Quantitative Markers of Phase Change, and Modelling the Size and Complexity of Trees

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Name of Chief Supervisor: Dr John Clemens Telephone Ext: 2570.

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ABSTRACT

Quantitative markers of ontogenetic phase change were sought to track the restoration of the adult state in plants of *Metrosideros excelsa* (pohutukawa) that had been rejuvenated by micropropagation (plantlets). The potential markers of leaf carbon isotope discrimination and tree architecture were examined in association with leaf morphology for plantlets, and juvenile and adult plants at a range of temperatures (32/24, 24/16 and 16/8 ^{o}C day/night). Changes in leaf morphology of plantlets and juvenile plants that were indicative of vegetative phase change were associated with a decrease in carbon isotope discrimination. Phase change, judged by these two markers, occurred most rapidly at 24/16 ^{o}C, and in plantlets faster than in juvenile plants. Adult plants showed long-term stability.

It was hypothesised that phase change could be quantified by changes in plant growth rate, expressed through canopy topological size and complexity parameters. A model of tree architecture (the Metrosideros Model) was developed that would allow tree size and 2D structural complexity to be recorded and analysed quantitatively. A further hypothesis was that juvenile plants and plantlets must attain a certain size and/or structural complexity before passing to the adult state and this was evaluated using the Metrosideros Model. Dynamics of growth and structural change were examined using both non-linear and linear analyses. The Metrosideros Model was successfully tested, confirming the hypothesis of quantitative differences between juvenile plants, plantlets and adult plants in structural complexity and branching patterns. The model was able to separate parameters of plant size from those of structural complexity. Complexity was indicative of ontogenetic state, and tracked the progress of phase change in juvenile plants and plantlets independently of temperature. Adult plant parameters of structural complexity, as δ^{13}C, also remained stable at all temperatures. On the other hand, the growth rate of size parameters was not associated with phase change, but was responsive to temperature.

It was concluded that while leaf morphology, carbon isotope discrimination and crown architecture can be used to track phase change, each relates to a program of

change that might occur largely independently of others. Crown architecture was less affected by temperature than were leaf characteristics, and was, therefore, the most reliable marker of phase change of those studied.

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List of Abbreviations

A_p	Partial Asymmetry
ANOVA	Analysis of variance
$\delta^{13}C$	Composition of carbon isotope ^{13}C
Δ	Carbon isotope discrimination against ^{13}C (Farquhar et al., 1989)
GLP	Growth lag period
MCON	Mean Centrifugal Order Number
PSAD	Proportional Sum of Absolute Deviations
PDB	Pee Dee belemnite
TAI	Tree Asymmetry Index
VPD	Vapour pressure difference

Plate 1 ***Metrosideros excelsa*** **(pohutukawa), the New Zealand Christmas tree**

CHAPTER ONE

1.1 General Introduction

Agricultural production plays an important role in the New Zealand economy. In recent decades there has been a significant shift towards the diversification of agricultural production away from the originally main areas of sheep farming or beef and milk production (Smith, 1984; Ulyatt et al., 1986). The increase in production from the horticultural sector has been an important feature of the New Zealand export trade. The introduction of the now well-known kiwifruit (*Actinidia chinensis*), but also of many other non-traditional horticultural products, contributes significantly to the New Zealand export economy. More attention has also been paid to the flower and ornamental plant market by both the farming sector and research and development organisations (Cameron and Clemens, 1996).

New Zealand's unique flora offers many interesting and potentially valuable new products especially in the lucrative market of ornamental plants. In this category belong the New Zealand native trees of the genus *Metrosideros*. Four closely related species in this genus, the northern and southern rata (*Metrosideros robusta* and *M. umbellata,* respectively), *M. kermadecensis* and *M. excelsa* (the pohutukawa) have attracted particular attention as ornamental crops. Because *M. excelsa* provides a spectacular flowering display in December, it is well known in New Zealand as the New Zealand Christmas tree. The focus of the current study is predominantly on this endemic New Zealand species.

A number of cultivars of *M. excelsa* are readily propagated vegetatively using *in vitro* techniques and proliferating shoot cultures initially established from adult plant material. However, the resulting plantlets exhibit juvenile leaf characteristics and have lost the ability to respond to signals that are florally inductive in adult plants. This thesis concentrates on an investigation into ontogenetic phase change in *M. excelsa*, addressing the need for quantitative

markers to track the changes occurring as rejuvenated plantlets and juvenile seedlings pass from the rejuvenated and juvenile states to the adult state.

1.1.1 *Metrosideros excelsa* (pohutukawa)

The genus *Metrosideros,* family Myrtaceae (Salmon, 1980) is represented in New Zealand by eleven species. Three of these are terrestrial trees and shrubs, while the rest are lianas (Dawson, 1972; 1974; 1985). *M. excelsa,* an evergreen tree endemic to New Zealand and occurring naturally only in the northern part of the North Island, is restricted naturally to coastal locations between Poverty Bay to the east, and a little north from Waitara, on the west coast (Salmon, 1980). The species is nowadays spread by cultivation around most of the North Island and to warmer places in the South Island. The tree grows up to 20 m in height, with a trunk up to 1 to 2 m in diameter. The trunk is usually short, with the main branches spreading into a broad crown. These main branches are often twisted and gnarled in appearance, some spreading almost horizontally and forming aerial roots that reach to the ground. These main branches further divide into much finer crown structures, following branching patterns described below

Allan (1961) assigned the following distinguishing attributes to the genus *Metrosideros*: flowers in terminal cymes or racemes, five imbricate sepals, five petals, infinite stamens, filiform filaments, anthers versatile, ovary 3-celled, style filiform, infinite ovules, capsules coriaceous with irregular dehiscence, seeds linear, and leaves opposite. Dawson (1968b) distinguished two groups of *Metrosideros* on the basis of vegetative and floral characteristics. Species Group A, to which *M. excelsa* belongs, together with both rata species and *M. kermadecensis*, exhibits a 'pseudo-dichotomous' branching habit, due to the abscission of the shoot apex during elongation. This habit, however, is 'irregular' (Dawson, 1968a). For example, only one of a pair of resulting distal axillary buds may be viable and form a branch. Also, juvenile forms of *M. excelsa* do not regularly exhibit such abscission of the apex; they can branch from any axillary position, or continue growth from the apical bud (Sreekantan et al., 2001).

In adult trees of *M. excelsa,* each over-wintering bud is protected by up to eight pairs of scales, which are shed as the shoot elongates, leaving a close-set series of scars at the shoot base (Dawson, 1968a). Several pairs of deciduous scales protect floral buds during the winter dormancy period. Deciduous bracts subtend the secondary axes, and the cymules have six deciduous bracts, one pair subtending the lateral flower, and two attached to its base (Dawson, 1968a). With respect to the floral structure, Dawson (1968b) further divided the species Group A into A1 and A2. The sub-Group A1 includes *M. excelsa, M. kermadecensis, M. umbellata* and *M. robusta.* In this group, all axes in the inflorescence, except the primary shoot terminate in cymules of three flowers, one terminal and two laterals. The inflorescences are usually in axillary pairs. In strongly flowering plants, there may be a second pair of inflorescences below the first. The flowers in *M. excelsa* are usually the biggest in size from the group. The terminal cymes rest on peduncles of 10 to 20 mm long. The pedicels, peduncles and calyces of the flowers bear tomentum. The most prominent parts of the flower are numerous red stamens, 20 to 50 mm in length. Red petals are ca. 5 mm long surrounded by green tomentose sepals, 3-5 mm long. These structures create a nectar cup with a 10-20 mm long style in the centre surrounded by the extended dark red stamens.

The leaves in *M. excelsa* were described by Salmon (1980) as deep green, with a stout petiole 10-12 mm long, elliptic, acute and coriaceous, with a glossy upper surface and tomentose lower surface. However, two morphologically distinguished types of foliage also exist in *M. excelsa.* The juvenile type is characterised by a larger size, pointed at the end and bearing no tomentum. In contrast, the adult leaves are smaller, rounded at the end and possess a downy tomentum on the abaxial leaf surface (Cockayne, 1928; Dawson, 1968a, Clemens et al., 1999).

1.1.2 Commercial propagation of *Metrosideros excelsa* and the loss of ability to flower

Numerous techniques are available for the vegetative propagation of adult woody perennials (Hackett, 1985; Maynard et al., 1991). Typically, growers in New Zealand have propagated selections of *M. excelsa* either by rooting cuttings or grafting using adult material. More recently, a micropropagation technique for multiplication and rooting has been developed (Oliphant and Loreti, 1988). As with many other plant taxa, the speed and efficiency of the *in vitro* technique are the main factors favouring its use over conventional nursery techniques (Maynard et al., 1991; Harry et al., 1994). Rejuvenation may result from the *in vitro* process (Durzan, 1990; Brand and Lineberger, 1992a,b). This may have some benefits, such as improved vigour and rooting ability. However, it can also have undesirable attributes, such as loss of adult leaf morphology and branching patterns, and a delay in flowering. This has been reported for *M. excelsa* (Oliphant et al., 1990; Clemens et al., 1999).

When adult material of selected *M. excelsa* varieties was initiated into sterile culture, high and effective shoot multiplication was achieved (J. Oliphant, pers.comm.). Rooted explants (plantlets) were transferred to a solid growing medium in the nursery, acclimatised and grown for the first few months in the greenhouse. These were then planted into plastic pots and transferred to nursery growing sites for further evaluation. Despite the adult status of the trees from which the original propagation material was taken (Oliphant and Loreti, 1988), plantlets exhibited juvenile leaf morphology and branching characteristic of juveniles (Oliphant et al., 1990; Clemens et al., 1999; employees of Duncan & Davies Nursery Ltd., New Plymouth, NZ, pers.comm.).

The most apparent change in shoot morphology in rejuvenated plantlets was the lack of shoot tip abscission during shoot elongation typical of adult plants of *M. excelsa* (Dawson, 1968a). This juvenile feature often resulted in

prolonged continuous growth of terminal sylleptic shoots in *M. excelsa* plantlets, similar to those reported by Borchert (1976) for juvenile forms of *Ulmus, Salix* or *Liriodendron.* Sylleptic shoots occasionally formed so-called 'terminal buds' described by Snowball (1989) during temporary cessation of growth between repeated growth flushes in *Citrus.* When these 'terminal buds' resumed growth, short internodes with unexpanded leaves also resulted in *M. excelsa.* Thus, the position of the growth pause on sylleptic shoots remained identifiable by the region of small leaves and nodes that had not elongated. It was also reported by growers that the rejuvenated plantlets of *M. excelsa* exhibited more 'vigorous' growth, i.e. they had longer periods of growth and more flushes of growth per season than adult plants. The *M. excelsa* rejuvenated plantlets exhibited monopodial growth associated with stronger apical dominance as described by Remphrey and Davidson (1992) in green ash (*Fraxinus pennsylvanica*), but showed little if any acrotony (Champagnat, 1978, quoted by Remphrey and Davidson, 1992). Acrotony, i.e. long lateral shoots arising near the end of parent shoots, was associated with the juvenile rather than adult state in green ash by Remphrey and Davidson (1992).

Similar to the ontogenetic changes occurring in seedlings of *M. excelsa,* micropropagated plantlets undergo a gradual transition from juvenile to adult leaf morphology. Thus, in the strict sense of Goebel (1900), *M. excelsa* exhibits homoblastic phase change characteristics.

Micropropagated plants of *M. excelsa* showed no sign of plagiotropic growth, unlike those in, for example, *Taxus* or *Araucaria* (Robbins, 1964). Thus, a tendency of some vegetatively-propagated plants to retain growth characteristics of the part of the tree from which they came, termed topophysis, was not observed in rejuvenated *M. excelsa* plantlets, which exhibited normal orthotropic growth.

The juvenile leaf and shoot growth features were reported by growers to persist in plantlets for two to three years after micropropagation. After this time, new leaves started to exhibit adult morphology. Flowering would not occur during the time of juvenile foliage growth. This was in contrast to grafted plants or cuttings rooted

directly from adult material. While grafting is prohibitively labour intensive, and rooting of cutting material has a low success rate, the inability of the micropropagated plantlets to flower had a negative effect on the sales of cultivars of this species (J. Oliphant, and employees of Duncan & Davies Nurseries Ltd, New Plymouth, NZ, pers. comm.).

Initially, experiments were planned, and one undertaken, to study floral induction in adult plants of *M. excelsa* and *M. collina.* Vegetative phase change in rejuvenated and juvenile plants in *M. excelsa*, tracked by changes in leaf morphology, carbon isotope discrimination and crown architecture, became the focus of this project. Therefore, this avenue was not pursued.

1.1.3 Phase change in *Metrosideros* species

These undesirable aspects associated with micropropagation of *M. excelsa* are examples of phase reversal. Therefore, hastening the process of phase change, and thereby restoring the potential for the induction of flowering in the rejuvenated plantlets, was sought by the ornamental industry. From an applied point of view, an ability to quantify the process of phase change would serve as a tool for the identification of an optimal treatment/s for hastening the return of rejuvenated plant material to the adult phase. In particular, the effect of temperature treatments on the maturation process was investigated in this study.

To address the phenomenon of phase change in *M. excelsa*, markers were sought that would enable the dynamics of this developmental process to be quantified. Quantification of the dynamics of phase change would then lead to the identification of a point at which phase change occurs in this species. However, reliable markers of plant developmental state are still lacking in the investigation of phase change phenomena in general (Hackett, 1985; Poethig, 1993; 1997a,b Greenwood, 1995).

Metrosideros excelsa was a convenient plant for research into phase change, because direct observation and quantification of leaf morphology allowed the progress of phase change to be tracked and used as a putative marker of the maturation process. As changes in leaf morphology might affect leaf gas exchange processes, which would be reflected in the degree of discrimination against ^{13}C, carbon isotope discrimination was considered as a potential marker of phase change. In addition, the distinction between patterns of shoot growth in rejuvenated plantlets and adult plants suggested that crown architecture might also be a worthy candidate as a marker of phase change.

Plate 2 Experimental plants of *Metrosideros excelsa*.

Top – Juvenile plants, left-hand side – Plantlet, right-hand side – Adult plant

1.2 Literature review

A number of comprehensive reviews on the phenomenon of phase change have been published (Wareing, 1959; Park, 1969; Zimmerman, 1972; 1983; Borchert, 1976; Hackett, 1985; Snowball, 1989; Durzan, 1990; Poethig, 1990; Haffner et al., 1991; Greenwood, 1995; Hackett et al., 1997; Michaels and Amasino, 2000). In this review a brief introduction to core terminology is given. Subsequently, the review covers aspects of phase change and focuses in detail on architecture and carbon isotope discrimination.

1.2.1 Terminology

During their life span, plants proceed through ontological development from seed germination, through a vegetative growth stage, to an adult stage of sexual reproduction (Durzan, 1990). The term 'phase change' is commonly used in plant developmental physiology to refer to the point or period of time when an individual plant ceases to exhibit its juvenile characteristics and satisfies one or more of the definitions of a adult plant, e.g. acquisition of the ability to flower.

In this thesis the terms used by Poethig (1990) have been adopted. He recognised three main post-embryonic phases, 'juvenile vegetative', 'adult vegetative' and 'reproductive'. 'Phase change' as used in this thesis will refer to the progression between the juvenile vegetative and adult vegetative states. The terms 'phase change', 'maturation' and 'ontogenetic ageing' are used synonymously in the literature (Greenwood, 1995). In this thesis, the terms 'phase change' and 'maturation' are used in the same sense as that of Hackett (1985), Poethig (1990), Greenwood (1995) and Sachs (1999), referring to processes localised in the meristem. In contrast, 'physiological ageing' or senescence is related to a long-term loss of vigour (Fortainer and Jonkers 1976; Corderso et al., 1985; Hackett, 1985; Greenwood, 1989; Poethig 1990). 'Tree vigour' was defined by Magnussen (1995) as 'a measure of how close a tree is to its maximum growth rate (RGR_{max})'. A further useful description used by Huang et al. (1995) is that the

main difference between 'ageing' and 'maturation' rests with the reversibility of maturation. This is shown in a model of plant development adopted from Durzan (1990) (Figure 1.1).

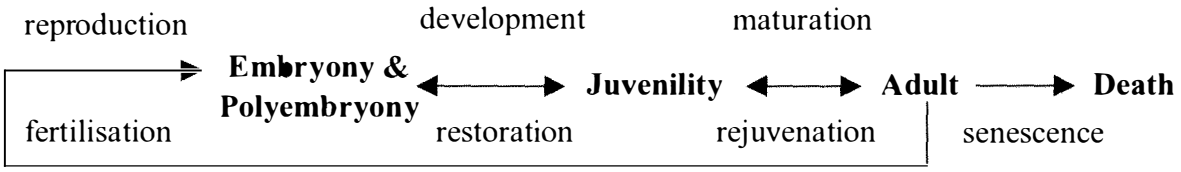


Figure 1.1 Model of plant physiological ageing, ontogeny and reversion of maturation (Reproduced from Durzan, 1990 p. 21).

Durzan (1990) uses the term 'rejuvenation' to describe the process of change that occurs during reproduction and the reversion from adult to juvenile. However, the term 'rejuvenation' is also used in the literature (Hackett, 1985; Greenwood, 1995) and in the micropropagation industry to describe the loss of adult characteristics during micropropagation. This term will be used in this thesis in both senses depending on context.

Consequently, three types of ontogenetic state were defined for the purposes of this thesis as follows: juvenile (in plants derived from seed and showing all characteristics typical of the juvenile state); plantlet (plants 'rejuvenated' by micropropagation of tissue taken from an adult plant and showing juvenile leaf morphology and growth (Section 1.1.2); and adult (in plants showing adult leaf morphology and growth and competence to flower).

1.2.2 Quantitative expression of phase change

A number of attempts in the past decades have been made to find for maturation, and phase change in particular, reliable quantitative markers. Suitable markers have been sought at the morphological, physiological, biochemical and molecular levels (Hackett, 1985; Hackett et al., 1990; Murray et al., 1994). In a review of phase change phenomena, Poethig (1990) presented a number of morphological

characteristics distinguishing the juvenile and adult states. These included phyllotaxy, leaf retention, growth habit, thorniness, adventitious root production, growth rate in terms of the plastochron, leaf thickness, epidermal cell shape, the presence of epidermal hairs, and cuticle thickness. A number of studies referred to different characteristics or morphological markers of the developmental phases. These included a change in leaf shape in *Hedera helix* (Hackett, 1985) and *Metrosideros excelsa* (Dawson, 1968a; Clemens, 1995, 1999), a decrease in thorniness in *Citrus* (Snowball, 1994a), the loss of the ability of cuttings to root (Monteuuis et al., 1995) and a decline in growth rate (Takemoto and Greenwood, 1993) associated with maturation.

Bostrack (1993) measured the size of the apical meristem and found a negative correlation with maturation in elm, while in other examined species no such correlation with ontogenetic state was found. Quantitative comparisons between leaves of juvenile and adult plants of the heteroblastic tree *Pseudopanax crassifolius* were presented by Clearwater and Gould (1993). They attributed the leaf differences to the accelerated growth of the primordial leaf axes. These authors also compared the size of shoot apical meristems and found that they were significantly larger in adult plants, i.e. positive correlation with maturation, in contrast to the findings of Bostrack (1993). Corderso et al. (1985) measured plastochron duration and found a longer duration in juvenile plants, while Marc and Hackett (1991) suggested that a change in phyllotaxy was a distinctive marker of ontogenetic state in English ivy.

At the morphological level, Snowball et al. (1994a,b) counted the number of nodes between roots and first flowers in *Citrus* and found that a minimum number of nodes was needed between the root and the position of the first flowers. The node counting hypothesis was reviewed by Sachs (1999), who argued that ontogenetic changes occur in individual apices rather than the plant as a whole, and stressed the physiological and ecological importance of 'node counting'. He hypothesised that node counting, at least in some plants, allowed for development independent of environment, presenting an example of *Senecio vernalis* plants that could complete an entire life cycle in a uniform environment. He also discussed the node counting hypothesis with respect to other hypotheses such as the number of

cell divisions, distance from roots, effect of leaves, interaction between the apices of the crown, and hypothesised of an abrupt versus a gradual cumulative mechanism affecting phase change. He believed that the distance from apex to the root was correlated to phase change, and was better measured in node numbers than in metric distance. In another recent study, an attempt was made to quantify and thus define separate developmental states, using a range of possible morphological and physiological markers of juvenility and maturity. McGowran et al. (1998) introduced a parameter of Discriminant Score (D) calculated from shoot diameters as observed in *in vitro*-cultured nodal segments, and they also used image analysis of leaves. These authors concluded that growth rate was highly variable with clone and time of culture and thus did not provide a suitable marker for juvenility/maturity in *Quercus* species. In that study, the growth rate was an expression of a multiplication rate rather than the growth in tree size or complexity.

Numerous attributes have been considered at the physiological and biochemical levels including the biological clock, the genetic stability of developmental states, carbon metabolism, carbon isotope discrimination, mineral concentrations, polyamines, phenolic compounds, chlorophyll content, photosynthetic efficiency, plant hormones, peroxidases and nucleic acid composition (reviewed by Haffner et al., 1991). Several of these have promise as markers.

In *in vitro* reinvigorated hazel (*Corylus avellana*) shoots, Rey et al. (1992; 1994a) observed higher endogenous polyamine concentrations than in adult leaves and concluded that polyamine concentrations may provide a simple assay for determining the juvenility of plant tissue. Interestingly, they noted that after 20 *in vitro* subcultures, the concentration of polyamine decreased, indicating maturation of rejuvenated tissue. Later, Rey et al. (1994b; 1994c; 1998) explored the content of endogenous polyamines in leaves and buds of hazelnut trees, inducing rejuvenation by reinvigoration through heavy pruning. They found that the putrescine/spermidine+spermine ratio increased in rejuvenated tissue. The authors believed that polyamine metabolism may have a role as a biochemical marker of juvenility and rejuvenation in woody plants.

Brand and Lineberger (1992a,b) studied both the biochemical and morphological differences between half-sib seedlings and micropropagated, grafted, and cutting-propagated adult-phase plants in *Betula* spp. The results from denatured protein banding patterns indicated that micropropagated plants were initially more like seedlings than adult plants. From morphological evaluations, rejuvenation of *Betula* appeared to occur during *in vitro* propagation, but the level of juvenility that was regained might not be equivalent to that of a seedling. They also concluded that not all morphological indicators of phase change were affected equally by *in vitro* rejuvenation. Changes in content of photosynthetic enzymes, such as Rubisco (Dai et al., 1993), of chlorophyll, photosynthetic activity (Bauer and Bauer, 1980), and even in the photosynthetic pathway occurring during the transition from the juvenile to reproductive phase (Popp et al., 1987; Nelson and Langdale, 1989; Wang et al., 1993) have all been reported.

Huang et al. (1996) experimented with *Sequoia sempervirens* and claimed rejuvenation through grafting of adult tips onto juvenile rootstock. After five repeated graftings, full rooting ability was restored, and also esterase and peroxidase isozymes associated with the adult state were replaced by isoesterases and isoperoxidases usually found in juvenile plant tissue. Thus the authors believed that esterase and peroxidase isozymes could be used as markers in phase change investigations.

The data for gibberellins appears contradictory. Gibberellins have been associated with reversion to juvenility in ivy and several New Zealand native species (Jameson et al., 1988; Horrell et al., 1990; Marc and Hackett, 1991). Conversely, in the maize mutants *dwarf1, 3, 5* and *anther ear1*, Evans and Poethig (1995) showed that endogenous gibberellins promoted the transition from juvenile to adult vegetative phases, and further to reproductive development.

At the molecular level various aspects have been considered (Evans and Poethig, 1995) such as the intrinsic differences in the DNA and RNA content of meristematic cells. Contrasting findings in the levels of RNA and DNA in juvenile versus adult tissue have been presented including a report that found no

quantitative difference between RNA and DNA related to phase change. However, later Mellerowitch et al. (1993) compared the nuclear and cytoplasmic content in cambium cells and Huang et al. (1995) compared the mitochondrial DNA, both finding differences between ontogenetic states. This suggested the genetic encoding of ontogenetic changes, and thus the possibility of marking phase change through differences in gene expression. Such a difference has been shown in terms of reduction in the expression of dihydrokaemferol 4-reductase (*DFR*) in adult ivy (Murray and Hackett, 1991).

Huang et al. (1995) suggested mitochondrial involvement in phase change on the basis of restriction fragment analyses of mitochondrial DNA. 4.0- and 3.6-kb *Bam*H1 mitochondrial DNA restriction fragments were present in juvenile shoots but absent in adult material and reappeared in rejuvenated (through repeated grafting) shoots of *Sequoia sempervirens*.

At the cellular level, Greenwood (1995) suggested, with respect to the stability of the adult state and rejuvenation, that the expression of maturity could be a function of the ratio of juvenile to adult cells in the apex at a given time. It was concluded that, whatever the nature of the intrinsic change, extrinsic factors, such as reaching a critical size, were usually required for expression of adult characteristics. Greenwood (1995) believed that criteria of reversion should not be confined to only some juvenile characteristics, such as increased regenerative ability or vigour (growth rate), since many maturational characteristics appeared to be independent of each other (Poethig, 1990). Consequently, one marker of rejuvenation, e.g. increased rooting ability may, not necessarily imply that other commercially important traits such as growth rate will also increase. Moreover, even if the juvenile characteristics of higher rooting ability or reinvigoration are exhibited, it may be a response of the plants to a change in cultural conditions, rather than true rejuvenation.

Many other authors studying phase change expressed the view that a minimum size and/or complexity must be attained by the plant in order for it to be able to respond to external, florally-inductive signals (e.g. Hackett, 1985; Evans and Poethig, 1995). Alternatively, this minimum size and/or complexity must be

attained before the plant is able to produce levels of endogenous substances, including carbohydrate, sufficient to switch on and/or express genes related to the progress towards reproductive development (Zimmerman, 1972; Bestford et al. 1996; Bongard et al., 1996). Such views highlight the multilevel and multifactorial basis of the phenomenon of phase change (Poethig, 1990).

Therefore, no universal marker of phase change has been identified. In this study, the possibility of identifying quantitative markers was attempted at two different levels. Firstly, at the level of the leaf and carbon fixation using analyses of carbon isotope discrimination. Secondly, at the level of the formation and growth of the whole crown through modelling of crown architecture. In both cases, the potential markers were examined in three ontogenetic states, viz. a true juvenile seedling, rejuvenated *ex vitro* plantlets and the adult state. These plants were exposed to well-defined environmental conditions in growth cabinets in order to observe the effect of temperature on plant development through these two levels of markers. A third marker, leaf morphology, which was known to be associated with phase change (Dawson, 1968a; Clemens et al., 1999), was also quantified in order to determine the time frame of phase change and its relationship to the potential markers.

1.2.3 Carbon isotope discrimination as a marker of phase change

1.2.3.1 Theoretical basis of carbon isotope discrimination in plants

The CO_2 in air contains a number of different carbon isotopes. Two non-radioactive isotopes are ^{12}C and ^{13}C, the latter being the less abundant at ca. 1.1% of total carbon. Plants take up and incorporate $^{12}CO_2$ preferentially to $^{13}CO_2$ as a result of their difference in molecular weight. $^{12}CO_2$ diffuses through plant tissue and reacts in photosynthetic reactions more rapidly than does $^{13}CO_2$ (Farquhar et al., 1982). Therefore, a ratio of $^{13}C/^{12}C$ (R) exists in plant material that is lower than the ratio of the natural abundance of these isotopes.

The value that is of interest in plant studies is the isotope fractionation, which represents the change in isotope content due to the physical, chemical, or biological processes involved in CO_2 capture (O'Leary et al., 1992). It can be assumed that in most experiments, all plants use CO_2 of the same ratio. Therefore, the actual value of R for the ambient CO_2 becomes irrelevant for comparisons within experiments. The value of R in the dry matter of leaves is predominantly determined by three factors. These are the primary carboxylating enzyme of the relevant photosynthetic pathway, the ratio of intercellular to atmospheric CO_2 partial pressure, and the composition of source CO_2, assumed in most studies to be constant at -8 ‰ (Farquhar et al., 1982; 1989).

