a quartz iodide auxiliary light for high intensities. Irradiance is under manual control and is varied by placing layers of shade cloth over the leaf chamber and by adjusting the height of the auxiliary lamp. Light intensity inside the leaf chamber is monitored by a small Lambda Instruments Company quantum sensor of 4% accuracy.

Photosynthetic rates were estimated by measuring the CO_2 concentration gradients across the leaf chamber. These concentration gradients were measured by a URAS 2T infra-red gas analyzer which samples air every fifteen seconds before and after it passes through the leaf chamber. Net photosynthetic rates were calculated by dividing the CO_2 concentration gradients by flow at a leaf area. Air was circulated at a flow rate of 10 litres min^{-1}, which was the lowest flow rate that could be maintained. A low flow rate was required to build up

Figure 4   Diagram of the Systems Controlling the Leaf
            Chamber Environment
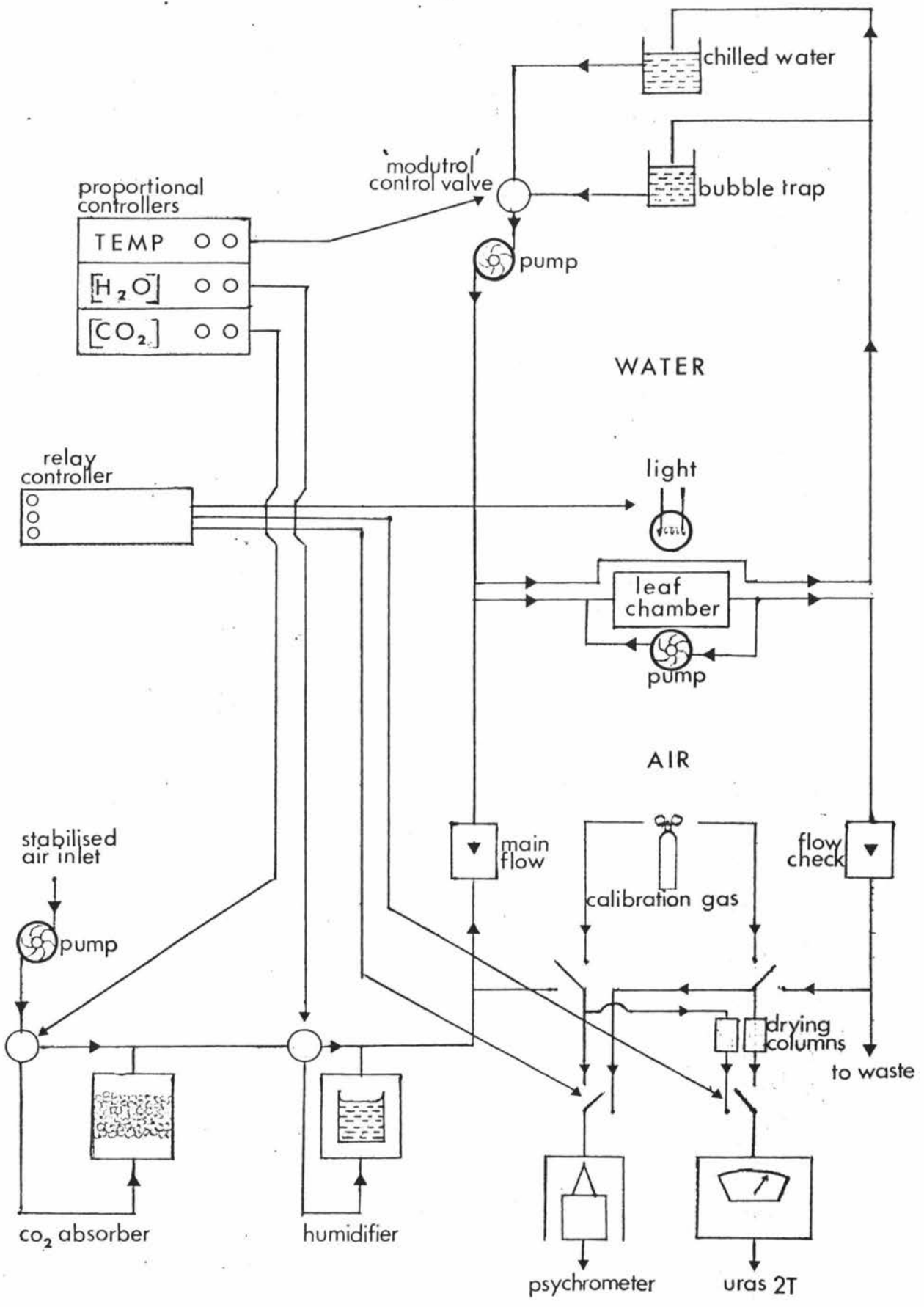
Figure 5   The Leaf Chamber
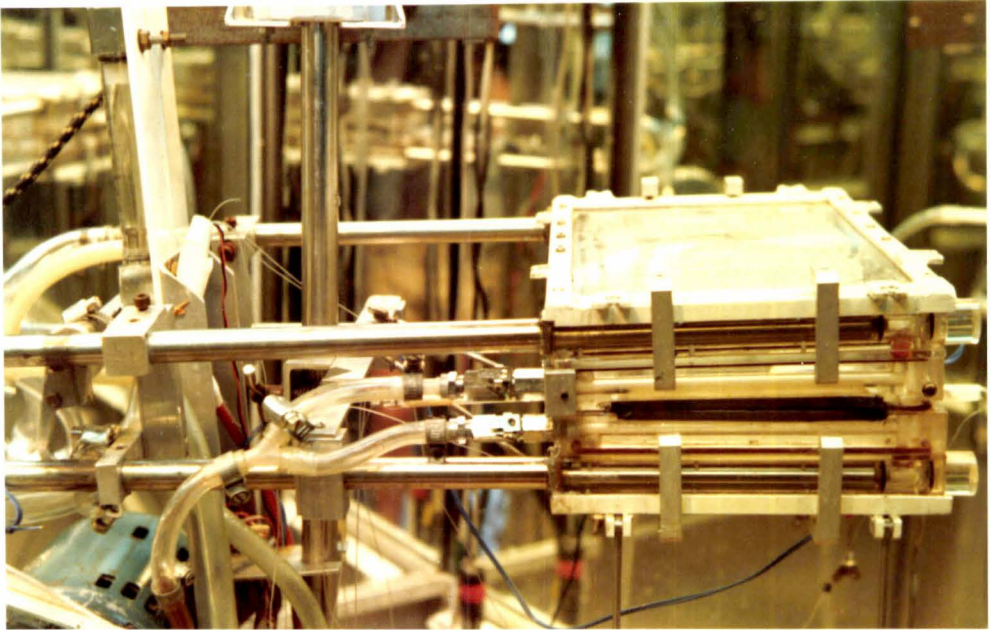
significant CO_2 concentration gradients across the leaf chamber. CO_2 was added via a manual control to the system to maintain the input concentration in the range of 500 p.p.m..

Vapour pressure deficit gradients were measured in a similar manner to CO_2 concentration gradients by a "wickless" psychrometer (McPherson et al., 1977, in publication). Transpiration rates, which were estimated by the gasometric method, were calculated using the same method as mentioned above for rates of photosynthesis. Vapour pressure deficit of the air entering the leaf chamber was controlled by proportional mixing of wet and dry air by an electrically controlled valve controling to a set point.

Leaf temperature was measured by a 0.002 inch copper-constantin thermocouple placed on the abaxial surface of a sample leaf. Temperature control was by electrical feedback from the leaf thermocouple which controlled the proportion of warm and cold water passing through the water jacket surrounding the leaf chamber.

The shape of the curves for the rate of photosynthesis as a function of, for example, temperature on light intensity often depends on the direction of which the environmental parameter is altered (Begg and Jarvis, 1968). Hysteresis was tested for by decreasing and then increasing light intensity and temperature. There were found to be no significant differences in the data obtained.

Readings were made after each shoot had equilibrated for 15-20 minutes. Data were collected on a Hewlett Packard logging system (Fig. 6 ,p. 22) which performed the control computation and printed out final results every 60 seconds. Each point on the graphs in Chapters 3 to 5 inclusive is the mean of at least ten readings, and was calculated on a Texas Instruments Programmable Calculator using the following formulae:

1. Standard Error:

$$S_{\bar{x}} = \frac{\sqrt{\sum\limits_{i=1}^{n} \dfrac{X_i^2 - n\bar{X}^2}{n-1}}}{\sqrt{n}}$$

Figure 6: Diagram of the Leaf Chamber Data Acquisition
System

transducers ⟷ control
(temp, $[CO_2]$, $[H_2O]$ etc)

analog signal

| 30 channel scanner (2740) | → | $1\mu v$ digital voltmeter 3490A |

keyboard and display ⟷ calculator controller 9830A → relay controller

clock →

magnetic tape cassette

page printer (9866A)
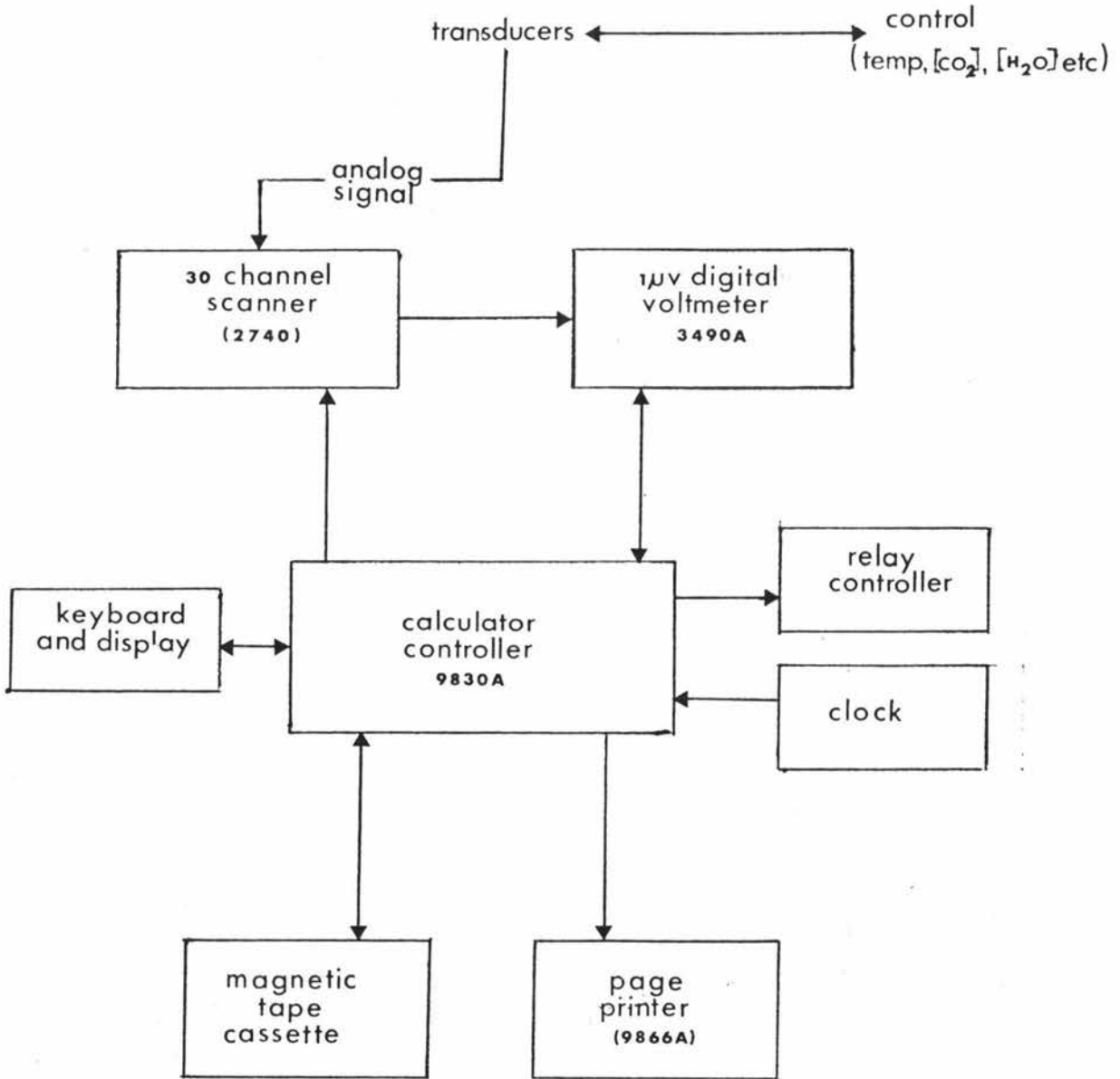
2. test for significant differences:
    (P > 5% is taken as significant)

$$f = \frac{\bar{x} - \bar{y}}{s\sqrt{\frac{1}{n_x} + \frac{1}{n_y}}} \qquad s = \sqrt{\sum_{i-1}^{n} \frac{X_i^2 - n\bar{X}^2}{n-1}}$$

n = number of measurements in sample

2:4   Description of Wind Tunnel

Wooding (1968) classified wind tunnels into three
categories: the closed circuit blower tunnel driven by a single
axial fan, the open circuit suction tunnel with the fans down-
stream of the working section and the open circuit blower tunnel
which often has a centrifugal fan upstream of a settling chamber.

Weatherley and Barrs (1959) described a climatological
tunnel of the same basic type as Wooding's closed circuit blower
which has subsequently been used in the investigation of plant
transpiration, e.g. Macklon and Weatherley (1965). Open circuit
tunnels have been used to investigate the effects of shelter
(Jensen, 1954; Shah, 1962) on growth as well as plant physio-
logical processes (references cited in section 6:1). Tunnels
of Wooding's class three are relatively few in number because
of the large cost involved in their construction. Tunnels of
this type have been used primarily in the study of microclimate
and turbulent diffusion processes.

The wind tunnel used in this investigation was an open
circuit suction type (Fig. 7 ,p. 25). It was constructed of a
galvanized steel cylinder four metres long with a diameter of
40 centimeters. A perspex cylinder six metres long, in which
the plants were placed, was connected to the end of the steel
cylinder farthest from the fan. The fan had eight 6 x 10 cm
blades and was powered by a ½ horse power electric motor. The
motor was wired to a rheostat which was adjusted to vary wind
velocity. The rheostat was calibrated with a manual anemometer.
Turbulence was reduced in the wind tunnel by two grids of
plastic tubing placed 0.5 m and 2.5 m from the fan. The light
source consisted of two Atlas 150 watt E. S. floodlights giving
a light intensity of 60 n Einsteins $cm^{-2}sec^{-1}$ PHAR at the centre
of the perspex cylinder when the plants were positioned.

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Figure 7   End-On View of Wind Tunnel
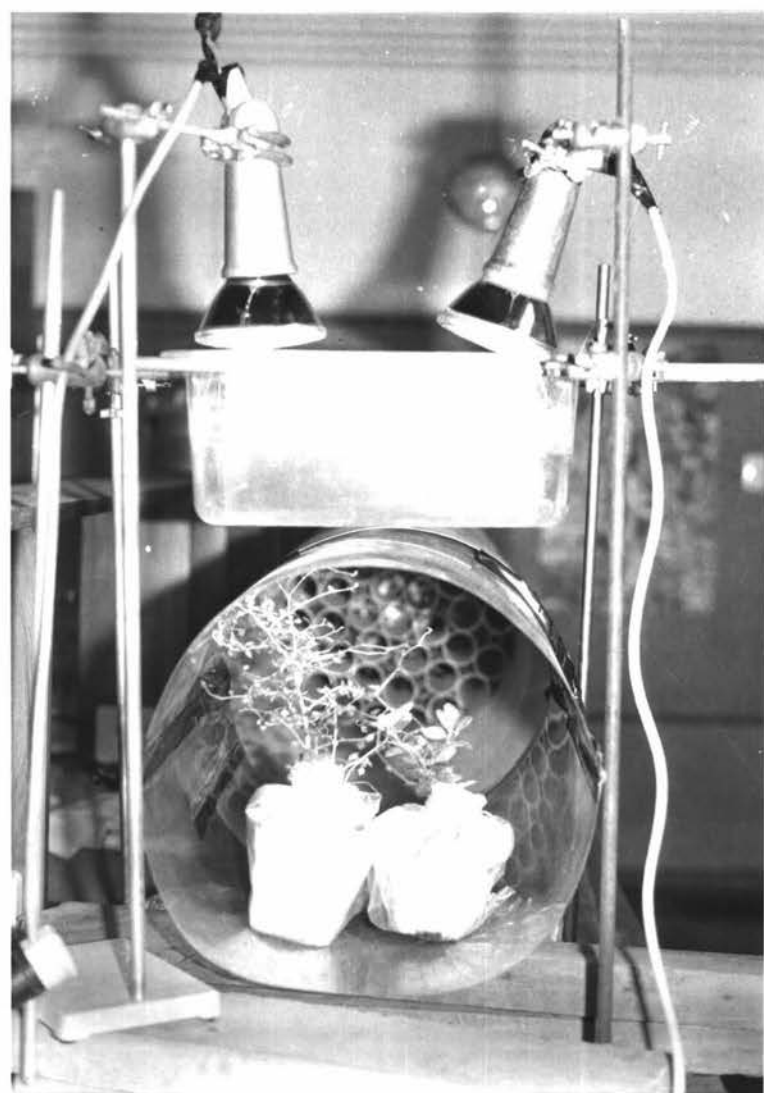
CHAPTER 3

ADAPTATION TO LIGHT INTENSITY

3:1   Effect of Low and High Light Intensity Pretreatments
        on Photosynthetic Activity in Juvenile and Adult
        PENNANTIA.

3:1:1   Introduction

One of the most important environmental variations to
which plants must adapt, and which directly and drastically
affects photosynthesis, is the difference in light intensity
between densely shaded and exposed habitats. It has been
known for some time that plants growing in deep shade often
have a considerably lower rate of photosynthesis at light
saturation than those growing in sunny locations (Böhning and
Burnside, 1956; Levitt, 1972). It is also well known that
photosynthetic characteristics, such as light compensation
and saturation levels, are strongly modified by the light
intensity under which a leaf develops (Björkman, 1968a).

The seedlings of Pennantia can be observed growing
in habitats varying from shaded lowland forest floors to
exposed river flats and forest margins. It has been suggested
that the juvenile of Pennantia might be adapted to the forest
floor environment (Wardle, 1963) in a similar manner to the
juvenile of Pinus taeda (Bormann, 1954). Bormann (1954)
looked at the ability of juvenile stages in Pinus taeda to
photosynthesize under low light intensities, comparing photo-
synthesis in sixteen week old and two year old seedlings. The
experiments indicated that the former reached the maximum
photosynthetic rate for the lowest light intensities. Bormann
concluded that this shift in photosynthetic response with
ontogeny explained why very young seedlings were able to
become established under dense weed cover.

In an attempt to determine whether the juvenile is
adapted to photosynthesize at a lower light intensity than

the adult, a comparative study of the ability of the photo-
synthetic apparatus of the two growth forms to adapt to low
and high light intensities was undertaken.

3:1:2   Materials and Methods

The pretreatment and growing conditions are described
in Section 2:2 and the system used to measure photosynthetic
rates in Section 2:3.

The photosynthetic rates were determined for four
adult and four juvenile shoots from four plants for both light
intensities pretreatments over a range of irradiances (i.e.,
giving four replicates per pretreatment) at a leaf temperature
of 20°C.

Leaf areas for the shoots adapted to low light
intensity were determined non-destructively by obtaining an
image of the shoot on low sensitivity photographic paper
(DS4.5L4) which was then cut out, weighed and the weight con-
verted to an area basis.

For the shoots adapted to high light intensity, leaf
areas were determined destructively by removing the leaves
and drying to constant weight at 80°C. Leaf area was then
determined by conversion of leaf dry weight data as above.

3:1:3   Results

The data obtained for the low and high light intensity
pretreatments are presented in Figure 8 , page 29.

For the "shade grown" plants, both juvenile and adult
had a net photosynthetic rate at the pretreatment light intens-
ity of approximately 5 ng CO_2 cm^{-2}sec^{-1}, while the rates at
each of the higher light intensities at which determinations
were made were found not to be significantly greater ($P > 5\%$).
The average rates of dark respiration were both approximately
2 ng CO_2 cm^{-2}sec^{-1} with no significant difference between the
juvenile and adult plants. (However the data obtained for
respiration rates have large standard errors, as the limits
of accuracy of the URAS were becoming significant at the low
CO_2 concentration gradients that were being sampled. Therefore
there may exist differences that were not detected.) The

Figure 8   Effect of Light Intensity on Rate of
            Photosynthesis of Four Juvenile (●)
            and Four Adult (▲) <u>Pennantia</u> <u>corymbosa</u>
            Shoots.

            A and B were pretreated for ten weeks at a
            light intensity of 4 n Einstein $cm^{-2}sec^{-1}$.

            C and D were pretreated for ten weeks at a
            light intensity of 40 n Einstein $cm^{-2}sec^{-1}$.

            (Each point is the mean of at least ten
             readings.)
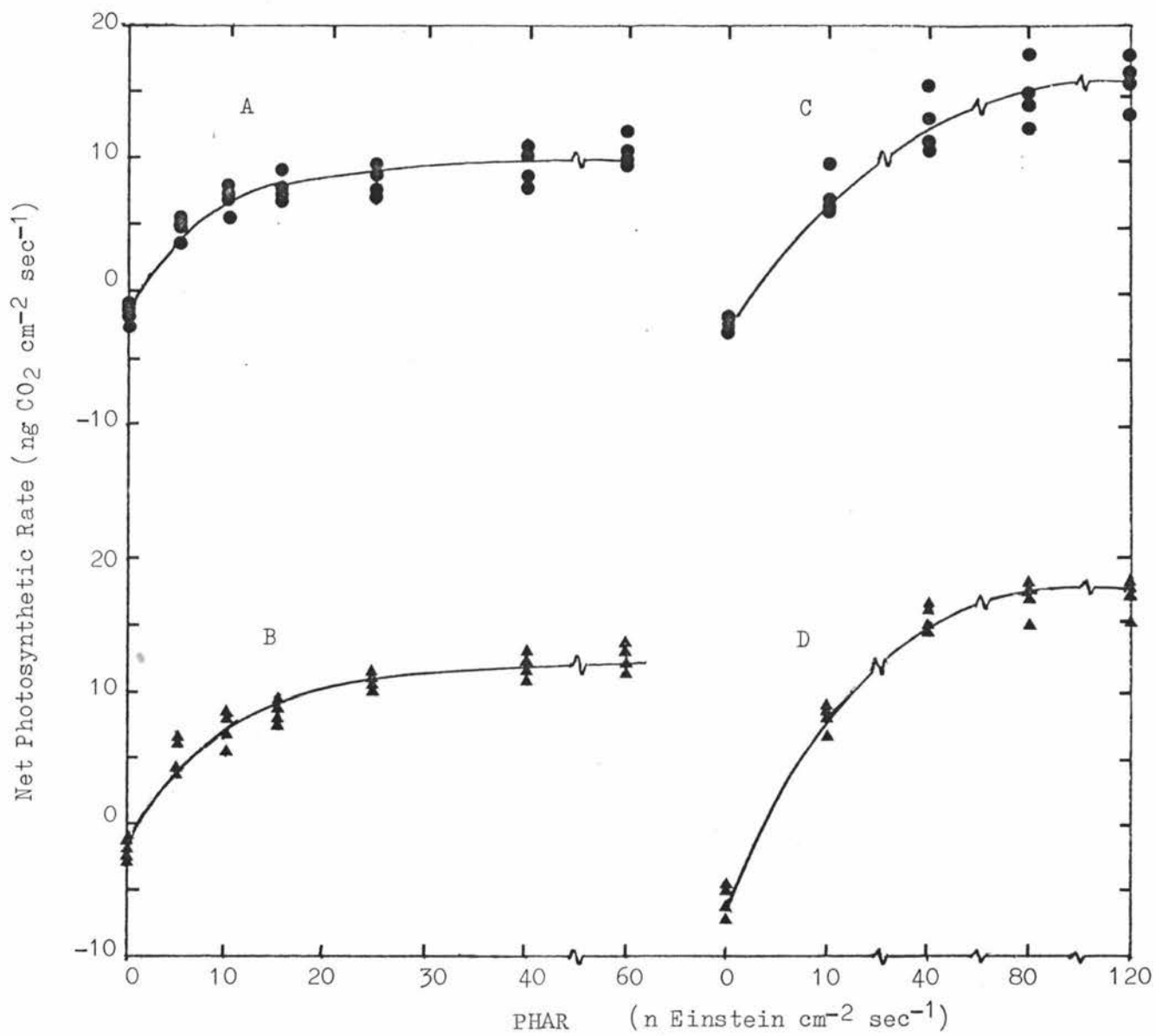
juvenile and adult plants both had a light compensation point
of approximately 1 n Einstein $cm^{-2}sec^{-1}$ as estimated by
interpolation.