O'Leary et al. (1992) reported that the majority of papers on carbon isotope discrimination gave values for isotopic composition ($\delta^{13}C$) relative to the ratio of the two isotopes in the Pee Dee fossil belemnite limestone formation (denoted R_{PDB}), and of value 0.01124.
This ratio can be used to determine values of $\delta^{13}C$ in a sample by the equation:

$$\delta^{13}C = [R_{sample}/R_{PDB} - 1] \times 1000 \text{ ('per mil' (‰), non-dimensional units)}$$

In using the above expression of discrimination against ^{13}C in plants, it was assumed that the $\delta^{13}C$ value of the source, the atmospheric CO_2, was constant at about -7.8 to

-8.0 ‰. However, this value can vary in practice, especially near to a source of CO_2 created by burning fossil fuels, when $\delta^{13}C$ can reach -32.5 ‰ (Gleason and Kyser, 1984, quoted by Farquhar et al., 1989).

Because $^{13}CO_2$ is discriminated against, $\delta^{13}C$ is always negative. To avoid confusion and for consistency of presentation, Farquhar et al. (1989) proposed discrimination be expressed by the parameter Δ, which is calculated from the difference between the isotope ratio δ_a in the source substrate (air) and that in the product (plant mass) δ_p, as follows:

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p)$$

where δ_a equals ca. -0.008 (-8 ‰) derived from the $^{13}C/^{12}C$ ratio of air (R_a= 0.011143) and the ratio of PDB (R_{PDB} = 0.011237) as follow:

$$\delta_{air}{}^{13}C = 0.011143/0.011237 - 1$$

Because the value of the denominator $(1+\delta_p)$ is always going to be close to unity in plant material, O'Leary et al. (1992) argued that it can be ignored. The discrimination against ^{13}C resulting from the carbon fixation by the plant from ambient CO_2 can therefore be calculated as:

$$\Delta = \delta_{air} - \delta_p$$

Since in most carbon isotope discrimination studies δ_{air} is assumed to be constant at -8‰, the main advantage of using the discrimination parameter Δ over the carbon isotope composition parameter $\delta^{13}C$ is the expression of data on a positive scale. Moreover, higher discrimination against ^{13}C is reflected in numerically greater positive values of Δ.

However, both Δ and $\delta^{13}C$ continue to be used to express carbon isotope discrimination in plants. For example, Valentini et al. (1994), Cordell et al. (1998), Damesin et al. (1997) and Waring and Silvester (1994) used $\delta^{13}C$ values. On the

other hand, Fleck et al. (1996), Donovan and Ehleringer (1994) and Hansen (1996) used the positive values of Δ to present results in their studies. Therefore, less negative values of carbon isotope composition ($\delta^{13}C$) and lower values of Δ refer to less discrimination against $^{13}CO_2$, and *vice versa.* Statistical differences between analyses using these two measures of carbon isotope discrimination are not likely to occur. Calculation of Δ is affected by the value of the divisor ($1+\delta_p$). However, this typically ranges in value from ca. 0.98 to 0.97 for C_3 plants ($\delta^{13}C$ being ca. -20‰). Thus, the relative differences between $\delta^{13}C$ and Δ values can reach a maximum of approximately one percent. The subtraction of the value for air$\delta^{13}C$ of -8‰ changes the absolute differences between δ and Δ, but has minimal effect on statistical analysis of these data.

Detailed reviews of the physical and chemical basis for carbon isotope discrimination in plants have been published by several authors (e.g. Craig, 1953; Bender, 1971; O'Leary, 1981; Farquhar et al., 1982; Farquhar et al., 1989; O'Leary et al., 1992). These show that the majority of discrimination against $^{13}CO_2$ in C_3 plants is accounted for by carbon fixation by Rubisco, at a value of ca. -20 ‰. However, the intercellular partial pressure of CO_2 also significantly affects discrimination during diffusion through stomata and membranes (Lin et al., Ehleringer, 1997). As the CO_2 taken up progresses through the leaf, the gradient in CO_2 concentration and transition into the liquid phase further affects discrimination, although this is less significant than that due to diffusion (O'Leary, 1981; Farquhar et al., 1982). Theoretical values of discrimination were calculated at -4.4‰ for diffusion of CO_2 through the stomatal pore (Craig, 1953), and at -2.9‰ for diffusion through the boundary layer to the stomata (Farquhar, 1983).

Carbon isotope discrimination in plant material has been used extensively in studies of photosynthetic pathways (Farquhar et al., 1989), and gas exchange and water use at the single leaf level (Robinson et al., 1993), and for communities of leaves (Vitousek et al., 1990; Donovan and Ehleringer, 1991,1994, 1998; Gutierrez and Meinzer, 1994; Stewart et al., 1995). Typically, in C_3 plants carbon isotope composition ($\delta^{13}C$) varies between -18 and -35‰ due to environmental or genetically-based influences. In CAM plants, the range of values of $\delta^{13}C$ was

reported to be -13 to -25‰ (Medina and Troughton, 1974, quoted by O'Leary, 1981). C_4 plants discriminate less against ^{13}C due to leaf anatomical and biochemical factors, with $\delta^{13}C$ ranging from -10 to -12‰ (O'Leary, 1981; Farquhar, 1982).

In C_3 plants, the level of discrimination is dependent largely on the ratio of intercellular to atmospheric partial pressure of CO_2 (p_i/p_a) prevailing in the leaf when carbon is assimilated (O'Leary, 1981; Farquhar et al., 1982; Gutierrez and Meinzer, 1994). As leaf conductance decreases, the lower p_i/p_a and discrimination become (O'Leary, 1981; Wang et al., 1997). In turn, discrimination is related to the ratio of assimilation (A) to stomatal conductance to water vapour (g), and to intrinsic water use efficiency (WUE) (Farquhar et al., 1989). The relationship between discrimination, A and g was expressed by Geber and Dawson (1990) and Gutierrez and Meinzer (1994) as instantaneous WUE = A/E, where E is transpiration, which is positively related to g. Similarly, leaf anatomy and/or morphology can affect discrimination through their effects on p_i/p_a and the availability of CO_2 at the site of carboxylation (O'Leary, 1981; Farquhar et al., 1982; 1989).

1.2.3.2 Factors responsible for variation of carbon isotope discrimination in leaves

The importance of considering external factors as well as intrinsic factors, such as leaf dimension and crown structure that in turn influence boundary layer conductance, have been discussed with respect to the interpretation of physiological functions of individual leaves and their ecological significance (Farquhar et al., 1989). A number of authors have addressed the differences in discrimination within a species caused by morphological, anatomical and physiological differences between leaves, or differences between positions in a crown (Koerner and Diemer, 1987; Geber and Dawson, 1990; Vitousek et al., 1990; Bostrack, 1993; Gutierrez and Meinzer, 1994; Waring and Silvester, 1994; Wang et al., 1997). In general, observed differences in discrimination were attributed to specific microenvironmental factors, such as temperature, position within the canopy, water and light availability, and salinity (Farquhar (1982; Farquhar et al., 1989). Some of

these studies examined differences in discrimination along environmental gradients, e.g. that within the tree crown (Waring and Silvester, 1994; Wang et al., 1997), an elevation gradient associated with leaf thickness in *Metrosideros polymorpha* (Vitousek et al., 1990), and a rainfall gradient (Stewart et al., 1995).

Vitousek et al. (1990), Geeske et al. (1994) and Cordell et al. (1998) found that pubescent leaves of *Metrosideros polymorpha* exhibited lower discrimination against $^{13}CO_2$ than did glabrous leaves of the same species. They attributed this difference to effects of altitude and water availability on the ratio of leaf mass to leaf area. Leaf mass/area ratio and leaf pubescence increased with altitude from 70 to 2350 m, pubescence accounting for up to 35 % of the leaf mass. Vitousek et al. (1990) concluded that lower discrimination resulted from increased internal resistance to CO_2 diffusion in thicker leaves found at higher elevations.

Genetically determined morphological differences, such as size of leaves, internodes, flowers and seeds, were linked to the rate of photosynthesis, leaf conductance, water-use efficiency (WUE) and leaf carbon isotope discrimination in the annual plant *Polygonum arenastum* (Geber and Dawson, 1990). Small-leaved families tended to have higher gas exchange, lower long-term WUE and higher carbon isotope discrimination. Discrimination was positively correlated with both assimilation rate and leaf conductance.

Wang et al. (1997) compared the physiology of two morphological types of *Populus euphratica* leaves, lanceolate (LL) and broad-oval (BOL), which were associated with particular positions within the tree canopy. The BOL leaves had lower stomatal conductance, resulting in lower transpiration rates, lower CO_2 concentration in intercellular spaces, and lesser discrimination against $^{13}CO_2$.

At the whole canopy level, Gutierrez and Meinzer (1994) examined the effect of seasonal changes in environmental conditions and leaf position within the developing canopy upon discrimination in leaves of coffee (*Coffea)* hedgerows. Differences in discrimination between leaves from different positions were ascribed to the effect of shading within the canopy.

Similarly, Waring and Silvester (1994) found that variation in discrimination within the crowns of *Pinus radiata* trees, which varied by as much as 6‰, could be best explained by the combined effects of shading (about 2‰) and relative branch hydraulic conductivity on stomatal conductance.

Damesin et al. (1997) compared discrimination in leaves of evergreen (*Quercus ilex*) and deciduous (*Q. pubescens*) oaks. They also attributed the differences in discrimination to the effect of environment and differences in leaf mass/area ratio, particularly in the evergreen *Q. ilex*. Later, Damesin et al. (1998) examined the long-term changes in discrimination in developing leaves of the two oak species, and found inter-specific differences were linked to the seasonal plant water potential. In a two year comprehensive study of *Pinus contorta, Populus tremuloides, Acer negundo* and *A. grandidentatum,* Buchmann et al. (1997) found a positive correlation between leaf area index (LAI) and discrimination in leaves of the deciduous trees, but not in the evergreen species (*P. contorta).*

An effect of soil water utilisation on carbon isotope discrimination has frequently been reported in the literature. For example, studying the effect of water utilisation among several species of conifer at two altitudes in the Italian Alps (1000 m and 1500), Valentini et al. (1994) reported that in the predominantly deep rooting *Larix decidua* ($\delta^{13}C$ = -29.0‰*)*, and in the surface water utilising *P. sylvestris* ($\delta^{13}C$ = -25.9‰), discrimination was principally affected by water availability.

Apart from other results, Aitken et al. (1995) found that trees of *Pseudotsuga menziesii* growing in a common garden experiment discriminated more against ^{13}C if grown without water stress, while stressed trees discriminated less and had a higher WUE. The average values for discrimination also appeared to be related to mean annual precipitation. Assessing the relative effects of annual precipitation and soil moisture availability, Stewart et al. (1995) measured discrimination in the leaves of 348 species from 12 plant communities along a 900 km-long rainfall gradient. The authors concluded that the average carbon isotope discrimination signature gave a strong indication of soil moisture availability. They also believed that because of its ability to integrate the effects of long-term conditions, carbon isotope discrimination provides a more meaningful measure of soil water availability than rainfall.

1.2.3.3 Carbon isotope discrimination and phase change

Phase change from the juvenile to the adult state is often accompanied by a range of morphological and physiological changes in leaf characteristics, as reviewed by Hackett (1985), Poethig (1990), Haffner et al. (1991) and Greenwood (1995) (see Section 1.2.2). Consequently, carbon isotope discrimination, which is affected by leaf morphology and physiology, might provide a useful tool for the investigation of phenomena associated with phase change. However, reports of research that explicitly set out to examine discrimination in relation to phase change are not common in the literature. The information concerning differences in carbon isotope discrimination between the juvenile or adult states and its possible relevance to phase change in *M. excelsa* is reviewed below.

Changes in leaf characteristics, such as the number and/or position of stomata (Bauer and Bauer, 1980), the thickness of epidermal and mesophyll tissue (Brand and Lineberger, 1992b; Bostrack, 1993; Clearwater and Gould, 1993; Hansen, 1996; Fleck et al., 1996; Wang et al., 1997; Day et al., 1997), and the development of tomentum have been reported to be associated with phase change in a number of woody perennial species, including *M. excelsa* (Cockayne, 1928; Dawson, 1968a; Clemens et al., 1999).

Diffusion of CO_2 into the leaf through surface tomentum and stomata, as well as diffusion within the leaf tissue, are also likely to be altered by phase change transformations of leaf thickness or stomatal distribution (Bauer and Bauer, 1980; Day et al., 1997). Longer-term changes in p_i and thus CO_2 availability at the site of carboxylation should then be reflected in values of carbon isotope discrimination (O'Leary, 1981; Farquhar et al., 1982). Bauer and Bauer (1980) compared photosynthetic and anatomical attributes of juvenile and adult leaves of *Hedera helix* and found that light-saturated net photosynthesis was 1.5 times higher in adult leaves than juvenile, owing partly to the lower stomatal and residual conductances to the CO_2 in juvenile leaves.

More directly, phase change and carbon isotope discrimination in leaves were examined in association with ecophysiological differences between juvenile and adult plants in an open field trial of several woody species (*Acer negundo, Artemisia tridentata, Chrysothamnus nauseosus,* and *Salix exigua*) by Donovan and Ehleringer (1991; 1994). These researchers concluded that the difference in discrimination between plants of the two ontogenetic states was probably related to plant size, and consequently to the soil moisture availability. They also found that a lower WUE in juveniles was accompanied by higher rates of photosynthesis and stomatal conductance than in adult plants. As expected, WUE was negatively correlated with carbon isotope discrimination. However, the effects of water availability and ontogenetic state were not separated in this experiment.

Rundel et al. (1999) also found that (presumed juvenile) seedlings of *Zygophyllum prismatocarpum* showed consistently higher discrimination than adult plants. In this study, however, no correlation between leaf morphology and carbon isotope discrimination was found.

Carbon isotope discrimination in juvenile and adult leaves was compared in *Acacia* by Hansen (1996) and in *Quercus* by Fleck et al. (1996). Hansen (1996) hypothesised that juvenile leaves of *A. koa* would possess suites of adaptations that would be consistent with rapid establishment in seedlings, such as high growth rate, while phyllodes borne by adult plants would exhibit attributes of drought resistance in response to unpredictable stress situations. The author used carbon isotope discrimination to quantify long-term physiological differences between these two types of photosynthetic organs. He concluded that leaf morphology, including the mass per unit area, accounted for differences in leaf physiological performance in juvenile leaves, which had higher discrimination. The author quoted Hansen and Steig (1993), who had earlier concluded that xeromorphic leaves have a lower p_i than mesomorphic leaves, which in turn lead to a lower discrimination against $^{13}CO_2$ (Farquhar et al., 1982).

Fleck et al. (1996) attributed an unexpectedly lower discrimination in resprouts (re-growth after tree felling and fire damage) compared to 40-year-old undisturbed trees

of *Quercus ilex* to the importance of underground organs, and a higher physiological adaptive capacity of resprouts in response to environment.

In the above studies, carbon isotope discrimination in true juvenile (Hansen, 1996) or rejuvenated resprouts (Fleck et al., 1996) was examined in comparison to that in adult leaves. However, differences were attributed to the role of underground organs (Fleck et al. 1996), the smaller size of juvenile leaves resulting in lower WUE, and to higher rates of photosynthesis and stomatal conductance (Donovan and Ehleringer, 1991, 1994; Hansen, 1996), rather than to the ontogenetic state of the plant itself.

The somewhat scattered studies of carbon isotope discrimination yield contradictory results in terms of an overall pattern of response in the juvenile *versus* the adult state. For example, rejuvenated sprouts had lower discrimination than adult material of the same tree (Fleck et al., 1996). Conversely, Donovan and Ehleringer, (1991; 1994), Hansen (1996) and Rundel et al. (1999) found the opposite, i.e. leaves of seedlings or rejuvenated plants had higher discrimination against $^{13}CO_2$ than those of adult plants.

Donovan and Ehleringer (1991) and Rundel et al. (1999) found that differences in carbon isotope discrimination due to ontogenetic state were more pronounced on wet sites, presumably as a result of juveniles being as water stressed as adults on drier sites. However, in the study of Rundel et al. (1999) a wet site was considered relative to other observed sites in an arid zone with ca. 100 mm of annual precipitation, while Donovan and Ehleringer (1991) considered a wet site to have 580 mm of annual precipitation. Consequently, what was considered in the report of Rundel et al. (1999) to be a relatively wet site, was a dry site in the study of Donovan and Ehleringer (1991).

Differences in discrimination between juvenile and adult leaves should arise from differences in p_i/p_a for CO_2 and/or the CO_2 concentration at the site of carboxylation. These arise from numerous changes in the morphology and anatomy of leaves that are associated with phase change in general (e.g. Bauer and Bauer, 1980; Hackett, 1985; Clearwater and Gould, 1993), and in leaf morphology of *M. excelsa* in particular (Dawson, 1968a; Oliphant et al., 1990; Clemens et al., 1999). Variation in

leaf thickness associated with phase change reported by Körner and Diemer (1987), Hansen (1996), and Day et al. (1997) is likely to alter CO_2 diffusion through the leaf, chlorophyll content and its distribution within leaf cells leading to differences in CO_2 assimilation (Bauer and Bauer, 1980; Woo et al., 1994; Fleck et al., 1996), anatomical changes, such as in thickness and composition of the mesophyll (Körner and Diemer, 1987; Clearwater and Gould, 1993; Day et al., 1997;Wang et al., 1997).

A number of papers reported on differences in discrimination between pubescent and glabrous leaves of *Metrosideros polymorpha* (e.g. Vitousek et al., 1990; Cordell et al., 1998). In these reports, the differences in discrimination were explained as an effect of the growth environment, but were also strongly correlated with the increasing leaf mass/area ratio of the pubescent leaves across all examined environments. Morphological differences between the leaves of juvenile and adult plants of *M. excelsa* (Cockayne, 1928; Dawson 1968, 1972; Clemens et al., 1999) provide a visual indicator of the progress of phase change. Moreover, the morphological differences of the leaves indicate that differences in leaf carbon isotope composition could be expected to exist between ontogenetic states of *M. excelsa*. There are no reports of a link between leaf anatomy and/or carbon isotope discrimination and ontogenetic changes in *M. excelsa*.

Summary

Based on the literature reviewed above, it was hypothesised that carbon isotope discrimination in leaves of *M. excelsa* would be affected by ontogenetic state. Thus, changes in discrimination would mark the progress of phase change. Moreover, if the hypothesis was verified, carbon isotope discrimination could be indicative of underlying physiological processes associated with phase change. Measuring carbon isotope discrimination would provide an integrated measure of the physiological processes associated with phase change over a relatively long time scale. However, Borchert (1976) considered that a more dynamic means of observation in ontogenetic studies was also necessary.

1.2.4 Plant architecture as a marker of phase change

1.2.4.1 Phase change, plant size and complexity

The correlation between the attainment of a certain minimal size and/or complexity and the process of phase change has frequently been reported in the literature (e.g. Davidson and Remphrey, 1990; Bostrack 1993; Evans and Poethig, 1993; Day et al. 1997; Sachs, 1999; Sachs and Novoplansky, !995; Eysteinsson and Greenwood, 1993; Gilmore et al., 1995; Hatta et al., 1999; Honda et al., 1997; Huhn and Kleinshchmit, 1993; McGowran et al., 1998; Nicolini et al., 1993; Powell, 1987; 1992; Powell et al., 1995; Snowball et al., 1994; Takemoto and Greenwood, 1993. However, while these studies are generally in agreement that a correlation between plant size and/or complexity and phase change exists, this conclusion tends to be intuitively based. Only limited biometrics and statistical proofs have been produced, as noted by Snowball (1989).

Because the juvenile and adult plant states were often clearly distinguishable, many earlier studies (e.g. Libby and Hood, 1976; Borchert, 1976) described these architecture parameters, such as the canopy shape and long/short shoot production, in colloquial terminology. However, this terminology was often appropriate only for a particular species, and was not always suitable for general descriptions of architectural differences associated with phase change (Borchert, 1976). Poethig (1990) concluded that tree architectural differences between ontogenetic plant states were often described in 'conventional' terminology, owing to the lack of methods to quantify tree architecture.

In the following review, a number of studies are noted that attempted to quantify plant architecture parameters so that statistical analysis could be applied to experimental data. However, the majority of these studies did not use common units to express plant size, often did not define rigorously the units used, and failed to address the complexity parameters of plant architectural structures. Various studies are reviewed with respect to their focus on examining the correlation between size and complexity and/or the switch from vegetative to the

reproductive plant state, consciously disregarding constraints of botanical classification.

Among the studies that attempted to quantify plant architecture, as opposed to giving qualitative descriptions of parameters, is that of Hanzawa and Kalisz (1993). These authors examined the relationship between age, size and reproduction in the herbaceous plant *Trillium grandiflorum*. Leaf area and rhizome volume were measured as parameters of plant size. It was concluded that these two size parameters were better predictors of plant reproductive status than plant age. Interestingly, these authors found that some plants that were above the reproductive size threshold did not flower. The authors maintained that while there was evidence of a certain size threshold for reproduction, other factors such as age or growth rate also affected the reproductive status of the plant. Young (1985) arrived at a similar conclusion while studying size, growth rate and reproduction in *Lobelia telekii*.

Comparable conclusions were also reported by Garcia and Antor (1995), who examined the age, size and reproductive status in populations of the long-lived *Borderea pyrennaica*. In this case, dry weight of tubers was used as a size parameter. The results indicated that juvenile plants lacked flowers because they were too small to reproduce, and not because they were too young to reproduce.

Bostrack (1993) compared morphological features of adult leaves and leaves on sucker (juvenile) branches in four deciduous trees. He found significantly greater leaf area on juvenile branches than on canopy (adult) branches. Bostrack (1993) hypothesised that the position of sucker branches in relation to the root system, and thus the probable difference in water balance, affected the development of a large surface area of leaves on juvenile branches. Also, total branch length, internode length and number of nodes per branch was significantly greater for juvenile branches than for adult branches.

Examining the trade-off between reproduction and growth in *Oenothera biennis*, Reekie et al. (1998) used the mass of harvested plants as a size parameter for correlation with reproduction. They concluded that mass was positively correlated

with reproduction. However, at high densities, stem elongation, another possible size parameter, appeared to be an equally important factor. Reekie (1998) believed reproductive allocation in plants to be strongly correlated with size, and quoted others who supported this view (Lotz, 1990; Mendez and Obeso, 1993). In perennial herbaceous plants of *Plantago major*, Reekie (1998) used the dry weight of vegetative and reproductive organs as expressions of plant size. He found that reproductive outputs showed no relationship with size, while reproductive allocation ratio decreased with size. He pointed out that this decrease might be a consequence of the increase in reproductive cost with size. In this study, controlled environment and photoperiod were used to manipulate the onset of reproduction.

Welham and Setter (1998) in their study of size-dependent reproduction in dandelion (*Taraxacum officinale)* used vegetative biomass as an expression of size. They found a positive linear relationship between vegetative mass and reproduction. Similarly, Reekie et al. (1997) categorised the canopies of *Oenothera biennis* into closed *versus* open, or of a small, medium and large biomass in their search for the trade-off between reproductive and vegetative growth.

Plant size and ontogenetic state was found to be correlated by Schmidt and Zotz (2001) in a study of a heteroblastic bromeliad (*Vriesea sanguinoleta)*, which these authors interpreted as a size-related management of water storage. Other changes in anatomical, morphological and physiological properties were emphasised in relation to the increase in size, change from juvenile to adult state and associated heteroblastic changes.

Robinson and Wareing (1969) believed that phase change was correlated with, but not dependent on, the attainment of a certain size, expressed as the distance of a shoot meristem from the root system. Later, Wareing and Frydman (1976) speculated that with the increasing distance of the apex from the roots, the effect of plant hormones produced in the roots declines, allowing for maturation to progress. Similarly, Snowball (1989) and Snowball et al. (1994a,b) measured the

number of vegetative nodes from the root, as a size parameter in single-stem *Citrus* plants, and found a correlation between size and phase change.

Poethig (1990) posed the question of how plants maintain the coexistence of different developmental phases in a system that is constantly increasing in size and complexity. In this respect, Westwood (1978) had envisaged tree growth and development and the coexistence of ontogenetic states within the tree structure as shown in Figure 1.

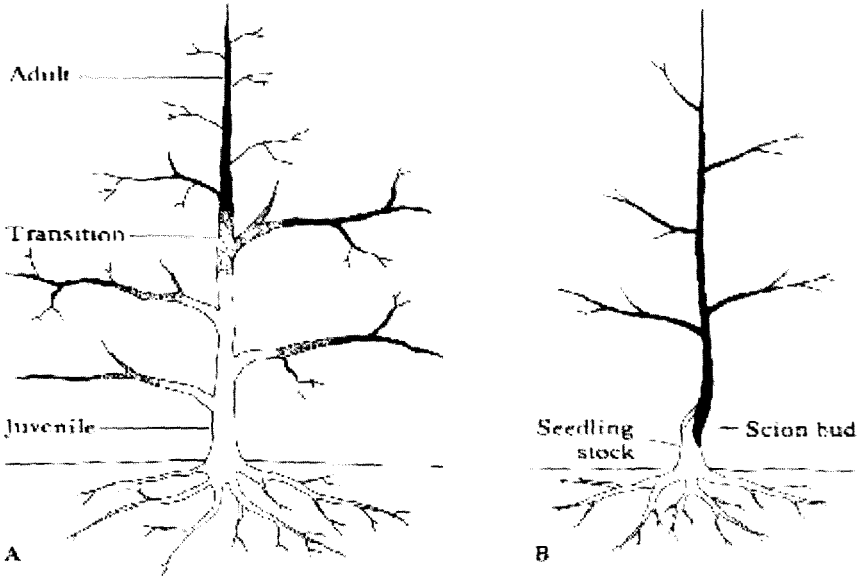


Figure 1.2 Representation of the coexistence of ontogenetic states within a tree structure A. A seedling tree with a juvenile zone at the base and lower part of the tree, a transition zone in the mid-region, and an adult zone at top and periphery. B. A grafted or budded nursery tree, which is entirely adult above the bud union. (Reproduced from Westwood, 1978).

Borchert (1976) made conclusions relating directly to tree architecture and phase change. Addressing the shoot growth patterns of juvenile and adult trees, he suggested that the reduction in shoot growth in adult trees could be a consequence of the increased complexity due to a larger numbers of shoots per tree. Focusing on the root/shoot ratio effect, he assumed that growth flushes would become

shorter and less frequent with increasing complexity, thus decreasing the vigour of adult trees.

Later, Borchert and Tomlinson (1984) studied crown geometry and other architecture parameters in the bifurcating *Tabebuia rosea*, examining shoot phenology, crown symmetry and shape. Erect, narrow, and multi-tiered crowns, usually associated with the juvenile state were more efficient than broad open crowns, which were associated with the adult state. These suggestions were based on computer simulations of tree crowns that illustrated higher mechanical support costs in broad open crowns. With respect to shoot phenology the authors observed that in *Tabebuia,* growth flushing was markedly asynchronous among individual young trees, in contrast to synchronised flushes in adult plants. Moreover, the number of flushes appeared to be negatively correlated with tree size. Both descriptive and quantitative methods were used to observe changes in tree architecture (Borchert and Tomlinson, 1984). This early report appeared to be the most comprehensive approach to the issue of recording the dynamics of crown architecture, and addressed directly the phenomenon of phase change through study of architecture parameters. Interestingly, the authors also noted that 'Quantitative data on the changes in efficiency occurring with increasing tree size are hard to obtain without destructive sampling, and few, if any experimental studies of these changes appear to exist' (Borchert and Tomlinson 1984, p. 964).

Some studies of crown architecture, show a correlation between phase change and tree crown characteristics indirectly, using parameters such as the loss of apical dominance, flower initiation, loss of vigour, or apical abortion (Hackett, 1985).