In the "sun" adult plants the rate of net photosynthesis
on an area basis at light saturation was approximately 16 percent
higher than in the "sun" juvenile plants, while the rate of dark
respiration was almost twice as great. The rate of dark respi-
ration per unit area in the "sun" adult is approximately twice
that of the "sun" juvenile, which corresponds to differences in
leaf dry weight (see below). Both juvenile and adult plants
reached light saturation between 20 and 30 n Einstein $cm^{-2}sec^{-1}$,
had light compensation points of approximately 4 n Einstein cm^{-2}
sec^{-1}, and increased their rates of light saturated photosyn-
thesis by approximately 50 percent when grown at the higher
level of irradiance.

The juvenile leaves have only approximately 0.6 times
the dry weight of the adult leaves per unit leaf area for "sun"
leaves and approximately 0.5 times for "shade" leaves (see
Table III,p. 31). When the data for photosynthesis are expressed
as net photosynthetic rates per unit of leaf dry weight the rate
in the juvenile is over 50 percent greater than that of the
adult (see Table IV,p. 32) at light saturation for both low and
high light intensity pretreatments. It is noted that the net
photosynthetic rates per unit leaf dry weight at light satur-
ation are approximately the same for the two light intensity
pretreatments for both the juvenile and adult plants (see Table
V, p. 33). Furthermore the increase in net photosynthesis per
unit area obtained after pretreatment at the high light intens-
ity is proportional to increases in leaf dry weight per unit
area (see Table III,p.   ).

Using the data obtained in Section 3:4 it is possible
to express photosynthetic activity in terms of CO_2 fixed per
unit chlorophyll content (see Table IV,p. 32). For the high
light intensity pretreatment the juvenile fixed 50 percent more
CO_2 per gram of chlorophyll than the adult, while at the low
light intensity pretreatment there is a 100 percent increase
from adult to juvenile.

TABLE III

Fresh and dry weights per unit leaf area for "shade" and "sun" leaves of juvenile and adult Pennantia.

	Juvenile		Adult	
	"Shade"	"Sun"	"Shade"	"Sun"
Fresh Weight gm cm^{-2}	11.8	18.7	18.0	27.3
Dry Weight gm cm^{-2}	3.2	5.0	6.1	8.7
Fresh Weight/ Dry Weight	3.7	3.7	3.0	3.1

TABLE IV

Mean Net Photosynthetic Rates of shoots of juvenile and adult
Pennantia at an irradiance of 80 n Einstein $cm^{-2}sec^{-1}$ PHAR and
a leaf temperature of $20^{o}C$ expressed on the basis of leaf area,
dry weight and chlorophyll content.

	Juvenile		Adult	
	"Shade"	"Sun"	"Shade"	"Sun"
Photosynthetic Activity per leaf area ($mg\ CO_2\ cm^{-2}sec^{-1}$) ($\pm$ 1 S.E.)	$10.6^{\pm}3.6$	$15.9^{\pm}1.2$	$12.2^{\pm}1.4$	$18.1^{\pm}0.95$
Photosynthetic Activity per leaf dry weight ($mg\ CO_2\ g^{-1}hr^{-1}$)	11.8	11.5	7.6	7.6
Photosynthetic Activity per chlorophyll content ($g\ CO_2/g$ chlorophyll)	0.82	0.93	0.42	0.6

TABLE V

Total chlorophyll contents as influenced by pretreatment
light intensities.

Chlorophyll Content	Juvenile		Adult	
	"Shade"	"Sun"	"Shade"	"Sun"
ng/g Fresh Weight ($\pm$ 1 S.E.)	1.12$\pm$ 0.11	0.93$\pm$0.08	1.58$\pm$0.13	1.08$\pm$0.07
mg/g Dry Weight	4.25	3.53	4.50	3.35
mg/ cm^{-2} ($\pm$ 1 S.E.)	0.013$\pm$0.01	0.017$\pm$0.001	0.029$\pm$0.002	0.030$\pm$0.002

3:2   Effect of Low and High Light Intensity Pretreatments
       on Chloroplast Ultrastructure.

3:2:1 Introduction

Light intensity has been observed to influence
chloroplast ultrastructure in a number of plant species
including Solidago (Björkman and Holmgren, 1963), Soybean
(Ballantyne and Forde, 1970), Solanum (Gauhl, 1970), Amaranthus
(Lyttleton, Ballantyne and Forde, 1971) and Paspalum (Forde,
Whitehead and Rowley, 1975).

In ecotypes of Solanum and Solidago adapted to exposed
and shaded habitats it has been reported that chloroplasts of
shade adapted ecotypes are more sensitive to solarization under
high light intensities. This effect is shown in the ultra-
structure by the reduction of granal stacking. As a further
investigation to determine whether the juvenile growth form
of Pennantia is adapted to photosynthesize under different
conditions of light intensity from the adult, chloroplast
ultrastructure was examined in juvenile and adult plants that
had been grown in simulated "sun" and "shade" environments.

3:2:2 Materials and Methods

To determine the effect of light intensity on
chloroplast ultrastructure, as a possible indicator of differ-
ential abilities of juvenile and adult plants to adapt to low
and high light intensities, chloroplasts were examined in
sample leaves removed from juvenile and adult plants after
low and high light intensity pretreatments (see section 2:2
for light regimes). Leaves were removed six hours after the
beginning of the daily light period in all instances.

Five blocks of tissue 2 mm x 5 mm were excised from
the mid-laminae of randomly selected young juvenile and adult
mature leaves under cold modified Karnovsky's fixative (2%
formaldehyde, 3% glutaraldehyde in 0.1M phosphate buffer,
pH 7.2), (Karnovsky, 1965).

The leaf tissue was placed in glass vials in fresh
fixative and vacuum infiltrated in a desiccator with a tap
vacuum pump. The fixative was replaced and the tissue was

stored at 4°C for three hours.

The tissue was given three buffer (0.1M phosphate buffer, pH 7.2) washes of 10, 20 and 30 minutes at 4°C, then placed in 1% OsO4 in 0.1M phosphate buffer for three hours at 4°C, after which the three buffer washes were repeated for the same durations, but at room temperature.

The tissue was dehydrated in a five step ethyl alcohol series: ten minutes in 25% alcohol, ten minutes in 50% alcohol, ten minutes in 75% alcohol, ten minutes in 95% alcohol, ten minutes in 100% alcohol and one final soak in 100% ethanol for 100 minutes.

Infiltration with 100% propylene oxide was achieved by placing the sections in two changes of 100% propylene oxide of ten minutes duration, after which propylene oxide was replaced by Fluka Durcupan-ACM Epoxy resin as follows:

```
25% Epoxy resin 75% propylene oxide, 1 hour
50%    "      "   50%    "        "     "   "
75%    "      "   25%    "        "     "   "
100%   "      "    -      -       -    7 hours
```

All steps were performed on a magnetic stirrer.

The tissue was immersed in fresh resin in gelatin capsules and cured for three days at 60°C.

Sections, which were cut with an LKB 'ultratome' using a diamond knife to a thickness of 0.8μm, were mounted on collodion-coated 3 mm 200 mesh copper grids, post-stained for five minutes in a saturated solution of uranyl acetate in 50% ethanol and five minutes in lead citrate (Venable and Coggeshall, 1965). All samples were examined with a Phillips EM-200 transmission electron microscope.

3:2:3   Results

Variation in light intensity had a major effect on chloroplast ultrastructure in both juvenile and adult plants (Figs. 9, 10, 11, 12, pp.37 & 39). However there was no marked difference in response between the juvenile and adult chloroplasts for any of the leaf segments examined. In all cases where the leaf developed under high irradiance, chloroplasts contained fewer and smaller granal stacks, while osmophilic bodies were more prevalent than in chloroplasts of leaves

Figure 9   Chloroplast From Juvenile "Shade" Leaf

(x 1.75 x 10^4)

GS = granal stack
SG = starch grain

Figure 10   Chloroplast From Adult "Shade" Leaf

(x 2.25 x 10^4)

GS = granal stack
SG = starch grain
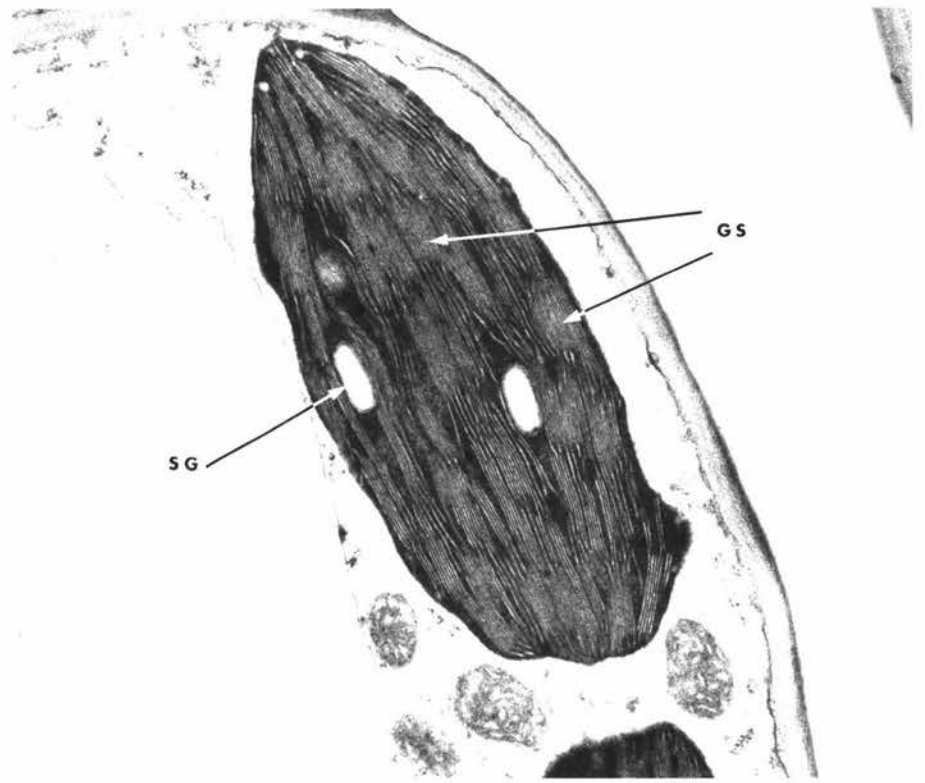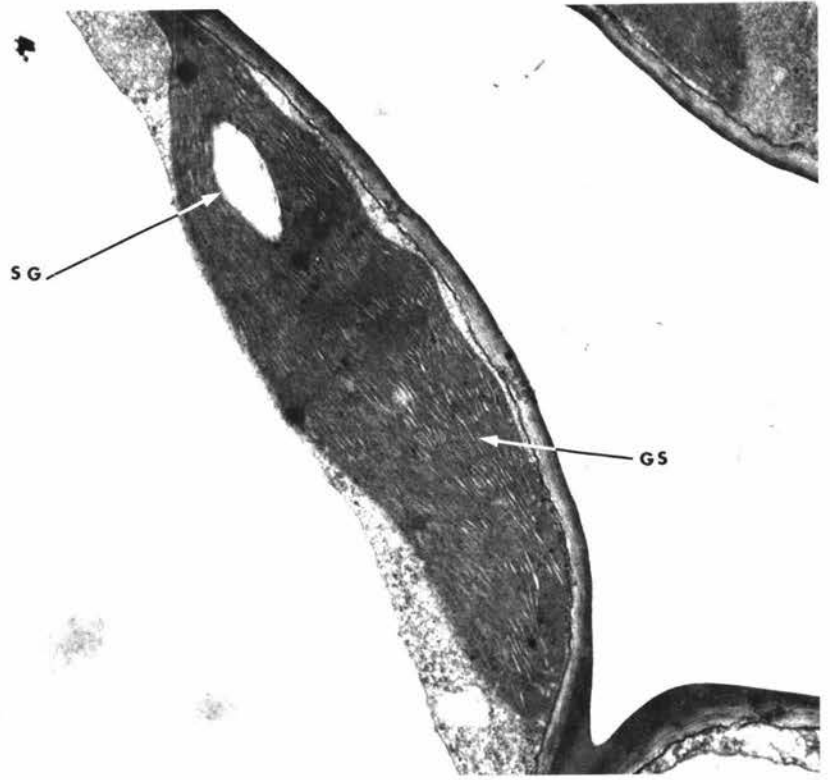
Figure 11   Chloroplast From Juvenile "Sun" Leaf
(x 1.75 x 10^4)

GS = granal stack
SG = starch grain
OB = osmiophillic body

Figure 12   Chloroplast From Adult "Sun" Leaf
(x 2.25 x 10^4)

GS = granal stack
SG = starch grain
OB = osmiophillic body
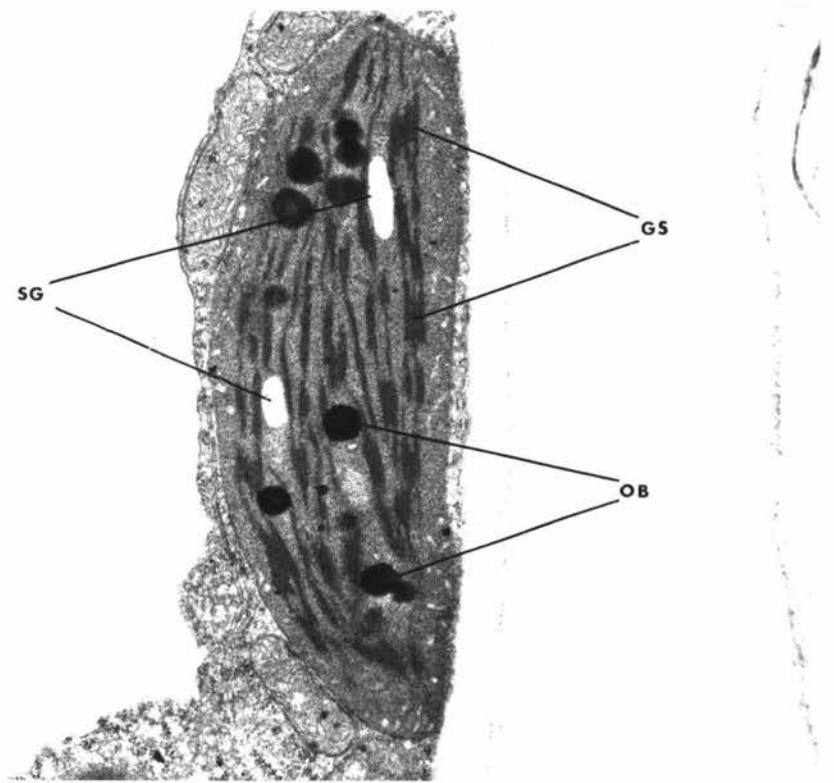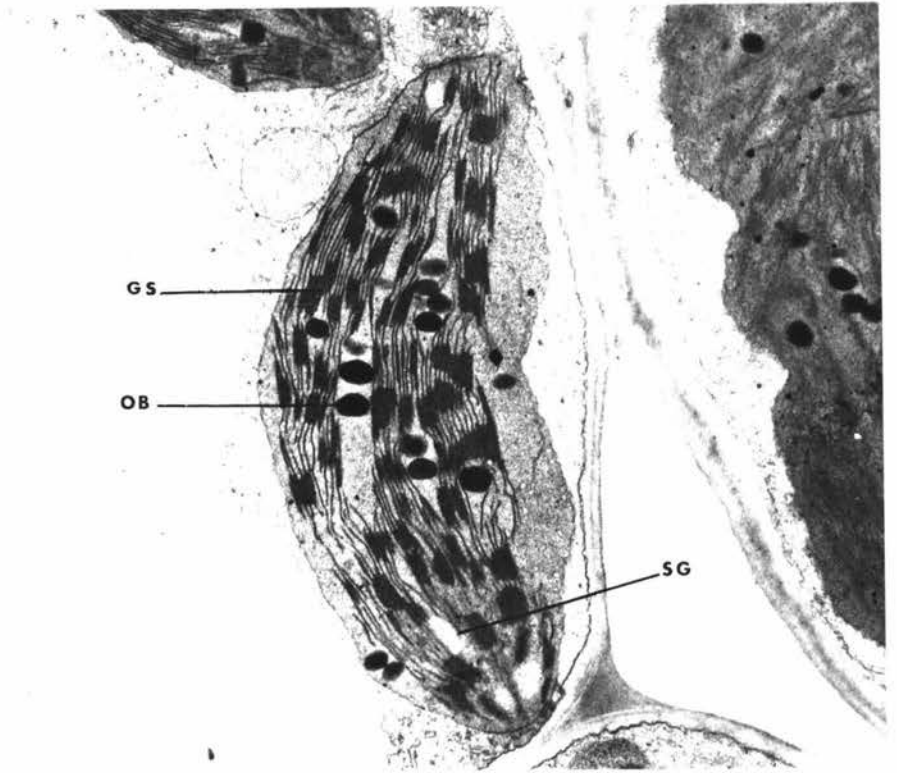
adapted to low light intensities.

Chloroplasts adapted to low light were densely packed with granal stacks, and starch grains were more evident. For some reason that is not clear, the grana appeared as negative images in the chloroplasts pretreated at low light intensities.

There was considerable variation in the degree of granal stacking in the same leaf and even in the same cell, particularly in the leaves adapted to high light intensity. A gradient of increasing complexity of granal organization from adaxial to abaxial epidermis as has been reported in Amaranthus (Lyttleton et al.,1971) and Paspalum (Forde et al., 1975) was not observed.

3:3   Effect of Low and High Light Intensity Pretreatments on Leaf Anatomy

3:3:1   Introduction

Differences in sun and shade leaves are found in respect of morphology and anatomy as well as physiology. Sun leaves are commonly thicker than shade leaves, usually because of greater elongation of the palisade parenchyma or by the formation of more layers of cells in the mesophyll of the leaf (Dufour, E., 1887; Pieters, 1962; Björkman and Holmgren, 1963). It is generally accepted that light is one of the main factors determining the relative development of mesophyll (Esau, 1962; Pieters, 1974). Xeromorphic leaves also have a more strongly developed palisade tissue than mesomorphic leaves (Shields, 1950; Esau, 1962).

It has been reported that "sun" leaves of adult Pennantia possess a hypodermis while those of the juvenile do not (R. G. Thomas, personal communication). An hypodermis is often found in the leaves of xerophytes (Cutter, 1971) and has been hypothesized to have a role in water storage (Fahn, 1974). It has also been suggested the hypodermis may play a role in protecting the chloroplasts in the mesophyll against solarization at high light intensities (Haberlandt, 1914).

3:3:2   Materials and Methods

To determine the effect of light intensity on the
development of the palisade mesophyll in juvenile and adult
Pennantia, sections were cut from the material prepared as
mentioned in Section 3:2:2, giving five replicates per growth
form for each light intensity. Sections were cut to a thick-
ness of $20 \mu m$ on a LKB ultratome, post-stained with toluidine
blue for ten minutes, and then viewed under a Reichart Diapan
compound microscope with bright field background. Represent-
ative sections were photographed at magnifications of 125x and
500x with a Nikon 35 mm SLR camera.

3:3:3   Results

The micrographs feature entire transverse sections of
leaves at a magnification of 125x (Figs. 13 & 14,p. 43 and Figs.
15 & 16,p. 45) and the adaxial epidermis together with three to
four layers of mesophyll at a magnification of 500x (Figs. 17 &
18,p.47 and Figs. 19 & 20,p. 49). In both the "shade" (Fig. 18,
p. 47) and "sun" (Fig. 20,p. 49) adult leaves a well developed
palisade layer two to three cells thick is evident. A hypodermis
was present in the adult "sun" leaf and cell wall thickening and
cutinisation of the epidermis was most evident here. The adult
"shade" leaf had a single layer of cells below the epidermis
similar in structure to the hypodermis of the adult leaf. How-
ever, the presence of chloroplasts indicate that it was not a
true hypodermis. There was a marked reduction in the number of
chloroplasts visible in the "sun" leaf when compared with the
"shade" leaf.