For example, in an attempt to address both size and complexity in adult male and female green ash trees (*Fraxinus pennsylvanica* var. *subintegerrima*), Remphrey and Davidson (1992) examined the relationship between branch length, branch order in relation to the trunk, and shape of the whole crown. They found that there was a negative correlation between branch length and increasing order, and that trees with sympodial growth (weak apical control) developed broader crowns than those with monopodial growth. They also elaborated on the correlation between increased branch complexity, competition for nutrients, and loss of vigour

followed by loss of apical dominance. The authors of this study loosely correlated the effect of tree architecture on bud conversion to the floral state. However, no direct conclusion with respect to phase change was reached. Apart from dealing with the general characteristics associated with phase change, this study also represents a rare attempt to include the parameter of crown complexity.

Earlier, Davidson and Remphrey (1990) used architecture parameters such as shoot length, numbers, diameter, and angle for comparisons between male and female trees of green ash. It was concluded that there are strong interrelationships between these architecture parameters and shoot tip abortion. Shoot tip abortion in *M. excelsa* is a distinct characteristic of the adult state (Dawson, 1968a), and thus quantification of these features would be desirable in ontogenetic studies in this (but also other) species.

Similarly, Leakey and Longman (1986) studied branching patterns as a function of apical dominance using decapitated 1 m tall *Triplochiton scleroxylon* trees. The authors concluded that branching, whilst not well understood, was a function of apical dominance. Two 'phases' of growth were identified: the 'dominance phase', in which uppermost shoots dominated and suppressed growth, and the 'sprouting phase' when many buds were released from inhibition. While their study was not directly related to ontogenesis, the growth type descriptions fitted some of the general characteristics of juvenile and adult phases of tree growth. Some of these characteristics cover those of *M. excelsa*, and thus relate to the issue of size and complexity expression in trees.

Similarly, Baltunis and Greenwood (1999) examined shoot elongation and phenology in larch (*Larix* spp.), and concluded that vigour, often also associated with juvenility (Hackett, 1985), seemed to be partly a function of late growth cessation and/or increased duration of shoot elongation.

1.2.4.2 The expression of plant architecture parameters

Despite the frequently expressed importance of the correlation between phase change and plant size and/or complexity, agreement has not been reached on how to measure and express plant size, let alone plant complexity. In most studies the complexity component was missing, overlooked or not quantified (P.W.Gandar, pers. comm.; Room et al., 1994). In others, the natural complexity of a plant canopy was eliminated by canopy treatments, e.g. pruning to a single shoot (Snowball et al., 1994), applied in order to simplify the recording of otherwise complex structures (Prusinkiewicz et al., 1994). This has made it difficult to draw general conclusions about plant canopy complexity from these fragmented studies of plant growth and development (Borchert, 1976; Borchert and Tomlinson, 1984). Consequently, only a few experimental results have been published dealing with crown architecture (Takemoto and Greenwood, 1993; Wilson, 1998).

The lack of availability of models and biometric methods for recording and analysis of architecture parameters has to some extent been overcome in the plant science literature by simplification of such parameters. Such simplifications include the use of the diameter of the shoot apex to predict and simulate the shape of bifurcating trees (Thornley, 1977), the number of nodes (Snowball et al., 1994), length of shoot (Leaky and Longman, 1986; Remphrey and Davidson, 1994), and allometry of plant reproductive biomass and stem diameter (Thornley and Johnson, 1990; Niklas, 1993). Other studies used the plastochron (Corderso et al., 1985), ordering of shoots (Day and Gould, 1997) or branches within the crown (Tomlinson, 1978; Prusinkiewicz et al., 1994; Godin et al., 1999), distinguishing various types of shoots and crowns (Borchert, 1976) or weighing the production of plant mass (Reekie, 1998).

Apart from the dubious value of representing plant architecture by such simplistic parameters, many of these methods led to the destruction of the plant, and/or provided only momentary measurements of plant growth and development. Borchert (1976) saw this as a problem. This author claimed that because plant development and phase change are dynamic processes, investigations of these

phenomena needed to be of a similar nature. However, experiments designed for making repeated, non-destructive measurements presented the logistical problem of recording the 3-dimensional tree architecture, which itself had not been adequately addressed even in a non-repeating manner (Prusinkiewicz et al., 1994). Consequently, the logistics of collecting data representative of the tree architecture, as well as the scarcity of methods for non-destructive growth measurement of the dynamic development of the plant (Gounot et al., 1989), have proved to be substantial hurdles.

To progress the elucidation of phase change, Borchert (1976) suggested that any future analyses should include quantitative descriptions of associated structural changes. These should be integrated with environmental effects into dynamic models. Lastly, extrapolation of structural complexity should be made from such models, and comparisons of these predictions made with observed growth patterns. Borchert also concluded that interactions between a tree and its environment were strongly dependent on size and complexity of the tree. Borchert (1976) believed that most published studies did not meet the conditions of a well defined means of measuring tree structure. Nor were growth conditions well enough defined for an adequate assessment to be made of the importance of size-related changes affecting the development of trees (Borchert, 1976).

Both the review of literature concerning phase change and the expression of plant architectural parameters showed that substantial progress remained to be made in these fields. However, with the increasing availability of computer power, modelling and simulations of plant architecture can begin to tackle the insufficiency of the biometrics of plant architecture and tree-like structures (Prusinkiewicz et al. 1994; Room et al., 1994; Remphrey and Prusinkiewicz, 1997; Godin et al., 1997; Honda et al., 1997; van Pelt, 1997; Yokozawa et al., 1998). The availability of new methods would inevitably lead to the kinds of experimental designs proposed by Borchert (1976).

Summary

It became clear from the literature that plant size measures were selected arbitrarily and differed between most studies. Other parameters of plant architecture, such as complexity measures, were rare and not applied consistently. Quantitative, dynamic monitoring of plant growth and development using architecture parameters has not been carried out, despite frequent claims of a correlation between phase change and plant size and complexity. Therefore, new methods of measuring and expressing plant architecture, that would also allow for repeated, non-destructive recording, needed to be developed. Such methods would then allow the evaluation of the hypothesis that phase change is associated with the attainment of a certain minimal tree size and/or complexity (The Size/Complexity Hypothesis), and that developmental states are associated with certain type of architecture, growth function and/or relative growth rate.

Since the development of these methods was a challenging but essential task, the literature from other scientific disciplines was searched for suitable models that could be adjusted for use on botanical trees, and particularly on those of *M. excelsa*.

1.2.4.3 Modelling plant architecture

A number of tree-like structures exist for which architectural models have been described. These include river or drainage systems (Werner and Smart, 1973), arborescent animals (Cheetham et al., 1980), dendrite trees (van Pelt and Verwer, 1985; Verwer and van Pelt, 1986) and other structures (Thornley, 1977). Because botanical trees are of a comparable complexity, these studies give an indication of modelling approaches to be adopted and the amount of information that would need to be collected to describe these structures (Prusinkiewicz et al., 1994).

Bell et al. (1979) noted that although the expression of the architecture of a plant is fundamental to its existence, this most apparent feature is little understood.

More recently, the discipline of plant architecture modelling and the methods used to express tree architecture, have progressed significantly, although they are still at an early stage of development (Hanan and Room, 1997; Godin et al., 1997; Remphrey and Prusinkiewicz, 1997; Godin et al., 1999; Pearcy and Valladares, 2000).

Although modelling tree architecture presents considerable challenges, there are advantages in using models as an experimental approach. Analytical or numerical solutions of the model functions can often contribute directly to the elucidation of a phenomenon, as well as enhancing the effectiveness of experimental design (Borchert, 1967; Honda, 1978; 1981; Honda et al., 1982; Thornley and Johnson, 1990; Buwalda, 1993). Because the tree-like structures consist of three-dimensional vectors, often a simplification of such structures is needed to enable the model, its parameters and recording to cope with the complexity involved. In this respect, the topological method is a common approach to modelling plant architecture.

1.2.4.4 Topological recording of plant architecture

According to a dictionary definition, "topology is a mathematical study of geometrical properties and spatial relations unaffected by the continuous changes of shape or size of figures" (Allen, 1990). On the other hand, Room et al. (1994) defined topology as the "relative arrangements of objects with respect to each other; which parts are connected to which others".

In general, topological methods break structures into units according to common simplified definitions that often disregard other properties (e.g. metric properties) of the units. Historically, topological methods were developed for analysis of river systems (Horton, 1945; Strahler, 1957; Leopold, 1971; Werner and Smart, 1973), which are two-dimensional in nature. In other tree-like structures, such as botanical trees (Day and Gould, 1997), bronchial networks (Weibel, 1963) or neurological systems (van Pelt and Verwer, 1986), the topological method was

used to simplify three-dimensional structures into two-dimensional topological maps. These showed the connectivity of the structural units, and thus determined the topological position of each unit in relation to other units. Recording, and the determination of the relative topological position of each unit, was done directly through visual observation of the structure itself, its drawing, or by digital encoding of each unit's position. Because of the system, units are grouped according to either their definition, or according to their topological distance from a particular point of the structure. In botanical trees, the topological distance is often regarded as the number of topological units between the root-stem junction and particular unit within the structure. This can be done in descending (e.g. Strahler's system) (Strahler, 1957) or ascending (e.g. Weibel's system) (Weibel, 1963) order from the root.

Other architecture properties, such as metric, geometric or morphological attributes, can also be accommodated within topological frameworks. Increasingly, there is demand for the dynamic observation of plant growth and development, as opposed to one-off measurements. Continuous changes can by a captured by a topological model through repeated measurements of the structure. In this respect, Porter (1994) claimed that focusing on the production of modular plant parts, i.e. topological units, was more appropriate for the quantification of the dynamics of whole plant architecture than was conventional growth analysis. Later, Room et al. (1994) pointed out that the latest availability of computer power made efforts to model the dynamics of growth and development of constituent plant parts more feasible. Similarly to Borchert (1976), these authors highlighted the need to focus on the effects of environmental factors on tree architecture when dynamic growth modelling was being studied.

1.2.4.5 Architecture units and recording

In order to accommodate the architectural descriptions of size and complexity of a tree, the crown has to be divided into a number of measurable variables (Fitter et al., 1991). These variables generally consist of one or more elementary units, such

as branches, metamers, nodes, junctions, segments, links, or leaves. Zeide (1993a) argued that the leaf presents a convenient and meaningful unit of the crown. However, Room et al. (1994) reviewed the terminology of crown units based on a node or meristem as the smallest unit, and presented a table of crown components and their definitions (Table 1.1).

Table 1.1 Definitions of components of individual plants in order of size. Components following metamer may contain many metamers; components following module may contain many modules (Reproduced from Room et al., 1994)

Component	Definition
Node	The most distal point of the junction between a stem and a leaf (or coincident whorl of leaves), just proximal to any subtended axillary bud(s) (Kurihara *et al.*, 1978)
Axillary/apical meristem	A cell or group of cells, specialized for mitosis, initiating or at the apex of a shoot. An axillary meristem becomes an apical meristem as soon as it starts to produce a shoot
Bud	An unextended, partly developed, shoot having at its summit the apical meristem which produced it; an unexpanded metamer or group of metamers (Bell, 1991)
Internode	A portion of stem between nodes i.e. from immediately distal to the junction of a leaf with the stem to the same position with respect to the next most distal leaf. The basal internode of a branch starts at the node of the leaf from whose axil the branch grew
Metamer	An internode, the axillary bud(s) as its proximal end and the leaf or leaves at its distal end but not any shoots resulting from growth of axillary buds (Kurihara et al., 1978)
Segment	The one or more metamers between nodes at which successive branches are attached. Equivalent to an edge in graph theory terminology (MacDonald, 1983)
Unit of growth	A morphologically discrete growth increment, the result of one episode of rhythmic growth by a module, i.e. extension of the preformed contents of a previously dormant apical bud followed by growth of neoformed leaves (if any) and formation of a new, dormant, apical bud (= unit of extension of) (Hallé et al., 1978)
Unit of morphogenesis	The product of a single episode of mitotic activity of an apical meristem having rhythmic growth (Hallé, 1986), extending from proximal to the oldest of a set of neoformed leaves to the most distal of the next distal set of preformed leaves
Shoot	A young stem which has grown from a single axillary/apical meristem and the leaves and buds which it carries; the young portion of a module
Short shoot	A shoot having shorter/fewer internodes which exploits ambient conditions and often bears flowers
Long shoot	A shoot having longer/more internodes which explores for new resources

Module	The product of one meristem; in shoots; a set of metamers originating from one axillary/apical bud (White, 1984); the smallest unit of morphology capable of producing daughter units and/or seeds (Maillette, 1982b)
Axis	A sequence of units of growth in the same general direction from one (monopodial) or more (sympodial) meristems
Branch	An axis other than the main stem plus any subordinate axes it bears
Ramet	The unit of clonal growth, capable of an independent existence if severed from the parent plant (Harper, 1977)
Individual	A physical coherent, structural individual (Vuorisalo and Tuomi, 1986)
Architectural Unit	The complete set of axis types and their relative arrangements found in a species – cannot be seen until an individual is old enough to have expressed its architectural model in full (Barthélémy, 1991)

Most topological models using these units would describe two dimensions of tree architecture. For three dimensional crown descriptions, further parameters, such as the length of segments and branching angles (Fisher and Honda, 1977; Godin et al., 1999) would be required.

The number of architecture models attempting to use these basic biological units is still limited (Prusinkiewicz et al., 1994; Room et al., 1994; Godin et al., 1997). This is mostly owing to the fact that any system endeavouring to record and describe architecture at the detailed level of, for example, the meristem, becomes too complicated with increasing tree size (Prusinkiewicz et al., 1994).

1.2.4.6 Tree architecture models

Over 30 years ago, when plant architecture was receiving renewed attention in botanical science, Borchert (1973; 1975) addressed simulations of rhythmic tree growth under constant conditions from the point of view of shoot/root ratio. A tree was considered to consist of three components: shoots, leaves, and roots. Total leaf area determined transpiration, while root size the capacity for water absorption. Growth was considered as a function of available water, and shoot and

leaf growth rates were dependent upon a favourable water balance. Consequently, the length of a growth period depended upon genetic characteristics governing shoot, root and leaf growth, and a set of environmental factors. With respect to the genetic control of growth, Borchert (1975) quoted Greathouse et al. (1971) who demonstrated that clonal material flushed synchronously, while growth flushes of non-clonal material were asynchronous. Moreover, in his review concerned with phase change, Borchert (1976) stated that tree seedlings showed growth patterns that differed from those of adult trees. These conclusions were, however, based on descriptive evaluation rather then quantitative measures of architecture parameters. Consequently, Borchert stressed the need for the development of more sophisticated architecture models to elucidate growth and developmental dynamics of the type reviewed earlier in this chapter.

Since then, a number of different approaches to the modelling of tree architecture have been described (Honda et al., 1997; Michalowicz, 1997). The majority of these models could be regarded as topological systems. However, the model assumptions, modelling unit definitions, and recording systems vary with almost every author, making it difficult to achieve a model categorisation.

Thornley and Johnson (1990) reviewed plant modelling, and also acknowledged the diversity of models and difficulties with categorisation. However, they divided models into three main types. Teleonomic or goal-seeking models examine lower levels of organisation to seek an understanding of higher levels of a system. Empirical models are associated with curve fitting, or applying mathematical formulae to experimental data without seeking or being restrained by the mechanism of the examined system. Mechanistic models, on the other hand, are concerned with the mechanism of the system; they reduce the complexity of the system and contribute to the understanding of it. The latter were sometimes called explanatory models. In their book on plant and crop modelling these authors also examined models dealing with plant architecture and branching, although no model suitable for the purposes of this study was found.

During the process of determining the most suitable model on which to base the botanical tree of *M. excelsa*, a number of different published models were

examined. Particular attention was paid to the model of Lindenmayer (1975), which presented developmental algorithms for multicellular organisms, known as L–systems. Hanan and Room (1997) and Prusinkiewicz et al. (1997) further developed this model. However, at that time the model was not capable of recording dynamic data for the whole tree crown at the meristem level owing to the complexity of such an exercise (Prusinkiewicz et al., 1994).

Similarly, models developed by Honda et al. (1982), Remphrey and Powell (1987) and Godin et al. (1997) were not capable of dynamic recording and the calculation of architecture parameters. In fact, Godin et al. (1997, p.81) stated with respect to the progress of their plant architecture growth modelling that 'based on these results, we can start a second step to consider more macroscopic scales such as the branching system or entire plant'.

De Reffye et al. (1996) introduced a new approach to plant growth modelling using AMAPpara software. The model simulated the architecture and growth of trees. De Reffye and Houllier (1997) believed that in the last few decades plant architecture modelling had developed along two major lines. These were the ecophysiological, which often lacked the structural parameters, and the simulation of virtual plants using morphogenetic models in stable homogeneous environments. Attempts to merge these two approaches into the production of a combined ecophysiological and architectural model were reported, and the work of AMAP (Atelier de Modelisation de l'Architecture des Plantes) presented (de Reffye et al., 1996).

Sinoquet et al. (1997) presented a 3D architecture model of walnut (*Juglans regia)*, including the simulation of leaf irradiance distribution within the crown at the shoot level. Using this model preliminary correlation analyses between shoot diameter and shoot location parameters were performed.

Godin and associates (Godin et al., 1997; Sinoquet et al., 1997; Sinoquet and Rivet, 1997; Godin et al., 1999) based their model on topological coding and 3D digitised recording of whole tree structures in multi-scale tree graphs, simultaneously describing topology, geometry and shoot morphology. Tailor-

written computer software AMAPmod then processed these recordings. Using this model, illustrations of virtual 1-4 year-old apple and 20 year-old walnut trees were presented. Recording methods and architecture analyses were presented. It was concluded, however, that data acquisition in the field was still tedious, because neither topological recording nor spatial co-ordinate measurements were automated (Godin et al., 1999). Moreover, the majority if not all examined parameters in this study were metric in nature. It should be noted that topological parameters of 2D branching structures would be expected to be unitless. Thus, it can be argued that the topological information on the structural connectivity and/or its mechanism extracted using this model would be limited. For example, the number of branches can be regarded only as one vector (size) of the 2D structure and its connectivity. While the number of branches indicates which tree is topologically bigger, it gives no information about the second vector that is needed to express how the branches were organised within the topological concept of a 2D structure. Similarly, Grace et al. (1998) acknowledged their attempt to model *Pinus radiata* branches as being empirical, rather than exploring the mechanism of branching.

In another model, Honda et al. (1982) used a topological ordering system to describe structural position, but also included metric properties, such as shoot length and branch angle. Two types of model of bifurcation were described in this paper. The '*H-model*' or '*Horizontal plane model*' was particularly useful for simulation of plagiotropic branch complexes that were flattened dorsiventrally (Honda, 1971). The '*P-model*', or '*Perpendicular plane model*', was a special case of the '*H-model*', in which the successive branch planes could be rotated at an angle of up to 90^{o} to reproduce spiral or opposite phyllotaxy. Because these geometric models included the branch angle property, the computer simulated tree models became more spatial or three-dimensional than models dealing with topological position only. These models, however, were more concerned with developing the strategies and theoretical basis for plant modelling, rather than with experimental data recording. In this respect, Robinson (1996) reviewed the symbolic framework for the description of architecture models and the 23 models proposed by Halle and Oldeman (1970) that became a 'classic' base for later

architecture model development. New theoretical models for pagoda and monocarpic tree architecture were also proposed in his paper. In a more goal focused and practical approach to plant architecture, several authors have attempted to model the architecture of heteroblastic plants with divaricate juvenile branching endemic to New Zealand to quantitatively distinguish between the juvenile and adult branching characteristics. Tomlinson (1978) attempted to do this by applying Horton's method (Horton, 1945). Horton (1945) expressed mathematically the first and second of 'Horton's' laws by calculating bifurcating ratio as $R_b = N_u/N_{u+1}$ and the segments length ratio as $R_l = l_u/l_{u+1}$, respectively. The bifurcating ratio R_b is calculated from the number of segments N_u, in the order u, and the number of segments N_{u+1}, in order $u+1$. Similarly, the segments length ratio R_l is calculated from the length of segments in a particular, subsequent order N_l/N_{l+1} (Horton, 1945). Although Tomlinson (1978) could not quantify the differences between juvenile and adult states of shrubs, such as *Pennantia corymbosa* and *Plagianthus betulinus*, he concluded that the bifurcation ratio R_b, which compares the number of segments in two subsequent centrifugal orders, increased with branch size and canopy complexity.

Later, similar attempts to quantify the architecture of divaricating and non-divaricating plants were made by Atkinson (1992). Using branch angle, branching order and branching density, Atkinson calculated the divaricating index as an architecture parameter.

Following the earlier work of Tomlinson and Atkinson, Kelly (1994) examined and measured 24 species of New Zealand divaricate and non-divaricate shrubs in order to provide a numerical definition for distinguishing architecture features. He processed measures of leaf shape and arrangements, branch frequency, length, and angles, and number of apices into an architecture parameter index. From this study a numerical index was constructed that correlated well with the perceived degree of divarication. Kelly believed that while his indexing method may not have been an advance on some of Atkinson's earlier work, it worked well for its purpose, and was easier to apply. He pointed out the higher discriminatory power of a branching order variable used by Atkinson, but also the difficulties of measuring branching density using ordering systems.

In a later study focused on phase change, McGowran et al. (1998) evaluated the potential of branch angle, tip diameter and mid-stem diameter as quantitative markers to discriminate juvenile and adult plants. From these architecture-related measures, values of discriminant score (D) were calculated as a parameter of ontogenetic development. Clones of *Quercus robur* from stump sprouts, designated as juvenile, yielded negative D. On the other hand, clones from hedges, grafted onto adult trees yielded positive D, indicating adult status, according to the authors. Multiplication rate and leaf shape did not appear to be suitable markers for juvenility or maturity in *Quercus* species due to high variability among clones. This study illustrated the awareness of the researchers of the limited capacity of models for experimental recording and analysis. This is of particular concern in developmental studies, where detailed observations at the meristem level may be critical.

Summary

The models built by Prusinkiewicz and associates, as well as Godin and his co-workers, appeared to be able to achieve some of the researchers' aims. They were built robustly to accommodate many aspects of architecture and research applications. Nonetheless, their use as a research tools was limited in terms of practical application and data recording technologies.

In terms of model applicability, it seemed that in order to accommodate the reality of a plant canopy, many of these models aspired to capture tree architecture in 3D space. While that was an admirable goal and significant results were achieved, data reduction of the highly complex structural systems through 2D topological parameters was not achieved. The goal of understanding branching mechanisms was somehow replaced by seeking a mechanism of virtual plant imitation. In this respect these models, despite their high level of sophistication, satisfy the criteria of teleonomic and empirical models, rather than those of mechanistic models. As a consequence, researchers are still seeking alternative, ready to use tools to study tree structures. For example, Day and Gould (1997), studying phase change,

attempted to use an architecture model developed for the classification and analysis of dendrite trees (van Pelt and Verwer, 1989). From the literature review presented above and personal advice (P.W. Gandar, pers.comm., 1993), the need to develop a tailored architecture model for the study of phase change in *Metrosideros excelsa* was warranted.

1.2.4.7 Methods for recording dynamic growth and development

Gounot et al. (1989) presented a non-destructive method to obtain dynamic growth curves of fresh weight for individuals of clones of *Dactylis glomerata.* This was a relatively rare example of the use of a repeated measurements. However, it was not suitable for the intended purposes of the current project, owing to the complexity of the planned factorial experiment. Moreover, this method measured fresh weight, which may be good parameter of plant size, but gives no information on crown architecture.

Wilson (1989) studied branching in five broadleaf and conifer tree species. He believed that an order numbering based on the natural branching process (ascending ordering, like Weibel's) would be more useful then Strahler's order numbering system (descending ordering) to record the dynamics of tree growth. Thus, for the purposes of the current study, topological ordering systems increasing with distance from the root were expected to be more suitable than those using Strahler's ordering system.

In forestry, dynamic models have often been used to simulate and predict the growth of tree parts, individual trees or tree stands. For example, Ford (1985) described a model and algorithm for the simulation of branch morphological development, concentrating on defining the optimum length of time for branch and, subsequently, trunk growth. Hasenauer et al. (1998) described regression techniques that simultaneously estimated tree growth parameters, such as diameter, height or crown ratio, in order to model individual tree growth. Ritchie (1999) reviewed the latest available forest growth models and simulators for the

yield of a number of forest species in the USA Pacific coast states. Five different types of yield simulators were distinguished, and two of these modelled individual tree growth. The main emphasis in forestry models was on accuracy of yield prediction rather than on mechanism of tree growth and development. However, these two aims are closely intertwined and thus many of the modelling procedures were common to the aim of this study, particularly the need for dynamic observation. At the same time, none of these models and/or methods for collecting non-destructive measurements was suitable for the purposes of quantifying crown architecture.

Day and Gould (1997) attempted to record the dynamic growth of branches and used architecture parameters based on the Dendrite Tree Model (van Pelt et al., 1989) to study ontogeny in the divaricating New Zealand endemic *Elaeocarpus hookerianus*. 'Divaricating' shrubs exhibit, amongst other attributes, an extreme form of bifurcation. Short and long branches were used as two basic units and centrifugal ordering applied to the branch structure. Day and Gould (1997) noted that there was a lack of quantitative evidence of the effect of environmental factors on the growth and form of divaricating species. Day and Gould (1997) believed that their moderate amount of information suggested the significance of environmental effects, as well as a relationship between ontogenetic state and architecture. However, because of the paucity of the information on the model itself, and the recording system, which involved hand drawing of structures, the use of this method was not suitable for the current project.

Similar constraints associated with the recording method existed in studies of the tree-like branches (dendrites) of tissue-cultured neurons (van Veen and van Pelt, 1992). However, in contrast to all other architecture growth models and methods, the Dendrite Tree Model developed by these authors had well defined topological units, the classification of architecture through topological parameters was mathematically advanced (Verwer and van Pelt, 1986), and numerous dynamic simulations were published in the literature (van Pelt, 1997). Conveniently, the centrifugal order-segment-length relation was also studied through dendrite tree growth simulations, and examples of experimental data analyses were presented (see Section 1.2.4.8 for more details).

Summary

Based on the information gained from the tree modelling literature and driven by the need for dynamic growth recording, the model built for the topological analysis of dendrite trees (van Pelt and Verwer, 1986) was selected. The Dendrite Tree Model was examined as a possible foundation for the dynamic observation of whole crown architecture and the development of a topological model for *Metrosideros excelsa*. The following section provides a detailed study of the Dendrite Tree Model.

1.2.4.8 Van Pelt's topological model and its relevance to botanical tree modelling

During the last three decades a topological model was developed for the observation of bifurcation in dendrite trees of Purkinje neuronal cells (Smart, 1969; Werner and Smart, 1973; Verwer and van Pelt, 1983, 1985, 1986, 1990; Verwer et al., 1987; 1992; van Pelt and Verwer, 1983, 1984a,b, 1985, 1986, van Pelt et al., 1986, 1989, 1992; Uylings et al., 1989; van Veen and van Pelt, 1992; 1993; 1994; van Pelt, 1997; Cannon et al., 1999). This model in its various forms is referred to below as the Dendrite Tree Model. To avoid confusion, the term 'botanical tree' refers to architecture model application in plants.

Of course, the van Pelt Dendrite Tree Model was not developed for the observation of botanical trees. There were, therefore, significant differences in the branching mechanisms between dendrite and botanical trees. However, the basic classification of structures (van Pelt and Verwer, 1984a,b) and the modelling processes are comparable across tree-like structures of different origin (Leopold, 1971; Bell, 1979; Borchert and Slade, 1981; P.W. Gandar, pers.comm., 1993; Day and Gould, 1997).

Compared with other architecture models applied to botanical trees, such as the *L-model* (Prusinkiewicz et al., 1994) or the AMAP modelling system (Godin et al.,

1999), the Dendrite Tree Model had the significant advantage of having been successfully used to collect data, and subsequently carry out statistical analyses based on the expression of architecture parameters (van Pelt et al., 1986; 1989). On the other hand, due to the relatively small scale of the dendrite trees studied by van Pelt and coworkers (up to 100 external segments), the topological measurements relied on manual reading from drawings or microscope slides of the Purkinje cells (Figure 1.3) (Verwer and van Pelt, 1986). However, recording of topological structures through drawings and the manual calculation of architecture parameters was not expected to be feasible for the generally much larger botanical trees.

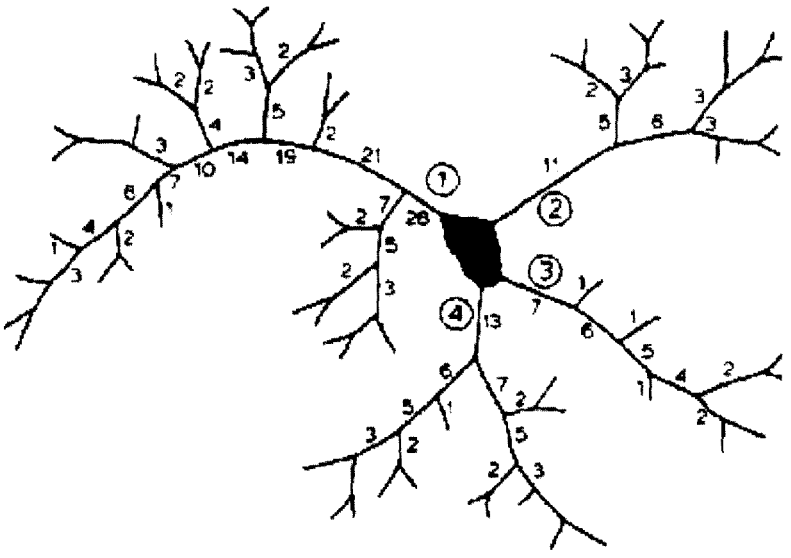


Figure 1.3 An illustration of 'an aspiny human neuron from the human striatum' showing (1-4) dendrites (Reproduced from Verwer and van Pelt, 1986, p 185).

As mentioned earlier, it is common for different terminology and topological units to be used by different authors, even in the same discipline. For example, in hydrological studies Werner and Smart (1973) used the terms 'channel network', 'sources', 'outlets', 'junction', 'exterior' and 'interior' 'links' or 'streams'. Since publications from non-botanical disciplines had some relation to the Dendrite Tree

Model, and thus to the model to be developed in the current study, some of the commoner terms and/or descriptions are reviewed.

Van Pelt and Verwer (1983) used the terms 'intermediate' and 'terminal segments' in the topological study of dendrite trees (Figure 1.4). Later van Pelt (1997) distinguished a number of tree elements such 'branch point', 'terminal tip', 'root', 'terminal' and 'intermediate segments'. In general an 'intermediate segment' in the Dendrite Tree Model represents the part of the structure between two branching (junction) points (Figure 1.4). A 'terminal segment' is the part of the structure of which only one side is connected to the structure at the branch (junction) point, while the other side terminates the structure at or by the 'terminal tip' (Figure 1.4b) (Verwer and van Pelt, 1986).

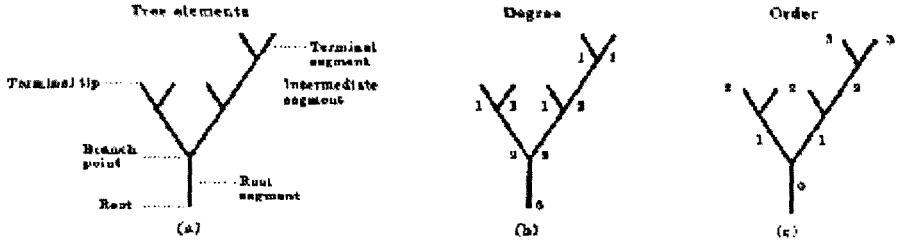
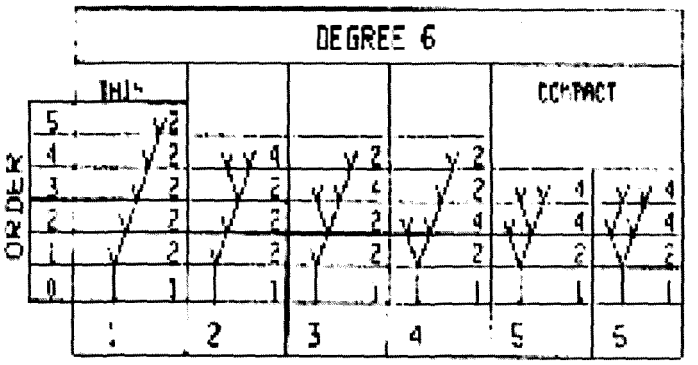


Figure 1.4 Elements of a topological tree. a) Tree elements b) labelling of segments according to degree, i.e. number of terminal segments, and c) centrifugal order . (Reproduced from van Pelt, 1997, p. 19).

In the initial stages of the development of the Dendrite Tree Model, Werner and Smart (1973) and Uylings et al. (1989) specified the *centrifugal order system* (Weibel, 1963) of topological tree elements (points or segments) as the number of *segments* between that element and the root point (Figure 1.4c).

Van Pelt et al. (1986) specified the tree *degree* as being the number of *terminal segments* (or equivalent *terminal tips)* in a tree or sub-tree, and denoted tree degree with a number, *n.* A bifurcating (or binary) tree of degree *n* therefore has *n-1* intermediate segments, and a total number of segments equal to *2n-1,* which can be used to denote the tree topological *size* (Figure 1.5). In this respect it was

noted that both tree *degree* (van Pelt, 1997) and total number of segments (van Pelt et al., 1989) was used to express tree *size*. The wording may be confusing for the reader but the decision to use either parameter to express topological *size* is arbitrary as the value will always be equal to *2n-1* or *vice versa* for *n*.



Figures 1.5 Assignment of centrifugal order to the segments of six trees of degree 6 (terminal segments). The number of segments at each order is identified. (Reproduced from van Pelt et al., 1989, p. 514).

Following the application of this centrifugal ordering system (Weibel, 1963), van Pelt and Verwer (1984a,b) developed a simple method of grouping trees of the same degree. In a further development of the Dendrite Tree Model, Verwer and van Pelt (1986) attempted to classify different types of dendrite tree structures in order to evaluate and analyse structural differences between trees, and thus define architecture parameters. However, the number of tree types became unacceptably large for trees of degree higher than about 11. Later, van Pelt et al. (1992) showed that about 10^{192} tree types exist for trees of degree 500, and concluded, therefore, that tree type frequency distribution was not appropriate for statistical analysis of tree architecture. In other words, too many different types of tree within each size group exist, preventing sensible statistical analysis that would distinguish between types or even across tree sizes.

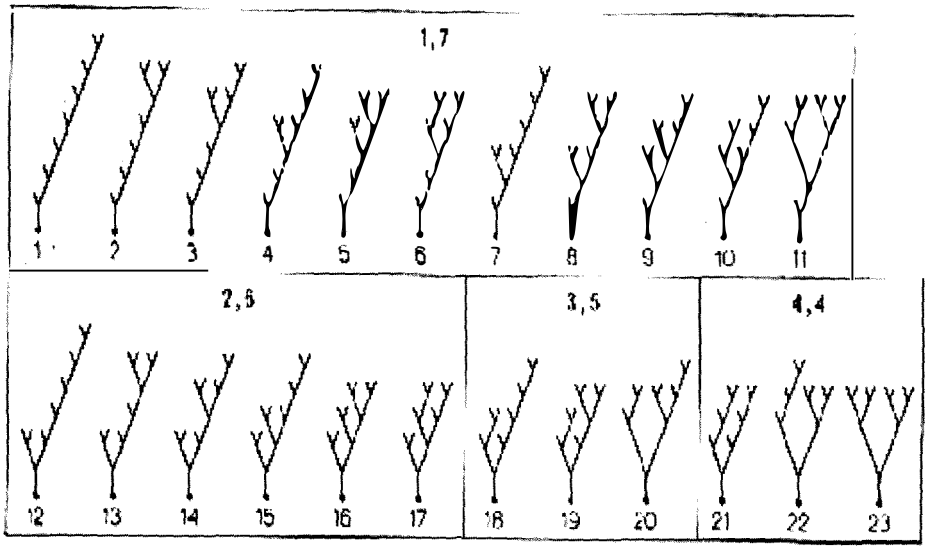


Figure 1.6 Display of all ambilateral types of tree degree 8. The blocks in the figure are labelled by (r,s) denoting the degrees of first-order subtrees with r≤s. (Reproduced from van Pelt and Verwer, 1983, p. 274)

From these basic structural specifications (Figure 1.6) followed the definition of a single number for complexity within a particular tree size called the *Mean Centrifugal Order Number* (van Pelt et al., 1986, 1989). With the need for statistical analysis of tree structures across tree sizes, a number of attempts were made to develop a parameter that would be less size-dependent, such as *Partial Tree Asymmetry* (van Pelt et al., 1992), from which *Tree Asymmetry Index* was calculated (van Pelt, 1997). To further the ability of the model to analyse and compare tree structures across sizes, methods of comparison using tree complexity against the tree degree (size), branching probabilities, and branching modes were also attempted (Verwer and van Pelt, 1986; van Pelt et al., 1989; Carriquiry et al., 1991; van Pelt et al., 1992; van Pelt 1997; Cannon et al., 1999). Some of these parameters, such as degree, Q-S model or tree asymmetry, were used for tree architecture growth simulations (van Pelt et al., 1992) and lately also for tree pruning simulations (van Pelt, 1997). Some of the more important Dendrite Tree Model parameters and methods for tree analysis are reviewed in more detail later in this section.

With respect to the adaptation of the model to the trifurcating botanical tree, it was noted that some attempts to adjust the Dendrite Tree Model for the occurrence of

trifurcating branching had been made by Berry et al. (1986), Sadler and Berry (1988) and Werver and van Pelt (1990). However, trifurcation was resolved only by regarding it as a close distance bifurcation event. Thus, the basic model assumption of bifurcation remained the same (Werver and van Pelt, 1990). However, the latter authors also applied the method of link-vertex analysis (Horsfield et al., 1987), which differs slightly from the original van Pelt model by focusing on the description and recording of junction points as opposed to the use of links-segments as basic units (Figure 1.7). MacDonald (1984) argued that there was significant statistical difference between segment or vertex systems of recording.

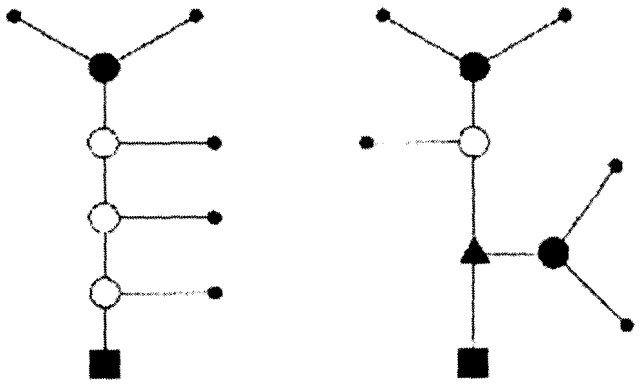


Figure 1.7 An illustration of a link-vertex recording system.
Terminal segments are represented by small dots, topological nodes by large dots, circles and triangles, and roots by squares. Both trees have $V_t = 5$ terminal segments. However, due to the differences between the segment–vertex systems, that on the left has $V_a = 1$, $V_b = 3$, and that on right has $V_a = 2$, $V_b = 1$ (Reproduced from MacDonald, 1984)

1.2.4.9 Architecture parameters

For every structural model, architecture parameters have to be designed so that they condense the collected architectural data into a size amenable to analysis, at the same time as retaining most of the architectural information gained through the model. This is a central feature to the process of architecture modelling

(Thornley and Johnson, 1990). The extraction of architecture parameters from a model is essential for the comparative analysis of different tree architecture types, and for examining the effect of experimental factors on tree architecture (van Pelt and Verwer, 1984a,b; Verwer and van Pelt, 1986). Some of the important parameters and methods for tree comparisons of the van Pelt Dendrite Tree Model are reviewed in more detail below.

Mean centrifugal order number

For the purpose of analysing tree architecture for larger data sets, van Pelt et al. (1989) introduced and described the parameter *mean centrifugal order number* $\overline{\gamma_\alpha}(n)$ as:

$$\overline{\gamma}_\alpha(n) = \frac{1}{2n-1}\sum_{\gamma=0}^{\gamma_m} \gamma s_\alpha(\gamma) \qquad (1.1)$$

where n is the tree degree (number of terminal segments), γ_m its highest order (highest topological distance between the root segment and the segment in the last centrifugal order), and $s_\alpha(\gamma)$ is the number of segments at order γ.

The authors also distinguished and mathematically defined the two architectural extremes of a *Thin Tree*, which always branches from only one point of a possible bifurcation, and a *Compact Tree*, in which the number of segments always doubles with each increase in order. For a particular tree degree, any other tree type would be expected to have a value for mean centrifugal order number between those of the *Thin* and *Compact Trees.*

The equations for these extremes were also derived (van Pelt et al. 1989). The mean centrifugal order number for the *Thin Tree* was expressed as :

$$\overline{\gamma}_{thin}(n) = \frac{1}{2n-1}\sum_{\gamma=1}^{n-1} 2\gamma = \frac{n(n-1)}{2n-1} \qquad (1.2)$$

And that for the *Compact Tree* was:

$$\bar{\gamma}_{compact}(n) = \frac{1}{2n-1}\left\{\sum_{\gamma=0}^{\gamma_m-1}\gamma 2^{\gamma} + \gamma_m\left(2n - 2^{\gamma_m}\right)\right\} \tag{1.3}$$

Partition asymmetry and Tree Asymmetry Index

In other publications the van Pelt group (van Pelt and Verwer, 1986; van Pelt et al., 1992; van Pelt, 1997) described the measure of *partition asymmetry* (A_p) as the absolute difference between the degrees of two sub-trees, divided by the absolute value of the maximal possible difference:

$$A_p = \frac{|n-2r|}{|n-2|} \tag{1.4}$$

or

$$A_p(r,s) = \frac{|r-s|}{r+s-2} \tag{1.5}$$

where n is the number of terminal segments of the whole bifurcation, r and s are the numbers of terminal segments of each sub-tree, and $0 \leq A_p \leq 1$.

An overall *Tree Asymmetry Index* (TAI) (van Pelt, 1997) can be calculated as the mean value of all the partition asymmetries in a tree. The Tree Asymmetry Index parameter provides important information about the tree architecture. Moreover, this parameter is almost independent of tree degree (and thus also of size) (Figure 1.8), and hence is suitable for comparisons between trees of different size (Verwer and van Pelt, 1986). The TAI can be zero (for a completely symmetrical bifurcating tree), and have values that approach unity for a completely asymmetrical tree (Figure 1.8).

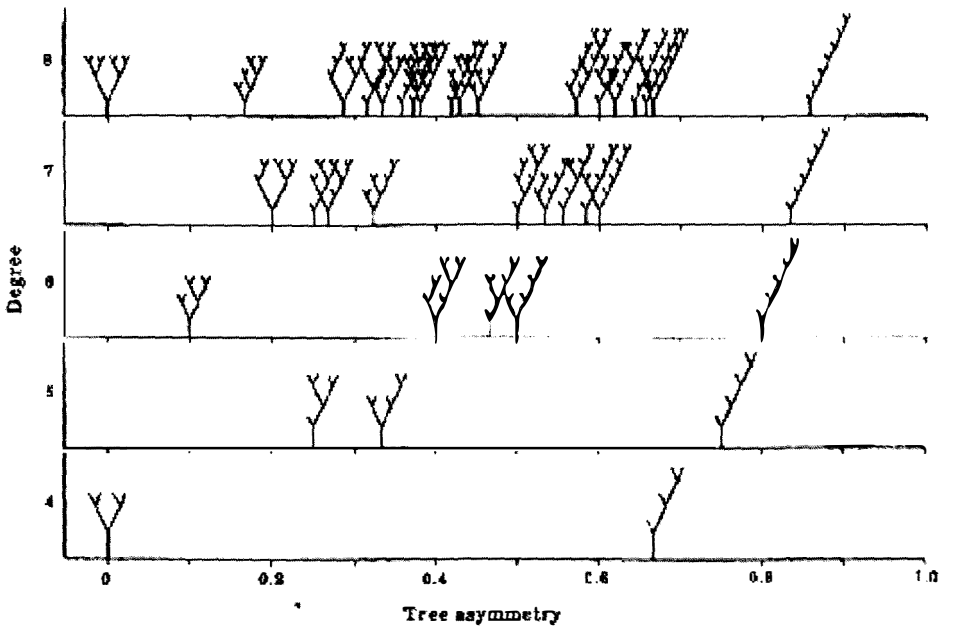


Figure 1.8 Illustration of Tree Asymmetry Index. Tree types with 4-8 terminal segments have been plotted against the value for the Tree-Asymmetry Index. (Reproduced from van Pelt, 1997)

Proportional sum of absolute deviations

Similar in properties to the Tree Asymmetry Index, Verwer and van Pelt (1986) introduced the parameter of *Proportional Sum of Absolute Deviations* (PSAD). This expresses how far the degree of each sub-tree deviates from the mean degree of the whole tree, and can be calculated as:

$$PSAD = \frac{m}{2(m-1)(n-m)} \sum_{i=1}^{m} \left| r_i - \frac{n}{m} \right| \qquad (1.6)$$

where m was the number of sub-trees (which is equivalent to the number of branching points); n is the degree of the whole tree, and r_i is the degree of the i-th sub-tree. This parameter calculates the whole tree asymmetry directly, as opposed to the partition asymmetry (A_p), which assesses the asymmetry of each sub-tree separately. Then the overall *Tree Asymmetry Index* can be expressed as the mean of each sub-tree asymmetry (van Pelt, 1997).

Because of the calculations, these two asymmetry parameters, TAI and PSAD, differ in value when the same tree is evaluated, and thus provide complementary information about the tree's architecture (Verwer and van Pelt, 1986). It is important to note that the partition asymmetry (A_p) calculations as presented by van Pelt and Verwer (1986) were based exclusively on trees exhibiting bifurcating branching. These would need to be adjusted for use in a trifurcating branching tree. On the other hand, the PSAD parameter was extended, according to the authors (Verwer and van Pelt, 1986) from bifurcating partitions to multiple partitions, and thus became partially independent of branching pattern.

Q-S growth modes

Van Pelt et al. (1986) claimed that a tree model is complete when the branching probabilities of all segments are specified. For this purpose they distinguished the branching probability based on segment *type* (internal or terminal), denoted *Q*. Similarly, the probability of branching with respect to the topological distance from the root, i.e. the effect of centrifugal order, was denoted *S*. These authors referred to architectures developing with such branching probability patterns as growth "modes".

In the same publication, the order distribution and its dependence on growth model were also discussed. Two growth models were recognised. Firstly, *random segmental growth* in which the branching probability (*Q*) depends on segment (sometimes called link) type, i.e. branching from either internal or terminal segments randomly through all centrifugal orders. And *random terminal growth* in which branching occurs from terminal segments only, and the probability of branching (*S*) is affected by the segment position in a particular centrifugal order of the tree. The overall branching probability, and thus the growth model, could then be expressed in terms of Q-*S* values.

In the Dendrite Tree Model (van Pelt and Verwer, 1985; van Pelt et al., 1986) separate branching probabilities for the internal segments (P_i) and for the terminal segments (P_t) were calculated. The parameter Q was then defined as:

$$Q = P_i/(P_i + P_t) \tag{1.7}$$

The branching probability depending on centrifugal order (S-mode of branching) was then expressed as the probability of branching from terminal segments (P_t) as:

$$P_t = C.2^{-S\gamma}, \tag{1.8}$$

where γ is centrifugal order, and C is a normalisation constant (van Pelt and Verwer, 1985; van Pelt et al., 1986) that adjusted the branching probability to the range from 0 to 1.

And for the probability of branching from an internal segment (P_i),

$$P_i = R.P_t, \tag{1.9}$$

Where R, in connection to the branching mode dependent on segment *type* (Q), is then expressed as:

$$R = Q/(1-Q). \tag{1.10}$$

In the extreme case called the *Q-model* (where $S = 0$), if also $Q = 0$, then $P_i = 0$, and thus only terminal segments can branch independently of centrifugal order. For $Q = 0.5$, branching probability is equal from all segments, and so, in the *S-model* (where $Q = 0$), branching depends only on the centrifugal order, and branching occurs from terminal segments only. Positive values of S then imply that the branching probabilities decrease with topological distance from the root (with increasing centrifugal order), while negative values of S mean that probabilities of branching increase with topological distance from the root.

Van Pelt (1997) used the *Q-S* model and the tree asymmetry index for extensive simulations of dendrite tree architectures under different simulated pruning regimes and *Q-S* growth modes.

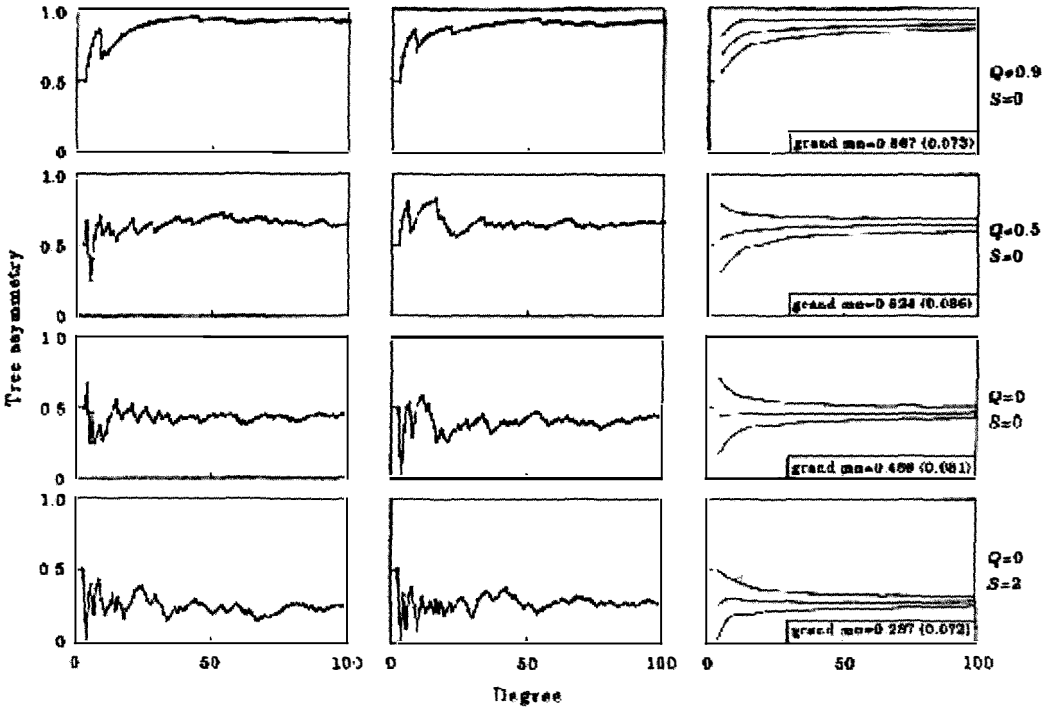


Figure 1.9 Computer simulated effects of Q-S growth modes on the Tree Asymmetry Index. The effects of four different growth modes (shown at right) on Tree Asymmetry Index in (first two columns) two individual trees. The third column shows the (smoothed) curves for the mean and standard deviation intervals for the asymmetry values obtained by repeating the growth process 100 times, thus for a population of 100 trees per degree. (Reproduced from van Pelt, 1997, p. 21).

The parameter of Tree Asymmetry Index is representative of the tree branching probabilities in relation to both the changes with centrifugal order and probabilities of segment branching (Figure 1.9) (van Pelt, 1997). This is a highly desirable feature for any architecture parameter, since a single number reflects the tree architecture. Consequently, such a parameter could be used for tree architecture analysis, and was essential for the current project.

1.2.5 Metric qualities of topological structures

While topological expression of plant architecture allows significant structural information of the plant to be captured, other measures can be required to comprehensively examine the effects of experimental factors, such as environment or those of genetic origin. One such measure is segment length, i.e. the metric length of internodes (Room et al., 1994) and its correlation to the topological complexity of the crown. However, accommodation of this length measure with the topological parameters of structural complexity is not a straightforward exercise, because a topological model usually omits metric properties by definition (e.g. in the Dendrite Tree Model; van Pelt et al., 1989). Therefore, approaches others have used for such an accommodation were examined in the available literature. The relevance of the analysis of segment length and methods to achieve such an analysis were then judged with respect to the aims of this study.

Horton (1945) examined the relationship between topological order and the length of topological segments for drainage basin structures. Horton's Second Law (Horton, 1945 quoted by Tomlinson, 1978), states that the average length of streams (segments) of a given order forms a constant geometric series with stream order. Tomlinson (1978) found that this law did not hold for New Zealand divaricating plants. However, the branch length ratio was not constant in divaricates, and indications were that it may be more stable in the adult state of trees.

Park (1985) applied Horton's Second Law in the analysis of topological data records of colonies of mycelial fungi. Strahler's ordering system, i.e. descending order number with increasing topological distance from the root segment, was used. Park concluded that, the length of branches tended to form a direct arithmetical, not geometrical, series with the branch order. He showed data of Barker et al. (1973) that also resulted in an arithmetical rather than a geometrical series of mean branch length with the branch order in the botanical trees of apple and birch. On the basis of further simulations, it was concluded that only when the

growth rate declined sufficiently steeply in a geometric fashion, would the mean branch length per order tend to form a direct geometric series with branch order (Park, 1985).

In another type of study, working on populations of twigs in five tree species (*Quercus coccinea, Acer rubrum, Picea rubens, P. abies,* and *Pinus strobus)*, Wilson (1989) examined the correlation between the length of parental branches and twig lengths. Although the examined tree species were different in their overall form, their branches had similar twig length distributions. In all apart from *Acer rubrum,* the twig length generally increased the further it was attached from the terminal twig, which could be considered as consistent with Horton's Second Law. The author pointed out that similar twig length distributions occurred in different species despite their very different rates of growth. He speculated that different growth rates give particular species (the fastest) an advantage under certain environmental conditions. In this respect, it was interesting to note that differences in growth strategies from the ecological point of view were also associated with the juvenile ontogenetic state (Waring, 1983; Borchert and Tomlinson, 1984; Hackett, 1985; Greenwood, 1989; Hansen, 1996; Hattenschwiler et al., 1996; Hattenschwiler and Koerner, 1996;.

Cheetham et al. (1980) examined relative growth rate in colonies of arborescent cheilostome bryozoans (*Cystisella saccata*). Growth rate was calculated from measures of the length of paired branch proportions ending in growing tips (relative growth ratio), and the length of branches between bifurcations (mean segment length and segment-length ratio measures), and concluded that relative growth was species-specific. These authors believed that although growth properties between taxonomic groups differed, models based on common properties could be applied to botanical trees as well as to arborescent animals, quoting Halle and Oldeman (1970) and their work on botanical trees for comparison. These findings highlighted the possible importance of changes in segment length in subsequent topological orders.

Using the Dendrite Tree Model, but Strahler's ordering, Woldemberg et al. (1993) examined the relationship of Purkinje neuron age with lengths of topologically

defined segments. The analysis of geometric mean length of links indicated that they followed a Fibonacci series, thus demonstrating the importance of the length-topological mechanism of biological structures.

Crawford and Young (1990) in a study on two species of oak (*Quercus*) used the Weibel centrifugal ordering scheme (Weibel, 1963), i.e. ascending order with topological distance from root segment. They found that a simple algorithm that exhibited power-law behaviour governed the distribution of branch length with respect to order. From further study of the botanical tree structures, they pointed out that genetic factors rather than factors of environmental origin were responsible for the structural design. With regard to the branch length and topological distribution, they quoted a number of attempts to relate the geometry of botanical trees to the strategies to maximise light interception (e.g. Honda et al., 1981; Borchert and Tomlinson, 1984), to optimise mechanical stability (McMahon and Kronauer, 1976) or both (Niklas and Kercher, 1984).