In the juvenile leaf the palisade was less structured
than in the adult leaf for both light intensity pretreatments.
In the juvenile "shade" leaf (Fig. 17,p. 47) there was no
distinct layer of palisade cells and there was no hypodermis
in either the "sun" (Fig. 19,p.49) or the "shade" leaves.
However, despite the lack of an hypodermis there were well
developed chloroplasts in the single layer of palisade cells
of the "sun" leaf. This suggests that the hypodermis in
adult Pennantia leaves is a xeromorphic structure rather than
an adaptation to prevent the solarization of chloroplasts at

Figure 13   Transverse Section of Juvenile "Shade" Leaf

(x 125)

M = mesophyll

Figure 14   Transverse Section of Adult "Shade" Leaf

(x 125)

M = mesophyll
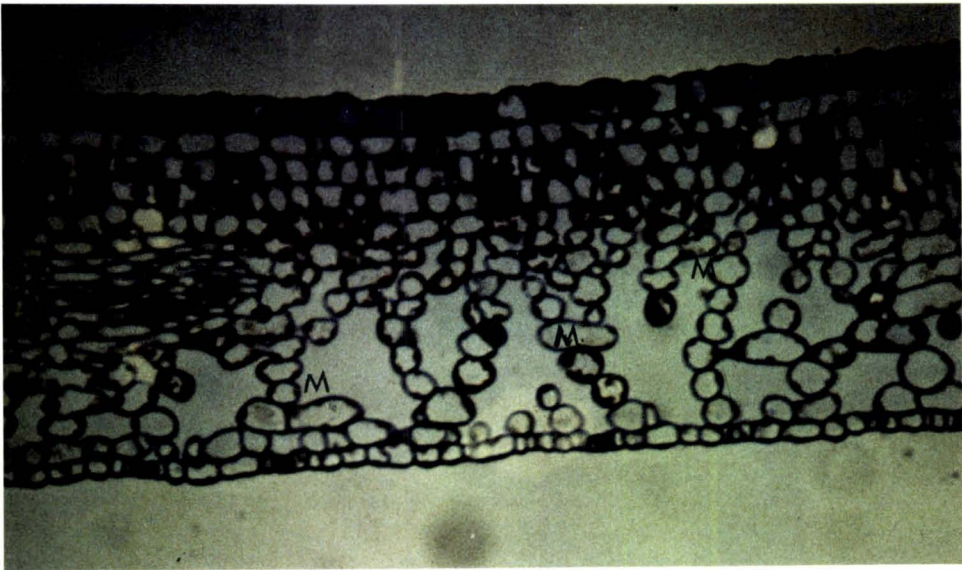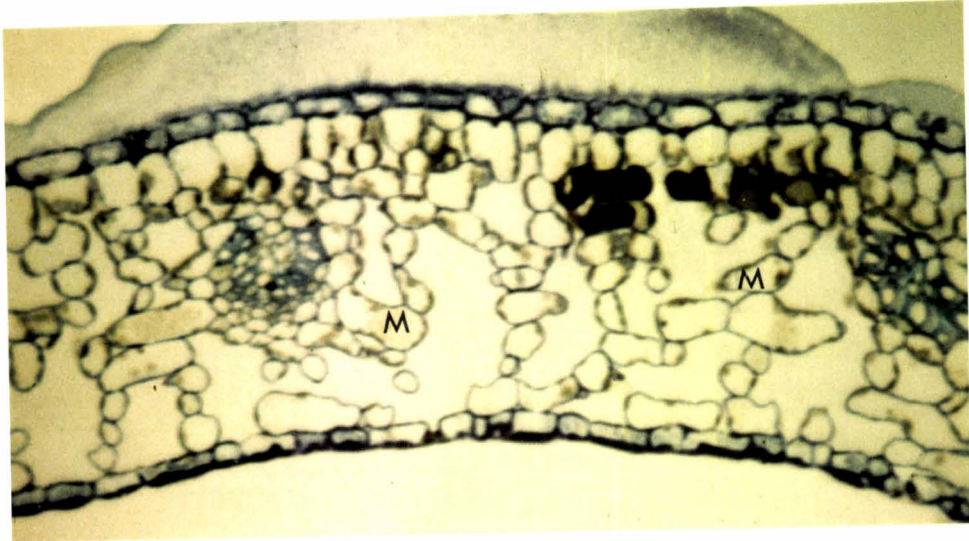
Figure   15   Transverse Section of Juvenile "Sun" Leaf

(x 125)

M = mesophyll

Figure   16   Transverse Section of Adult "Sun" Leaf

(x 125)

M = mesophyll
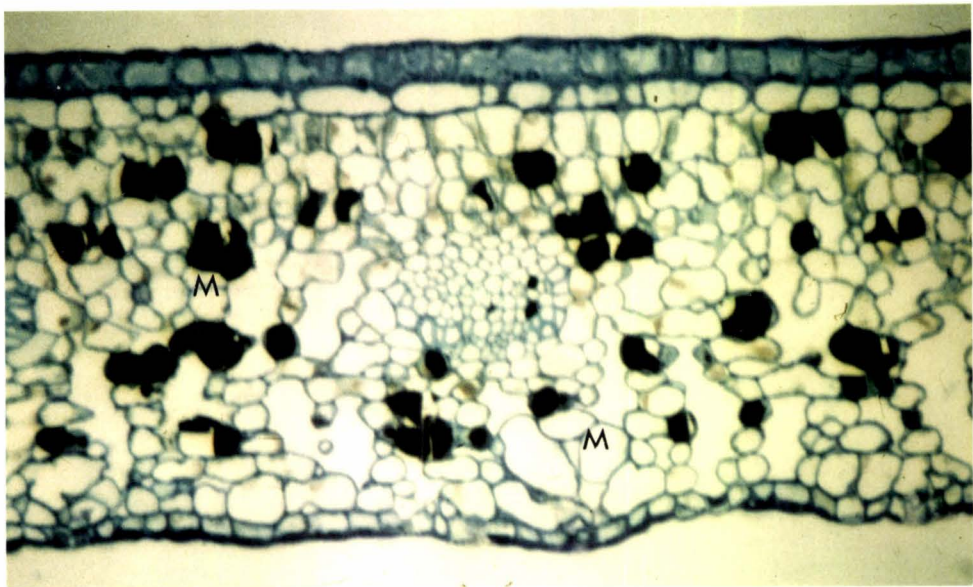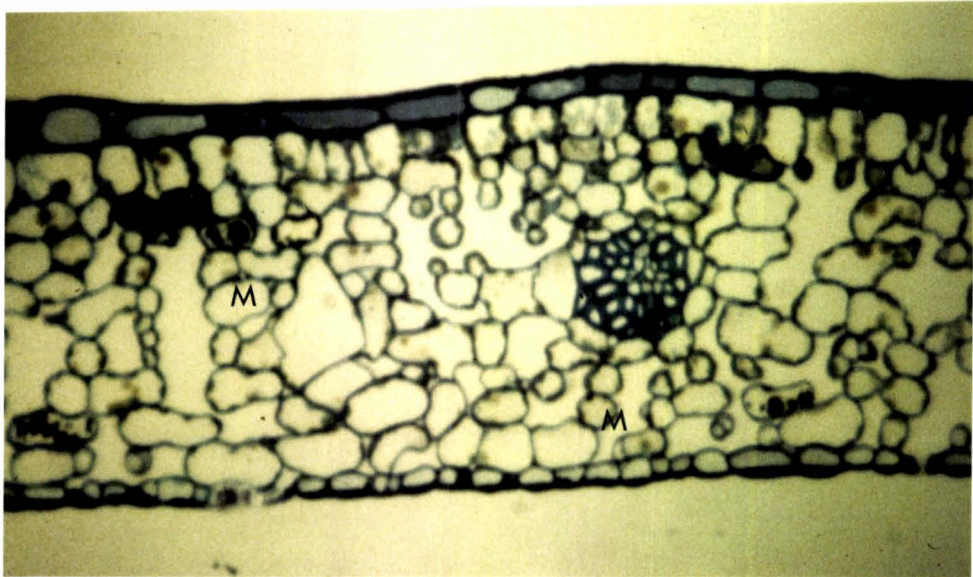
Figure   17   Adaxial Region of Juvenile "Shade" Leaf

(x 500)

C = chloroplast
E = epidermis
P = palisade mesophyll
S = spongy mesophyll

Figure   18   Adaxial Region of Adult "Shade" Leaf

(x 500)

C = chloroplast
E = epidermis
P = palisade mesophyll
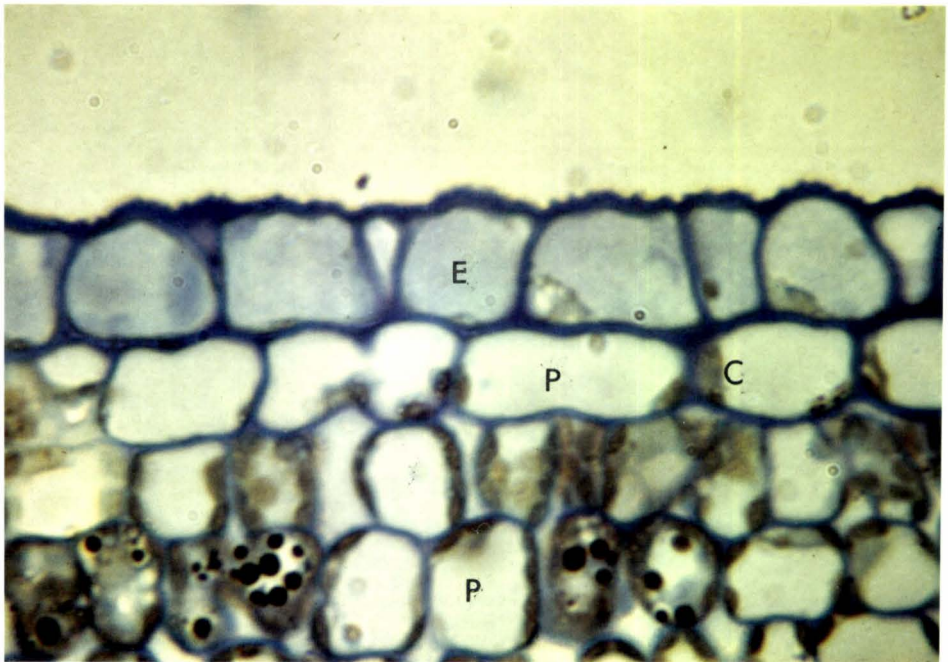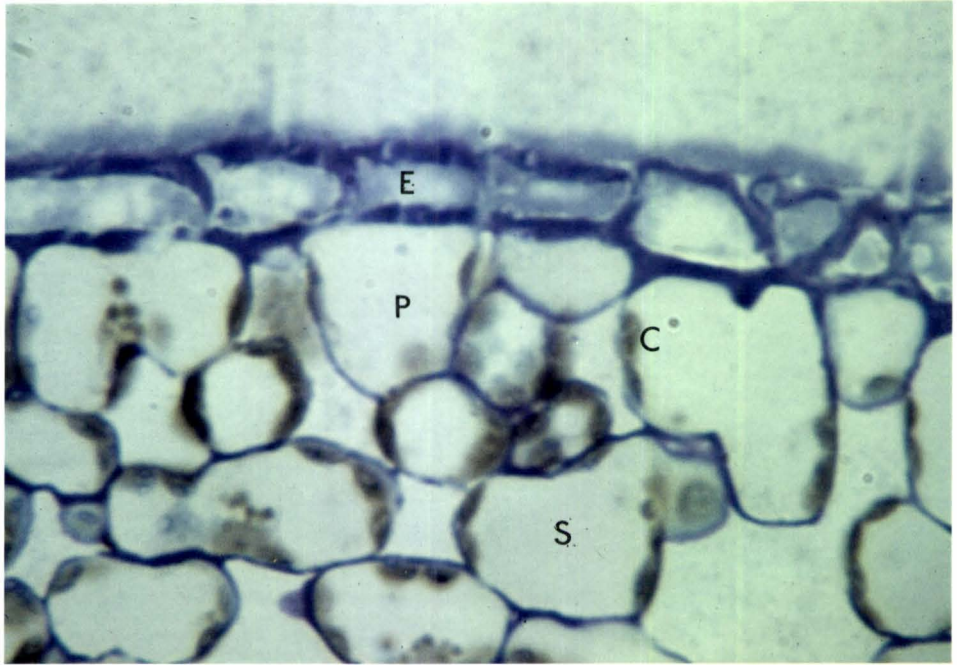
Figure 19 Adaxial Region of Juvenile "Sun" Leaf

(x 500)

C = chloroplast
E = epidermis
P = palisade mesophyll
S = spongy mesophyll

Figure 20 Adaxial Region of Adult "Sun" Leaf

(x 500)

C = chloroplast
E = epidermis
H = hypodermis
P = palisade mesophyll
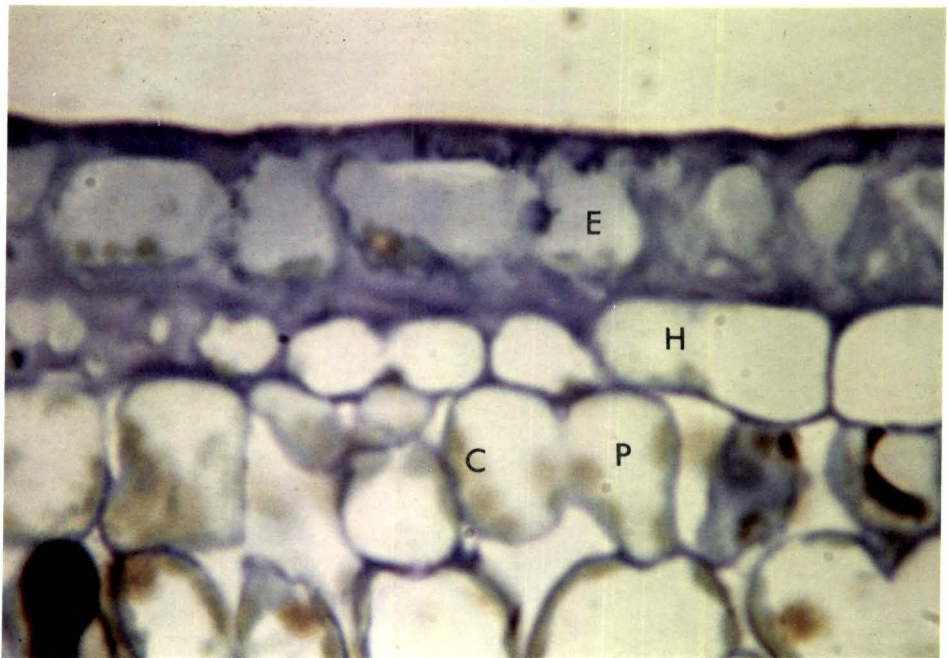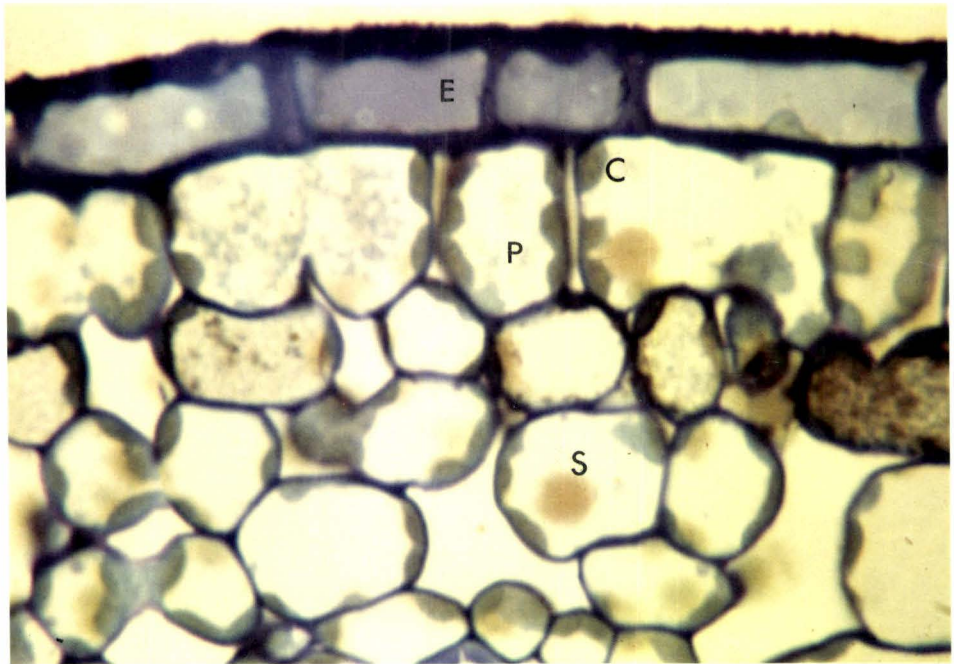
high light intensities. There appear to have been more
chloroplasts per unit area of each cross section in the juv-
enile "sun" leaf than in the adult "sun" leaf. This is further
suggested by the chlorophyll analysis data (Table V,p. 33)
which show a greater amount of chlorophyll per unit dry weight
in the juvenile "sun" leaf.

For both light intensity pretreatments the mesophyll
of the juvenile leaves was more diffuse and the cells were
larger. This could explain the greater fresh weight/dry weight
ratios of the juvenile leaves and the greater dry weight per
unit leaf area of the adult leaf.

3:4   Effect of Low and High Light Intensity Pretreatments
         on Total Chlorophyll Contents

3:4:1   Introduction

Leaf chlorophyll content has been shown to depend on
light intensity and a great many other conditions such as
temperature, age, nutrition and water availability (Pieters,
1974).

The relationship between chlorophyll content and
irradiation is unclear. The general observation is that the
chlorophyll content of leaves decreases with increasing light
intensities at least on a leaf weight basis, although the
opposite has also been reported. Björkman and Holmgren (1963)
found opposing reactions to increasing irradiance in plants
from different habitats. These observations support the
division by Montfort and Kress-Richter (1950) of plants into
categories they term photolabile and photostable. Plants
adapted to exposed habitats (photostable types) increase their
chlorophyll content with increasing light intensities (on a
leaf area basis) while those adapted to shaded habitats
(photolabile types) show the opposite reaction.

Björkman (1968a) found in genetically "shade adapted"
species a higher chlorophyll content expressed on a fresh
weight basis than in species genetically adapted to exposed
habitats. Therefore, if the juvenile and adult growth forms
of Pennantia are ontogenetically adapted to "shade" and "sun"

habitats respectively, differences in their total chlorophyll contents could be expected. Furthermore, the responses of chlorophyll contents to pretreatments at low and high light intensities could differ for the two growth forms.

3:4:2   Materials and Methods

The effect of light intensity on chlorophyll content in "sun" and "shade" adapted juvenile and adult Pennantia plants expressed on a fresh weight basis was determined for the plants pretreated as described in Section 2:3.

The technique used is that described by Arnon (1949), with the formula given by Holden (1965) to estimate the total chlorophyll content spectrophotometrically.

Five 200 mg leaf samples, taken at random with a 0.5 cm diameter cork borer were used for each determination. Pigments were extracted by grinding the leaf samples in 5 ml of precooled acetone using a chilled mortar and pestle. The filtered extract was then added to a separating funnel along with 2 ml of petroleum ether to which was then added an equal volume of distilled water. The aqueous layer containing the acetone and non-chlorophyllous pigments was discarded. The petroleum either was then washed twice with an equal volume of distilled water. Any remaining water was removed from the petroleum ether solution by adding approximately 5 gms anhydrous Na_2SO_4. The volume of petroleum ether was made up to 5 ml in a volumetric flask. Extracts were stored in the dark at $4^{\circ}C$.

Absorbance spectra were read on a Hitachi Recording Spectrophotometer and total chlorophyll contents calculated using the following formula:

$$\text{Total Chlorophyll (mg/1)} = 7.12 \log\left[\frac{I_o}{I}\right] 660 + 16.8 \log\left[\frac{I_o}{I}\right]$$

where $\log\left[\frac{I_o}{I}\right] 660$ is the absorbance at 660 nm and $\log\left[\frac{I_o}{I}\right] 642.5$ is the absorbance at 642.5 nm.

3:4:3   Results

The results are given in Table V, p. 33. Because of the differences in fresh and dry weights per unit area (Table III, p. 31) the extent of the changes of chlorophyll contents with

increasing irradiance and the extent of differences between juvenile and adult leaves depends on the basis on which chlorophyll contents are expressed. Nevertheless the trends remain the same.

Using the criteria of Montfort and Kress-Richter (1950), from the chlorophyll data expressed on a leaf area basis it is evident that the juvenile is more photostable than the adult. There was a significant increase ($P < 1\%$) in chlorophyll content per unit leaf area for the juvenile from low to high light intensities while the change for the adult was not statistically significant ($P > 5\%$). When expressed on a leaf fresh weight basis there are significant reductions in the chlorophyll content for both growth forms, with approximately a 10 percent reduction per unit fresh weight for the juvenile and a 50 percent reduction for the adult with increasing light intensity. These reductions result from the greater fresh weight per unit area of "sun" leaves. The chlorophyll data, regardless of the basis on which they are expressed, indicate that the total chlorophyll content was greater in the adult when compared with the juvenile at both light intensities.

3:5   Ribulose-1,5-diphosphate (RuDP) Carboxylase Activity in Juvenile and Adult Pennantia

3:5:1   Introduction

There is strong evidence that the activity of certain photosynthetic enzymes may be a major factor in determining the light saturated rate of photosynthesis in leaves. In particular ribulose-1,5-diphosphate carboxylase, the enzyme that catalyses the fixation of CO_2 in C-3 plants, is believed to be of importance (Björkman, 1968; Gauhl, 1970). Studies on the activity of RuDP carboxylase have revealed that cell-free leaf extracts from "sun" species have much higher levels of the enzyme than "shade" species (Bjorkman, 1968a).

Bjorkman (1968b) found that ecotypes of Solidago virgaurea adapted to sunny habitats had a higher activity of RuDP carboxylase than ecotypes from shaded habitats. It was therefore hoped to clarify for Pennantia whether or not the

juvenile was biochemically better adapted to photosynthesize under high or low light intensities than the adult by determining the activity of the RuDP carboxylase enzyme.

3:5:2   Materials and Methods

To determine the level of RuDP carboxylase activity in juvenile and adult _Pennantia_ leaves the technique described by Björkman (1968) was used.

Five samples of leaf tissue from both growth forms were taken from plants pretreated at the high light intensity mentioned in Section 2:3. Leaves used for the enzyme assays were prepared by grinding 200 mg freshweight of leaf tissue using a chilled mortar and pestle in 5 ml of a mixture of 0.1 M Tris-HCl pH 7.8, 0.01 M $MgCl_2$, 0.25 mM EDTA, and reduced glutathione (GSH). The supernatant, obtained after spinning the extract at 30,000 g for 20 minutes in an MSE High Speed 18 refrigerated centrifuge, was used for the enzyme assays without further purification. All preparative procedures were carried out at $0^{\circ}C$ to $2^{\circ}C$.

The enzyme assays were consistently made forty to sixty minutes after the leaf tissue was removed from the plant. The reaction was started by the addition of 0.2 ml of the enzyme extract to 0.4 ml of a freshly prepared mixture of 2.5 μm of $NaH^{14}CO_3$ (20 μm curie/μmol), 0.15 μmol ribulose-1,5-diphosphate, 1.25 μmol GSH, 0.1 μmol EDTA, 8 μmol Tris-HCl and 2.5 μmol $MgCl_2$.

After ten minutes incubation in a waterbath at $23^{\circ}C$ the reaction was stopped by the addition of 0.1 ml 6M acetic acid. A 0.2 ml aliquot was pipetted into a liquid scintillation counting vial and dried at $90^{\circ}C$ for two hours. Then 0.1 ml of water was added to each vial, followed by 10 ml of scintillation fluid. The radioactivity measurements were made with a Packard Tri-Carb Model 2111 Liquid Scintillation Spectrometer.

3:5:3   Results

The results for the RuDP carboxylase activity determinations are expressed in Table VI as counts per minute of

TABLE VI

RuDP carboxylase activities for juvenile and adult <u>Pennantia</u> leaves.

	Juvenile	Adult
c.p.m. mg fwt^{-1} ($\pm$ 1 S.E.)	1,592 $\pm$ 215	293 $\pm$ 35
ng CO_2 mg fwt^{-1} min^{-1} ($\pm$ 1 S.E.)	0.088 $\pm$ 0.12	0.016 $\pm$ 0.002
g CO_2/g Chlorophyll min^{-1}	0.094 $\pm$ 0.014	0.015 $\pm$ 0.002

^{14}C and nanograms CO_2 fixed per gram of chlorophyll. These data strongly indicate that the level of RuDP carboxylase activity was higher in the juvenile than the adult when pretreated at the same light intensity. The data obtained indicate a rate of activity in the juvenile that is over five times the rate of enzyme activity in the adult when expressed on a basis of CO_2 fixed per unit leaf fresh weight and per unit chlorophyll content (0.088 cf. 0.016 $ngCO_2$ mg $fwt^{-1}min^{-1}$ and 0.094 cf. 0.015 g CO_2/g chlorophyll).

3:6   Discussion

Both the juvenile and adult plants responded in a similar manner to the two pretreatment light intensities used in this investigation. Although the biochemical and photo-synthetic data did show some significant differences, the trends were the same for the two growth forms. It has been reported (Gauhl, 1970) that when plant ecotypes adapted to shaded habitats are subjected to prolonged exposure to high light intensities, the light-saturated rate of photosynthesis is reduced, while ecotypes that are adapted to sunny habitats increase their light-saturated rates of photosynthesis when transferred from a low to a high light intensity.

Both juvenile and adult _Pennantia_ increased their light saturation points from approximately 25 to 80 n Einsteins $cm^{-2}sec^{-1}$ when pretreatment light intensities were increased from 4 to 40 n Einsteins $cm^{-2}sec^{-1}$. Light saturated rates of photosynthesis on a leaf area basis increased by 50 percent when plants were transferred from the lower to the higher light intensity, thus indicating a genetic "preference" for photosynthesis at the higher light intensity. However, as the higher light intensity was only 20 percent of full sunlight the results must be treated with some caution. It is possible that there may be greater differences in response to even higher light intensities between juvenile and adult plants. Other workers have tended not to quote the photoperiods used during investigations into the effects of light intensity pretreatments and on the photosynthetic apparatus, thereby

making it impossible to compare total light energies over a twentyfour hour period. However, Schulze (personal communication) found that the leaves of _Fagus_ exposed to the sun in European beech forest received a daily average light intensity that was only 20 percent of full sunlight, which suggests that the high light intensity used in this investigation is not unrealistically low.