Crawford and Young (1990) claimed that most models of tree structure adopted an empirical rather than a mechanistic approach. Empirical models were species-specific, and thus more descriptive than informative. From their mechanistic approach, the authors concluded, it appeared that some basic principle of organisation and evolutionary stability could exist that may be common to many complex biological structures.

Summary

The mechanism governing segment length in biological structures of dynamically increasing complexity has received significant attention in the literature. Different methods for the incorporation of metric properties into topological models of biological structures were examined, and a comparable method was later developed for shoot length-topological order analysis in the Metrosideros Model.

1.2.6 Mathematical and dynamic modelling of growth and development

1.2.6.1 Data suitability

In order to model plant growth mathematically, parameters need to be measurable in a non-destructive manner, so that the dynamics of growth can be reconstructed and analysed with respect to the independent variable of time. The Dendrite Tree Model (van Pelt, 1997; van Pelt et al., 1989) addressed tree architecture through topological recording and parameter expression. Previously researchers had used repeated measurements of shoot length (Karlsson and Heins, 1994) or of fresh weight of clonal sympatric (growing under the same conditions) replications (Gounot et al., 1989). These were used to reconstruct and analyse the dynamics of plant growth through the fitting of repeated measurements to a sigmoid function. In forestry where dynamic growth observations are used for modelling and yield forecasting, for example, the stem diameter and height of a tree were commonly used (Zeide, 1993a,b; Ritchie, 1999).

With respect to the units of plant growth measurement, Porter (1988) argued that plant growth is not only expressed as an increase in plant mass or some other continuous variable, but also involves changes in the number of plant parts. This author showed that study of the production of modular plant parts is more relevant to the quantification of the architecture of whole plants than conventional growth analyses (Porter, 1988). Moreover, Thornley and Johnson (1990) argued that if growth parameter functions are defined so that they are biologically meaningful, the process of growth modelling may have significant value in plant and crop research in its own right. The deterministic approach to growth modelling, as opposed to the empirical, can thus go beyond being just a test of the statistical accuracy of fit to the data (Thornley and Johnson, 1990).

1.2.6.2 Mathematical functions of biological growth

A number of mathematical functions are available to describe biological growth. For comprehensive reviews, the reader is referred to Hunt (1982), Ratkowsky, (1990), Thornley and Johnson (1990) and Zeide (1993b).

Due to the sigmoid function of plant growth (Hunt, 1982), relative growth rate changes during the growth period. While the first derivative of the biological growth function, i.e. the growth rate, is always positive (Richards, 1959), it is not linear. Thus, any comparative analysis between individual plants should be done in a time dependent manner (Thornley and Johnson, 1990). This feature can make comparative statistical analyses intricate when dynamic measurements of more than one plant, or even of a shoot representing the growth of one plant, is concerned (Hunt, 1982; Zeide, 1993b).

The possibility exists of analysing plant growth through its growth rate over time by comparing the so-called intrinsic or inherent growth rate coefficient (Richards, 1959; Thornley and Johnson, 1990). The value of the growth rate coefficient depends on the size at time zero (Richards, 1959), the shape of the function, and the value of the upper asymptote, i.e. the final size of the plant.

Consequently, the estimated values of the growth rate coefficient are not strictly time dependent as is the actual relative growth rate. Thus, the growth rate coefficient could in some instances be more suitable for growth rate comparisons of repeated measurements made during growth than instantaneous relative growth rate calculated at a certain point of growth. This feature of the growth rate coefficient could be particularly applicable in developmental studies of genetically homogeneous or related material where examination of intrinsic differences rather than different underlying functions of growth may be more appropriate. This feature of the inherent growth rate coefficient, to our knowledge (P.W. Gandar, pers. comm.) has not been recognised. Consequently, no reports were found using this coefficient for analysis of growth.

The preciseness of the estimation of the growth rate coefficient depends on selection of the growth function, the fitting procedure, and the number of points in the experimental data set used for function fitting (Zeide, 1993b). Notwithstanding the previous criteria, it would not be impossible to construct an equation that would go through all data points. However, the value of such a contribution to modelling without biologically meaningful parameters would be questionable (Thornley and Johnson, 1990).

Ratkowsky (1990), in his book on non-linear regression models, reviewed a vast number of one- to five-parameter growth functions from the point of view of their mathematical and statistical characteristics. Because of the use of the three-parameter function of Chapman-Richards in this project (Chapter 4), it should be mentioned here, that Ratkowsky (1990) argued that the Richards growth model, from which the Chapman-Richards function was derived, had high intrinsic non-linearity that made the function less suitable for plant growth modelling. On the other hand, Causton and Venus (1981) used the function and obtained satisfactory results, e.g. for predicting sunflower harvest data. The use of data sets with an insufficient number of data points, rather than to the unsuitability of the function in itself, could lead to unrealistic estimates of growth parameters (Ratkowsky, 1990).

Zeide (1993b) reviewed a number of available growth functions, and discussed the advantages and shortcomings of plant growth modelling for each specific function. He concluded that growth functions, when properly applied, have an important role as research tools and predicted that their use would increase with the increasing availability of computer modelling power. In defining the basic assumptions of growth models, Zeide (1993b) quoted Medewar and Medewar (1983) and their two basic postulates of biological growth:

1. Fundamentally, growth is multiplicative. That which results from biological growth is itself, typically, capable of growing.

2. Relative growth is always decreasing (Minot's law).

In the ontogenetic literature (or that concerned with ontogeny), the second postulate has been used, often subconsciously, by authors to distinguish the growth of the juvenile plant from that of the adult, the greater 'vigour' of the former being assumed without experimental quantification. This was pointed out, for example, by Snowball (1989). Over the period of a plant's life-span this assumption may be correct. However, any direct correlation of ontogenetic phase change and a particular point of decreased growth rate, either at the overall level or within a particular growth period (flush), was absent from most studies, including those of Borchert (1976) and Borchert and Tomlinson (1984), which were otherwise comprehensive juvenile-adult growth comparisons.

Moreover, it appeared that misinterpretations of the second postulate were often projected into the discussion regarding ontogenetic changes *versus* physiological ageing (Fortanier and Jonkers, 1976). Intuitively, both cases involve growth rate decrease. However, by examining the biological growth function, three points can usually be identified where the growth rate changes distinctly (Richards, 1959; Ratkowsky, 1990; Thornley and Johnson, 1990; Zeide, 1993b). None of these points has yet been related to the phase change when the life-span of the plant was concerned. In this respect the feature of decreasing relative growth rate is a continuous process as is the process of physiological ageing, of which ontogenetic phase change could be regarded as a particular period, during which more abrupt changes of growth characteristics could be expected.

Similarly, comparisons of biological growth, whether modelled as separate growth periods, seasonally or over the life-span of the plant, are not frequent in the literature, and their relation to plant ontogeny appeared to be even more infrequent (Hanzawa and Kalisz, 1993). For example, Pearson et al. (1994) in their model of the effect of temperature on the growth and development of cauliflower (*Brassica oleracea)* did not consider juvenility 'as this is still a poorly understood phenomenon.'

A mathematical approach to the investigation of the maturation process, therefore, provides a distinct opportunity to develop a new tool for research (Thornley and Johnson, 1990).

1.2.6.3 Mathematical growth modelling and the expression of size

With respect to the expression of tree size, the module-based models of crown growth (such as those based on the meristem) (Porter, 1988; Room et al. 1994) should represent biological growth in a more mechanistic manner (Thornley and Johnson, 1990) than, for example, observations of single shoot growth, as studied by Karlsson and Heins (1994) in chrysanthemum *(Dendranthemum grandiflora)*. Consequently, models based on crown modules should allow for more comprehensive analyses and conclusions about plant growth and development.

If the basic assumptions of different models for measuring and describing size are biologically sound, then derived experimental data should yield sigmoid curves, even though the shape of the growth function would be expected to vary with the measured units of size (Hunt, 1982; Thornley and Johnson, 1990).

In the module-based models, the shape of the growth function will be affected by the increasing numbers of the model modules (segments), as well as by the timing of the growth of individual shoots (e.g. bud break), and their synchronisation within the canopy. With respect to plant ontogeny, synchronisation of shoot growth within the crown or between individual trees was reported to vary between developmental states (Borchert, 1973; 1976; Tomlinson, 1978; Bortchert and Slade, 1981; Borchert and Tomlinson, 1984; Fernandez and Wagner, 1994).

Summary

From the information contained in the literature concerned with plant architecture modelling, it appeared that a model based on topological units (modules) at the level of the meristem would be desirable. It would then be feasible to use such a model to record the architectural growth in *Metrosideros excelsa* trees in a dynamic manner. Further, it would be warranted to use experimental data from such a model to test its biological suitability to represent plant growth. If biological suitability were confirmed, the model would be used to study the effect

of ontogenetic state on both the dynamics of crown growth, and detailed architectural parameters.

1.2.6.4 Applications of dynamic growth models

In order to assess the current state of dynamic growth analysis and examine how others have applied growth functions, the relevant literature has been reviewed in this section.

To evaluate variation in the pattern of lateral shoot elongation in larch hybrids (*Larix kaempferi x decidua* and *L. laricina x decidua*), Baltunis and Greenwood (1999) successfully fitted shoot elongation data over 120 days with the three-parameter Weibull-type of growth function (Zeide, 1993b). The estimates of the function parameters were calculated for each tree, and then the time of growth initiation, the time of the start of linear growth, growth duration, and growth rate were analysed for the hybrid effect. Further analyses between individual growth parameters were also performed in order to assess correlation between the parameters. Amongst other things, they concluded that the greatest growth and growth rate were correlated with late cessation of elongation.

Karlsson and Heins (1994) modelled the growth of chrysanthemum (*Dendranthemum grandiflora*) using the three-parameter Richards function (Richards, 1959), applied to a set of five randomly selected lateral shoots. From the modelled growth parameters of relative maximal shoot length, the shape of the growth curve and mean relative growth rate were calculated. The results of the model were used to forecast the time from apical shoot pinching to flowering in this greenhouse crop.

Some architecture parameters, but also dynamic elongation of shoots in young trees of *Cedrus atlantica* were recorded weekly and modelled at the end of the growing season by Sabatier and Barthelemy (1999). The modelled growth parameters, such as final shoot length, duration of growth and growth rate, were

related to the total number of sylleptic shoots produced, and their position within the tree crown. It was suggested that type and number of axillary shoots were related to the final length and extension rate of parent shoots. On the other hand, it was concluded that the date at which a shoot started to elongate was not related to its architectural position, while daily growth rate and final length decreased with branching order.

Pearson et al. (1994) used the so-called thermal time to model curd growth rate, and to determine the optimal temperature for growth and development of cauliflower. These authors also mentioned the juvenile period and the gap in our understanding of phase change and the insufficiency of its quantitative expression beyond the requirement of a certain number of leaves. Elaborating further, they hypothesised that the relative growth rate declines throughout ontogeny as a linear function of thermal time. Examining juvenility in cauliflower as a function of leaf number, Hand and Atherton (1987) concluded that the chronological duration of juvenility was strongly influenced by temperature.

Summary

In reviewing the literature, no growth modelling using architecture parameters was found. Most studies examining dynamic growth in plants deal with size expression by measuring stem diameter or other continuous variables, such as length. There were a number of growth models that forecast tree growth in forestry using established modelling practices, predominantly from repeated measurements of stem diameter and tree height. Despite some similarities between the growth modelling methods, these models were not reviewed in detail here because the aim of forestry dynamic growth models was to empirically forecast yield, as opposed to the mechanistic modelling of growth in order to capture growth differences in relation to ontogenetic development.

1.3. Research aims and objectives

The successful development of a new crop to satisfy an identified market often relies on a good understanding of developmental and physiological processes, which in turn allows for predictable and effective management of the desired features of the crop. In the case of *Metrosideros excelsa*, effective propagation of selected varieties has been achieved through *in vitro* techniques. However, this brought with it undesirable rejuvenation, manifesting itself in juvenile growth habit and leaf morphology, and loss of competence to respond to florally inductive signals.

The aim of this research was to explore the possibility of identifying a quantitative marker or markers signalling or determining the onset of the adult ontogenetic state, through examination of leaf morphology, carbon isotope discrimination and tree architecture parameters.

It was the intention to quantifying carbon isotope discrimination in the leaves of juvenile seedlings, rejuvenated plantlets, and adult plants. It was hypothesised that changes in leaf morphology occurring during phase change in *M. excelsa* plants would affect leaf gas exchange processes, which would be reflected in the degree of carbon isotope discrimination (O'Leary 1981; Farquhar et al. 1983).

It was also an objective to explore tree architecture as a marker of phase change by developing a model that would allow the architecture of a tree crown to be recorded quantitatively, and the changes in architectural parameters to be calculated and analysed as growth occurred. It was hypothesised that by collecting architectural growth data from the plants in the three ontogenetic states, and by modelling and analysing the changes occurring in crown architecture parameters, phase change could be tracked with respect to growth rate, size and complexity parameters.

If this were the case, then it would be possible to test the Size/Complexity Hypothesis of phase change, that juvenile (and rejuvenated) plants must attain a certain size and/or architectural complexity threshold before passing to the adult ontogenetic state.

The objective of identifying the usefulness of carbon isotope discrimination and tree architecture as markers of phase change was examined under a range of temperature conditions. This was in order to gain an indication of the temperature that would be optimal for advancing phase change in juvenile plants and rejuvenated plantlets.

CHAPTER TWO

Carbon isotope discrimination as a marker of phase change

2.1 Introduction

The ratio between the carbon isotopes ^{12}C and ^{13}C in the leaf depends on physiological and biochemical processes associated with CO_2 acquisition (Farquhar et al., 1989). It could, therefore, be indicative of the developmental state of the plant, since morphological, physiological and biochemical changes were reported to occur in relation to phase change, as shown from the relevant literature (Chapter 1). If carbon isotope discrimination showed potential as a quantitative marker of plant developmental state, the data could be used to model the dynamics of the phase change process. In such a way the method could become an effective tool for determination and forecast of phase change, and thus contribute to the elucidation of phase change phenomena.

Foliar carbon isotope discrimination was measured in *Metrosideros excelsa* plants of distinct developmental state (juvenile seedlings and adult plants that had flowered in earlier seasons), and for plants exhibiting juvenile morphology that had been rejuvenated by micropropagation (plantlets). It was hypothesised that leaves of juvenile plants would exhibit greater discrimination against ^{13}C than those of adult plants, reflecting higher stomatal conductance and lower long-term water use efficiency (see Section 1.2.3). It was expected that values for discrimination in juvenile plants would approach those of adult plants if they were to progress towards the adult state, and that that of adult plants would remain stable. It was further hypothesised that plantlets would exhibit a discrimination signature intermediate between juvenile and adult plants. To alter the rate at which the adult state might be attained in juvenile plants and plantlets, plants were grown under four contrasting temperature regimes.

These hypotheses were based on reports of carbon isotope discrimination in wild populations of juvenile and adult plants growing in the field. However, in these field studies, adult plants exploited soil horizons in a manner consistent with their

long-term drought resistance and survival, whereas juvenile plants tended to exhibit high stomatal conductance and transpiration rates. Nor had adult and rejuvenated plants of the same genotype been contrasted in a study of this type. At the time of the current study, reports that directly associated changes in carbon isotope discrimination with ontogenetic phase change in container grown plants under well-watered conditions had not been published.

To test these hypotheses the following objectives were:

To determine whether differences exist in carbon isotope composition ($\delta^{13}C$) in leaves of seedlings and adult plants, and plantlets of *M. excelsa*.

To examine whether and how leaf $\delta^{13}C$ correlates with phase change in juvenile plants and plantlets, the latter being assessed by quantification of changes in leaf morphology.

To determine how carbon isotope composition and leaf morphology are affected by temperature.

2.2 Material and Methods

2.2.1 Plant material

Juvenile seedlings, adult plants and plantlets (arising from micropropagation of adult *M. excelsa*) were used. Seedlings were derived from open-pollinated parent plants. They were 10 months old from germination, exhibited juvenile leaf morphology, had not flowered, and were not expected to flower for several years. Adult plants were derived from rooted cuttings taken from adult trees of *M. excelsa* cv. Scarlet Pimpernel. They were 22 months old from rooting, exhibited adult leaf characteristics, and had flowered the previous year. Plantlets were micropropagated plants of *M. excelsa* cv. Scarlet Pimpernel. They were in a rejuvenated state, exhibited juvenile leaf morphology, and had not flowered.

Seedlings and plantlets were supplied by Lyndale Nurseries Auckland Ltd. Adult plants were supplied by Duncan & Davies Nursery Ltd., New Plymouth. A bark-based potting mix with controlled release fertiliser was used for growing the plants.

Four weeks before the experiment commenced, the plants were re-potted from their original 0.5 litre square plastic pots (seedlings and plantlets), and four litre plastic bags (adult plants), to 4.5 litre square and six litres round plastic pots, respectively. Whole root balls were transferred with minimal disruption into new pots, which were filled with North Carolina potting mixture consisting of gravel 'pea metal', vermiculite and pumice (70: 15: 15 v/v). This mixture allowed for the drainage of surplus water, and provided good root aeration in each treatment during the experiment. After re-potting, the plants were held in a shade house on the premises of the Horticulture and Food Research Institute of New Zealand (HortResearch), Palmerston North for two and half weeks before experimentation began.

2.2.2 Temperature treatments and growth conditions

Four rooms in the National Climate Laboratory, HortResearch, Palmerston North were used to apply four temperature regimes (Table 2.1) upon the plants of the three developmental states.

Table 2.1 Treatment temperature regimes and corresponding relative humidities.

Temperature regime (Day/night °C)	Relative Humidity (%)
32/8	87/66
32/24	87/77
24/16	77/76
16/8	60/66

Each of the four temperature rooms fitted six 1.20 m x 0.80 m mobile trolleys. Within each room, 12 juvenile plants and 12 plantlets were placed on each of two trolleys, and six adult plants on each of the two remaining trolleys. The total number of plants in each room was 60, i.e. 12 adult plants and 24 each of juvenile plants and plantlets.

A 4 x 3 factorial design was used to assess the effect of the four temperature regimes upon the three sets of plants of different developmental state. Efforts were made to keep all other environmental factors, except the temperature treatments, similar for each room. To achieve this, trolleys positions were rotated within each room, and the containers holding juvenile plants and plantlets (which were of smaller statue relative to adult plants) were raised so that all plant canopies were equally distant from the light source.

The duration of the changeover between night and day temperature was 1 h, except in the 32/8 °C treatment for which the changeover between temperatures was 1.5 h. Humidity was set at a vapour pressure deficit (VPD) for all treatments of -7/-4 MPa (day/night), corresponding to relative humidities for each treatment as shown in Table 2.1.

Leaf surface temperature for plants in the 24/16 °C treatment was measured using a LI-6200, LI-COR, Inc., Lincoln, NE. From these records the difference between ambient temperature and leaf surface temperature was calculated.

The experiment started on the 21 March and finished 21 July 1994. The plants were therefore exposed to the treatments for 18 weeks. Time of new leaf appearance was recorded during the course of the experiment, and was taken as the time when a particular leaf was 5 mm in length on the day of observation.

Irradiance

Irradiance was maintained at 700 μmol m^{-2} s^{-1} at 40 cm above the canopy height. That was equivalent to ca. 750 and 600 μmol m^{-2} s^{-1} in upper and lower parts of the canopies of adult plants, respectively, and for juvenile plants and plantlets, ca. 700 and 580 μmol m^{-2} s^{-1} at the top and bottom of the plant canopies. Rapid decrease in irradiance within the canopy due to mutual leaf shading was noted. Photosynthetically active radiation (PAR) was monitored using a LI 6200 light monitor (LI-COR, Inc., Lincoln, NE). As the plants grew, their canopies received higher levels of irradiance, the uppermost not exceeding 800 μmol m^{-2} s^{-1}.

Daylength

A long day of 16 h was applied to plants in all treatments. During the light period a full intensity irradiance level was applied, as described in previous section. Day and night temperatures corresponded with the time of the light and dark periods.

Ventilation

Farquhar et al. (1989) stressed the importance of adequate ventilation for plant material used in carbon isotope studies. This is to ensure that ambient CO_2 in a growth cabinet as well as within the plant canopy remains close to the assumed value at 8 ‰ at all times and not affected by other factors. For the calculations of carbon discrimination in this experiment, it was assumed that the ambient ratio of $^{13}C/^{12}C$ in ambient CO_2 remained at 8‰ at all times. The effect of re-assimilation due to the more enclosed canopy as reported by Medina and Minchin (1980) was considered negligible in this experiment due to there being sufficient ventilation.

Irrigation

Irrigation was by a microtube watering system, each of the 4.5 litre and the 6 litre pots being fitted with two and four micro-tubes, respectively. Irrigation was

supplied to excess daily. Because the containers were irrigated to container capacity, soil moisture was self-regulated by the properties of potting mixture by free draining of excess irrigation.

Nutrition

One quarter strength Hoagland's solution was applied two weeks before the experiment started, and a half strength solution supplied during the experiment once a day. During the experiment a supplement of iron (as chelated iron) was sprayed twice in each treatment (3 June and 8 June 1994). Insufficient of this element was noted as yellowish spots along the leaf veins, similar to a problem reported by Starrett et al. (1993).

Disease control

Ridomil 25G was applied as directed on three occasions to all plants.

2.2.3 Collection of leaf samples for analysis of carbon isotope discrimination and leaf morphology

2.2.3.1 Carbon isotope composition analysis

At the end of the four months experimental period, leaf samples were destructively harvested from all the temperature treatments for carbon isotope composition analysis. However, after drying and grinding, the leaf sample from the 32/8 ^{o}C treatment weighed less than the minimum required for carbon isotope analysis. Fully expanded leaves that were born by the plants before the start of the experiment, and that expanded after two and four months of experimentation were sampled from the upper part of the plant canopies. Samples were collected from a population of six plants in each treatment. Within each plant, leaves were pooled from three successive weeks in the middle (after c. 2 months) and the last three weeks of the experimental period (after approximately 4 months). Leaves that had become fully expanded before the start of the experiment, after two months and four months were designated T_1, T_2 and T_3 samples, respectively.

Samples were dried at 75 °C for 24 h, finely ground and analysed for carbon isotope composition with respect to the standard values in PDB at the Stable Isotope Unit of the University of Waikato, Hamilton, New Zealand.

2.2.3.2 Image analysis

Original leaves, i.e. those that were fully expanded before the start of the experiment, and the most recently fully expanded leaves grown during the experiment from the16/8, 24/16 and 32/24 °C treatments were destructively harvested at the end of the experiment. The harvested leaves were placed abaxial surface downwards upon a normalised colour manifold and pressed flat with a glass sheet. Care was taken during the measurements that the image was captured before evapotranspiration from the leaves could affect the quality of the image by fogging the cover glass. Leaf morphology was quantified by capturing leaf images with a video camera, and analysing by a computer program that measured and calculated optical parameters (colour saturation, lightness, and hue), and dimensions (length, width, length/width ratio, area, perimeter and roundness). Bailey and Hodgson (1988) designed the image analysis program used. The quantified leaf attributes were analysed by two-tailed t-test using statistical calculations from Microsoft Excel 97 (Microsoft).

2.2.4 Statistical analysis

Data were analysed with a factorial design with three levels of ontogenetic state (juvenile, plantlets and adult), three levels of temperature (regimes 24/16, 32/24 and 16/8 °C) and three levels of time of emergence (T_1, T_2 and T_3) using ANOVA (SAS, Institute, 1995). The temperature and other conditions under which leaves in the T_1 had developed was unknown. Therefore, after the overall experiment analysis, the data for these samples were analysed separately from other experimental data.

Following the overall analysis, separate analyses were carried out for comparison of discrimination in plants of the three states at time T_1, i.e. in leaves that had expanded before the start of the experiment. Data for T_2 and T_3 were also analysed separately from those for T_1 in a three states x three temperatures x two times (T_2, T_3) factorial design. Further detailed three-way LSMeans analyses were performed since an overall significant effect of the interaction between Temperature, State and Time was observed. For each of the analysed factors there were six replications, variation of which was used to evaluate the statistical significance of differences between means by ANOVA.

2.3 Results

2.3.1 Overall analysis of leaf carbon isotope composition

There was a statistically significant interaction between the effects of all three factors (Temperature, State and Time) on $\delta^{13}C$. However, in a separate analysis of $\delta^{13}C$ for the leaves originally present on the plants before the experiment started (T_1), there were no significant differences between states (Table 2.2). Since the environmental factors (including temperature) under which these leaves developed were unknown, data from this sampling time (T_1) were excluded from further analyses.

Table 2.2 Initial leaf carbon isotope composition for plants of three ontogenetic states. Sampled leaves had expanded before the start of the experiment (sampling time T_1).

State	$\delta^{13}C$ (‰)	LSMeans test
Adult	-26.3550 (n = 6)	ns
Plantlets	-27.1783 (n = 6)	ns
Juvenile	-26.5716 (n = 6)	ns

ns - not significantly difference at P ≤0.05

2.3.2 Carbon isotope composition in leaves that expanded during the experiment

There was a highly significant ($P < 0.01$) three-way interaction between the effects of State, Temperature and Time on $\delta^{13}C$ for leaves that expanding during the experiment. This indicated that discrimination in plants of different state responded differently to temperature after two months (T_2) and four months (T_3). These responses are presented in graphical form (Figure 2.1).

Values for $\delta^{13}C$ were in the range –23‰ to –27.8‰ across treatments. After both two months (T_2) and at the end of the experiment (T_3), $\delta^{13}C$ was lower at 32/24 °C than at 16/8 °C for plants of all states, except for adult plants at T_3. The difference between $\delta^{13}C$ at 32/24 °C and 16/8 °C was more marked in juveniles (2-3‰) than in plantlets and adult plants (1-2‰).

Although there was a significant decrease in $\delta^{13}C$ for adult plants with each increase in temperature at T_2, values were always ≤ 25.4‰, and no temperature effect remained at T_3 for these plants. Similarly, $\delta^{13}C$ in juvenile plants decreased with each increase in temperature at T_2. However, after four months, there was a significant increase in $\delta^{13}C$ for juvenile plants (~2‰) between 16/8 °C and 24/16 °C, and a decrease (by >4‰) at 32/24 °C. The response of plantlets resembled that of juvenile plants, except that plantlets showed an increase in $\delta^{13}C$ at 24/16 °C (cf. 16/8 °C) after

only two months, an effect that became more marked at T_3. There was no significant difference between $\delta^{13}C$ for juvenile plants and plantlets grown at 24/16 °C for four months (Figure 2.1).

2.3.3 Leaf morphology and optical properties

The optical properties of leaves of plantlets contrasted strongly with those of adult plants at the start of the experiment. For instance, the initial values for lightness and saturation were ca. 60% higher and 50% lower for leaves of adult plants than for those of plantlets, respectively (Figure 2.2; Figure 2.3). However, at 24/16 °C, these differences became less marked as the experiment progressed. This was because there was a significant increase in leaf lightness (by ca. 40% of the initial value), and a decrease in leaf saturation (by ca. 60%) for plantlets at this temperature. Moreover, the values for these leaf attributes in adult plants did not change over the course of the experiment (Table 2.3; Figure 2.2; Figure 2.3).

There were similar differences between the leaves of juvenile and adult plants at the start of the experiment. Lightness and saturation of leaves on juvenile plants responded similarly to those of plantlets at 24/16 °C, although the magnitude of the changes were not as great (Figure 2.2; Figure 2.3; Table 2.3).

Significant changes also occurred during the experiment in leaf hue, and several leaf shape and dimension attributes in both plantlets and juvenile plants grown at 24/16 °C. The changes were always towards the values of the attributes in the leaves of the adult plants, which generally did not change significantly (Table 2.3).

As at 24/16 °C, initial and final values of leaf attributes in adult plants were similar at the other two temperatures. The changes occurring in leaves of plantlets and juvenile plants at 24/16 °C were generally not as evident at 16/8 °C or 32/24 °C (Figure 2.2 Figure 2.3 and Appendix I. for other attributes).