Plants genetically adapted to shade have lower compensation points and therefore can accumulate photosynthate at lower light levels than can "sun" plants (Levitt, 1972). On the other hand they show a light saturation at lower light levels than in the case of "sun" plants (Björkman, 1968a). It is evident that the lower compensation points result from lower rates of respiration (McCree and Troughton, 1966). In the "shade" adapted _Pennantia_ there were no significant differences in respiration rates and compensation points between juveniles and adults, but the respiration rate in the "sun" adapted adult was significantly greater than in the juvenile. This latter finding correlates with differences in the leaf fresh weight per unit area and suggests that the thinner juvenile leaf can maintain a rate of net photosynthesis comparable with the adult leaf by reducing the dark respiration rate.

The activity of RuDP carboxylase, which was much higher in the juvenile leaf, did not correlate with the photosynthetic rates of the two growth forms. Other workers have generally found that RuDP carboxylase activities are proportional to photosynthetic activities (Björkman, 1968b; Gauhl, 1970), but this is not always so (W. Laing, personal communication).

Ecotypes adapted to sunny habitats are reported to have higher rates of RuDP carboxylase activity than those genetically adapted to shade (Björkman, 1968b). Thus the results of the RuDP carboxylase assays indicate that the juvenile has the enzymatic capability to photosynthesize at a faster rate than the adult; possibly this only occurs at light intensities greater than that used for the pretreatment regime.

The possible significance of a high rate of RuDP carboxylase activity in the juvenile will be further discussed

in Section 6:3 with regard to physiological adaptations to
high wind velocities.

It may be questioned whether the reactions to high
light intensities as described by Björkman and Holmgren (1963)
for sun and shade ecotypes are a direct effect of light intens-
ity or a secondary effect resulting from sun-induced xeromorphy
brough about by changes in the water and ionic balances.
Daubenmire (1959) published a list of properties of sun, shade,
mesomorphic, and xeromorphic leaves which shows the similarity
between the properties of sun leaves and xeromorphic leaves to
be striking. Thus the development of a hypodermis in the adult
leaf need not necessarily indicate an adaptation to high light
intensities that is not present in the juvenile. It has been
suggested (Haberlandt, 1914) that the hypodermis might act to
screen out excess light and reduce the solarization of chloro-
phyll. However, from the chlorophyll content data (Table V,
p. 33) it is apparent that chlorophyll breakdown at the higher
light intensity is greater in the adult when compared to the
juvenile despite the presence of a hypodermis, which supports
the hypothesis that the hypodermis is a xeromorphic structure
rather than an adaptation to reduce solarization.

The greater reduction in chlorophyll content in the
adult per unit leaf dry weight at the higher light intensity
than in the juvenile is most likely caused by a decrease in
the number of chloroplasts rather than a decrease in the
amount of chlorophyll per chloroplast. The breakdown in the
granal stacking was similar for both the juvenile and adult
chloroplasts (Section 3: 2) but from the anatomical studies
(Section 3: 3) it is apparent that the number of chloroplasts
per unit cross sectional area is less in the adult leaf.
Furthermore the development of the hypodermis (which is devoid
of chloroplasts by definition) and also the greater cell wall
thickening in the epidermis of the adult leaf could account
for the greater reduction in chlorophyll content on a dry
weight basis.

From the standard description of genetically adapted
"sun" and "shade" species it is not possible to define the
juvenile as a "shade" or a "sun" plant compared with the

adult. Both the juvenile and adult are physiologically
adapted to photosynthesize better at the higher pretreatment
light intensity used in this investigation as the light satu-
rated rates of photosynthesis increased by approximately 50
percent for both growth forms when the pretreatment light
intensity was raised from 4 to 40 n Einsteins $cm^{-2}sec^{-1}$. At
the higher light intensity the juvenile was more photostable
(see Section 3:4:3, p. 52) than the adult and therefore it
could be predicted that with a further increase in pretreat-
ment light intensity a reduction in photosynthetic activity
would occur sooner for the adult than the juvenile. It is
pertinent to note here that the high intensity pretreatment
light intensity used in preliminary trials ws 80 n Einsteins
$cm^{-2}sec^{-1}$ and that this was reduced to 40 n Einsteins $cm^{-2}sec^{-1}$
for the main experiments because of the chlorosis that was
evident in the adult leaves (but less markedly so in the
juvenile) at the higher intensity.

Further investigations (with a longer pretreatment
period to ensure adequate growth) to determine the light
intensity at which photo-inhibition of photosynthesis occurs
for the juvenile and adult growth forms would be required to
ascertain whether the juvenile is physiologically adapted to
photosynthesize at higher light intensities than the adult.
However, the greater photostability and the higher carboxy-
lase activity tentatively indicate that the juvenile is
better adapted to open situations than the adult, but it is
also capable of growing in shaded conditions.

CHAPTER 4

EFFECT OF LEAF TEMPERATURE ON NET PHOTOSYNTHESIS
IN JUVENILE AND ADULT Pennantia

4:1   Introduction

It has been mentioned already (see Chapter 1) that
some workers (Cockayne, 1911; Rattenbury, 1962) have suggested
that the divaricating growth form evolved as an adaptation to
cold, dry Pleistocene climates.

Rattenbury (1962) stated that "cold climatic conditions
coupled with water-logged soils and strong winds...would
greatly reduce the absorptive efficiency of the roots and at
the same time cause considerable evaporation from the leaf
surface." The prevalence of xeromorphic forms at altitudes
where winter soil temperatures are close to freezing is used
by Rattenbury to support the hypothesis that low temperatures
can induce physiological drought.

Since the publication of Rattenbury's paper consider-
able evidence has been collated to show that low temperatures
(particularly those approaching $0^{\circ}C$) are an important agent
in inducing edaphic water stress (Levitt, 1972). Thus it
appears that Rattenbury's suggestion that low soil temperatures
and poor root absorption, rather than low soil moisture, could
bring about excessive transpiration losses relative to uptake
gains and therefore account for the evolution of the divari-
cating growth habit, warrants consideration.

There is however some disagreement as to the severity
of the Pleistocene climate. Dumbleton (1967) suggested that
the low incidence of winter deciduousness could result from
the Pleistocene cold climate being markedly less severe than
in comparable latitudes in the Northern Hemisphere. Bussell
(1968) found that both low temperature and shortening days
were necessary to induce winter inactivity in the New Zealand

flora, while for Northern Hemisphere species winter dormancy
is usually a response to short days alone (Wareing, 1956). A
winter dormancy mechanism that responds to short days alone
permits dormancy in anticipation of low temperatures while a
low temperature prerequisite for dormancy does not allow growth
to stop before the oncoming seasonal low temperatures.

The only published research on the effect of temperature
on indigenous species is that of Scott and co-workers. They
examined the effect on the relative growth rates of seven species
of native grasses (Scott, 1970a) and looked at the relationship
between temperature and net photosynthesis for Celmisia
spectabilis, Chionochloa rubra and Nothofagus solandri, (Scott,
1970b; Scott and Menalda, 1970). The relative growth and photo-
synthetic rates of the high altitude species had temperature
maxima that were lower than those of the lowland species
Chionochloa rigida and the high altitude ecotype of Festuca
novae-zelandiae had maximum relative growth rates at $9^{o}C$ and
$12^{o}C$ respectively, while lowland species had temperature optima
of $18^{o}C$ and greater. Chionochloa rubra and Celmisia spectabilis
had photosynthetic temperature maxima at $4^{o}C$. Maximum rates
of photosynthesis per unit dry weight were only 5 ng $g^{-1}hr^{-1}$
for the two alpine species compared to rates of 35 mg $g^{-1}hr^{-1}$
for Lolium perenne, Trifolium repens and Lycopersicon esculentum
during the same investigation.

While it is a general observation that seedlings are
less tolerant of low temperatures than the corresponding adult
plants, there is little published research to support this
statement. In the indigenous flora it is believed that the
southern range of species such as Agathis australis and
Metrosideros excelsa is limited by the frost tenderness of the
seedlings, (R.G. Thomas personal communication). There is
also a paucity of published research on the responses of photo-
synthetic activity to temperature at different stages of
ontogeny. Went (1945) reported that the growth rate of tomato
seedlings has a higher temperature optimum than the adult
plants and DePuit and Caldwell (1975) found that low temper-
ature depression of photosynthetic activity was greater in
the early phenological stages of Agropyron spicatum var. inerone.

If the divaricating growth form of Pennantia is physio-
logically adapted to low temperatures, a lower temperature
optimum for the juvenile would be predicted. To determine the
temperature optima for juvenile and adult Pennantia, photosyn-
thetic activities over a range of temperatures were obtained
for both growth forms.

4:2   Materials and Methods

Photosynthetic rates at a temperature range of $10-30^{\circ}C$
were measured in the open-system leaf chamber described in
Section 2:3. Pretreatment conditions were described in Section
2:2. Plants pretreated at the higher light intensity (40 n
Einsteins $cm^{-2}sec^{-1}$) were used in all cases. Replication was
achieved by measurements on four shoots from two plants for
both adult and juvenile growth forms. Leaf chamber light
intensity was maintained at 80 n Einsteins $cm^{-2}sec^{-1}$, which
was the light saturation point for both juvenile and adult.

All shoots were equilibrated for 15-20 minutes at
each temperature. Preliminary measurements showed an inhibition
of photosynthesis at a leaf temperature of $30^{\circ}C$ after 10-15
minutes. Consequently measurements of photosynthetic rates
were made at the lowest temperatures first in order to prevent
irreversible damage to the photosynthetic apparatus.

4:3   Results

The data are expressed in terms of absolute rates of
net photosynthesis and as a percentage of net photosynthesis
at $20^{\circ}C$ (Figs. 21, p. 63 and 22, p. 65).

The responses of net assimilation rates to temperature
for juvenile and adult Pennantia indicate that both growth
forms are sensitive to temperature variations at light satur-
ation.

Pronounced temperature optima of net photosynthesis
were evident for both juvenile and adult. Some studies on
other species have reported little effect of changes in temp-
erature on net assimilation (Warren Wilson, 1966; Connor and

Figure 21   Effect of Leaf Temperature on the Rate of
            Photosynthesis for Juvenile (●) and Adult
            (▲) <u>Pennantia</u>. (Each point is the mean
            of at least ten readings.)
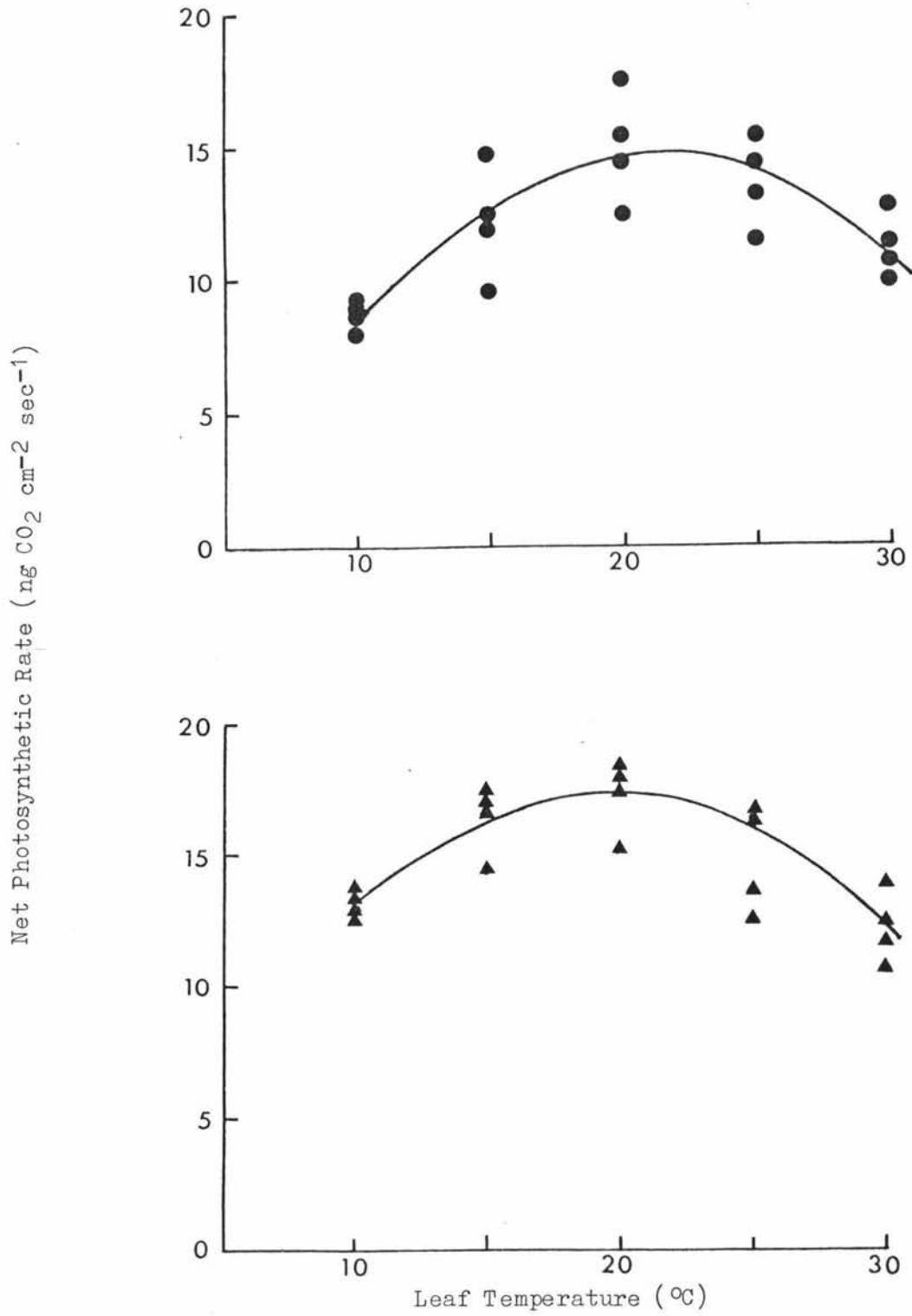
Figure 22   Relationship Between Photosynthesis and
             Temperature for Juvenile (●) and Adult (▲)
             Pennantia Expressed as a Percentage of Net
             Photosynthesis at 20°C.

Relative Photosynthetic Rate (%)
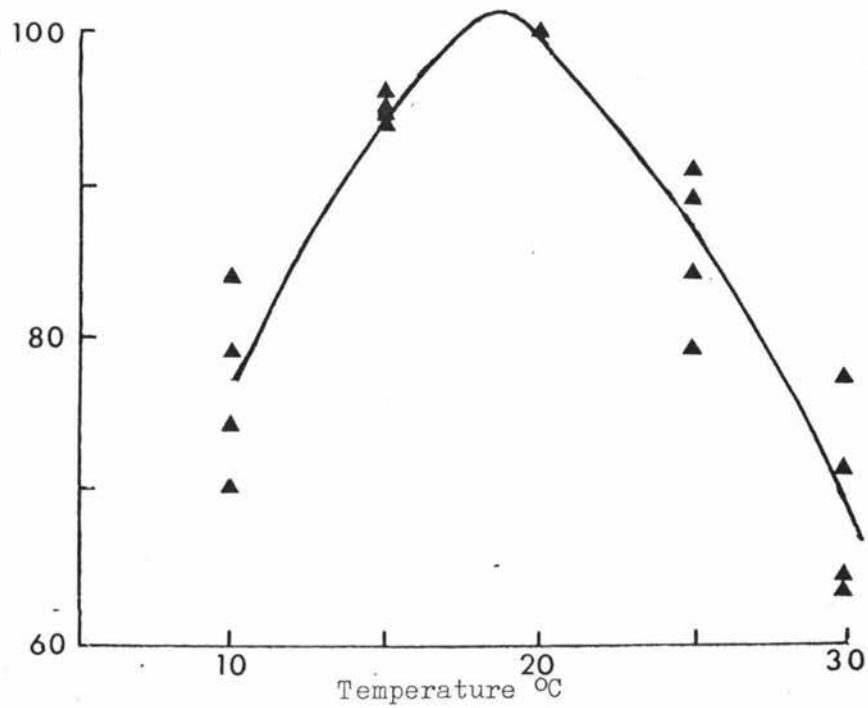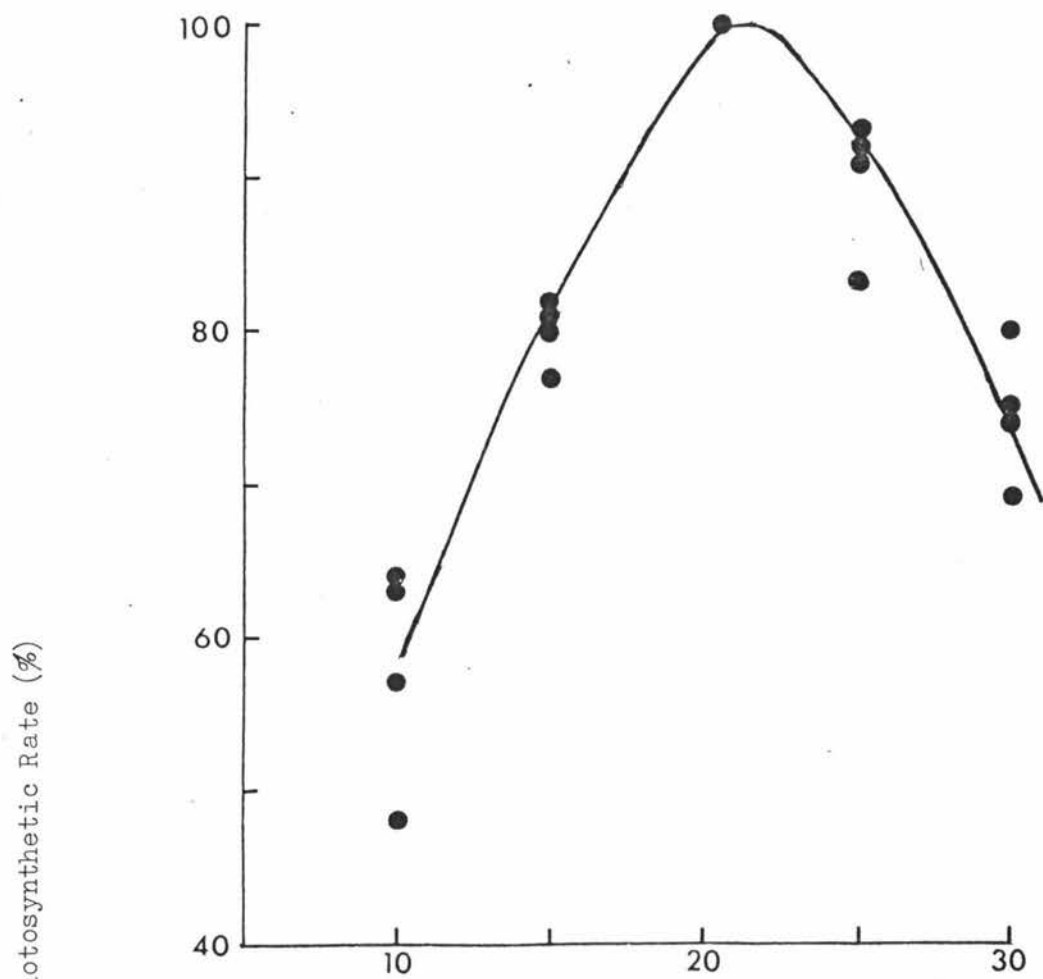
Temperature °C

Cartledge, 1970), while the occurrence of temperature optima
for net assimilation has certainly been recognized by others
(e.g. Scott, 1970b; Björkman et al., 1972; McPherson and
Slatyer, 1973; DePuit and Caldwell, 1975). With a pretreatment
temperature regime of $18^{\circ}C/13^{\circ}C$ the juvenile growth form had an
average temperature optimum of about $21^{\circ}C$, with individual
shoot optima varying from approximately $20^{\circ}C$ to approximately
$22^{\circ}C$. The adult growth form had a slightly lower optimum of
about $18^{\circ}C$ with individual shoot optima varying from approxi-
mately $17^{\circ}C$ to approximately $20^{\circ}C$. The two populations of
individual shoot optima was found to be significantly different
$(P< 5\%)$.

Low temperature depression of photosynthetic activity
was significantly greater in the juvenile $(P< 2\%)$. For the
adult the rate of photosynthesis at $10^{\circ}C$ was approximately
80 percent of the rate at $20^{\circ}C$, while for the juvenile the rate
was only approximately 60 percent. There was no significant
difference in the temperature response of photosynthetic
activity between the juvenile and adult at $25^{\circ}C$ and $30^{\circ}C$
$(P> 5\%)$.

4:4   Discussion

It is not clear to what extent the pretreatment
temperature has influenced the data obtained. It is known
that some plants can show an acclimation effect, with the
optimum temperature for net assimilation being dependent on
the temperature regime to which the plant has been exposed
(Robertson, 1970). For some species higher acclimation temp-
eratures have been found to induce higher net assimilation
rates, (El-Sharkaway and Hesketh, 1964; Mooney, 1973). Net
assimilation rates of other species may be adversely affected
by warmer growing temperatures (Treharne and Eagles, 1970;
DePuit and Caldwell, 1975). However, acclimation effects in
the temperature optimum for net assimilation do not occur in
all species (Helmuth, 1969, 1971; DePuit and Caldwell, 1975).

It is possible, therefore, that the data obtained were
influenced by the pretreatment temperature regime. Nevertheless,

as a comparative study to test for differential response for the juvenile and adult growth forms the investigation remains valid and the significant differences found are still meaningful.

The greater inhibition of photosynthetic activity at low temperatures and the higher temperature optimum of the juvenile growth form are similar to the observations of Went (1945) and DePuit and Caldwell (1975) and suggest that generally, as plants age, there is a decrease in physiological temperature optima. Certainly the juvenile is not adapted to continue growing at the low temperatures reported by Scott (1970a) and Scott and Menalda (1970) for indigenous alpine species.

The repression of physiological processes such as photosynthesis at low temperature could in itself assist adaptation to cold. It has been reported that the cessation of growth in the autumn may confer low temperature hardiness (Larcher, 1954; Sakai, 1955). In many cases, before the onset of winter in temperate regions in the Northern Hemisphere plants enter a non-growing rest period, and freezing tolerance has frequently related to the length of this period (Levitt, 1956). When plants are growing rapidly, frost hardening is generally difficult (Rivera and Cornelli, 1931; Dexter, 1932) whereas treatments that retard growth increase hardening (Levitt, 1972).

Where growth and development are controlled by photoperiod there is often a close relationship between photoperiod and freezing tolerance (Levitt, 1972). Hardening is improved in short days both in the case of woody plants (Bogdanov, 1935) and in herbaceous plants (Dexter, 1935; Ahring and Irving, 1969).

In Pennantia growth is not inhibited by short days alone; both juvenile and adult plants will grow under glasshouse conditions throughout the year. It is therefore likely that winter inactivity in Pennantia is a temperature response with perhaps a photoperiodic response also a prerequisite for winter inactivity, as suggested by Bussell (1968). It is thus possible that a greater freezing tolerance is conveyed in the

juvenile than in the adult by an earlier cessation of growth in the autumn.

As the family to which Pennantia belongs, the Icacinaceae, is considered to be tropical in origin, it is reasonable to suggest that rather than a photoperiodic dormancy mechanism having evolved in Pennantia, the duration of the ontogenetic stage that confers the greatest degree of frost tolerance has been extended.