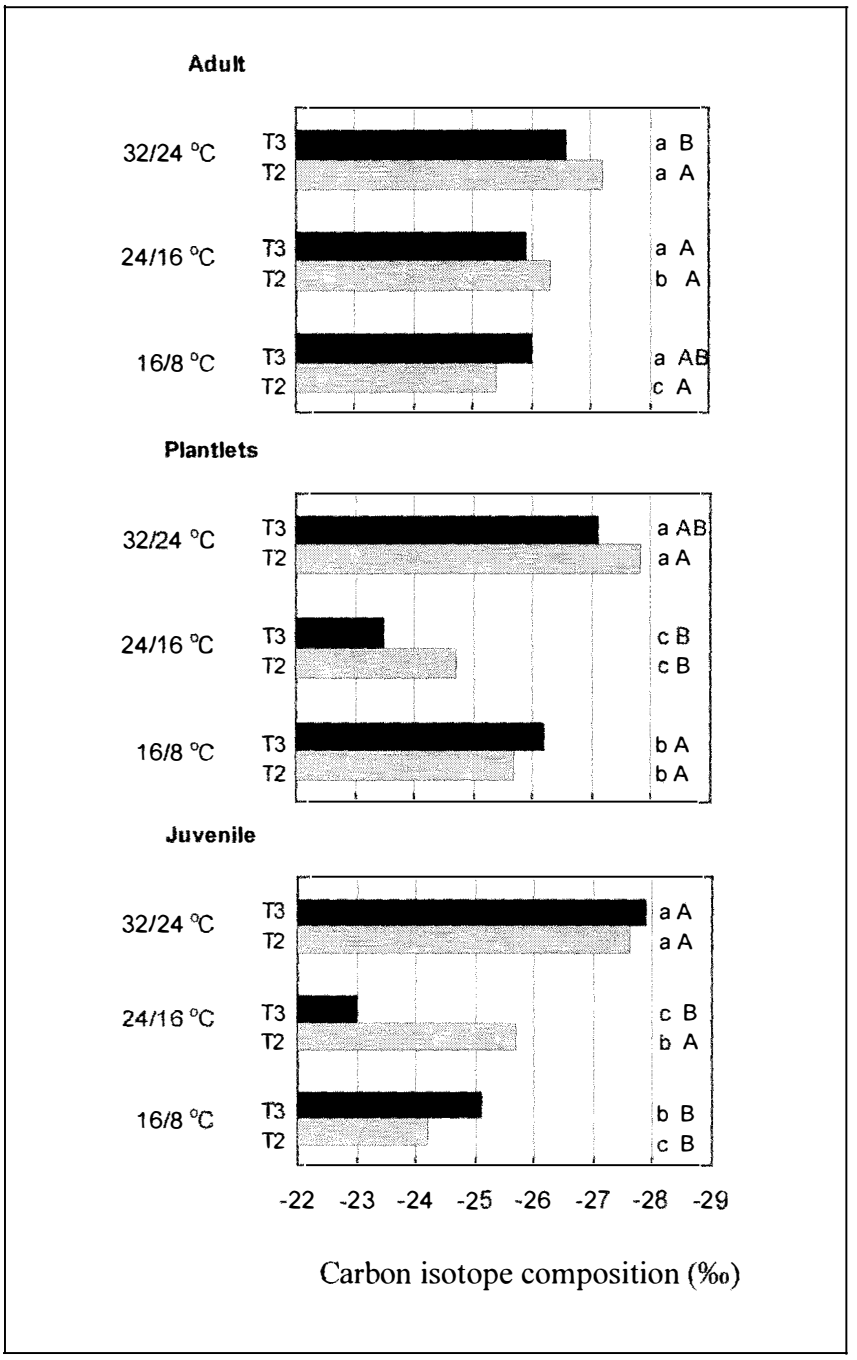


Figure 2.1 Effect of ontogenetic state and temperature on carbon isotope composition in leaves of *M. excelsa* expanding during experiment. Samples were taken for analysis after two (T2) and four (T3) months. Means annotated with the same letter were not significantly different ($P < 0.05$) for (lower case) comparisons between temperatures within ontogenetic states, and (upper case) comparisons between ontogenetic states within temperatures, both within each sampling time.

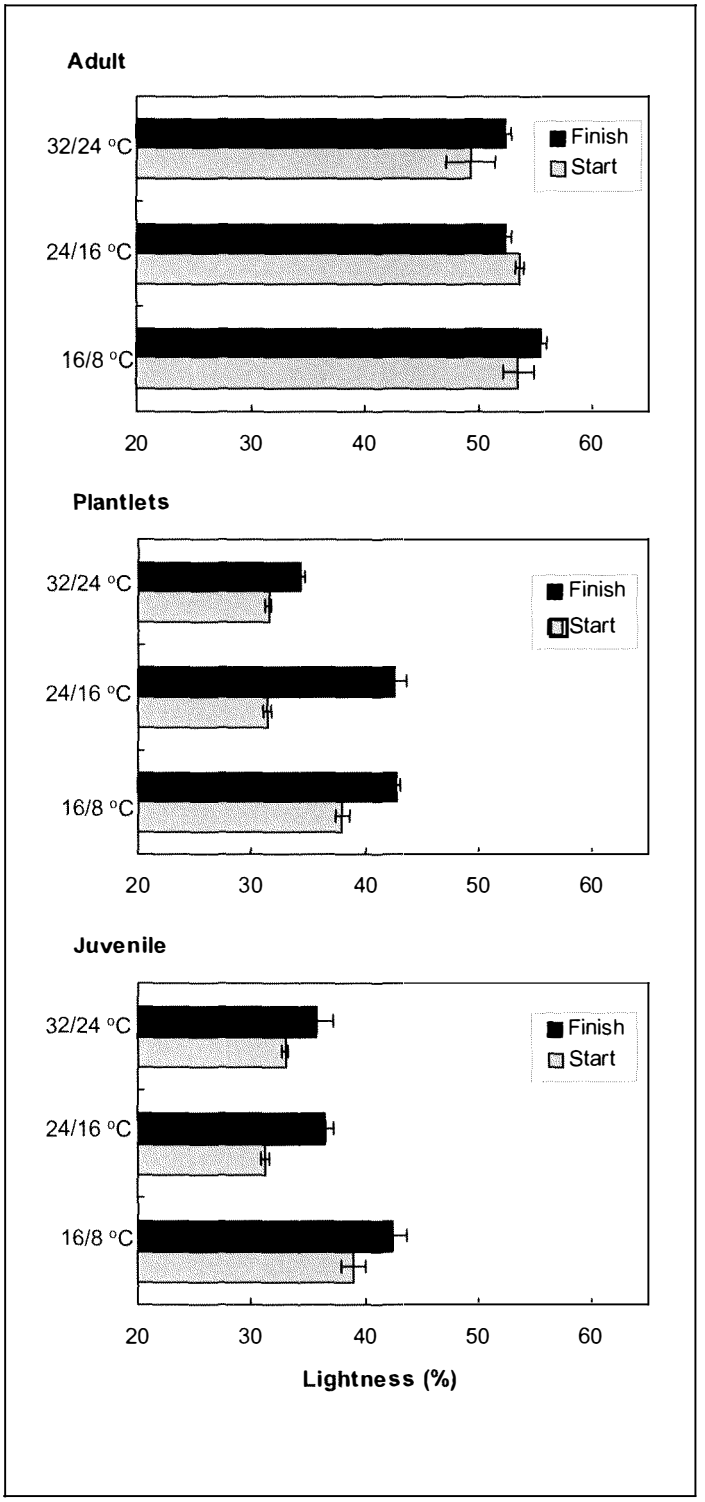


Figure 2.2 Effect of ontogenetic state and temperature on lightness (mean ± se) of the abaxial leaf surface. Analyses were for leaves that were the most recently fully expanded at the start and at the finish of the experimental period.

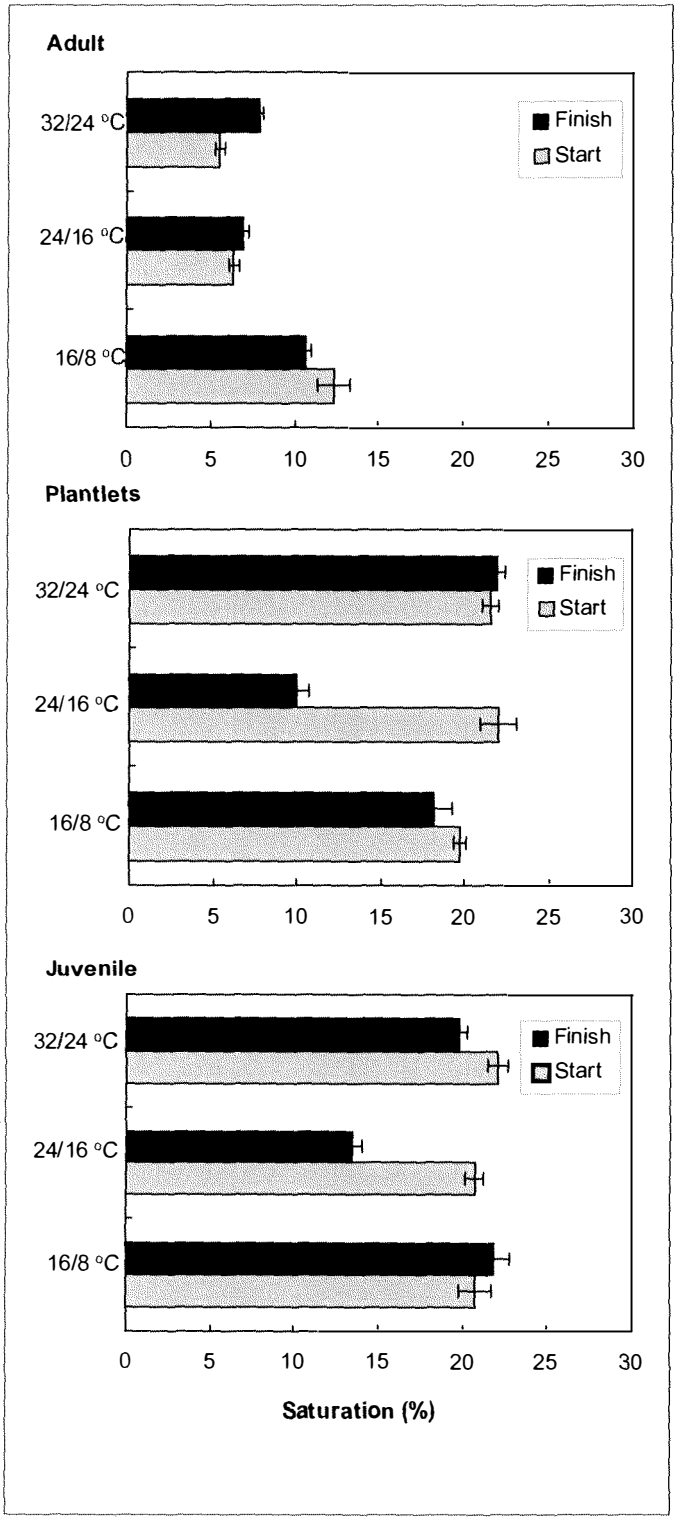


Figure 2.3 Effect of ontogenetic state and temperature on colour saturation (mean ± se) of the abaxial leaf surface. Analyses were for leaves that were the most recently fully expanded at the start and at the finish of the experimental period.

Table 2.3 Initial and final optical and dimensional attributes in fully-expanded leaves. Measurements were made for leaves present at the start of the experiment (initial) and after four months growth (final) under a day/night temperature regime of 24/16 ^{0}C.

		Ontogenetic state		
Attribute	**Harvest time**	Juvenile	Plantlets	Adult
Colour saturation (%)	Initial	20.70	22.07	6.38
	Final	13.45	9.99	6.94
		**	**	NS
Lightness	Initial	31.20	31.36	53.61
	Final	36.48	42.62	52.46
		**	**	NS
Hue	Initial	48.58	48.31	27.56
	Final	45.11	36.99	28.48
		**	**	NS
Length	Initial	44.69	43.12	27.60
	Final	33.70	25.79	25.81
		**	**	NS
Width	Initial	21.10	21.77	24.65
	Final	18.21	24.22	20.13
		*	NS	*
Length/Width	Initial	2.13	2.00	1.12
	Final	1.86	1.08	1.31
		*	**	NS
Area	Initial	670	663	524
	Final	443	484	396
		**	*	*
Perimeter	Initial	112.2	110.0	90.5
	Final	89.5	86.7	78.9
		**	**	NS
Roundness	Initial	19.09	18.65	15.71
	Final	18.51	15.72	16.18
		NS	**	NS

Two levels of significance are marked for P≤0.05 (*) and P≤ 0.01(**), and NS for statistically non-significantly different.

2.4. Discussion

2.4.1 Phase change and $\delta^{13}C$ in plantlets and juvenile plants

Cockayne (1928) was one of the first observers to describe adult plants of *Metrosideros excelsa* as having a characteristic downy tomentum on the abaxial surface of the leaf blade. Recently, the distinctive light grey character of leaves in adult *M. excelsa* plants was quantified by Clemens et al. (1999), who identified the significance of leaf colour saturation and lightness attributes for their "mealy" appearance. These authors also studied rejuvenated plants of the same species, and tracked the changes in leaf colour, dimension and shape attributes that occurred as plantlets progressed through vegetative phase change. In the current study, the changes reported for leaf lightness and colour saturation (Figure 2.2; Figure 2.3), and other leaf attributes (Table 2.3) in plantlets (and to a lesser extent, juvenile plants) at 24/16 ^{o}C were generally consistent with those occurring during the vegetative phase change in this species, as reported by Clemens et al. (1999).

This was best shown, for example, by leaf colour saturation in rejuvenated plants and adult plants, which were ca. 20% and 6-10% in the current study, respectively, compared with 15-20% and 5-6% in the respective plants in the study of Clemens et al. (1999). Similarly, leaf lightness at the start of the experiment described in the current study was 31-38% and 50-54% in rejuvenated and adult states, compared to the reported 35-40% and 45-50%, respectively (Clemens et al., 1999).

The leaf colour, dimension and shape attributes in plantlets were similar to those in juvenile seedlings, indicating that the process of rejuvenation in the sense of Hackett (1985) and Greenwood et al. (1987) that had been caused by micropropagation was complete, as assessed by these measures. Conversely, values for leaf colour saturation and lightness in plantlets undergoing vegetative phase change approached those in adult plants, although the change was not complete. This was presumably because the experiment in the current study lasted only four months, which was not long enough for the gradual process of vegetative phase change to reach completion in this homoblastic (Goebel, 1900)

species. The study of vegetative phase change in *M. excelsa* described by Clemens et al. (1999) lasted nine months.

Vegetative phase change that occurred in plantlets and juvenile plants at 24/16 ^{o}C, was paralleled by increases in $\delta^{13}C$. Therefore, there was less discrimination against ^{13}C as the abaxial leaf surface gained the downy tomentum that gave them their characteristic light grey appearance. These observations are consistent with increased boundary layer resistance and reduced c_i (O'Leary, 1981) in leaves of plantlets and juvenile plants as leaves became more adult.

Overall, the decrease in ^{13}C discrimination was in the order of 3‰ in both plantlets and juvenile plants. Farquhar (1983) reported effects on discrimination of a similar magnitude due to effects on CO_2 diffusion through the leaf boundary layer. Theoretical values for discrimination associated with diffusion of CO_2 through stomata, and diffusion through the boundary layer to the stomata were reported to be 4.4‰ and 2.9‰, respectively (Farquhar et al., 1989). Hansen (1996) found significant differences in boundary layer conductance between juvenile and adult leaves in *Acacia koa*, and concluded that decreased conductance in adult leaves limited CO_2 uptake, which led to lower c_i and lower discrimination.

Comparisons of juvenile and adult plants in leaf $\delta^{13}C$ and gas exchange characteristics have been made in several ecophysiological studies, e.g. of *Acacia koa* (Hansen, 1996), *Metrosideros polymorpha* (Cordell et al., 1998) and a number of North American shrub species (Sandquist et al., 1993; Donovan and Ehleringer, 1994). The findings of these authors suggest that juvenile seedlings are able to avoid soil moisture deficit during their establishment by developing a root system for effective water extraction. As plants age (and presumably undergo phase change) under long-term field conditions, they become more water-use efficient, and less susceptible to environmental stresses (Donovan and Ehleringer, 1991; 1994; Fleck et al., 1996; Hansen 1996). Values for $\delta^{13}C$, therefore, tend to be higher in adult than in juvenile plants, reflecting their reduced ability to discriminate against ^{13}C. In this

respect, the increases in leaf $\delta^{13}C$ as phase change progressed in plantlets and juvenile plants of *M. excelsa* grown at 24/16 °C are consistent with these studies.

However, ontogenetic ageing and adaptation to site conditions were confounded in the ecophysiological studies noted above. No report has appeared in the literature that compared carbon isotope discrimination in juvenile and adult woody plants grown in containers under well-watered conditions. Because these conditions applied in the current study, it is suggested that the observed results arose directly from the effects of ontogenetic state and its interaction with temperature. They did not arise from effects that might complicate field studies, e.g. moisture availability, rooting depth, and the long-term effects of moisture stress on the attrition seedling populations and adaptation of adult plants (Sandquist et al., 1993; Donovan and Ehleringer, 1994; Hansen, 1996; Cordell et al., 1998). Moreover, the current study was also novel in that it allowed comparisons to be made between juvenile plants (at least to the extent that micropropagated plantlets did indeed represent a rejuvenated state) and adult plants of the same genotype, as opposed to within wild, seed-derived populations.

The agreement between the onset of vegetative phase change, as assessed by image analysis, and the progressive reduction in ^{13}C discrimination in plantlets and juvenile plants of *M. excelsa* grown at 24/16 °C was consistent with theoretical and experimental studies. Farquhar et al. (1982; 1989) pointed out that any factor that causes a decrease in leaf conductance, relative to the photosynthetic capacity, will tend to decrease c_i/c_a and, therefore, increase $\delta^{13}C$. However, the divergence of leaf $\delta^{13}C$ in these plants away from the values for $\delta^{13}C$ measured in adult plants was unexpected. This was all the more remarkable because leaf $\delta^{13}C$ in adult plants remained relatively constant over the four-month experimental period across all three temperature regimes. Surprisingly stable internal CO_2 partial pressures in range environments and for different species was reported by Wong et al. (1979) and O'Leary (1981), even

though the ontogenetic state of the examined plants was not discussed by these authors.

Since the leaves of plantlets undergoing phase change were becoming morphologically more similar to those of adult plants, the seemingly anomalously low values of $\delta^{13}C$ in adult plants might have resulted from a reduced photosynthetic capacity in these adult leaves (Farquhar et al., 1982; 1989; J. Clemens, P. Bannister pers. comm.). Thus, as phase change progressed to completion in plantlets and juvenile plants of *M. excelsa*, $\delta^{13}C$ would be expected to approach that of adult plants because of the acquisition of both the photosynthetic capacity and the morphology of their leaves.

The range of carbon isotope discrimination values exhibited by leaves of *M. excelsa* (-23 to -28‰) was similar to those for vascular plants from desert floras, e.g. woody species of the Sonoran Desert (Ehleringer and Cooper 1988, Ehleringer 1993, Rundel et al. 1999). In addition, values of $\delta^{13}C$ for *M. excelsa* were generally higher than the mean global average for vascular plants (Farquhar et al., 1982; O'Leary, 1988), and similar to those for long-lived growth forms in general (Stewart et al., 1995).

2.4.2 Effects of temperature on $\delta^{13}C$

Notwithstanding the progress of phase change in plantlets and juvenile plants at 24/16 ^{o}C, which resulted in a decrease in discrimination against ^{13}C, higher temperatures generally increased carbon isotope discrimination in plants of all ontogenetic states. *Metrosideros excelsa* occurs naturally in parts of New Zealand with a warm temperate climate, and its gas exchange physiology, e.g. stomatal conductance, could be expected to respond positively to higher temperature. Higher stomatal conductance would lead to increased c_i/c_a, and greater discrimination (Farquhar et al., 1982; 1989).

In other studies of both C_3 and C_4 plants, $\delta^{13}C$ also became more negative (up to -2‰) with increasing temperature (Smith et al., 1976 quoted by O'Leary 1981; Farquhar, 1982; 1989).

Other workers, however, have failed to find such a linear relationship of temperature with $\delta^{13}C$, according to O'Leary (1981). Wong et al. (1979) reported that internal CO_2 concentration increased at both high and low temperature. Consequently, ^{13}C discrimination would be expected to exhibit a similar response (O'Leary, 1981). In the current study, plantlets and juvenile plants of *M. excelsa* showed a non-linear correlation of $\delta^{13}C$ with temperature, similar to that reported by Wong et al. (1979) and O'Leary (1981). However, there was a positive linear effect of temperature on $\delta^{13}C$ in adult plants after two months, and the absence of an effect at four months. It was, therefore, concluded that the response of the plantlets and juvenile plants was due to the physiological changes intrinsic to phase change rather than to an effect of temperature. Such a conclusion was also supported by the observed changes in leaf morphological attributes at 24/16 °C. Furthermore, it was unlikely that the range of the temperature treatments used in the current study was great enough for a non-linear correlation as presented by Wong et al. (1979) to be displayed.

2.4.3 Carbon isotope discrimination as a marker of phase change

With respect to the treatment effect of ontogenetic state and the progress of phase change, and of temperature, the results are internally consistent, and in keeping with published research on carbon isotope discrimination and phase change. Therefore, within the constraints of the defined experimental procedures described in the current study, carbon isotope discrimination was an informative and reliable marker of phase change at 24/16 °C. However, discrimination was also responsive to temperature, and phase change was not apparent in *M. excelsa* when grown for four months at the higher and lower temperatures. Moreover, there was a critical lack of significant difference in carbon isotope discrimination between the plants of different ontogenetic state before the experiment started. Whatever, the

conditions under which the plants were grown before purchase, it had overridden effects of ontogenetic state to the extent that their $\delta^{13}C$ signature could not be used as a marker for this property.

2.5 Conclusion

In conclusion, carbon isotope discrimination could be used as a marker of phase change in *M. excelsa* under controlled experimental conditions. However, discrimination in leaves of plants of distinct ontogenetic state grown before the start of the experiment did not differ. Therefore, because of interactions between environment and ontogenetic state, carbon isotope discrimination is not a property intrinsic to ontogenetic state. Therefore, the hypothesis that carbon isotope discrimination can be used as a marker of phase change was substantiated provided its use is confined to the experimental situation, such as that reported in the current study.

CHAPTER THREE

Development of the Metrosideros Model

3.1 Introduction

The architectural structure of a tree crown is a combined expression of genetic information, physio-ecological and ontogenetic processes as these are affected by the environment during plant growth and development. It was assumed, therefore, that by capturing this architectural expression mathematically through time, the developmental state of the plant could be quantified by tree architecture parameters (Tomlinson 1978; Borchert and Tomlinson, 1984; Poethig, 1990; Day and Gould, 1997).

A number of methods to express plant size exist (see Section 1.2.4) but no method to quantify plant architecture was available (Buck-Sorlin and Bell, 2000; Pearcy and Valladares, 2000). Room et al. (1994) argued that because plants are modular organisms, their structure can best be captured using basic morphological units or modules (Prusinkiewicz et al., 1994; Room et al., 1994). Consequently, if tree architecture could be modelled in response to different controlled environments as proposed by Bortchert (1976), the hypothesis that phase change between the juvenile and adult states is dependent on the attainment of a minimum size and/or a certain complexity could be tested (see Section 1.2.2). To test this Size-Complexity Hypothesis was one of the principal objectives of this project.

Therefore, in order to quantify architecture and growth difference between ontogenetic states and to thus quantify the progress of phase change, the new topological model for the trifurcating *Metrosideros excelsa* plant described in this chapter was developed. This was done by defining basic units, assumptions, topological parameters, a recording system and processing software. By defining the model in terms of modular units as proposed by Room et al. (1994), a new system of topological recording was developed. Trees were accommodated that were far greater in size and at a level of detail far greater than had been achieved by other authors in modelling tree-like structures (e.g. van Pelt, 1997). This topological model is referred to as the Metrosideros Model.

This model enabled the accommodation of the logistics of measuring the structural complexity of *Metrosideros excelsa* trees, as well as the dynamics of growth during the experiment described in Chapter 2 in an architecturally, mathematically and statistically sound manner.

Initially, only the number of segments, terminal segments, mean centrifugal order number as architecture parameters were mathematically expressed for the trifurcating branching pattern of *Metrosideros excelsa.* Nevertheless, the initial evaluation (in this Chapter), dynamic growth analysis (Chapter 4) and detailed architecture analysis (Chapter 5) all contributed to the evaluation of the model's suitability to describe tree architecture and its dynamics in a mechanistic fashion. This model characteristic was in contrast to either purely empirical models (Thornley and Johnson, 1990; Grace et al., 1998) or simpler measures of growth in size, such as height, weight, elongation of individual shoots (Karlsson and Heins, 1994) or node counting (Snowball, 1989; Sachs, 1999) used in previous studies of plant architecture.

3.2 Methods

3.2.1 Methodology for building the Metrosideros Model

The Metrosideros Model was built by simultaneous consideration and integration of a number of tasks. Initially, a tree model with a suitable ordering system (Weibel, 1963; Werner and Smart, 1973) and a developed theoretical basis for structural classification was selected from the available literature (see Section 1.2.4). This was the Dendrite Tree Model of van Pelt and coworkers (van Pelt and Verwer, 1986). However, units used, the method for architecture recording, and the most basic assumptions of the bifurcating dendrite tree needed to be extended and modified for them to be relevant to botanical tree modelling, and to *Metrosideros excelsa* in particular.

The tree-like networks of Purkinje cells described by the Dendrite Tree Model (van Pelt et al., 1989) were similar in some respects to the branching structures of botanical trees. Most importantly, however, the Dendrite Tree Model addressed some theoretical issues of classification of branching patterns, and attempted to statistically

compare the effect of treatments on tree architecture (van Pelt and Verwer, 1984a,b; Verwer and van Pelt, 1986; van Pelt et al., 1986).

However, crucial differences between the structures of dendrite trees and botanical trees had to be addressed in order to utilise the mathematical and statistical theories of the Dendrite Tree Model. Initially, basic model assumptions and suitable morphological units of a botanical tree had to be identified and defined. In this respect, the adjustment from a model of bifurcation to one of trifurcation needed to be made, as well as accommodation of pre-determined sites of branching at apical and axillary meristems. These units had no analogues in the dendrite tree structure. Topological parameter definitions and their calculation had to be examined for the feasibility of their adjustment to trifurcating branching. Similar attempts were made earlier (Berry et al., 1986; Verwer and van Pelt, 1990) with only limited success.

In general, *Metrosideros excelsa* can grow from three points at each potential branching position. However, it also behaves more like a bifurcating system in its adult state because of the frequent abortion of the apical bud (Dawson, 1968a; Clemens et al., 1999). In the juvenile state, plants normally retain three buds at the end of each shoot, and often the apex resumes growth after a short growth pause (see Section 1.1.1). From the topological point of view it was defined that growth from the apical bud was not a new branch, but a continuation of an existing axis (model approximation to the descriptive definition of sylleptic shoot). However, when branching occurred from the distal axillary buds after apex abortion in adult plants, that was considered as new branching in the Metrosideros Model.

It was also assumed by definition that one intermediate segment always exists between the botanical root system and the above-ground structure, denoted centrifugal order '0'. This was important for parameter calculation.

3.2.2 Units and construction of the Metrosideros Model

With respect to the basic units suitable for modelling a botanical tree, the latest literature was reviewed in Chapter 1 (e.g. Room et al., 1994). It was essential to the success of the work that basic units, model assumptions, recording system and the

extraction of architectural parameters be defined simultaneously. This would result in a model that could serve as a practical tool for the measuring and mathematical classification of tree architectures, and their statistical analyses. This was a challenging task, previously attempted in vain by others (see Chapter 1).