Mark (1975) considered that the evergreen habit coupled with low energy demands is an effective adaptation to cold, windy environments. He found that alpine snow tussocks had slow photosynthetic and growth rates, and low rates of dark respiration. The dark respiration rates of the juvenile Pennantia plants pretreated at the higher light intensity were lower than for the corresponding adult plants (Section 3:2:1).

Photosynthetic rates per leaf dry weight at light saturation and a leaf temperature of $20^{\circ}C$ were 11.8 and 7.6 ng CO_2 $g^{-1}hr^{-1}$ for "sun" leaves of juvenile and adult plants respectively (see Table IV ,p. 32). On a leaf area basis the rates were higher in the adult leaf (approximately 18 ng CO_2 $cm^{-2}sec^{-1}$ c.f. approximately 16 ng CO_2 $cm^{-2}sec^{-1}$). These rates are higher than those reported by Scott (1970a) and Scott and Menalda (1970) for the indigenous alpine species studied (5 ng CO_2 $g^{-1}hr^{-1}$) but considerably lower than the rates reported by Scott and Menalda (1970) for non-alpine exotic species (35 ng CO_2 $g^{-1}hr^{-1}$) (see Section 4:1, p. 60). However it is difficult to make comparisons of the published absolute photosynthetic rates of other species because of differences in pretreatment regimes and environmental conditions under which photosynthetic rates are estimated.

The juvenile of Pennantia, as a result of the greater repression of photosynthesis at low temperatures and the low respiration rate, could therefore be better adapted both to seasonally cold conditions and to more exposed habitats in which heat loss brought about by radiation is relatively high.

CHAPTER 5

EFFECT OF DECREASING SHOOT WATER POTENTIAL
ON PHOTOSYNTHETIC AND TRANSPIRATION RATES
IN JUVENILE AND ADULT Pennantia

5:1   Introduction

Over the past twenty years or so numerous reviews have
been published on the effects of water stress on the physio-
logical and metabolic processes of plants and their growth in
general (Kramer, 1959; Stocker, 1960; Slatyer, 1967; Kramer,
1969; Levitt, 1972; Hsiao, 1973; Vieira da Silva, 1976; and
Hsiao et al., 1976).

Photosynthesis and transpiration rapidly decrease with
increasing water stress, as the first effect of water stress
in leaves is a partial or complete stomatal closure (Iljin,
1923). Hsiao (1973) observed that an increase in stomatal
resistance through closure of the stomata need not necessarily
cause a proportional decrease in transpiration because leaf
temperature could rise concurrently with a consequent increase
in the water vapour concentration inside the leaf. Some thres-
hold values of leaf water potential above which stomatal
opening is constant are -7 to -9 bars for tomato, -10 to -12
bars for soybean and -12 to -16 bars for grape (Hsaio, 1973).
A steady decrease in stomatal aperture of leaf water potentials
below the threshold values is observed. This markedly slows
down the movement of carbon dioxide into, and water out of, the
assimilating leaves. The result is a reduction of the photo-
synthetic rate by two to tenfold, according to the amount of
water lost and the sensitivity of the plant.

It has been shown by the early work of Stalfelt
(1929) on herbaceous plants and by later work on woody plants
(e.g. Pisek and Winkler, 1956) that stomatal closure
is responsible for a slowing but not necessarily a

complete halt in transpiration. The effect of stomatal closure
on photosynthetic activity may differ from the effect on trans-
piration, thus perhaps influencing the rate of dry matter pro-
duction under water stressed conditions and therefore being
important to competition and survival in certain xerophytic
plants (Larcher, 1965).

Maximov (1929) classified xerophytes into two distinct
types of drought avoiders:
1. the water savers, that avoid drought by water conservation
and
2. the water spenders, that avoid drought by absorbing water
sufficiently rapidly to keep up with the extremely rapid
water loss. Both kinds of adaptation maintain the plants in
the turgid high water potential (i.e., stress avoiding) state
under drought conditions.

True xerophytes are usually thought to be plants that
can withstand physiological drought of the soil or a high rate
of evaporation into the air without cessation of the living
processes (Huber, 1924; 1935).

Plants may develop structural characteristics that
appear to be adaptations to arid habitats. Such character-
istics are termed xeromorphic. Xeromorphism, however, is not
confined to xerophytes, and not all xerophytes exhibit xero-
morphic characters (Fahn, 1974).

Stocker (1960) considered the effects of soil dryness
and air dryness on plants to be similar with both resulting
in the development of xeromorphic characters:

      1. Smaller but thicker leaves,

      2. Shorter internodes

      3. Increased root/shoot ratios,

      4. Increased amount of vascular tissue and

      5. Increased number of stomata per unit area.

The view that xeromorphic features are invariably
adaptations to arid habitats may be questioned in the light
of experimental findings, which have shown, for example, that
transpiration is not necessarily reduced in leaves with abun-
dent hairs or sunken stomata (Cutter, 1971). The fact that
some xeromorphic characters develop in response to dry

environments as shown by Stocker (1960) suggests that there may be a causal, rather than adaptive, relationship between environment and structure, although one does not necessarily preclude the other.

It has been suggested that divaricating juveniles are more xerophytic than their adult counterparts (Cockayne, 1911; Rattenbury, 1962; Wardle, 1963), while other workers (Fitzgerald, 1923; Johnston, 1948; Rumball, 1961; Keen, 1970) have indicated that divaricating juveniles have xeromorphic characteristics.

Fitzgerald (1923) quoted by Rumball (1961) stated that the juvenile leaf of _Pennantia_ is more xeromorphic than the adult. The criteria for xeromorphy used by Fitzgerald are not known.

In this investigation the effects of increasing water stress on the physiology of juvenile and adult _Pennantia_ were determined. Shoot water potential was used as an estimation of water stress (see Section 5:1:1 below). Changes in both net photosynthetic and transpiration rates were monitored.

5:1:1   Techniques for Measuring Water Stress

Recent reviews of techniques for measuring water stress include those by Kramer and Brix (1965), Slatyer (1967), Slatyer and Shmueli (1967), Barrs (1968), Boyer (1969), Kramer (1969) and Sullivan (1972).

Plant water deficits are generally described by either one or both of the parameters, water content and water potential.

The water content of a leaf can be expressed on dry weight, fresh weight, full turgor weight and area bases but full turgor weight is the only satisfactory base because dry weight fluctuates diurnally, fresh weight minimises large changes, and area decreases when water stress is high (Barrs, 1968).

Water potential can be measured by liquid exchange, vapour equilibrium, thermocouple psychrometry, freezing point depression, and pressure bomb techniques (Sullivan, 1972). Thermocouple psychrometry is currently considered to be the most accurate method of measuring plant water potential (Cary and Fisher, 1971; Sullivan, 1972) but the technique is limited

to laboratory operation, while the pressure bomb developed by
Scholander et al.(1964) is considered the most practical as
it can be used in the field as well as the laboratory. Some
of the errors and operating problems associated with thermo-
couple psychrometers are discussed by Barrs (1968).

Results from pressure chamber determinations do not
always closely agree with thermocouple psychrometer values
(Cary and Fisher, 1971; Sullivan, 1972). Cary and Fisher
(1971) reported discrepancies between pressure bomb and
freezing point measurements and suggested that pressure
chambers tend to measure the water potential in the xylem
elements while the psychrometer measures the potential of
water in the cell walls and intercellular spaces. Talbot
et al. (1974) found that agreement between the two techniques
is good when whole leaves are used in the psychrometer chamber
and suggest that disagreements could be caused by the effects
of wounding on the values obtained from the psychrometer.

Other techniques which can be used for the measure-
ment of water stress are osmotic potential, stomatal closure
and leaf diffusive resistance (Sullivan, 1972).

Barrs (1968) concluded that both water content and
water potential measurements were useful criteria while
Kramer (1969) considered that water potential is the better
parameter because it is apparently more closely related to
the physiological and biochemical processes which control
growth. In this investigation water potential measurements
made with a Scholander pressure bomb were used to estimate
plant water status, because of the efficiency and accuracy
($\pm$ 1 atm) of the method and the comparative nature of the
study.

5:2   Materials and Methods

Two juvenile and two adult plants pretreated under
the conditions given in Section 2:3 at a light intensity of
40 n Einsteins $cm^{-2}sec^{-1}$ PHAR were used for the investi-
gation.

Water was withheld over a period of two weeks during which time shoot water potential was determined with the Scholander pressure bomb. Shoots about 4 cm long were excised and placed in the bomb cylinder within five seconds of excision. Atmospheric pressure was increased gradually in the bomb cylinder until moisture appeared at the cut surface of the protruding section of shoot. At this point it was considered that the atmospheric pressure in the cylinder was equivalent to the water potential of the excised shoot and the pressure recorded.

When significant decreases in shoot water potential were registered, net photosynthetic and transpiration rates were measured with the leaf chamber described in Section 2:4. Leaf temperatures were maintained at $20^{\circ}C$.

Photosynthetic and transpiration rates were determined for four juvenile and four adult shoots at a range of shoot water potentials. These were determined before and after the leaf chamber measurements, and the mean values were plotted against the photosynthetic and transpiration data.

5:3   Results

Transpiration rates per unit leaf area (Fig. 23,p. 75) in the juvenile at all shoot water potentials measured were greater than those in the adult until both transpiration and photosynthesis (Fig. 24,p. 77) virtually ceased at around -20 bars for both growth forms. The transpiration rate declined more rapidly with decreasing shoot water potential in the adult. There was no significant reduction in transpiration rate in the juvenile until a shoot water potential of approximately -10 bars, while at this water potential in the adult transpiration was reduced by almost 50 percent.

Photosynthetic activities were higher in the adult at high potentials above -10 bars. As potential decreased further the rate of net photosynthesis dropped to a greater extent in the adult. Thus from -10 to -18 bars photosynthetic activities were higher in the juvenile. At the lowest potentials there was no significant difference between the photo-

Figure 23   Effect of Shoot Water Potential on Transpiration
             Rates for Juvenile (●) and Adult (▲) <u>Pennantia.</u>

             (Each point is the mean of at least ten readings.
             Vertical lines represent twice the standard
             error.)

Transpiration Rate (ng H_2O cm^{-2} sec^{-1})

Shoot Water Potential (Bars)

Figure 24. Effect of Shoot Water Potential on the Rate of Photosynthesis for Juvenile (●) and Adult (▲) <u>Pennantia</u>.

(Each point is the mean of at least ten readings. Vertical lines represent twice the standard error.)

Shoot Water Potential (Bars)
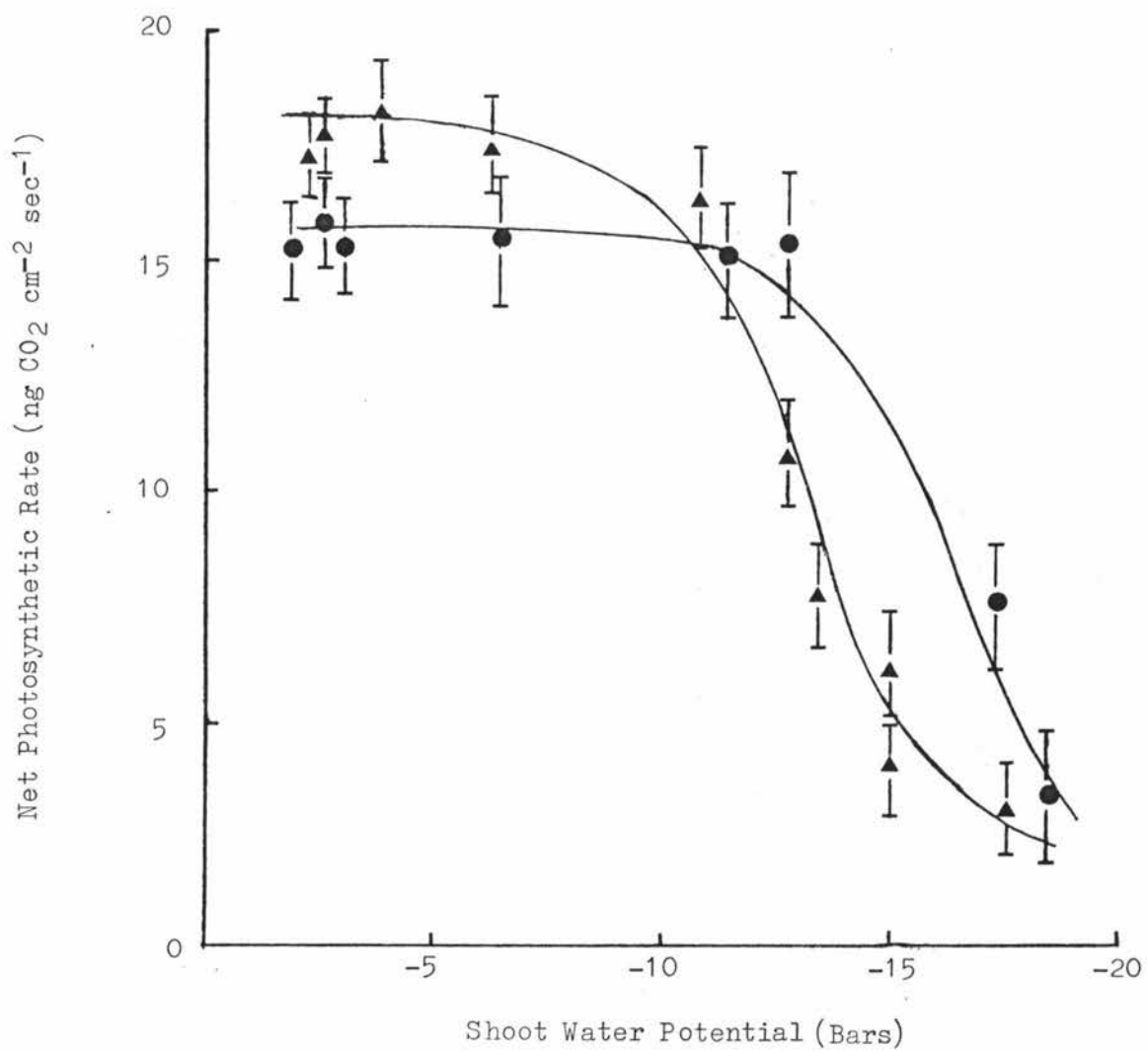
synthetic activities of the juvenile and adult plants.

Water use efficiency (mg CO_2 fixed/ g H_2O transpired) (Fig. 25 ,p. 80) was significantly greater in the adult down to -15 bars. Below -15 bars, because of the proportionally larger standard errors associated with the low transpiration rates the data are difficult to interpret. Nevertheless, a convergence of water use efficiencies is apparent at low shoot water potentials.

5:4   Discussion

The differences in juvenile and adult transpiration and photosynthetic rates at low shoot water potentials suggest that the juvenile is better adapted to grow under drought conditions.

The higher transpiration rates per unit leaf area at high shoot water potentials in the juvenile are in keeping with the higher transpiration rates recorded by Johnston (1948) for the small-leafed, semi-divaricating juvenile of Carpodetus serratus. Keen (1970) compared the transpiration rates in small-leafed and large-leafed species from the Coprosma, Melicope and Plagianthus genera and also observed that the transpiration rates per unit leaf area were greater in the small-leafed plants.

Keen (1970) found in the small-leafed species of the three genera that she investigated that stomatal closure did not occur until a greater leaf water stress (estimated by determining relative water content - see Section 5:1:1) than in the corresponding large-leafed species. Using transpiration rate as a measurement of leaf resistance it is indicated that the stomata in the juvenile do not close until a lower shoot water potential, which is in agreement with Keen's findings for other indigenous leptophylls. That the stomata stay open at lower shoot water potentials in the juvenile is further borne out by the data on photosynthesis (Fig. 24,p. 77) which show the repression of photosynthetic activity with increasing water stress to be greater in the adult; stomatal behaviour generally parallels photosynthetic behaviour (Brix, 1962;

Figure 25   Effect of Shoot Water Potential on Water Use
             Efficiency for Juvenile (●) and Adult (▲)
             Pennantia.

             (Each point is the mean of at least ten
             determinations. Vertical lines represent
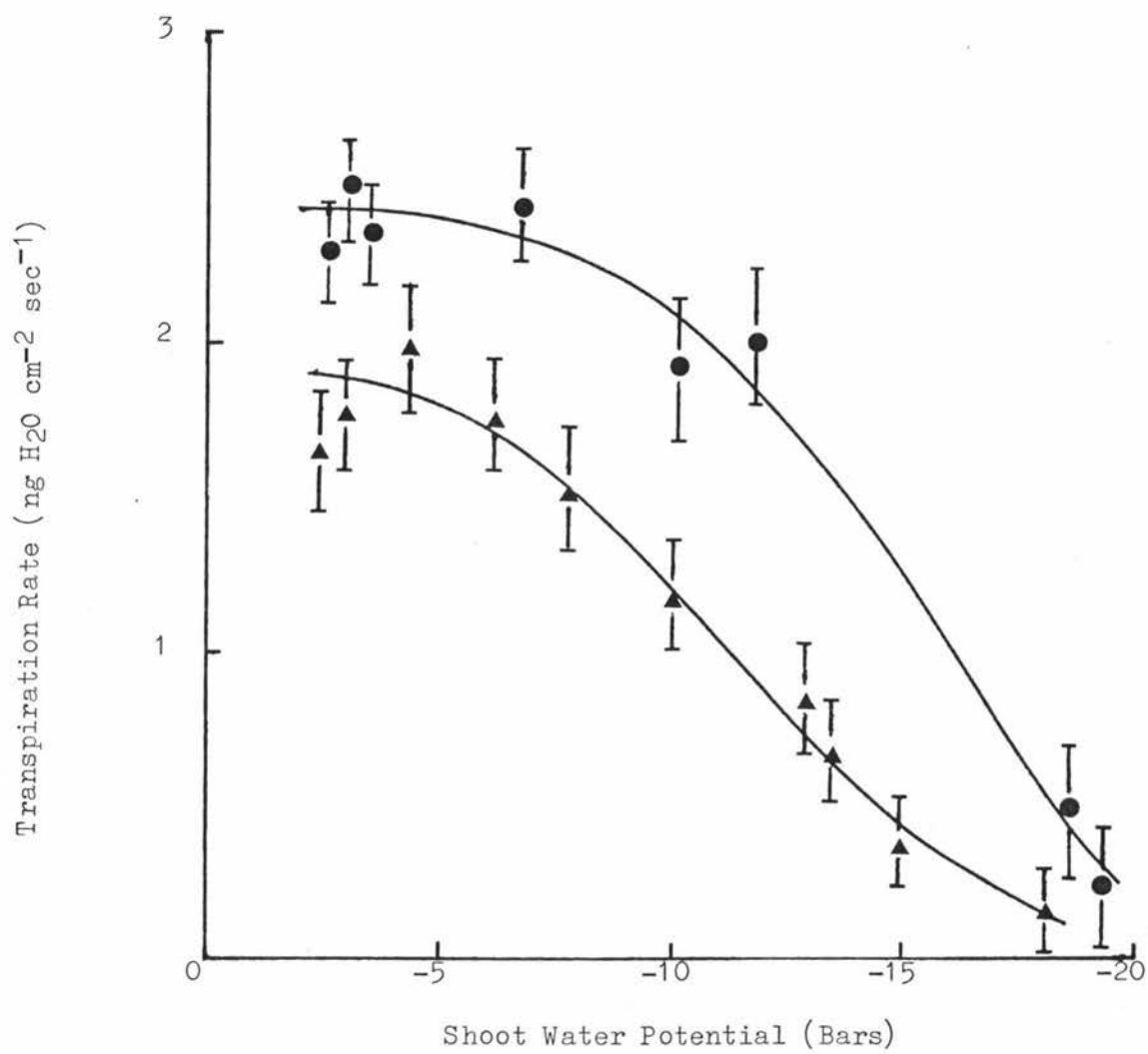   twice the standard error.)
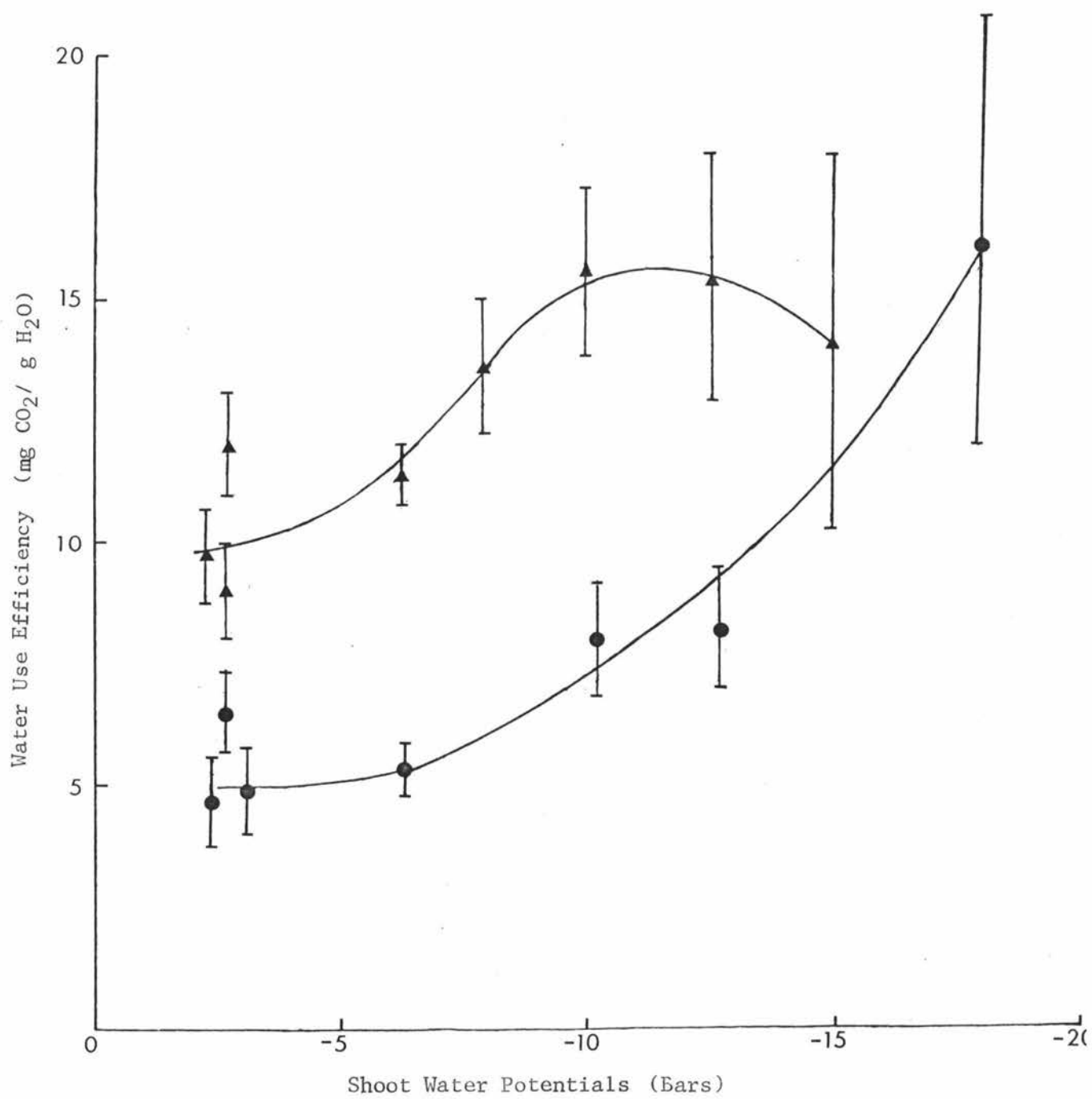
Boyer, 1970).

The juvenile leaf, while being smaller and having a higher stomatal frequency (Section 6:3, p. 91) than the adult, which are both xeromorphic characters (see Section 5:1, p. 70), has anatomical characteristics that are regarded as being meso-morphic. The juvenile leaf, unlike the adult, does not develop a hypodermis, while the palisade tissue is better developed cell wall-thickening is greater and the volume of intercellular spaces is less in the adult (see Figs. 19 & 20, p. 49). Thus indi-cating that anatomically the adult leaf is more xeromorphic than the juvenile leaf (Turrell, 1936; Shields, 1950) contrary to the findings of Fitzgerald (1923).

However, xeromorphic features do not necessarily indicate a xerophyte (Cutter, 1971). Using the criteria of Huber (1924, 1935) the juvenile of _Pennantia_ is physiologically more xerophytic than the adult, as it can remain physiologically more active at lower shoot water potentials.

The graph of water use efficiency as a function of shoot water potential (Fig. 25, p. 80) indicates that on a leaf area basis the juvenile can be classified as a "water spending" xerophyte, at least relative to the adult. However, although water loss is greater in the juvenile per unit leaf area, on a whole plant basis water loss would be less in the juvenile because of the smaller leaf size. Therefore by reducing leaf area the juvenile can reduce overall water loss while maintaining a high transpiration rate per unit leaf area and thereby continue to function under conditions of edaphic water stress.

CHAPTER 6

EFFECT OF WIND ON TRANSPIRATION RATES AND LEAF DIFFUSION
RESISTANCES IN JUVENILE AND ADULT Pennantia

6:1   Introduction

Leonard Cockayne (1911) was the first to suggest that
wind was an important ecological factor in the present day
New Zealand environment. His evidence came from observations
of the effect of wind on plant growth habits. He noted that
on windy sites there is often a convergent epharmonic reaction
resulting in compact, prostrate bushes of a similar habit to
that resulting from divarication. However, when postulating
an explanation for the prevalence of divarication in the
indigenous flora Cockayne found it necessary to describe dry
steppe Pleistocene climates to which divarication was an
epharmonic adaptation. Rattenbury (1962) supported Cockayne's
hypothesis of divarication as an adaptation to harsh Pleis-
tocene climates and suggested that the growth form would act
as an effective wind break.

Since Cockayne's elaborate hypothesis several workers
have suggested that divarication may be an adaptation to the
present environment (Bulmer, 1958; Wardle, 1963; Denny, 1964).
Bulmer (1958 suggested that the strong winds may result in
conditions which approach the continental "Steppe" climate,
while Wardle (1963) suggested that divarication is an adap-
tation to still existing fairly dry forest environments.

Denny (1964), looking at habit heteroblastism in
Sophora, suggested that low rainfall combined with wind, as
found in Canterbury and Central Otago, may have resulted in
the evolution of the divaricating growth form. She considered
that this evolution might have commenced in the Pleistocene
as suggested by Cockayne (1911) and Rattenbury (1962) but
that the divaricating plants are adapted to a climate that

still exists.

Mark (1975), (see also section 4:4) suggested that in the New Zealand flora "the evergreen habit coupled with low energy demands is an effective adaptation to cold, windy environments."

It has recently been suggested that much of the palaeontological evidence for a dry-cold climate during the Pleistocene can be reinterpreted in terms of increased windiness (M. McClone, personal communication), while Dumbleton (1967) hypothesised that the low incidence of winter deciduousness indicates that the Pleistocene cold climate was markedly less severe than in comparable latitudes in the Northern Hemisphere.

Wind therefore could have been an important ecological agent during the Pleistocene. As for the present day climate, meteorological data show that New Zealand does not experience exceptionally high mean wind speeds but, because of the terrain, the gustiness of the wind is marked. Thus, some parts of the country, especially about and east of the main ranges and near Cook Strait have a large number of days with high wind gusts. For example, on average, wind gusts exceeding 18 m/sec occur in Wellington on about 150 days of the year and in very exposed places wind gusts may exceed 65 m/sec (A. Tomlinson, personal communication). It is therefore possible that the magnitude and frequency of wind gusts, perhaps combined with periods of severe drought which occur in New Zealand about every five years (Richard and Fitzgerald, 1969) (see Section 5:1), could confer a degree of adaptive significance to divarication with regard to the contemporary environment.

Wind effects plants by causing mechanical damage, scattering disseminules, and altering transpiration (Martin and Clements, 1935; Daubenmire, 1959). The effects of wind on transpiration and internal water balance are complex and depend to a large degree on wind velocity, air temperature and humidity (Kramer and Kozlowski, 1960; Gates, 1968). Wind increases transpiration by removing layers of humid air which tend to accumulate adjacent to leaf surfaces. An important effect of this is a steepening of the vapour pressure gradient

between leaf and air. Eventually internal water deficits in plants are thereby increased even if roots are growing in wet soil (Whitehead, 1963a).

Whitehead (1963b) listed the anatomical and morphological changes that occur as a result of wind exposure. These features are similar to those that develop as a response to soil water deficits as listed by Stocker (1960) (see Section 5:1, p. 70). This indicates that anatomical and morphological responses to wind are primarily moisture stress effects.

To determine whether the semi-divaricating juvenile of Pennantia is better adapted to a windy environment than the orthotropic adult the responses of transpiration and stomatal resistances to wind for both growth forms was monitored.

Davies et al. (1974) found that variations among species of woody plants in transpiration capacity and in the effects of wind on stomatal opening and closing correlated with distinct anatomical differences of the stomatal structures. Stomatal anatomy was therefore examined and stomatal frequency determined for juvenile and adult leaves.

6:2   Materials and Methods

6:2:1   Wind Tunnel Investigations

Artificial winds have been used for some time to study the effects of wind on transpiration (Martin and Clements, 1935; Jensen, 1954; Shah, 1962; Macklon and Weatherley, 1965; Kalma and Kuiper, 1966; Tinkler and Weatherley, 1968; Davies et al., 1974).

Artificial wind generated in the wind tunnel described in section 3:4 was used to study the relationship between wind velocity, whole plant transpiration rate and leaf resistance for the two growth forms of Pennantia.

Reviews on the measurement of whole plant transpiration include those of Slatyer (1967), Slatyer and Shmueli (1967) and Kramer (1969).

The four methods of measuring transpiration which are

commonly used are given by Kramer (1969) as:

1. phytometer (potted plant) method
2. water vapour loss (gasometric method)
3. cut shoot (rapid weighing method), and
4. diffusion porometry.

The phytometer method is the most widely used (Franco and Magalhares, 1965) despite the limitations of confining the root system to a small space and that sealing the container can affect water absorption by the roots through depletion of oxygen in the soil.

The cut shoot method has received considerable criticism and is not recommended as a good technique (Slatyer, 1967). Increases in stomatal aperture and a concurrent surge in transpiration following the removal of a twig or leaf from a plant have been reported (Slatyer, 1967).

Water vapour loss techniques are generally regarded as very useful in studies carried out in the laboratory (Franco and Magalhares, 1965; Slatyer, 1967; Kramer, 1969) (see Section 2:3, p. 20).

Diffusion porometers such as the gaseous diffusion porometer (Slatyer and Jarvis, 1965), the differental transpiration porometer (Meidner and Spanner, 1959), and the sensor element diffusion porometer proposed by Walligan (1964) will measure transpiration. The sensor element diffusion porometer has been modified and developed into a portable instrument suitable for field use (Kanemasu et al., 1969).

The phytometer method was chosen to measure transpiration rates because of the non-destructive nature of the method and also because of the need to measure water loss while the plant is growing in the wind tunnel.

Prior to initial weight determinations, the plants were watered to saturation and allowed to drain for three to four hours. Two plastic bags were then placed around each plant pot and tied to the lower plant stem. Weight of the pot and plant was measured with a top weighing Mettler balance. The pots were removed from the plastic bags after each wind tunnel run, but to further minimize possible root damage as a consequence of oxygen depletion any individual plant was used only

on alternate days.

Because of the variability of the transpiration data obtained from day to day, possibly due to variation in temperature and humidity, transpiration is expressed as relative transpiration rate, i.e., as a percentage of the transpired water loss per plant in still air rather than as water loss per leaf area. Furthermore, in order to determine absolute transpiration rates, an estimation of leaf area is required, which would have required destruction of the plants. The wind tunnel investigations were conducted over a period of three months, during which time the plants were actively growing; thus an estimation of leaf area at the end of the wind tunnel investigations would have been of no use in determining the absolute transpiration rates for the majority of the wind tunnel runs. Transpiration rates for the juvenile and adult leaves were determined during the leaf chamber investigations and these can be used to estimate the effect of wind velocity or water loss on a leaf area basis.

For every wind tunnel run the plants were placed in the wind tunnel for one hour in still air. Weight loss of the pot plus plant was considered to represent transpirational water loss. The plants were then returned to the wind tunnel for a further three hours at the required wind velocity, after which water loss was again determined. Only one wind tunnel run was undertaken daily, between the hours of 11 a.m. and 4 p.m.. For juvenile and four adult plants were used to obtain replication and at least six determinations were obtained for each wind velocity at which transpiration was measured.

The effect of wind velocity on leaf diffusion resistances was determined with a Lambda Instruments Kanemasu leaf diffusive resistance meter (Kanemasu, 1969) using a horizontal sensor. Leaf diffusion resistance gives an estimation of stomatal aperture.

As the area of the horizontal sensor was greater than the area of the juvenile leaf in all cases, the entire leaf was inserted into the sensor aperture, and the leaf resistance value obtained was multiplied by the ratio of the area of the sensor aperture to the area of the leaf. Readings were taken

at fifteen minutes and forty-five minutes after plants were inserted into the wind tunnel. Five readings on the five juvenile and five adult leaves were made for each wind velocity.

To determine whether differences in relative trans-piration rates were caused by boundary layer or stomatal effects, the wind tunnel runs were repeated at night when measurements made with a Kanemasu leaf diffusive resistance meter showed that the stomata were closed. Any significant differences in night transpiration rates of juveniles and adults at various wind velocities would thus most probably be the result of the greater boundary layers of the larger adult leaves rather than of differences in degree of stomatal opening.

6:2:2   Examination of the Stomatal Anatomy and Determination of Stomatal Frequency

The grosser features of leaf surfaces and structures are readily observed by optical microscopy, but many attri-butes of physiological significance such as stomatal structure, are difficult to interpret because of the limited resolution of the light microscope, the small depth of focus of the instrument, and the transparency of the materials involved. By employing a Scanning electron microscope it is possible to obtain three-dimensional images of resolutions up to 100-150 A. Scanning electron microscopy as a method of studying leaf structure was first employed by Y. and J. Heslop Harrison (1969) who examined leaf surfaces and experimented with a number of drying and fixing techniques.

In this investigation sections of tissue were removed from the leaves of juvenile and adult Pennantia under cold modified Karnovsky's fixative as described in Section 2:5. The tissue was kept in fixative for five days at $3^{o}C$ and then given two buffer (0.1 M Phosphate buffer, pH 7.2) washes of thirty minutes duration. After which they were given three washes in distilled water, also of thirty minutes duration.

The specimens were thin freeze-dired and sputter-coated with gold in a Film-Vac Inc. minicoater to a 100-150

Å thickness. Specimens were viewed with a Cwikscan 100 field
emission scanning electron microscope.

Stomatal frequencies were calculated for juvenile and
adult leaves by removing strips of the abaxial epidermis
(stomata are found only on the lower epidermis in Pennantia)
and counting the number of stomata per field of view under an
Olympus compound microscope. Stomata half or more in the
field were included in the count. Stomatal frequencies for
ten juvenile and ten adult leaves were estimated on a leaf
area basis.

6:3   Results

The graph of relative transpiration rates during the
day as a function of wind velocity for juvenile and adult
Pennantia is given in Fig. 26,p. 90. In the adult the increase
of relative transpiration rate is significantly greater for wind
velocities up to 10 m sec^{-1}, with the maximum relative trans-
piration rate of 195 percent being reached at 6 m sec^{-1}. The
juvenile reached a maximum of 150 percent at the highest wind
velocity tested (12 m sec^{-1}). At wind velocities greater than
6 m sec^{-1} there is a convergence of juvenile and adult relative
transpiration rates, with no significant difference ($P > 5\%$)
between the two growth forms at 12 m sec^{-1}.

Assuming that the transpiration rates in the wind
tunnel and leaf chambers were comparable, from data obtained
during the leaf chamber investigation (Fig. 23,p. 75) it is
possible to estimate transpirational water loss per unit leaf
area for the wind tunnel investigations. Using a mean non-
water stressed transpiration rate of 2.4 ng H_2O $cm^{-2}sec^{-1}$ for
the juvenile leaf and 1.8 ng H_2O $cm^{-2}sec^{-1}$ for the adult leaf,
absolute transpiration rates can be calculated from the data
expressed in Figure 26, page 90. The juvenile and adult
transpiration rates at a wind velocity of 6 m sec^{-1} are 3.3
and 3.5 ng H_2O cm^{-2} sec^{-1} respectively, while at a wind
velocity of 12 m sec^{-1} juvenile and adult rates of 3.6 and
2.7 ng H_2O $cm^{-2}sec^{-1}$ respectively are calculated.

Figure 26   Effect of Wind Velocity on Transpiration Rate
             for Juvenile (●) and Adult (▲) <u>Pennantia</u>.

             (Transpiration rate is expressed as a percentage
             of the transpiration rate in still air.)

             (Each point is the mean of at least six
             determinations. Vertical lines represent
             twice the standard error.)
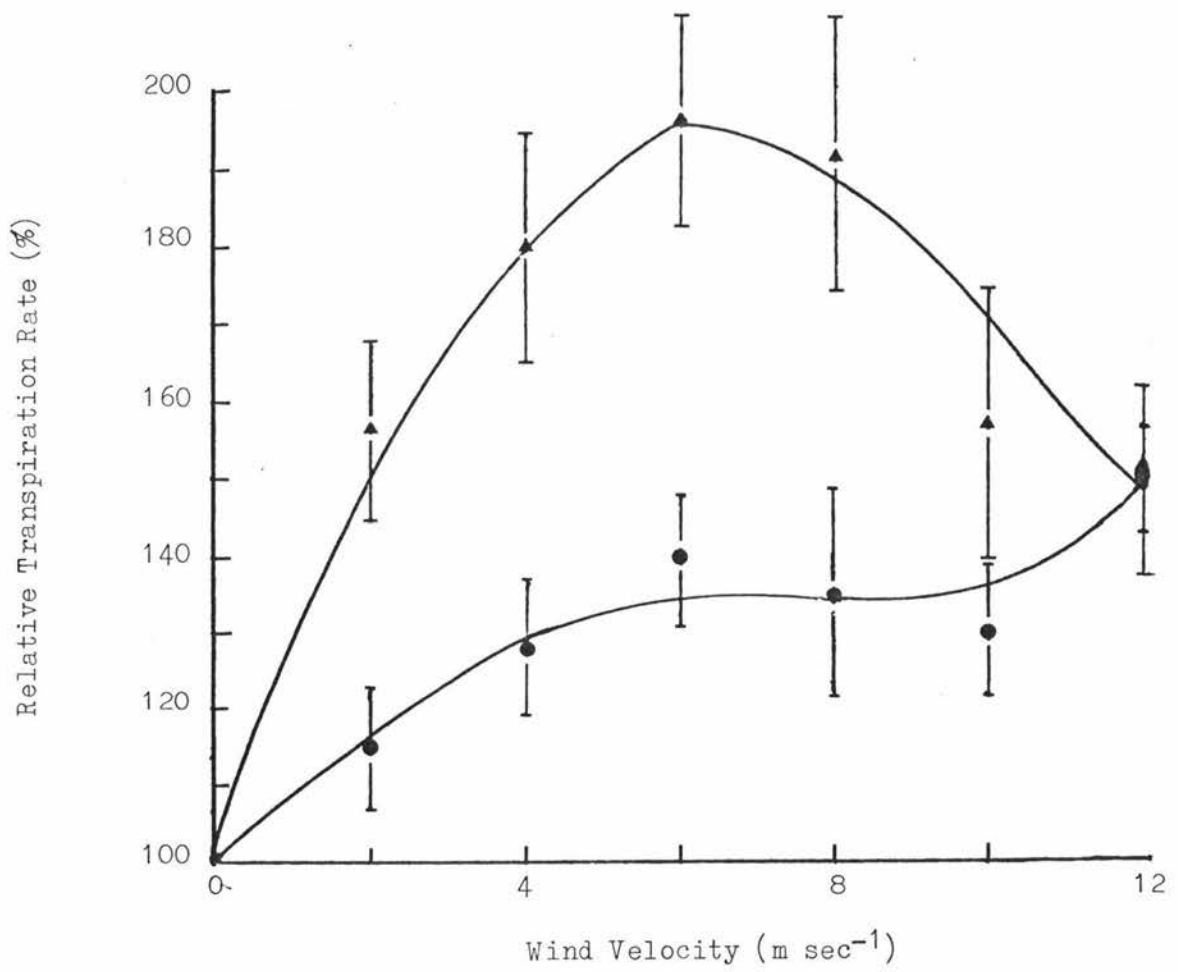
The graph of relative transpiration rates during the night as a function of wind velocity is given in Figure 27, p. 93. There were no significant differences in the relative transpiration rates of the juvenile and adult plants in the dark at the wind velocities tested.

Figures 28, p. 95 and 29, p. 97 give the leaf diffusion resistances after 15 and 45 minutes exposure to wind velocities up to 8 m sec^{-1} and 6 m sec^{-1} respectively. It was only possible to measure leaf resistances of up to 30 sec cm^{-1} with any accuracy because of the length of time required to measure larger resistances.

At wind velocities below 6 m sec^{-1} for the 15 minute exposure period and below 4 m sec^{-1} for the 45 minute exposure period the leaf diffusion resistances were significantly lower $(P > 5\%)$ in the juvenile than the adult. At low wind velocities a lower leaf diffusion resistance for the juvenile would be predicted because of the higher transpiration rates found for the juvenile leaf under low soil water conditions (Fig. 23, p. 75).

At a wind velocity of 6 m sec^{-1} after 15 minutes exposure the leaf diffusion resistance in significantly greater $(P > 5\%)$ for the juvenile leaves while after 45 minutes at 4 m sec^{-1} there is no significant difference $(P > 5\%)$ between the leaf diffusion resistances for the two growth forms.

Scanning electron microscope studies of stomatal structure revealed no differences in stomatal anatomy (Figs. 30 and 31, p. 99). In both juvenile and adult leaves the stomata are of the same size and architecture. The stomata feature a prominant cuticular ridge and an associated eisodial pore.

Stomatal frequency was found to be significantly greater for the juvenile leaf $(P < 2\%)$, stomatal frequencies with standard errors being 503   49 cm^{-2} and 392   50 cm^{-2} for the juvenile and adult leaves respectively.

6:4   Discussion

The wind tunnel investigations have revealed marked differences between the juvenile and adult growth forms of

Figure 27   Effect of Wind Velocity on Night Transpiration
              Rate for Juvenile (●) and Adult (▲) <u>Pennantia</u>.

              (Transpiration rate is expressed as a percentage
              of the transpiration rate in still air.)

              (Each point represents the mean of at least
              six determinations. Vertical lines represent
              twice the standard error.)
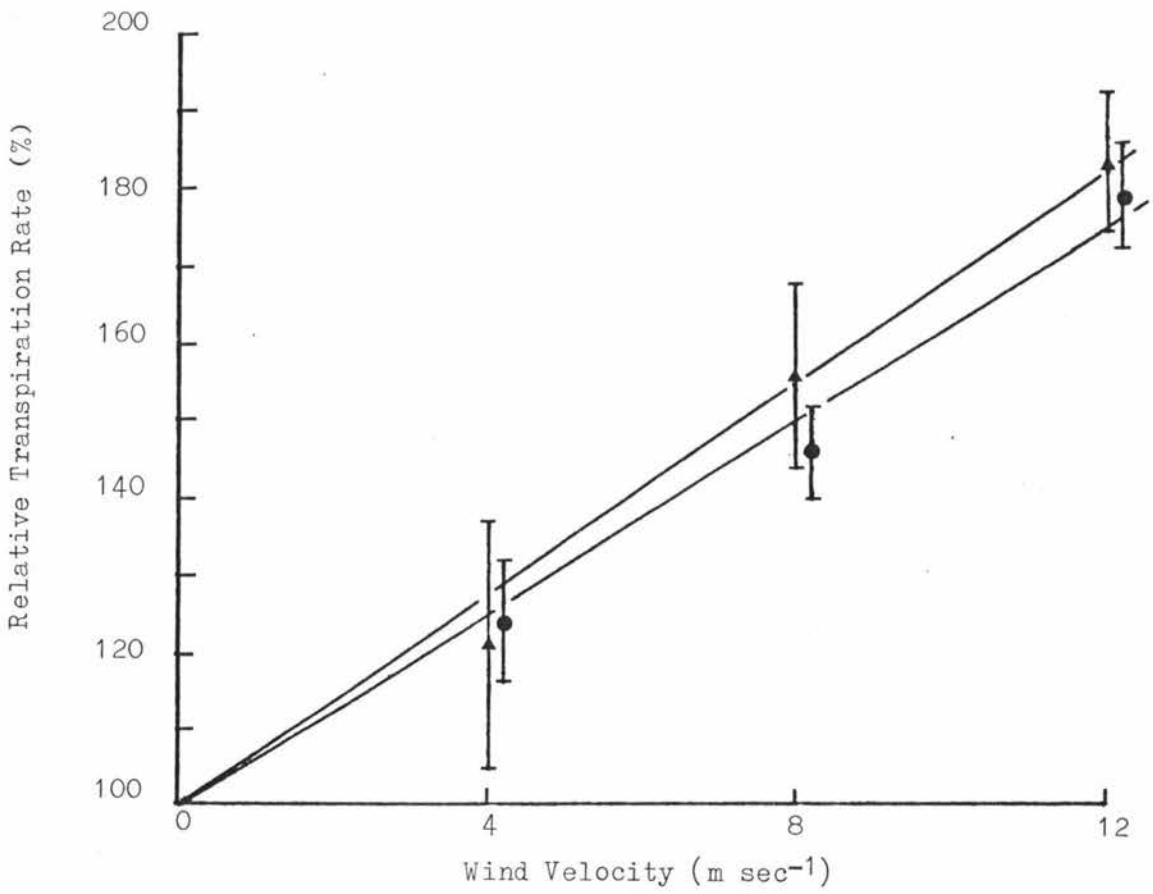
Figure 28   Effect of Wind Velocity on Leaf Diffusion
Resistance in Juvenile (●) and Adult (▲)
Pennantia after 15 minutes Exposure.