From the botanical point of view, a branch represents the final product of the branching process. However, in the process of building the Metrosideros Model, it was first assumed that meristems represent potential sites of branching. Thus, a model based on the meristem unit would be capable of representing the mechanism of plant architecture development more realistically than a model based only on shoots or segments of shoots (axes) (Room et al., 1994; Day et al., 1997). It was anticipated that such a model would be able to reflect tree architecture through the probabilities (modes) of branching events in response to plant state and environment (Borchert, 1976; Remphrey and Prusinkiewicz, 1997). van Pelt et al. (1986) considered the mathematical expression of branching probabilities essential to the completion of any tree architecture model.

Two basic units

The units of the Dendrite Tree Model (van Pelt et al., 1989) appeared to be a suitable basis for modelling a botanical tree, and were applied by Day and Gould (1997) in order to assess branch architecture. However, Day and Gould (1997) did not define botanical units. Rather, they simplified the branching structure, so that it seemingly complied with the assumptions of the Dendrite Tree Model, and calculated topological parameters without further consideration of branching dynamics. Such an empirical approach was also helped by the application of the Dendrite Tree Model to a botanical tree of bifurcating pattern (Day and Gould, 1997).

The basic morphological units of the Metrosideros Model had to be identified and defined in a manner that would accommodate assumptions of branching probabilities as well as a recording system for experimental testing. The suitability of a unit was judged in two ways. Firstly, the scale at which the plant was going to be modelled was considered, and the logistic feasibility of the experimental recording exercise. Secondly, the selected unit had to represent the mechanism of branching

dynamically. In other words the units had to be observable, able to be recorded, and part of the tree branching mechanism.

A meticulously detailed level of tree structure dissection is desirable when model units are to be selected. However, this can lead to an unacceptably laborious recording procedure (Prusinkiewicz et al., 1994). The coding of topological position of each unit needs to be done in such a manner that the position of each unit with respect to all other units of the two-dimensional tree structure can be obtained within the centrifugal ordering. This was essential for further data processing and parameter calculation, e.g. of Mean Centrifugal Order Number or Tree Asymmetry Index.

For the purposes of modelling *Metrosideros excelsa*, the topological tree was constructed from two types of basic unit. These were called internal (or intermediate) and terminal (or external) segments. While these terms are similar in name to those used in the Dendrite Tree Model (van Pelt et al., 1989), their meaning in the Metrosideros Model was substantially different. In the Metrosideros Model an internal (intermediate) segment corresponded to what could be described in botanical terms as that part of a shoot that is positioned between adjacent axillary buds (or between an axillary bud and a contiguous apical bud at the end of the shoot). A terminal (external) segment, on the other hand, represented either an axillary or an apical bud.

In contrast to the Dendrite Tree Model, a terminal segment in the Metrosideros Model represented a morphologically defined potential branching point. This ability of the Metrosideros Model to account for the probability of branching at terminal segments had important implication for the modelling of dynamic tree growth on a mechanistic basis. The ability of the Metrosideros Model to capture the tree branching mechanism was also in contrast to the Dendrite Tree Model, where both terminal and internal segments are defined as a branch (i.e. a product of branching) that bears unspecified potential branching points (van Pelt et al., 1989). Consequently, little difference exists between the two basic units in the Dendrite Tree Model, which is able to address branching only through empirical modelling (Thornley and Johnson, 1990).

Dynamic growth

The two basic units (internal and terminal segments as defined above) appeared to be sufficient to model the topological structure of a botanical tree, and its development through branching in a mechanistic type of model. However, to model the tree branching mechanism, the model has to be able to accommodate dynamics of growth of the tree structures.

The branching mechanism was built into the model by the assumption that each terminal segment could become an internal segment through growth and development. The newly grown internal segment (botanical internode) could connect the position of the original terminal segment with at least three or more new terminal segments (apical bud and two axillary buds at the end of the shoot, plus pairs of axillary buds along the shoot axis). By repeated addition of internal and the appropriate number of terminal segments, growth of new metamers (internode with a set of nodes, according to Room et al., 1994) and shoots occurs. The dynamics of growth can be captured through repeated recording and addition of segments as the growing tree produces these. These records are added to the topological record of the tree model, from which the dynamics of the growth can be reconstructed. The reconstruction can be made either in computer visualisations in 2D or 3D form as achieved by others, or numerically by using calculated values of architecture parameters, the new approach and research tool described in this thesis.

Metric properties

Because the Metrosideros Model was built up in detail from axillary and apical meristems, it was capable of considering architecture at the level of the node, branch, or centrifugal order, or a combination of topological and metric properties of the tree crown.

Owing to the physical and physiological importance of some metric properties, such as the length of the tree structures and leaf area (see Section 1.2.5), the inclusion of these properties in the model was also considered. Length of each shoot was repeatedly measured during observations, as well as the number of leaves on each

shoot. Parallel recording of these parameters within the framework of the tree topological records enabled the processing software to assign metric properties to the branches of known topological position. Thus, it was possible to carry out analysis of experimental data with regard to Horton's Second Law (see Section 1.2.5). This is described in Chapter 5.

Branching and probabilities

The amount of branching within the centrifugal orders of the tree structure was expressed as the probability of branching in each centrifugal order, and calculated in the Metrosideros Model from the topological database record. Probability was calculated, as a ratio of the number of internal segments (branching points) to the number of terminal segments in each centrifugal order. Probability of branching for the whole tree crown was also calculated, using total numbers of internal and terminal segments of the tree, i.e. in a way similar to that in the Dendrite Tree Model (van Pelt et al., 1989).

Calculation of branching probabilities in each centrifugal order was in contrast to the Dendrite Tree Model, in which branching points were not specified, and branching occurred randomly at any part of the segment length. Consequently, the branching probability had to be expressed empirically or through simulations in the Dendrite Tree Model through the values of the *Q*-*S* branching modes (see Section 1.2.4.9). In the Metrosideros Model the *Q*-*S* branching probabilities were set by definition to $Q = 0$. Hence, in the Metrosideros Model the probability of branching from any other than from the terminal segment was equal to zero. Thus, in relation to the *Q*-*S* model, as defined in the Dendrite Tree Model, branching probability relating only to the centrifugal topological position (order) was pertinent in the Metrosideros Model, i.e. the values of the *S* branching mode. However, the *S* branching mode value can be calculated from experimental data in the Metrosideros Model.

Therefore, in the Metrosideros Model the branching probabilities of any terminal segment (p_t) could be expressed in accordance with the van Pelt model, but adjusted for a trifurcating tree, as:

$$p_i = C \cdot 3^{S\gamma} \qquad (3.1)$$

where C is a normalisation constant restraining the probability values between 0 and 1, S is the mode of branching with respect to the centrifugal order, and γ is the centrifugal order of the examined terminal segment.

3.2.3 Topological recording

As mentioned previously, it was assumed that in every modeled tree there was at least one intermediate segment between the root and the tree structure. This root – canopy intermediate segment was called 'Base' in the recording database, and was defined as being of order '0'. The whole subsequent branching hierarchy was linked directly or indirectly to this Base. The hierarchy linkage and the position of each additional segment was recorded using the following:

A – The sequential number of the terminal segment on its original (mother) branch (from junction point up by each node), from which the new branch (intermediate segment) originated.
B – The number of that original (mother) branch as it was assigned by the database when first recorded.
C – The number of the new branch assigned by the database as identification number.
D – The number of nodes on that branch

For example, a small botanical tree may be numerically captured as follows:

A	B	C	D
Base	0	0	7
3	0	1	8
5	1	2	7
5	2	3	6

The above example refers to a tree, which had one stem and 3 branches arranged in particular manner. The initial Base had 7 internal segments (nodes), the first, at order zero, connected to the root system. At the third node of the stem (3-0) is branch # 1 with 8 nodes. From node # 5 of branch #1, branch # 2 originates (5-1). The last branching point occurred at node # 5 of branch # 2, and was assigned # 3.

This type of recording can be applied theoretically to trees of unlimited size. In order to help with orientation within the canopy during measurements, branches were tagged with their identification number corresponding to the branch number assigned to the branch by the database. Moreover, recording of each branch length and number of leaves was recorded in parallel to topological position. These, or other, desired measurements could easily be added to each branch in a separate row of the recording spreadsheet and column for each repeated measurement.

Using the Metrosideros Model topological recording system, topological measurements as well as the number of leaves and lengths of branches were recorded weekly for the first 11 weeks, and fortnightly for the last four weeks of the experiment described in Chapter 2. These repeated, non-destructive measurements of tree architecture were recorded in a database using QPRO Version 2 (Borland, 1993, USA). Calculations of topological parameters for the trifurcating Metrosideros Model were made using tailor made software. Additional metric data (number of leaves and lengths shoots) were also collected and accommodated in the model. An electronic version of the database and calculation program is available on disk.

3.2.4 Expression of topological parameters in the Metrosideros Model

In agreement with the definition of trifurcating assumption for the Metrosideros Model, there were three potential internal segments at each centrifugal order. Consequently, considering the definitions of architecture parameters in the bifurcating Dendrite Tree Model of van Pelt and Verwer (1986) (see Figure 1.6, Chapter 1) the total number of terminal and internal segments (T) in the Metrosideros Model was as follows:

$$T = \left(\frac{3n-1}{2} \right) - 1 \tag{3.2}$$

where n denotes the total number of terminal segments as defined earlier.

The number of terminal segments (n) (sometimes called tree *degree*) can be calculated as:

$$n = \frac{2T+3}{3}. \tag{3.3}$$

Further to the topological parameter degree (n), van Pelt et al. (1989) introduced the parameter of Mean Centrifugal Order Number. For a trifurcating tree of degree n, denoted as $\alpha^{(n)}$ with $s_\alpha(\gamma)$ segments at order γ, and with a highest order γ_{max}, the Mean Centrifugal Order Number for the Metrosideros Model was defined as:

$$\bar{\gamma}_\alpha(n) = \frac{1}{\left(\frac{3n-1}{2} \right) - 1} \cdot \sum_{\gamma=0}^{\gamma_{max}} \gamma s_\alpha(\gamma) \tag{3.4}$$

As for a bifurcating dendrite tree (van Pelt et al., 1989), there were also two extremes of branching mode for a trifurcating tree: branching from every potential branching point (*Compact Tree*), and branching from only one branching point of the three potential points (*Thin Tree*).

Therefore, in a trifurcating *Compact Tree,* the Mean Centrifugal Order Number was calculated from:

$$\bar{\gamma}_{compact} = \left(3^1 \cdot 1 + 3^2 \cdot 2 + 3^3 \cdot 3 + \ldots 3^{\gamma_{max}} \cdot \gamma_{max}\right) \cdot \frac{1}{3^1 + 3^2 + 3^3 + \ldots 3^{\gamma_{max}}} =$$

$$= \left(3^1 \cdot 1 + 3^2 \cdot 2 + 3^3 \cdot 3 + \ldots 3^{\gamma_{max}} \cdot \gamma_{max}\right) \cdot \frac{1}{\frac{3^{(\gamma_{max}+1)} - 1}{2} - 1} =$$

$$= \frac{2\left(3 + 3^2 \cdot 2 + 3^3 \cdot 3 + \ldots n \cdot \gamma_{max}\right)}{3n - 3} = \tag{3.5}$$

$$= \frac{2}{3n-3} \cdot \sum_{\gamma=0}^{\gamma_{max}} \gamma \cdot 3^{\gamma}$$

where $3^{\gamma_{max}} = n$; n being the tree degree, or number of terminal segments.

In a trifurcating *Thin Tree,* Mean Centrifugal Order Number was calculated from:

$$\bar{\gamma}_{thin} = \frac{3\left[\gamma_{max} + (\gamma_{max} - 1) + (\gamma_{max} - 2) + \ldots 1\right]}{3\gamma_{max} + 1} =$$

$$= \frac{3\left\{\gamma_{max} \cdot (\gamma_{max} + 1)\right\}}{2} \cdot \frac{1}{3\gamma_{max} + 1} \tag{3.6}$$

where the *Thin Tree* degree $n = 2\gamma_{max} + 1$. Therefore, $\gamma_{max} = \frac{n-1}{2}$ and the mean order could be expressed and calculated from the highest order only (as above) or from the number of terminal segments as:

$$\bar{\gamma}_{thin} = \frac{3\left[\frac{n-1}{2} \cdot \left(\frac{n-1}{2} + 1\right)\right]}{2} \Big/ \left(3\left(\frac{n-1}{2}\right) + 1\right) \tag{3.7}$$

As assumed in the Dendrite Tree Model (van Pelt and Verwer, 1986), mean centrifugal order number of any botanical tree of a given size should lie between the two extremes of the *Compact* and *Thin Trees.*

3.2.5 Computerised calculations of topological parameters

Routinely recording of two-dimensional tree structure was done by drawings (van Pelt et al. 1989; Day and Gould, 1997). For the Metrosideros Model a hand drawing was initially attempted for calculation of architecture parameters (Figure 3.1)

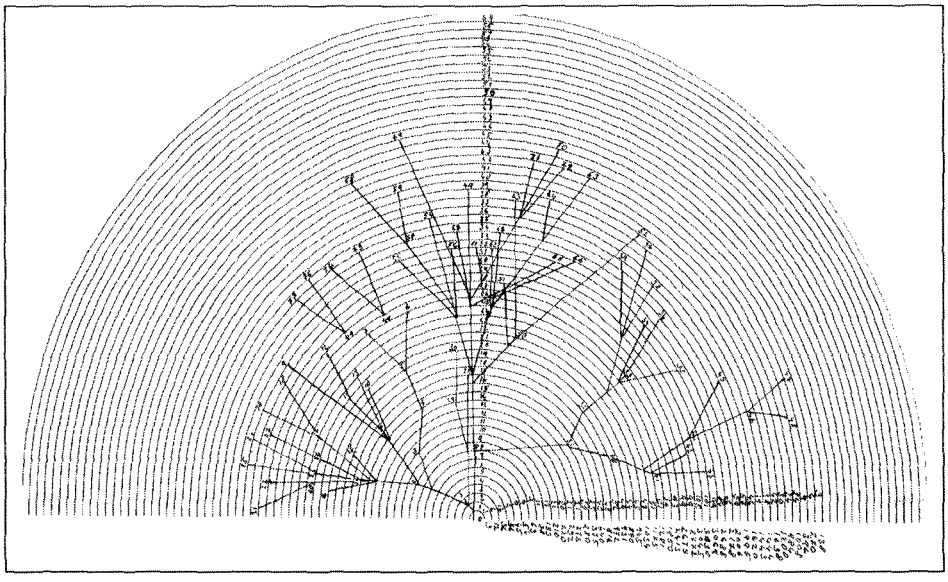


Figure 3.1 A hand-drawing of topological tree at one point in time. Over a 1000 such drawings would be needed to calculate the two basic topological parameters size and size-complexity used in this study. (Note: The two 'un-rooted' branches originate from branch No. 49, as marked).

However, the size of experimental plants and the need for repeated measurements prohibited manual approach to calculations. Moreover, a base for computerized calculations was laid through successful numerical recording of experimental trees architecture.

Initially, a simple computer program was developed that processed the recorded data and calculated the first three topological parameters. Later, another program was developed to calculate a number of additional architecture parameters, such as tree asymmetry, branching probabilities, and also metric properties, in relation to their ordering position within the tree structure. This program was more sophisticated and followed the definitions of the model rigorously for the purposes of asymmetry

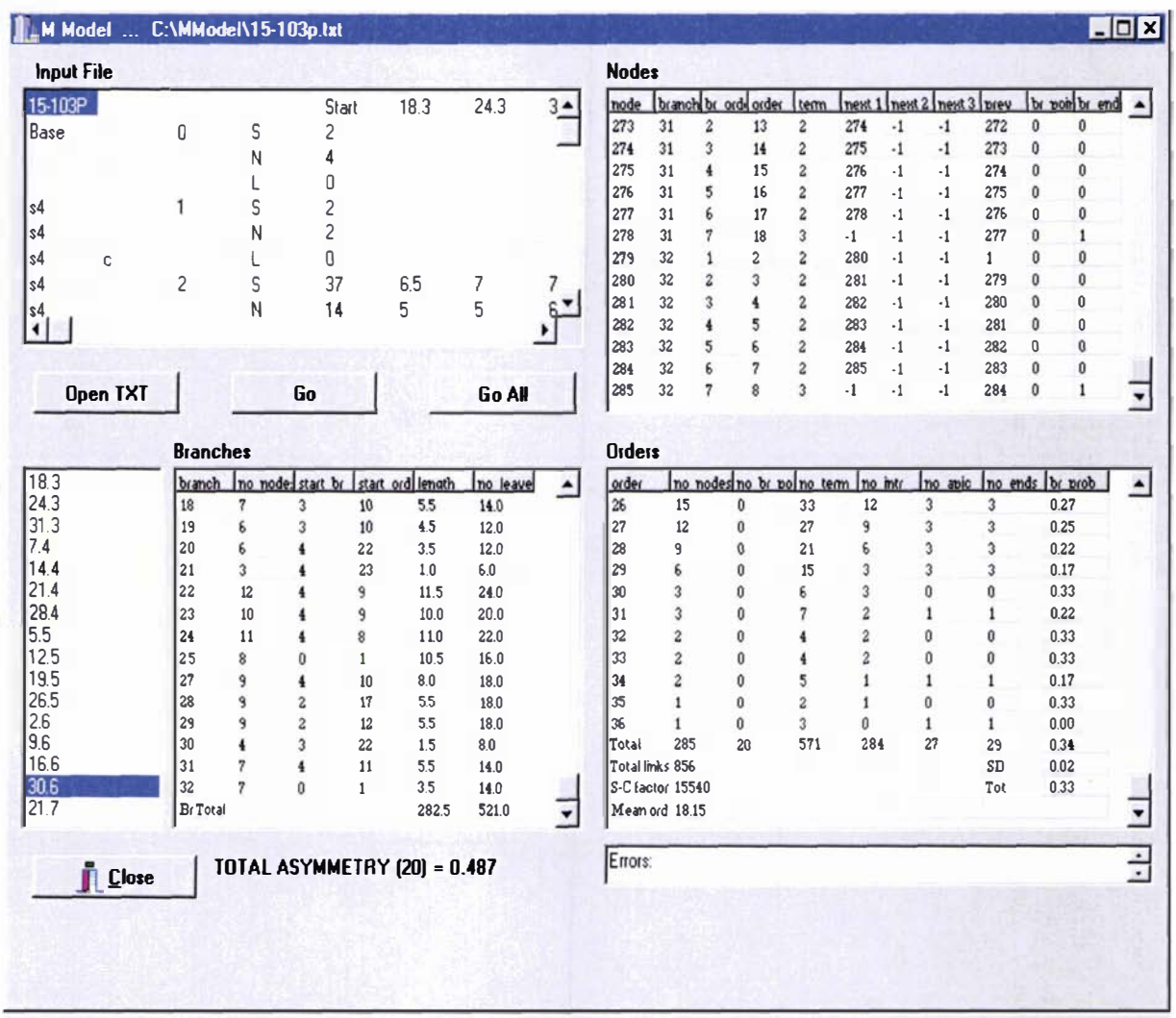
calculations. Consequently, it was able to distinguish branching points and their topological position, and hence was able to calculate advanced architecture parameters, such as the Partition Asymmetry (A_p) and Tree Asymmetry Index (TAI). Moreover, the program processed the recording datasheet for any recording errors, and required these to be corrected before calculation of parameters from the recording database. Correction of recording errors is an important desirable feature of any architecture model (Godin et al., 1999).

The tailor made programs called 'Mmodel' calculated the following parameters for each recording time:

- Topological size (total number of segments)
- Topological size-complexity factor (order weighted sum of segments)
- Topological complexity factor (mean centrifugal order number)
- Topological height-distance from root (highest order number)
- Topological degree (total number of terminal segments)
- Total number of internal segments
- Total number of branching points
- Total number of branches
- Total number of leaves
- Total length of branches
- Number of all segments per each order
- Number of internal segments per each order
- Number of apical segments per each order
- Metric length, topological length and position of branches
- Probability of branching per each order
- The mean of probability of branching per order ± SD
- Probability of branching of the whole canopy
- Partition symmetry of each sub-tree branching
- Tree Asymmetry Index (mean of partition asymmetries ± SD)
- PSAD index (proportional sum of absolute deviations of multiple partitions of the terminal segments over all sub-trees)

The Metrosideros Model software for each experimental tree calculated these parameters at each of the repeated observation (Table 3.1).

Table 3.1 A view of a computer screen interface. The windows show for one plantlet on 30.6. the input file , and calculations of parameters sorted for nodes, orders and branches



Calculated topological results from experimental repeated measurements were automatically saved by the Metrosideros Model program into two Microsoft Excel 97 files, examples of which are shown in Table 3.2 and Table 3.3.

Table 3.2 An example of a file with calculated topological parameters for observations. Repeated experimental measurements were made starting on 18.3.

18.3

Order #	no_nodes	no_br_point	no_term	no_intr	No_apic	br_probabi
1	1	1	0	3	0	1
2	3	0	6	3	0	0.33
3	3	0	7	2	1	0.22
4	2	0	4	2	0	0.33
5	2	1	4	2	1	0.33
6	2	0	4	2	0	0.33
7	2	1	2	4	0	0.67
8	4	0	8	4	0	0.33
9	4	0	9	3	1	0.25
10	3	0	6	3	0	0.33
11	3	0	7	2	1	0.22
12	2	0	4	2	0	0.33
13	2	0	4	2	0	0.33
14	2	0	6	0	2	0
Total	35	3	71	34	6	0.36
Total links	106			SD		0.05
S-C factor	807			Tot		0.32
Mean order	7.61					

Total Branches 7

Branch #	no_nodes	start_br	start_ord	length	no_leaves
0	5	-1	0	2	0
1	4	0	1	7	8
2	2	0	1	3	4
3	9	0	5	18	16
4	4	0	5	3	0
5	7	4	7	15	13
6	4	4	7	4.5	7

24.3

Order #r	no_nodes	no_br_point	no_term	no_intr	no_apic	br_probabi
1	1	1	0	3	0	1
2	3	0	6	3	0	0.33
3	3	0	7	2	1	0.22
4	2	0	4	2	0	0.33
5	2	1	4	2	1	0.33
6	2	0	4	2	0	0.33
7	2	1	2	4	0	0.67
8	4	0	8	4	0	0.33
9	4	0	9	3	1	0.25
10	3	0	6	3	0	0.33
11	3	0	7	2	1	0.22
12	2	0	4	2	0	0.33
13	2	0	4	2	0	0.33
14	2	0	6	0	2	0
Total	35	3	71	34	6	0.36
Total links	106			SD		0.05
S-C factor	807			Tot		0.32
Mean order	7.61					

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Total Branches 7

Branch #	no_nodes	start_br	start_ord	length	no_leaves	
0	5	-1	0	2	0	
1	4	0	1	7	8	
2	2	0	1	3	4	
3	9	0	5	18	16	
4	4	0	5	3	0	
5	7	4	7	15	13	
6	4	4	7	4.5	7	

31.3

Order #	no_nodes	no_br_point	no_term	no_intr	no_apic	br_probabi
1	1	1	0	3	0	1
2	3	0	6	3	0	0.33
3	3	0	7	2	1	0.22
4	2	0	4	2	0	0.33
5	2	1	4	2	1	0.33
6	2	0	4	2	0	0.33
7	2	1	2	4	0	0.67
8	4	0	8	4	0	0.33
9	4	0	9	3	1	0.25
10	3	0	6	3	0	0.33
11	3	0	7	2	1	0.22
12	2	0	4	2	0	0.33
13	2	0	4	2	0	0.33
14	2	0	5	1	1	0.17
15	1	0	3	0	1	0
Total	36	3	73	35	6	0.35
Total links	109			SD		0.05
S-C factor	852			Tot		0.32
Mean order	7.82					

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Total Branches 7

Branch #	no_nodes	start_br	start_ord	length	no_leaves	
0	5	-1	0	2	0	
1	4	0	1	7	8	
2	2	0	1	3	4	
3	10	0	5	18.5	16	
4	4	0	5	3	0	
5	7	4	7	15	13	
6	4	4	7	4.5	7	

Table 3.3 An example of a file with calculated tree asymmetry parameters. Repeated experimental measurements were made starting on 18.3.

18.3									
Branch	0(1)	57	9	5	71	0.706	0.765	0.059	0.51
Branch	0(5)	19	31	1	51	0.25	0.375	0.625	0.417
Branch	4(2)	5	15	9	29	0.385	0.154	0.231	0.256
TOTAL ASYMMETRY (3) = 0.394									
TOTAL PSAD = 0.882352948188782									

24.3									
Branch	0(1)	57	9	5	71	0.706	0.765	0.059	0.51
Branch	0(5)	19	31	1	51	0.25	0.375	0.625	0.417
Branch	4(2)	5	15	9	29	0.385	0.154	0.231	0.256
TOTAL ASYMMETRY (3) = 0.394									
TOTAL PSAD = 0.882352948188782									

31.3									
Branch	0(1)	59	9	5	73	0.714	0.771	0.057	0.514
Branch	0(5)	21	31	1	53	0.2	0.4	0.6	0.4
Branch	4(2)	5	15	9	29	0.385	0.154	0.231	0.256
TOTAL ASYMMETRY (3) = 0.390									
TOTAL PSAD = 0.878571450710297									

7.4									
Branch	0(1)	59	9	5	73	0.714	0.771	0.057	0.514
Branch	0(5)	21	31	1	53	0.2	0.4	0.6	0.4
Branch	4(2)	5	15	9	29	0.385	0.154	0.231	0.256
TOTAL ASYMMETRY (3) = 0.390									
TOTAL PSAD = 0.878571450710297									

14.4									
Branch	0(1)	61	11	5	77	0.676	0.757	0.081	0.505
Branch	0(5)	23	31	1	55	0.154	0.423	0.577	0.385
Branch	4(2)	5	15	9	29	0.385	0.154	0.231	0.256
TOTAL ASYMMETRY (3) = 0.382									
TOTAL PSAD = 0.851351380348206									

22.4									
Branch	0(1)	75	13	5	93	0.689	0.778	0.089	0.519
Branch	0(5)	35	33	1	69	0.03	0.515	0.485	0.343
Branch	3(1)	29	5	1	35	0.75	0.875	0.125	0.583
Branch	3(2)	23	5	1	29	0.692	0.846	0.154	0.564
Branch	4(2)	5	17	9	31	0.429	0.143	0.286	0.286
TOTAL ASYMMETRY (5) = 0.459									
TOTAL PSAD = 1.16477262973785									

28.4									
Branch	0(1)	123	13	5	141	0.797	0.855	0.058	0.57
Branch	0(2)	113	5	5	123	0.9	0.9	0	0.6
Branch	0(3)	107	5	1	113	0.927	0.964	0.036	0.642
Branch	0(5)	65	39	1	105	0.255	0.627	0.373	0.418
Branch	3(1)	53	5	7	65	0.774	0.742	0.032	0.516
Branch	3(2)	41	11	1	53	0.6	0.8	0.2	0.533
Branch	3(3)	27	7	7	41	0.526	0.526	0	0.351
Branch	3(4)	21	5	1	27	0.667	0.833	0.167	0.556
Branch	4(2)	5	19	13	37	0.412	0.235	0.176	0.275

TOTAL ASYMMETRY (9) = 0.496
TOTAL PSAD = 2.40340900421143

5.5

Branch	0(1)	187	17	5	209	0.825	0.883	0.058	0.589
Branch	0(2)	177	5	5	187	0.935	0.935	0	0.623
Branch	0(3)	171	5	1	177	0.954	0.977	0.023	0.651
Branch	0(4)	161	5	5	171	0.929	0.929	0	0.619
Branch	0(5)	99	61	1	161	0.241	0.62	0.38	0.414
Branch	3(1)	89	9	1	99	0.833	0.917	0.083	0.611
Branch	3(2)	69	11	9	89	0.674	0.698	0.023	0.465
Branch	3(3)	47	11	11	69	0.545	0.545	0	0.364
Branch	3(4)	25	11	11	47	0.318	0.318	0	0.212
Branch	4(2)	5	39	15	59	0.607	0.179	0.429	0.405
Branch	5(1)	29	9	1	39	0.556	0.778	0.222	0.519
Branch	5(7)	11	7	1	19	0.25	0.625	0.375	0.417

TOTAL ASYMMETRY (12) = 0.491
TOTAL PSAD = 3.09275507926941

From these files the parameter values to be further analysed were organised into tables in accordance with the 3x4 factorial design, and analysed statistically using SAS in Chapter 4 and Chapter 5, where the methods of analysis are described.