(Each point is the mean of five readings.
Verticle lines represent twice the
standard error.)
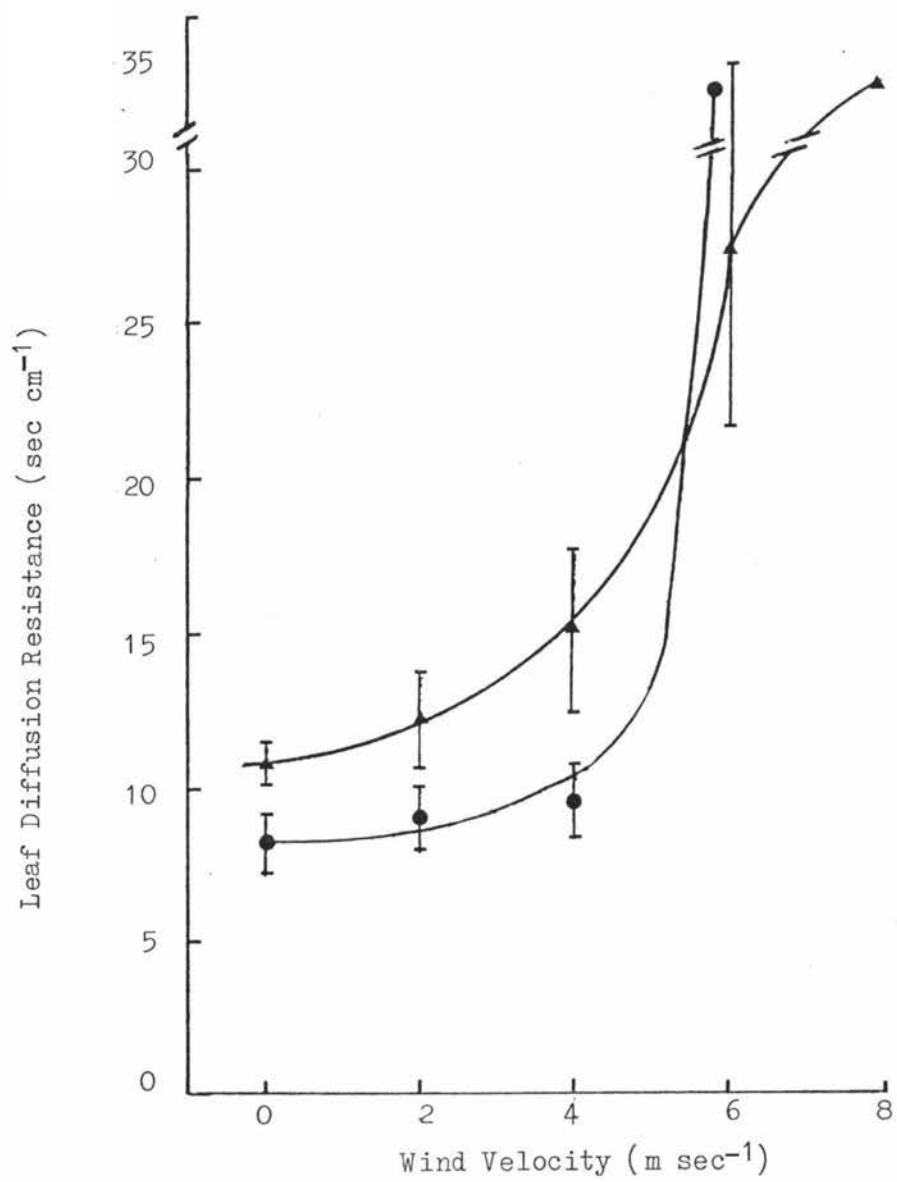
Figure 29   Effect of Wind Velocity on Leaf Diffusion
             Resistance in Juvenile (●) and Adult (▲)
             <u>Pennantia</u> after 45 minutes Exposure.

             (Each point is the mean of five readings.
              Verticle lines represent twice the standard
              error.)
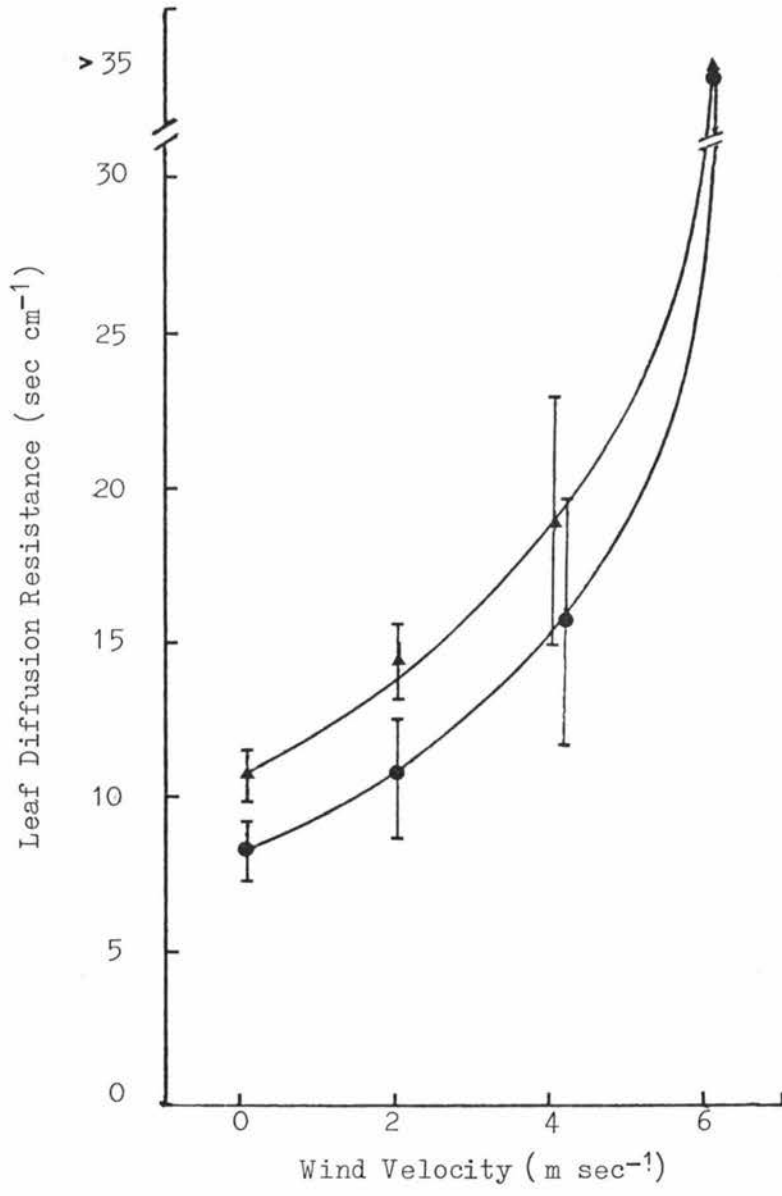
Figure 30   Scanning Electron Micrograph Of A
             Surface View Of A Closed Stoma On The
             Abaxial Epidermis Of A <u>Pennantia</u> Leaf

             (x 7000)
             EP = eisodial pore

Figure 31   Scanning Electron Micrograph Of A
             Transverse Section Of A Closed Stoma
             Of <u>Pennantia</u>

             (x 7000)
             CR = cuticular ridge
             GC = guard cell
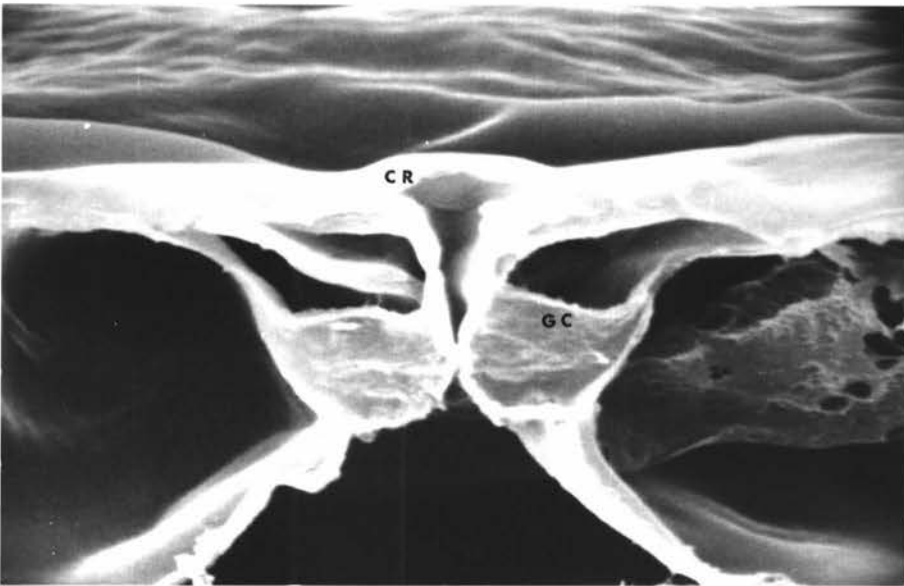
Pennantia in their physiological responses to wind. During the
day both the relative transpiration rates and leaf diffusion
resistances differed between the juvenile and adult plants at
various wind velocities.

The graph of relative transpiration rates during the
night as a function of wind velocity (Fig. 27,p. 93) indicates
no significant difference in the responses of the juvenile and
adult. The very high leaf diffusion resistances indicate that
at night the stomata are closed in the leaves of both growth
forms and therefore the only other component of leaf resistance
that could respond to changes in wind velocity would be the
boundary layer* resistances. Any differences in mesophyll and
cuticular resistances to water vapour movement from inside
the leaf to the ambient air would remain constant and there-
fore have no effect on the relative transpiration rates.

As there were no differences in response to wind at
night, the differences in relative transpiration rates during
the day must have been caused by some other factor or factors.
If the differences in boundary layer resistance, which is
proportional to leaf size, had a significant effect on the
transpiration rates it would be predicted that for the two
wind velocities tested the relative transpiration rates during
the night would be greater for the adult plants. This is a
consequence of the marked reduction of the thickness of the
boundary layer with increasing wind velocity. Thus the
thickness of the juvenile and adult boundary layer will con-
verge, the reduction of the boundary layer being greater for
the adult because its greater initial thickness. As a conse-
quence the reduction in leaf resistance of the adult leaf
would be greater than for the juvenile leaf as wind velocity
increases.

Further evidence that the differences in boundary
layer resistances are not significant fomces from the calcu-
lation of boundary layer resistances. Using the formula
derived by Nobel (1970):

- - - - - - - - - - - -

* See Glossary for definition

$$R_ja \cong 0.4 \frac{\sqrt{\frac{l}{v}}}{D_j}$$

R_ja = boundary layer resistance

l = length of leaf (cm) in the direction of wind flow

v = wind velocity ($cm^{-2}sec^{-1}$)

D_j = water vapour diffusivity into air

it can be calculated that at 20°C for a juvenile leaf 1 cm long and an adult leaf 6 cm long the boundary layer resistances in a 2 m sec^{-1} wind will be 114 sec cm^{-1} and 278 sec cm^{-1} respectively. When these values are compared to the total leaf resistances (Fig. 28, p. 95), which after 15 minutes at wind velocity of 2 m/sec are approximately 8 sec cm^{-1} for the juvenile leaf and 12 sec cm^{-2} for the adult leaf, they are found to be insignificantly small (under 2 percent of the total leaf resistances).

At a wind velocity of 12 m sec^{-1} it is evident from the leaf resistance meter readings that the stomata close rapidly. However, although the relative transpiration rates at this wind velocity are not significantly different, the calculated absolute transpiration rate in the juvenile leaf is higher than the adult (3.6 of 2.7 ng H_2O $cm^{-2}sec^{-1}$). Assuming the stomatal resistances to be similar and the boundary layer resistances to be insignificant it appears that the cuticular resistance is lower in the juvenile leaf. This could also help explain the higher rate of transpiration found for the juvenile during the water stress investigation (see Section 5:3, Fig. 23, p. 75). Further evidence for a lower juvenile cuticular resistance comes from the micrographs of sections of juvenile and adult leaves (Figs. 19 & 20, p. 49) where a thicker cuticle on the adult leaf is apparent.

From the leaf diffusion resistance data (Figs. 28 and 29, p. 95 and 97), it seems that the guard cells of juvenile _Pennantia_ were apparently more sensitive to increases in wind speed than the adult and responded by closing sooner. The explanation for the greater sensitivity of the juvenile guard

cells is unclear. Scanning electron micrographs of guard cells show no significant anatomical differences between adult and juvenile (Figs. 30 & 31,p. 99). However, the more diffuse nature of the mesophyll in the juvenile leaf (Fig. 19,p. 49) and a thinner cuticle might allow for a greater freedom of guard cell movement.

It is also possible that the initial leaf diffusion resistance and consequential higher transpiration rate (probably caused by the higher stomatal frequency and thinner cuticle) in the juvenile could result in a more rapid reduction in leaf turgor and ultimately guard cell turgor.

Whether the differences in stomatal response and cuticular resistance alone are adequate to explain the magnitude of the differences in transpiration rate is unclear. It must be remembered that the effect of the divaricating growth habit has not been investigated. It is possible even in the small plants used for the wind tunnel investigation that a degree of sheltering may have occurred in the juvenile because of the branching habit that may have reduced air movement in the vicinity of the stomata, particularly at the lower wind velocities. If a sheltering effect alone had been significant the graphs of the relative transpiration rates for the juvenile and adult plants would be expected to be displaced, with the shape of the graphs being similar, but with a lower relative transpiration rate being recorded for the juvenile at a given wind velocity. However, from the graphs given in Figure 26 p. 90, it is evident that the graph of the juvenile transpiration data is not merely a displacement of the graph of the adult transpiration data. Therefore, although a sheltering effect may have contributed to the lower relative transpiration rates for the juvenile leaves, a greater stomatal sensitivity to air movement is also considered important.

CHAPTER 7

CONCLUSION

The responses of juvenile and adult plants of
Pennantia to various environmental parameters used in this
investigation have resulted in several interesting observations.

First, it is apparent that the juvenile and adult
react in a similar manner to low and moderately high light
intensities. There is no physiological evidence that the
divaricating juvenile is better adapted to grow at low irra-
diances despite the presence of juveniles in shaded lowland
forest communities. The parallel increases in the rates of
light saturated photosynthesis when grown at the higher light
intensity and the photostability of the chlorophyll content
suggest that the juvenile and adult are both genetically adapted
to grow at least at an average daily light intensity of twenty
percent of full sunlight. The slightly lesser degree of solar-
ization and the greater rate of RuDP carboxylase activity
suggest that the juvenile is adapted to grow at higher light
intensities than the adult.

The higher temperature optimum lends further support
to the hypothesis that the juvenile is adapted to sunny habitats
which would result in higher leaf temperature than shaded hab-
itats. As discussed in Section 4:4, p. 67, a higher temperature
optimum could also result in a greater frost tolerance for the
juvenile which could also be an adaptation to more exposed
habitats where low temperatures are more extreme.

The responses of the two growth forms to edaphic
water stress show a greater tolerance by the juvenile, which
was able to maintain a lower stomatal resistance than the adult
at comparable low shoot water potentials. As a consequence the
juvenile has a greater photosynthetic activity than the adult
at low shoot water potentials.

The higher rates of transpiration result in a lower water use efficiency for the juvenile, which can be classed as a xerophytic "water spender " (Maximov, 1929, see Chapter 5 Section 1). However, because of the considerably smaller area of the juvenile leaf (Fig. 3 ,p. 9) on a whole plant basis the juvenile undoubtedly loses less water than the adult. As a consequence of a reduction in leaf size the juvenile can photosynthesize at low shoot water potentials without excessive loss of water via transpiration.

Therefore the adult plant, because of a greater leaf area, is better adapted on a whole plant basis to non-drought conditions. The greater leaf area resulting in a greater dry matter production. The juvenile growth form, because of a higher rate of photosynthesis at low shoot water potentials, is better adapted to grow under water stressed conditions.

The stomatal responses to wind at first appear to contradict the stomatal responses to low shoot water potentials. The stomata of the juvenile leaf were observed to remain open at lower shoot water potentials than those of the adult leaf, while the juvenile stomata were more sensitive than the adult stomata to the desiccating effect of air movement at the leaf surface. Thus the juvenile may be classed as a xerophylic "water saver" (see Section 5:1) in response to atmospheric water stress when compared with the adult. The juvenile growth form has apparently evolved to employ diametrically opposed strategies in adapting to soil and atmospheric water stress.

The anamalous behavior of the stomata may be explained by the observation of other workers that the stomata may act independently of the water status of the leaf. Schulze et al. (1972) reported that the stomata of the mesomorphic leaves of Prunus amenicana, the xeromorphic stems of Hammada scoparia, and the succulent leaves of Zygophyllum dumosum, responded to changes in air humidity. They found that the stomata opened at high humidities despite a decrease in leaf water content, thus excluding a reaction via the water potential in the leaf tissue and demonstrating that the stomatal aperture responds directly to the evaporative conditions of the atmosphere.

A greater independence of the water status of the leaf
in the juvenile could explain the differences in response
between the juvenile and adult stomata. If the adult stomata
were more dependent on the bulk leaf water status they would
close earlier with a decrease in leaf water potential and
respond less rapidly to atmospheric water stress than the
juvenile stomata. These differences in stomatal response could
be a consequence of the more diffuse nature of the mesophyll
of the juvenile leaf (see Fig. 13,p. 43), which would result
in less intercellular contact and therefore less intercellular
movement of liquid water. The thinner cutical and thinner
epidermal cell walls of the juvenile leaf could also allow for
greater flexibility of movement of the stomatal guard cells.

It is possible that the greater RuDP carboxylase
activity of the juvenile leaf is an adaptation to photosynthesis
under conditions of atmospheric water stress. Photosynthesis
at high stomatal resistances, caused by movement of relatively
dry air at the leaf surface, would be limited by the low carbon
dioxide concentrations in the vicinity of the leaf mesophyll.
Therefore by increasing the activity of the carboxylating
enzyme a steeper carbon dioxide concentration gradient between
the outside of the leaf and the site of carbon dioxide fixation
would be realized. As a consequence a greater overall amount
of carbon dioxide would be fixed and a higher rate of photo-
synthesis maintained.

The plastic response of divaricating juveniles to
growth in shaded and open habitats further supports the hypo-
thesis that divarication is an adaptation to exposed conditions.
Denny (1964) found that the external environment is capable
of altering the degree of divarication in juvenile plants of
Sophora microphylla, with shaded conditions giving the least
divarication while dryness and high light intensity (perhaps
acting via the induction of physiological water stress in the
leaves) both increased divarication. Philipson (1963) reported
that in sheltered and shaded conditions the twigs of the juvenile
phase of Paratrophis microphylla are loosely woven together,
whereas in exposed coastal situations the branches are locked

together with extreme tightness. Field observations indicate that the juveniles of Pennantia respond in a similar manner. Thus although the divaricating growth habit is genetically fixed for several years in species with divaricating juveniles, the degree of divarication may still be a plastic response to the environment in which the juvenile is growing. The observations that exposure tends to increase the extent of divarication supports the physiological evidence presented in this thesis that the divaricating juvenile is adapted to open habitats.

Denny (1964) investigated populations of Sophora microphylla from Auckland and Canterbury and found that the juveniles of the Canterbury plants were genetically more divaricating than the juveniles from the Auckland population. There was little difference in the spread of roots between the two populations which suggests that divarication is not an adaptation to edaptic water stress.

Talbot (personal communication) conducted a survey of leaf size and geographical distribution in the New Zealand flora which indicated that leaf size generally decreased with increasing latitude. It has also been reported that mean wind speeds over New Zealand increase with increasing latitude, the steepest gradient occuring over the summer months with a mean summer wind speed at Auckland of 4 m sec^{-1} and at Canterbury of 7 m sec^{-1} (Tomlinson, 1975). Thus there exists a positive correlation between degree of divarication in populations of Sophora microphylla, microphylly, and mean summer wind velocities.

The plasticity of the degree of divarication as noted by Philipson (1963) and Denny (1964) is indicative of a more general physiological plasticity which was made apparent by the responses of the juvenile of Pennantia to a range of light intensities and shoot water potentials. This adaptive flexibility would be advantageous to a species such as Pennantia, that is found in succession communities and on forest margins and river flats where seed may fall on shaded or open ground. Divarication would only be of advantage to the plants growing on open ground. Presumably as the plant grows the advantage of divaricating decreases. This would occur during a forest

succession were there would develop a progressive increase in
shelter. For a plant growing to maturity on an open site the
need for divarication would also lessen with an increase in
size, as this would allow for an increase in self-protection
from climatic extremes. The development of a larger root
system would further ameliorate the effects of atmosphere and
edaphic water stress.

Thus the investigations presented in this thesis
tend to indicate that the divaricating juvenile of Pennantia
is adapted to a sunny, windy, dry, environment with warm summer
cool winter temperatures. While the attenuation of the juvenile
phase may have occurred as a response to a Pleistocene climate,
the evolution of ecotypes whose distribution correlates with
factors in the present day environment suggests that the divari-
cating growth form is still of adaptive significance.

The question remains as to the adaptive advantage of
expressing a divaricating growth habit the first twenty to sixty
years of development, rather than evolving the ability to
divaricate as a plastic response to an exposed environment.
Cockayne (1911) noted that species such as Leptospermum scoparum
adapt a compact growth habit similar to that of a divaricating
plant when growing on exposed windy sites. It is suggested that
the advantage of incorporating divarication as part of a develop-
mental sequence allows an anticipation of the environmental
extremes that induce divarication as a plastic response. By
anticipating a harsh environment it is possible to minimize
physiological and mechanical damage to the plant. Drought occurs
in New Zealand only once every five years on an average (Rickard
and Fitzgerald, 1969), and while the mean wind speeds are not
exceptionally high, the New Zealand climate is characterized by
periods of high wind gusts (Tomlinson, personal communication.
Thus it is possible that a combination of edaphic drought (which
would occur during the summer when the gradient in wind speeds
from North to South is the greatest) and high wind gusts could
supply the degree of selection pressure that has led to the
convergent evolution of the divaricating growth habit and juven-
ility in the New Zealand flora.

It will be apparent to the reader that certain aspects of the results obtained in this investigation require further study for a fuller understanding of the adaptive significance of the divaricating phase of development.

Measurement of the responses of juvenile and adult _Pennantia_ to very high irradiances would ascertain whether the juvenile is adapted to grow at higher light intensities than the adult. Field research into the onset of winter inactivity and concurrent tests for frost hardiness would determine whether the juvenile is better adapted to withstand periods of cold. A controlled environment wind tunnel would be of value in obtaining information on the effect of wind velocity on absolute transpiration rates, thus giving a more realistic picture of the physiological responses of the two growth forms to wind. Long term wind tunnel growth experiments would also be very useful. Furthermore, similar investigations using other species with divaricating juveniles, or genera with divaricating species would test the validity of some of the generalizations made during the writing of this thesis.

Finally, it is hoped that the information contained in this thesis will give an indication of the areas in which further research into the physiology of the New Zealand flora is needed to solve one of the enigmas of the indigenous biota.

GLOSSARY OF TERMS

Boundary layer:

> Defined as a transfer zone of gas or liquid in
> contact with an object in which the temperature,
> vapour pressure, or velocity of the gas or liquid
> are influenced by the object.

Divaricating:

> Pertaining to branching at an angle of 90° or
> greater.

Heteroblastic development:

> A term used to describe the change of leaf form
> in a single plant during its ontogeny.

Hypodermis:

> A specific layer of cells beneath the epidermis,
> which differ structurally from the tissue below
> them and are usually devoid of chloroplasts.

Leptophyllous:

> Pertaining to a leaf-size class in which the blade
> area is not more than 25 square millimeters.

Mesophyll:

> Parenchymatous tissue between the upper and lower
> epidermal layers in foliage leaves.

Mesophytic:

> Pertaining to the requirement of moderate amounts
> of water for optimum growth.

Nanophyllous:

> Pertaining to a leaf-size class in which the blade
> area is between 25 and 225 square millimeters.

Orthotropic:

> Pertaining to the tendency of a plant to grow with
> the longer axis oriented vertically.

Plastochron:

> The period of time between the initiation of two
> successive leaf primordia.

Palisade cell:

> A columnar cell of the mesophyll usually found below
> the adaxial epidermis.

Xerophytic:

> Pertaining to the adaptation to survive with a
> limited water supply.

AHRING, R.M. and IRVING, R.M. (1969)
A laboratory method for determining cold hardiness in
bermuda grass. Cynodon dactylon (L.). Pers. Crop. Sci.9:
615-618.

ALLEN, H.H. (1961)
Flora of New Zealand. Volume I. Government Printer,
Wellington. 1085 p.

ARNON, D. (1949)
Copper enzymes in isolated chloroplasts Polyphenol-oxidase
in Beta vulgaris. Plant Physiol. 24: 1-15.

BALLANTINE, J.E.M., and FORDE, B.J. (1970)
The effect of light intensity and temperature on plant
growth and chloroplast ultrastructure in soybean. Am. J.
Bot. 57: 1150-1159.

BARRS, H. D. (1968)
Determination of water deficits in plant tissues. p. 235-368.
In Kozlowski, T.T. ed   Water Deficits and Plant Growth,
Volume I. Academic Press, New York.

BARTLETT, D., and BARTLETT, J. (1976)
Where two worlds meet. National Geographic 149: 298-320.

BEGG, J.E. and JARVIS, P.G. (1968)
Photosynthesis in Townsville Lucerne (Stylosanthes
humilis H.B.K.). Agric. Met. 5: 91-109.

BJÖRKMAN, O. (1968 a)
Carboxydismutase activity in shade-adapted and sun-adapted
species of higher plants. Physiol. Plant. 21: 1-10.

BJÖRKMAN, O. (1968 b)
Further studies on diferentiation of photosynthetic properties
in sun and shade ecotypes of Solidago virgarea. Physiol.
Plant. 21: 84-89.

BJÖRKMAN, O. and HOLMGREN, P. (1963)
Adaptability of the photosynthetic apparatus to light
intensity in ecotypes from exposed and shaded habitats.
Physiol. Plant. 16: 889-911.

BJÖRKMAN, O., NOBS, M. and HIESEY, W. (1970)
Growth, photosynthetic and biochemical responses of contrasting
mimulus clones to intensity and temperature. Carn. Inst. Wash.
Year Book 68: 614-620.

BJÖRKMAN, O., PEARY, R.W., HARRISON, A.T. and MOONEY, H.A. (1972)
Photosynthetic adaptation to high temperature in Death Valley,
California. Science, N.Y. 175: 786-789.

BOGDANOV, P. (1935)
Photoperiodism in species of woody plants. Preliminary
contribution Exp. Sta. Rec. 73: 22.

BÖHNING, R.H. and BURNSIDE,C.A. (1956)
The effect of light intensity on rate of apparent photosynthesis
in leaves of sun and shade plants. Amer. J. Bot. 43: 557-561.

BORMAN, F.H. (1954)
Ecological implications of changes in the photosynthetic
response of Pinus taeda seedlings during ontogeny. Abstr.
Bull. Ecol. Soc. Amer. 1955. 36: 81.

BOYER, J.S. (1969)
Measurement of the water status of plants. Ann. Rev. Plant
Physiol. 20: 55-59.

BOYER, J.S. (1970)
Differing sensitivity of photosynthesis to low leaf water
potentials in corn and soybean. Plant Physiol. 46: 236-239.

BRIX, H. (1962)
The effect of water stress on the rates of photosynthesis and
respiration in tomato plants and loblolly pine seedlings.
Physiol. Plantarum 15: 10-20.

BROWN, K.W. (1968)
Experimental considerations for the measurement of photosynthetic
rates by means of carbon dioxide exchange in leaf chambers.
Nebraska Coll. Agr. Home Econ., Agr. exp. Sta. Lincoln,
Progr. Rep. 66: 1-40.

BULMER, G.M. (1958)
A key to the divaricating shrubs of New Zealand. Tuatara 7:
48-61.

BUSSELL, W.T. (1968)
The growth of some New Zealand trees. I, Growth in natural
conditions. N.Z. Journal of Botany 6 (1): 63-75.

CARLQUIST, S. (1965)
Island Life. National History Press, New York   660 p.

COCKAYNE, L. (1899)
An inquiry into the seedling forms of New Zealand phanerogams,
Part I. Trans. N. Z. Inst. 31: 354-398.

COCKAYNE, L. (1900)
An inquiry into the seedling forms of New Zealand phanerogams
and their development, Part III. Trans. N. Z. Inst. 32:
83-94.

COCKAYNE, L. (1901)
An inquiry into the seedling forms of New Zealand phanerogams
and their development, Part IV. Trans. N.Z. Inst. 33: 265-298.

COCKAYNE, L. (1911)
Observations concerning evolution derived from ecological
studies in New Zealand. Trans. N.Z. Inst. 44: 1-50.

COCKAYNE, L. (1929)
Flora and Vegetation of New Zealand. N. Z. Forest Service
Circular 27.

CONNOR, D.J. and CARTLEDGE, O. (1970)
Observed and calculated photosynthetic rates of Chloris
gayana communities. J. appl. Ecol. 7: 335-375.

CUTTER, E.G. (1971)
Plant Anatomy: Experimentation and Interpretation. Part 2,
Organs. Edward Arnold, London. 343 p.

DANSEREAU, P. (1964)
Six problems in New Zealand vegetation. Bull. Torrney Bot.
Club 91: 114-140.

DARLINGTON, P.J. Jr. (1965)
Biogeography of the Southern End of the World. Cambridge,
Mass. 236 p.

DAUBENMIRE, R.F. (1959)
Plants and Environment. Wiley, New York. 422p.

DAVIES, W.J., KOZLOWSKI, T.T. and PEREIRA, J. (1974)
Effect of wind on transpiration and stomatal aperture of
woody plants. In Biedeski, R.L. et al. eds. Bull. 12, Roy.
Soc. N. Z. Wellington p. 433-438.

DENNY, G.A. (1964)
Habit heteroblastism of Sophora microphylla Ait. Thesis
M. Sc., University of Canterbury.

DEPUIT, E.J. and CALDWELL, M.M. (1973)
Seasonal patterns of net photosynthesis of Artemisia
tridentata. Am. J. Bot. 50: 426-435.

DEPUIT, E.J. and CALDWELL, M.M. (1975)
Gas exchange of three cool semi-desert species in relation
to temperature and water stress. J. Ecol. 63: 835-858.

DEXTER, S.T. (1932)
Investigations of hardiness of plants by measurement of
electrical conductivity. Plant Physiol. 7: 68-78.

DEXTER, S.T. (1933)
Growth, organic nitrogen fractions and buffer capacity in
relation to hardiness of plants. Plant Physiol. 10: 149-158.

DUFOUR, L. (1887)
Influence de la lumiére sur la forme et la structure des
feuilles. Paris.

DUMBLETON, L.J. (1967)
Winter dormancy in the New Zealand biota and its
paleoclimatic implications. N. Z. Journal of Botany
5: 211-222.

EL-SHARKAWAY, M. A. and HESKETH, J.D. (1964)
Effects of temperature and water deficit on leaf photosynthetic
rate of different species. Crop Sci. 4: 514-518.

ESAU, K. (1962)
Plant Anatomy. John Wiley and Sons, Inc., New York. London.
735 p.

FAHN, A. (1974)
Plant Anatomy. 2nd ed. Pergamon Press, Oxford. 611 p.

FITZGERALD, M. S. (1923)
Rickanton Bush, a degenerating remnant of the ancient swamp
forest of the Canterbury Plains. Thesis, M. Sc., Canterbury
University.

FLEMING, C. A. (1962)
New Zealand biogeography a paleontologist's approach.
Tuetara 10: 53-108.

FORDE, B. J., WHITEHEAD, H. C. M., and ROWLEY, J. A. (1975)
Effect of light intensity and temperature on photosynthetic
rate, leaf starch content and ultrastructure of Paspalum
dilatum. Aust. J. Plant Physiol. 2: 185-195.

FRANCO, C. M. and MAGOLHARES, A. C. (1965)
Techniques for the measurement of transpiration of individual
plants. p. 211-241. In Eckardt, F.E. ed Methodology of Plant
Eco-Physiology. Arid Zone Research XXV, U.N.E.S.C.O.

GAFT, D. F. and CARR, D. J. (1964)
An examination of the refractometric method for determining
the water potential of plant tissues. Annals of Botany (N.S.)
28: 351-368.

GATES, D. M. (1968)
Transpiration and leaf temperature. Am. Rev. Plant Physiol.
19: 211-238.

GAUHL, E. (1970)
Leaf factors affecting the rate of light saturated photosynthesis
in ecotypes of Solarium dulcamora. Carn. Inst. Washington Year
Book 68: 633-636.

GREENWOOD, R. M. and ATKINSON, I. A. E. (1977)
Evolution of divaricating plants in New Zealand in relation
to moa browsing. Proc. N. Z. Ecol. Soc. 24: 14-28.

HARBERLANDT, G. (1914)
Physiological Plant Anatomy. MacMillan Company, London. 777 p.

HELLMUTH, E. O. (1969)
Eco-physiological studies on plants in arid and semi-arid
regions in Western Australia II. Field physiology of Acacia
craspedocarpa. J. Ecol. 57: 613-634.

HELLMUTH, E. O. (1971)
Eco-physiological studies on plants in arid and semi-arid
regions in Western Australia III. Comparative studies on
photosynthesis, respiration and water relations of ten
arid zone and two semi-arid zone plants under winter and
late summer climatic conditions. J. Ecol. 59: 225-260.

HESLOP-HARRISON, Y. and HESLOP-HARRISON, J. (1969)
Scanning electron microscopy of leaf surfaces. Scanning
Electron Microscopy Proceedings of the 2nd Annual Scanning
Electron Microscope Symposium.

HOLDEN, M. (1965)
Chlorophylls. p. 461-488 In Goodwin, T.W. ed Chemistry
and Biochemistry of Plant Pigments. Academic Press, London.

HOOKER, J. D. (1853)
The Botony of the Antarctic Voyage, Vol II, Flora Novae
Zealandiae, London.

HSIAO, T. C. (1973)
Plant responses to water stress. Ann. Rev. Plant Physiol.
24: 519-570.

HSIAO, T. C. , FEREREO, E., ACEVEDO, E. and HEDERSEN, D. U.(1976)
Water stress and dynamics of growth and yield of crop plants.
p. 281-303 In Lange, L.O., Kaplan, L. and Schulze, E.D. ed
Water and Plant Life Problems and Modern Approaches.
Springer-Verlag, Berlin.

HUBER, B. (1924)
Die Beunteilung des Wasserhaushaltes der Pflanzen. Jahrb.
Wiss. Botan. 64: 1.

HUBER, B. (1935)
Xerophyten. In Handworterbuch der Naturwissenschaften. 2nd ed.
Volume 10. p. 702-720. Fischer, Jena.

ILJIN, W. S. (1923)
Der Einfluss des Wassermangols auf die Kohlenstoff -
assimilation durch die Pflanzen. Flora (Jena) 116: 360-378.

JARVIS, P. P. (1969)
Characteristics of the photosynthetic apparatus derived from
the response to natural complexes of environmental factors.
In Prediction and Measurement of Photosynthetic Productivity
Proceedings of the IBP/PP Technical Meeting, Trebon, 14 - 21
September, 1969. Wageningen Centre for Agricultural Publication
and Documentation. 1970.

JENSEN, M. (1954)
Shelter effect. The Danish Technical Press, Copenhagen. 264 p.

JOHNSTON, J. G. (1948)
A comparison of adult and juvenile foliage of Carpodetus
serratus Forest with special reference to xeromorphic
characters. Thesis,M. Sc., Canterbury University.

KALMA, J. D. and KUIPER, F. (1966)
Transpiration and growth of Phaseolus vulgaris L. as
affected by wind speed. Landbonwhogschool Wagengin
Mededel 66 (8): 1-8.

KARNOVSKY, M. J. (1965)
A formaldehyde - glutaraldehyde fixative of high osmolality
for use in electron microscopy. J. cell Biol. 27: 137A-138A.

KEEN, L. R. (1970)
Significance of leaf size for water relationships in some
closely related species. Thesis, M. Sc., University of
Auckland.

KIRK, T. (1889)
Forest Flora of New Zealand.Wellington, New Zealand. 345 p.

KOLLER, D., and SAMISH, Y. (1964)
A null-point compensating system for simultaneous and
continuous measurements of net photosynthesis and transpiration
by controlled gas-stream analysis. Bot. Gaz. 125: 81-88.

KRAMER, P. J. (1959)
Plant and soil relationships. p. 607-726. In Steward, F.C. ed
Plant Physiology, Vol. II. Academic Press, New York and London.

KRAMER, P. J. (1969)
Plant and soil relationships: A modern synthesis. McGraw and
Hill Book Co, New York. 482 p.

KRAMER, P. J. and BRIX, H. (1965)
Measurement of water stress in plants. p. 343-351. In Eckardt,
F. E. ed. Methodology of Plant Eco-Physiology. Arid Zone
Research XXV. U.N.E.S.C.O.

KRAMER, P. J. and KOZLOWSKI, T. T. (1960)
Physiology of Trees. McGraw-Hill, New York. 642 p.

LARCHER, W. (1954)
Die Kalteresistenz mediterraner Immergruner und ihre
Beeinjlussbarkeit. Planta 44: 607-635.

LARCHER, W. (1965)
The influence of water stress on the relationship between CO_2
uptake and transpiration. p. 184-193. In Slavik, B. ed
Water Stress in Plants. Proc. Symp. Prague, 1963. Junk, The
Hague.

LARCHER, W. (1969)
Physiological approaches to the measurement of photosynthesis
in relation to dry matter production by trees. Photosynthetica
3: 150-166.

LEVITT, J. (1956)
The Hardiness of Plants. Academic Press, New York.

LEVITT, J. (1972)
Responses of Plants to Environmental Stresses. Academic Press.
New York and London. 697 p.

LOWRY, O. H., ROSEBROUGH, N. J., FAM, A. and RANDALL, R. J. (1951)
Protein measurement with Folin phenol reagent. Jnl. Biol. Chem.
193: 265.

LYTTLETON, J. W., BALLANTYNE, J. E. M. and FORDE, J. J. (1971)
Development and environment studies on chloroplasts of
Amaranthus lividus. p. 447-452. In Boardman, N.K., Linnane,
A.W. and Smillie, R.M. eds. Autonomy and Biogenesis of
Mitochondria and Chloroplasts. North Holland Publishing Co.,
Amsterdam.

MACKLON, F. S. and WEATHERLEY, P. E. (1965)
Controlled environmental studies of the nature and origins of
water deficits in plants. New Phytol. 65: 414-427.

MARK, A. F. (1975)
Photosynthesis and dark respiration in 3 alpine snow tussocks
(Chiono chloa spp.) under controlled environments. N. Z.
Journal of Botany 13: 93-122.

MARSHALL, J. K. (1967)
The effect of shelter on the productivity of grasslands and
field crops. Field Crop Abstracts 20: 1-14.

MARTIN, E. U. and CLEMENTS, F. E. (1935)
Studies on the effect of artificial wind on growth and
transpiration of Helianthus annuus. Plant Physiol. 10: 613-636.

MAXIMOV, N. A. (1929)
The Plant in Relation to Water. Allen and Urwin, London. 751 p.

MC CREE, K. J. and TROUGHTON, J. H. (1966)
Prediction of growth rate at different light levels from
measured photosynthetic and respiration rates. Plant Physiol.
41: 559-566.

MC PHERSON, H. G. and SLATYER, R. O. (1973)
Mechanisms regulating photosynthesis in Pennisetum typhoides.
Aust. J. biol. Sci. 26: 329-339.

MEIDNER, H. and SPANNER, D. C. (1959)
The differential transpiration parameter. J. Exp. Bot. 10:
190-205.

MONTFORT, C. and KRESS-RICHTER, I. C. (1950)
Reversible photochemische chlorophyll zerstorungen in
vesonnten. Roubblattern von Aurea formen und i hoe
Beziehunzen zer Strahlung Sklina und Erbgut. Planta 38:
516-520.

MOONEY, H. A. and SHROPSHIRE, F. (1967)
Population variability in temperature related photosynthetic
acclimation. Oecologia Pl. 2: 1-13.

NOBEL, P. S. (1970)
Introduction to Biophysical Plant Physiology. W.H.Freeman.
San Francisco. 488 p.

ORCHARD, B. and HEATH, O. V. S. (1964)
Carbon assimilation at low carbon dioxide levels. I.
Apparatus and technique. J. exp. Bot. 15: 314-330.

PIETERS, G. A. (1962)
Some aspects of the problem of sun and shade leaves. p. 393-399.
In Proceedings XIIIth International Horticultural Congress,
Volume IV.

PIETERS, G. A. (1974)
The growth of sun and shade leaves of Populus euramericana
"Robusta" in relation to age, light intensity and temperature.
Mededelingen Landbonwhogeschool, Wageningen, Nederland. p. 74-111.

PHILIPSON, W. R. (1963)
Habit in relation to age in New Zealand trees. J. Indian Bot.
Soc. 42: 167-179.

PISEK, A. and WINKLER, E. (1953)
Die Schliesbewegung der Stomata bei o kologisch verschiedenen
Pflanzentypen in Abhangigkeit vom Wassersatigungszustand der
Blatter und vom Licht. Planta 42: 253.

RATTENBURY, J. A. (1962)
Cyclic hybridization as a survival mechanism in the New Zealand
forest flora. Evolution 16: 348-363.

RICKARD, D. S. and FITZGERALD, P. D. (1969)
The estimation and occurrence of agricultural drought. J. Hydrol.
8: 11-16.

RIVERA, U. and CORNELI, F. (1931)
Rarsegna die casi fitopatologici osservati rel 1929 (danni da
freddo e da critto game). Rev. Patol. Veg. 21: 65-100.

ROBERTSON, P. A. (1976)
Photosynthetic and respiratory responses of natural populations
of Koeleria cristata grown in three environmental regimes.
Bot. Gaz. 137: 94-98.

ROSENBERG, N. J. (1967)
The influence and implications of windbreaks in agriculture in
dry regions. In Shaw, R.H. ed Ground Level Climatology
Am. Assc. Advan. Sci. Symp.

RUMBAL, W. (1961)
A study of heteroblastism. Thesis, Ph.D., University of
New Zealand.

SAKAI, S. (1955)
The seasonal changes of the hardiness and the physiological
state of the cortical parenchyma cells of mulberry tree.
Low temp. Sci. Ser. B 13: 33-41.

SCHOLANDER, P. F., HAMMEL, H. T., BRADSTREET, E. D., and
HEMMINGSEN   (1964)
    Sap pressure in vascular plants. Science (Wash.) 148: 339-345.

SCHULZE, E. D., LANGE, O. L., BUSCHBOM, U., KAPPEN, L. and
EVENARI, M. (1972)
    Stomatal responses to changes in humidity in plants growing
    in the desert. Planta 108: 259-270.

SCOTT, D. (1970a)
    Relative growth rates under controlled temperatures of some
    New Zealand indigenous and introduced grasses. N. Z. Journal
    of Botany 8: 76-81.

SCOTT, D. (1970b)
    Comparison between lodgepole pine and mountain beech in
    establishment and CO_2 exchange. N.Z. Journal of Botany 8:
    357-360.

SCOTT, D. and MENALDA, P. H. (1970)
    CO_2 exchange of plants. 2. Response of six species to
    temperature and light intensity. N.Z. Journal of Botany 8:
    361-369.

SÉSTAK, L., CÁTSKY, J. and JARVIS, P. S. (eds) (1971)
    Plant Photosynthetic Production Manual of Methods. Dr. W.
    Junk N. V. Publishers. The Hague.

SHAH, S. R. (1962)
    Studies on wind protection. Meded. 60 Inst. toegep. biol.
    Onderz. Nat. 113 p.

SHIELDS, M. (1950)
    Leaf xeromorphy as related to physiological and structural
    influences. Bot. Rev. 16: 399-446.

SLATYER, R. O. (1967)
    Plant - Water Relationships. Academic Press, London and New
    York. 399-446.

SLATYER, R. O. and JARVIS, P. G. (1966)
    Gaseous diffusion porometer for continuous measurement of
    diffuse resistance of leaves. Science 151 (3710): 574-576.

SLATYER, R. O. and SHMUELI, E. (1967)
    Measurement of internal water status and transpiration. p.
    337-353. In Hagan, R.M. et al. ed Irrigation of Agricultural
    Lands Agronomy No. 11. Am. Soc. Agron. Madison, Wisconsin.

STÅLFELT, M. G. (1929)
    Die Abhangigkeit der Spaltoffnungsreaktion von der Wasserbilanz.
    Planta 8: 287.

STOCKER, O. (1960)
    Physiological and morphological changes in plants due to water
    deficiency. In Plant Water Relationships in Arid and Semi-
    Arid Conditions. Arid Zone Research XV: 63-104. U.N.E.S.C.O.

STURROCK, J. W. (1973)
Wind and crop water use. Proc. Soil and Water Symp. N. Z.
D.S.I.R. Info. Series No. 96: 31-37.

SULLIVAN, C. Y. (1972)
Techniques for measuring drought stress. p. 1-18. In
Larson, K.L. and Eastin, J.D. eds. Drought Injury and
Resistance in Crops. Crop Sci. Soc. Am. Special Publ. No. 2.

TALBOT, A. J. B., TYREE, M. J. and DAINTY, J. (1975)
Some notes concerning the measurement of water potentials
of leaf tissue with specific reference to Tsuga canadensis
and Picea abies. Can. J. Bot. 53: 784-788.

TINKLIN, R. and WEATHERLEY, P. E. (1968)
The effect of transpiration rate on the leaf water potential
of sand and soil rooted plants. New Phytol. 67: 605-615.

TOMLINSON, A. I. (1975)
Structure of wind over New Zealand. New Zealand Meterological
Service Technical Information Circular No. 147. 24 p.

TREHAVNE, K. J. and EAGLES, C. F. (1970)
Effect of temperature on photosynthetic activity of climatic
races of Dactylis glomerata. Photosynthetica 4: 107-117.

TUCKER, J. M. (1974)
Patterns of parallel evolution of leaf form in new world
oaks. Taxon 23(1): 129-154.

TURRELL, F. M. (1936)
The area of the internal exposed surface of dicotyledon
leaves. Am. J. Bot. 23: 255-264.

VENABLE, J. H. and COGGESHALL, R. (1965)
A simplified lead citrate stain for use in electron microscopy.
J. Cell. Biol. 25: 407-408.

VIEIRA DE SILVA, J. (1976)
Water stress, ultrastructure and enzyme activity. p. 207-221.
In Lange, L. O., Kaplan, L. and Schulze, E. D., eds. Water
and Plant Life Problems and Modern Approaches. Springer-Verlag.
Berlin.

WALLIGON, E. F. (1964)
Modification and use of an electric hygrometer for estimating
relative stomatal aperture. Plant Physiol. 39: 86-90.

WARDLE, P. (1963)
Evolution and distribution of the New Zealand flora, as
affected by Quaternary climates. N. Z. Journal of Bot. 1:
3-17.

WARREN WILSON, J. (1966)
Effect of temperature on net assimilation rate. Ann. Bot. 30:
753-761.

WAREING, P. F.   (1956)
  Photoperiodism in woody plants.   Ann. Rev. Plant Physiol.
  7: 191-214.

WAREING, R. H. and CLEARY, B. D.   (1967)
  Plant moisture stress: evaluation by pressure bomb.   Science
  155: 1248-1254.

WEATHERLEY, P. E. and BARRS, H. D.   (1959)
  A climatological wind tunnel.   Nature 183: 94.

WENT, F.   (1971)
  Parallel evolution.   Taxon 20: 197-226.

WHITEHEAD, F. H.   (1963a)
  The effects of exposure on growth and development.   In
  Rutter, A.J. and Whitehead, F.H. eds.   The Water Relations
  of Plants.   British Ecological Society.   London.   394 p.

WHITEHEAD, F. H.   (1963b)
  Experimental studies on the effect of wind on plant growth
  and anatomy.   IV.   Growth substances and adaptative anatomical
  and morphological changes.   New Phytol.   62: 86-90.

WOODING, R. A.   (1968)
  A low wind speed tunnel for model studies in micrometeorology.
  C.S.I.R.O., Plant Ind. and Tech. Paper 25: 1-39.