3.3 Results

3.3.1 Testing the Metrosideros Model parameters

The validity of the model assumptions, representation and usefulness of parameters, and hence the whole new model needed to be tested. That is usually done by comparison of the model with reality, either through application, or predictions that are compared with observed reality (Thornley and Johnson, 1990). In the case of the Metrosideros Model, the model was developed in order to test the Size-Complexity hypothesis of phase change attainment. Hence, the model was first tested for biological correctness and its ability to quantify difference between the 2D structures of different ontogenetic states of *Metrosideros excelsa*. The ability of the model to represent tree growth was based, firstly, on the initial evaluation of the dynamic growth data for topological size, size-complexity and complexity in this chapter. Secondly, it was assessed in non-linear statistical modelling and analyses of experimental data (Chapter 4), and lastly by detailed experimental tree architecture analyses (Chapter 5).

Initial evaluation of three topological parameters

The obtained dynamic architecture parameters showed two types of result that required separate analysis. These were results firstly, for the growth lag phase, and secondly, for the growth period itself, i.e. the shape of the growth function and the rates of growth during the growth period. While detailed analysis of the latter will be described in Chapter 4, an initial evaluation is provided below. Some of the phenological information gained for the growth lag period is also presented in this chapter. The lag period refers to the time between the experiment commencement and when an individual plant began to grow. During the lag period all architecture parameters retained their original values, and thus from a strictly mathematical point of view, these data should not be considered as part of the growth function, which always has increasing values. For this reason and because of the additional information these data provided with respect to plant phenology, lag period data were examined separately from the overall growth data later in this chapter.

Using the dynamic repeated measurements of the trees' topological growth during the 15 weeks of the experiment, topological size, size-complexity and complexity factors could be presented graphically. Representative examples are provided for one-side normalised data, as used for the growth modelling analysis, double normalised, and non-normalised data in juvenile plants, plantlets and adult plants growth at 24/16 ^{o}C, for topological size (Figure 3.2), size-complexity (Figure 3.3) and complexity (Figure 3.4).

The initial assessments of the model, and the suitability of the derived topological parameters to represent tree architecture growth determined the direction of further analyses, as described in Chapter 4. The choice of mathematical growth function, function fitting and analysis of architectural growth will be described there.

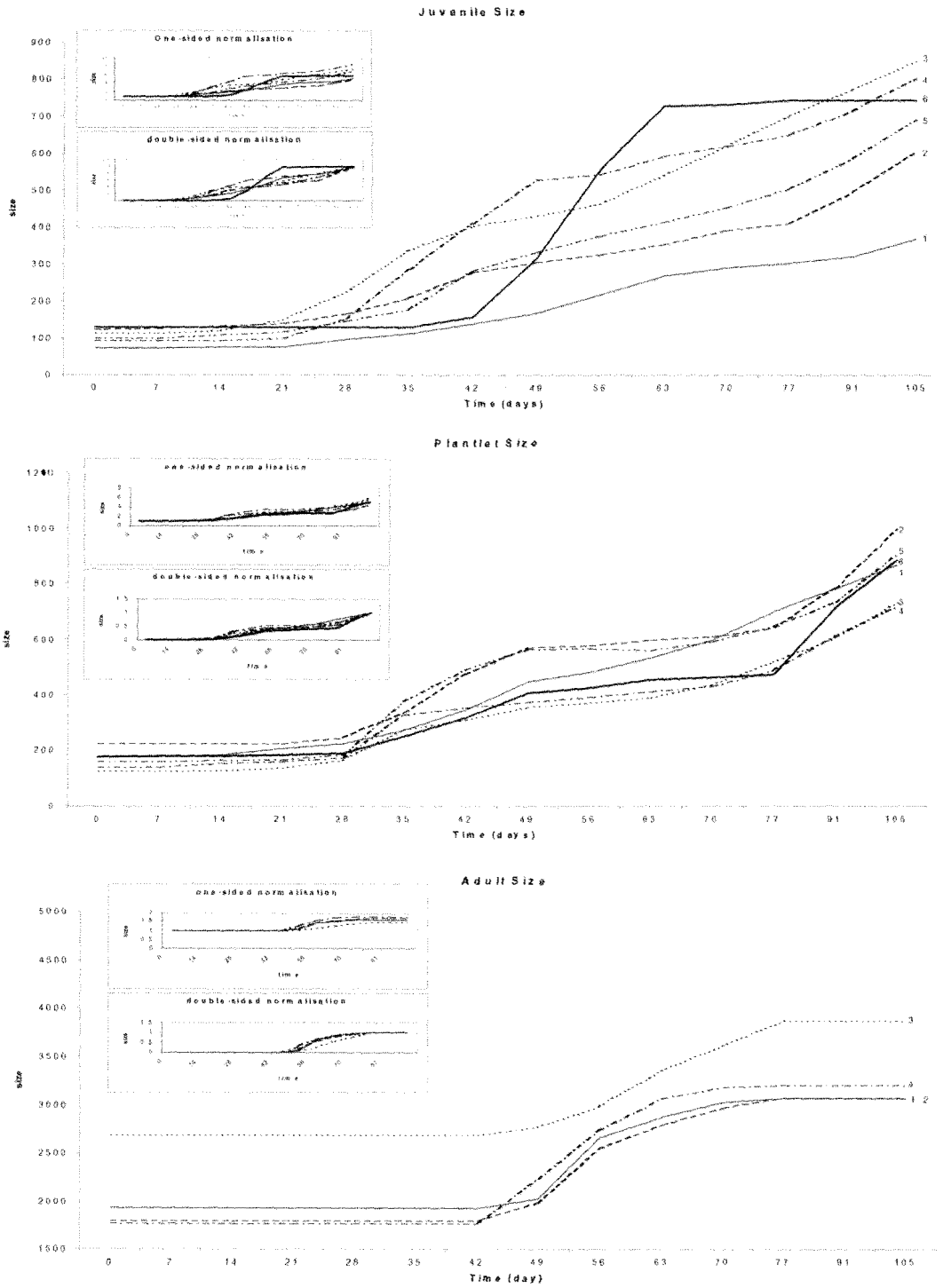

Figure 3.2 Topological growth for tree size in plantlets, juvenile and adult plants. The main panel for each plant state shows plot of growth in topological size (original data), and inserts showing plots of one-sided and double-sided normalisations.

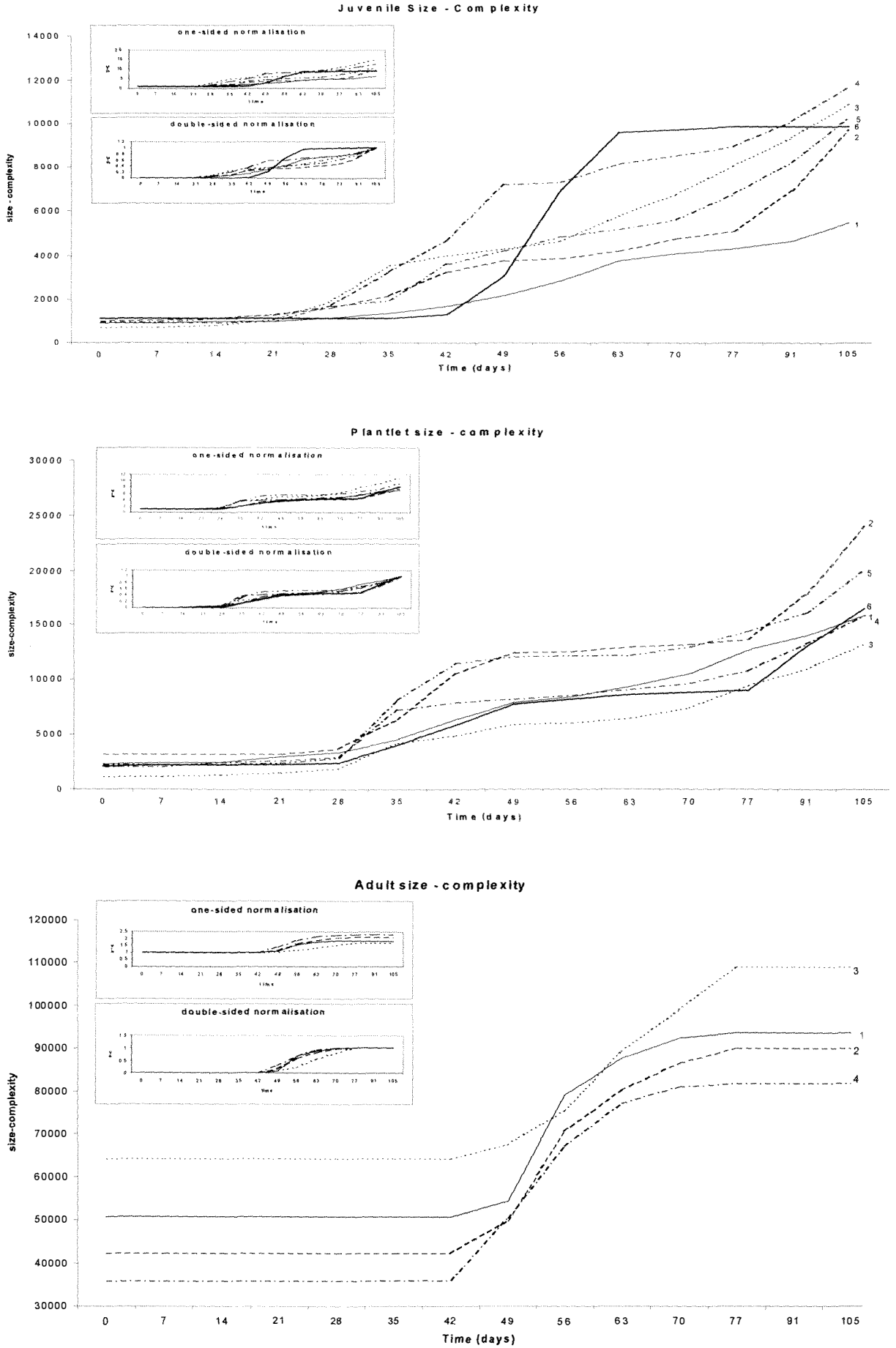

Figure 3.3 Topological growth for tree size-complexity in plantlets, juvenile and adult plants. The main panel for each plant state shows plot of growth in topological complexity (original data), and inserts showing plots of one-sided and double-sided normalisations.

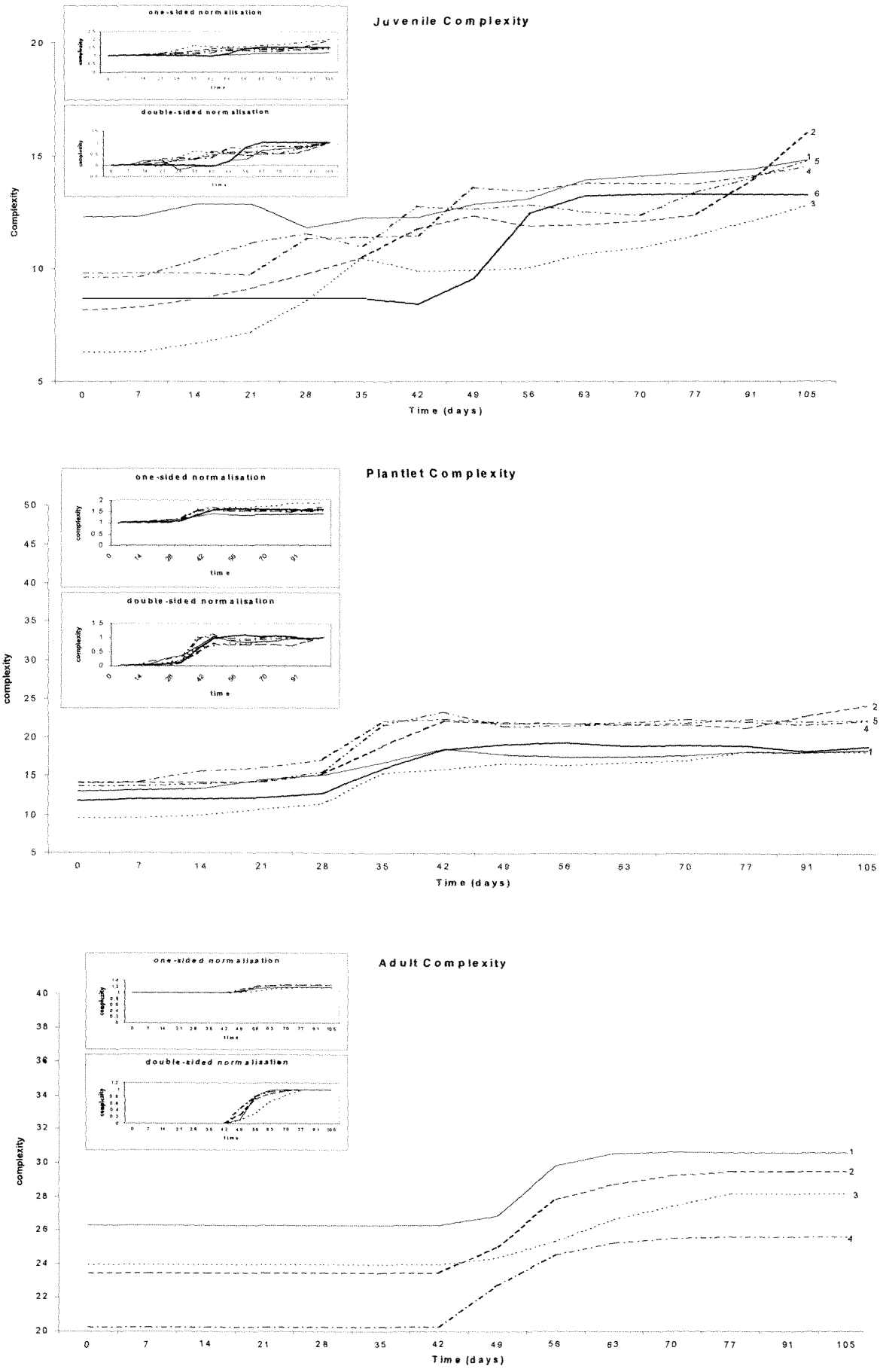

Figure 3.4 Topological growth for tree complexity in plantlets, juvenile and adult plants. The main panel for each plant state shows plot of growth in topological size-complexity (original data), and inserts showing plots of one-sided and double-sided normalisations.

Evaluation of the growth lag period

The duration of the lag period was affected by temperature, as well as by developmental state (Table 3.4). Overall, plantlets initiated growth in the shortest time after the experiment commenced. In the adult plants, on the other hand, lag period was the longest. However, the variation between individual adult plants within treatments was minimal, and at 16/8 and 24/16 °C, all adult plants initiated growth simultaneously at 70 and 42 days, respectively.

In contrast to adult plants, juvenile plants showed the highest variation between individual plants in all treatments (Table 3.4).

Table 3.4 Duration of the growth lag period (weeks) in juvenile, plantlets and adult plants of *M. excelsa* growth under four temperature regimes

Plant	Temperature regimes (Day/night) (°C)			
State	16/8	24/16	32/24	32/8
Juvenile	5.7 ±2.7[1]	2.0 ±1.8	3.2 ±1.3	1.0 +0.9
Plantlets	4.2 ±2.4	1.0 ±1.1	0.8 ±0.8	2.2 ±1.9
Adult	10 ±0.0	6.0 ±0.0	5.3 ±0.6	8.7 ±0.6

[1] Means ±SE

3.4 Discussion

The growth curves were generally of the logistic s-shape (sigmoid) for all architecture parameters in plantlets and adult plants as would be expected for biological growth of size (Hunt, 1982; Zeide, 1993b). They thus represented the dynamic growth in measured experimental trees in a biologically sound manner. From this initial evaluation of the parameters, it appeared that the 2D structural information was captured by the calculated parameters in a condensed quantitative form. Thus the Metrosideros Model, through its basic assumptions and definitions of units, was representative of the 2D topological growth of the *Metrosideros excelsa* trees. This was a significant advance and achievement in comparison to the current

state of dynamic tree architecture modelling as represented, for example, by the work of Godin et al. (1999), Prusinkiewicz et al. (1994), Thornley and Johnson (1990) or Honda et al. (1982).

The assumption of trifurcating branching in *Metrosideros excelsa,* and its application through dynamic development of the basic terminal units into internal units, which, in turn, bear growing numbers of new terminal units represented the mechanism of modular growth and branching in *Metrosideros excelsa* well. Similarly, the supporting assumption of representation of the biological hierarchy of basic units by the ascending centrifugal ordering system appeared to work well by being able to separate difference of complexity growth from that of representing biological growth of size.

With respect to the similarities in the size and size-complexity parameters growth functions shape, a considerable effect of size in the size-complexity factor parameter was not surprising since complexity is naturally size-dependent. This was projected into the size-complexity factor through its calculation as the sum of the centrifugal order weighted number of topological segments, i.e. topological size. Van Pelt et al. (1989) also acknowledged the effect of size in other architectural parameters. There are no previous reports for dynamic modelling of plant structural complexity (Buck-Sorlin and Bell, 2000; Pearcy and Valladares, 2000).

In all three parameters (except for complexity in juveniles) juvenile seedlings and plantlets showed a growth reduction and subsequent a second flush growth in the 24/16 ^{o}C treatment. It was noted that while the growth slowed considerably, no complete growth cessation was recorded between the growth flushes. This may be somewhat different to the observations of growth in single shoots, where complete cessation of growth between flushes may occur (Baltunis and Greenwood, 1999). However, the growth and/or elongation between subsequent flushes may not necessarily cease completely even in single shoots (Snowball, 1989). In fact, in accordance with a mathematical growth function, growth is continuously approaching its upper asymptote, and thus growth never ceases completely. Due to the nature of this study, the continued growth between two flushes could be accounted for either by the lack of synchronisation of shoot growth initiation within the crown shoot population (differing lag period), or by prolonged elongation of

individual shoots. From additional notes taken at the end of the experiment, it appeared that, for example, in the 24/16 ^{o}C treatment, about half of the shoots in the crown ended their growth and elongation while the rest continued to grow, and new branching occurred.

Baltinus and Greenwood (1999) examined the importance of variation in the timing of shoot growth initiation and its duration in terms of the overall growth performance in full-sib families and inter-specific *Larix* hybrids. Similar to the approach adopted in the current study, they fitted and analysed individual shoot growth, and analysed the time of growth initiation and cessation separately. They found a significant correlation between the overall growth performance and shoot phenology. In particular, growth rate increased with later time of growth cessation (Baltinus and Greenwood, 1999). This showed the importance of the lag period and its inclusion in the growth analysis as observed in this study, as opposed to studies where the lag period was eliminated from growth analysis (e.g. Richards, 1959; Karlsson and Heins, 1994). Moreover, the two different approaches with respect to the lag period in growth analysis also emphasised some of the difficulties of growth analysis, and justified the approach used in the current study, in which growth analyses including and excluding the lag period were carried out (Chapter 4).

In a related study, Borchert and Tomlinson (1984) measured seasonal growth and branching in the tropical tree *Tabebuia rosea* and found variation in lag period due to tree size. There were markedly asynchronous growth flushes among young (presumable juvenile) trees, growing at the same site as older (adult) trees, which flushed more synchronously.

The growth analyses of the Borchert and Tomlinson (1984), and Baltinus and Greenwood (1999) studies were comparable with the results of the current study. However, both Borchert and Tomlinson (1984) and Baltinus and Greenwood (1999) examined the growth of randomly selected individual shoots, while the results in this study represented the growth of the whole crown, i.e. all shoots in the population. In addition, two different measures of growth were used, i.e. metric measurements in previous studies, compared to the topological measurements in this current study. Deriving similar results from both types of study meant that the topological method in the current study appeared to be comparable to the more established metric

measurement of growth. Consequently, that indicated a sound biological base for the new architectural method developed in the Metrosideros Model.

Addressing the initial result with respect to ontogenetic states, there was little or no variation in the lag period between individual adult plants within each temperature treatment. This was in contrast to plants in the juvenile state, where individual plants showed high variability with respect to lag period within each temperature treatment. To a lesser extent, this was also valid for the plantlets. These results are consistent with those of Borchert and Tomlinson (1984) regarding the effect of size and developmental state on shoot phenology.

Overall the length of the lag period was negatively correlated with the increasing temperature of 16/8, 24/16 and 32/24 °C. The fourth temperature regime (32/8 °C), with its high difference of day and night temperatures, yielded a lag phase similar to that of the 24/16 °C treatment, which was not unexpected as the mean temperature in both regimes was 20 °C. Clearly, the timing of shoot growth initiation in the *Metrosideros* plants decreased as temperature increased, indicating that low temperature was not required to overcome physiological bud dormancy (Chuine et al., 1999).

With respect to the preliminary evaluations of experimental factors, as in other literature reports (Borchert, 1976; Borchert and Tomlinson, 1984), plants in the adult state showed a stability relative to the other plant states in their response to the environment. This was seen in the high level of synchronisation of their growth, and thus their whole crown phenology. Moreover, from initial evaluations of the complexity parameter it appeared that the growth processes were governed by different functions in the juvenile and adult states. In this respect the response of the plantlets, was similar in its sigmoid shape to the adult state, rather than to juvenile plant response. This revealed that the changeover of the governing functions between ontogenetic states, at least for complexity, is likely to be gradual and perhaps independent at the size and complexity levels of crown architecture. Such results support those of Poethig (1990) when considering developmental phase change in general, and Evans and Poethig (1993, 1995, 1997) in studying the phenomenon in maize.

Even though the Metrosideros Model was based on recording segments (links), as opposed to vertices (see Section 1.2.4.6), from the recording database the number of branching points for each tree could easily be derived (see Tables 3.1 and 3.2). The number of branching points in the Metrosideros Model was equivalent to the number of so called 'c – nodes' in an alternative Link-Vertex growth model used by Berry et al. (1986) and MacDonald (1984) (see Chapter 1, Figure 1.7). It is argued therefore, that while the two recording systems differ in the information that they carry, as pointed out by MacDonald (1984), they could be used to extract more information from a model. However, care has to be taken to distinguish between the two types of results. Conveniently, in the current project both types were used as described in Chapter 5 where Mean Centrifugal Order Number calculated on the basis of the number of segments was replaced with the calculation of Mean Centrifugal Order Number based on branching points. The latter parameter was then successfully used for relative architecture comparisons, whereas Mean Centrifugal Order Number calculated on the basis of number of segments was unsuccessful in doing this.

The Metrosideros Model also holds the possibility of incorporating adventitious branching. However, at this stage adventitious branching was disregarded in the Metrosideros Model by basic model assumption. Despite this, practical experience of using the Metrosideros Model for recording experimental data, showed that the model was able to handle adventitious branching, and it appeared that the possibility might exist for adjustment of the model to multifurcating branching.

A similarity existed between the assumption of branching in the Dendrite Tree Model and the definition of its probabilities and adventitious branching in botanical trees. As in the Dendrite Tree Model, the position and amount of adventitious branching would be difficult to define precisely, and could be expressed only as a probability of branching from a particular segment. Consequently, recording and quantification of adventitious branching could be achieved only after the branching had actually eventuated. Such an insufficiency in the basic assumption regarding adventitious branching would have severely compromised the development of a dynamic recording system for botanical tree.

At this stage, the Metrosideros Model addressed this issue in two ways. Firstly, it clearly distinguished between apical/axillary and adventitious branching by

definition of the terminal segments. Secondly, because the topological position of each internal segment was recorded, the model could theoretically allow for the potential inclusion of adventitious branching from internal segments in the recording system. The topological position of each adventitious branch could be identified. Therefore, under special conditions, which partially suppress the assumption of trifurcating branching, the model could allow for calculation of some limited topological parameters, such size or complexity. However, calculation of advanced architecture parameters, as those described in Chapter 5 would not be possible because they were based strictly on axillary/apical branching and the trifurcating assumption. Alternative ways of accommodating adventitious branching were explored during this project, and mathematical solutions possibly exist. However, such solutions were beyond the scope of this project, and thus were not further explored. If a mechanism of an 'extreme' type such as adventitious branching were the interest of study, then further exploration of the model possibilities would be desirable. In this project, however, the dynamics of apical and axillary buds were the main focus.

3.5 Conclusion

In the Metrosideros Model the essential assumption of trifurcation was upheld and the topological parameters were successfully expressed in mathematical form. These topological parameters represented growth in *M.excelsa* plants in a biologically sound manner, and thus the initial evaluation confirmed the suitability of the new Metrosideros Model for modelling the architecture of botanical trees.

CHAPTER FOUR

Testing the suitability of the Metrosideros Model for dynamic plant growth analysis

4.1 Introduction

Thornley and Johnson (1990) reviewed extensively the usefulness of modelling in plant physiology, the types of models available at the time, and a number of physiological topics with relevance to modelling approaches. They advocated a focus on dynamic deterministic models that would address the mechanism of a biological process at different levels, as opposed to empirical models that would purely evaluate experimental data without seeking the underlying mechanism. The need for dynamic recording and analysis at different plant levels is especially useful in developmental research due to the dynamic nature of the phenomenon itself (Borchert, 1976).

Experimental, mathematical and some statistical requirements and methods of growth modelling have been examined in detail by Hunt (1982), Causton (1986), Ratkowsky (1990) and Zeide (1993b). These studies identified two main obstacles that have to be addressed in order to proceed with dynamic tree growth modelling, and which often prevent wider use of this otherwise effective research tool. These are firstly, the development of a method for recording representative parameter/s of tree growth and development. Such a method needs to be non-destructive so that repeated measurements over time can be obtained. Secondly, a suitable growth model and statistical analyses have to be selected or developed in order to analyse these repeated measurements of plant growth with respect to the biological assumptions of the model.

In the current study, the issue of biometrics was addressed in the previous chapter, in which the topological Metrosideros Model, dynamic data recording and architecture parameter calculations were described. This chapter is concerned with the second part of dynamic growth modelling. It describes how a suitable growth function model

was selected that would fit the experimental data. From the modelled growth function, growth parameters were estimated and these were statistically analysed with respect to the effects of experimental factors.

The growth modelling exercise presented an opportunity to test the Metrosideros Model. Because the topological, two-dimensional method of measuring growth was new, as opposed to the more typical single-dimension metric measure (Thornley and Johnson, 1990), its suitability to represent plant growth and architecture of the whole canopy was also examined.

The Metrosideros Model and its parameters combined two factors of biological growth. It recorded the growth in terms of topological segments (units, equivalent to meristems in botanical terms) as well as the synchronisation (or its absence) of appearance of these units within the population of individual shoots within the crown. Both of these factors affect the character of the biological growth multiplicity (Ratkowsky, 1990) and thus the shape and growth rate (Causton, 1991; 1994) of the resulting growth function. Moreover, the parameters of complexity and size-complexity factor also accommodated a structural dimension of crown growth. In their 'two-dimensional' character, the examined topological growth parameters were different from the growth parameters used elsewhere. For example, they differed from those used by Karlsson and Heins (1994) in modelling shoot elongation, or by Garcia and Antor (1995) in modelling dry weight as a plant size parameter. Therefore, the dynamic behaviour of the topological parameters may not necessarily be identical to one-dimensional size parameters, such as weight or length (Thornley and Johnson, 1990). Thus, the effect of dimensionality and in fact the ability of the model to quantify the architectural information was tested in this chapter through the biological growth modelling and analysis of experimental data.

Lately, examples of dynamic modelling of shoot growth have become more common in the literature (Leakey and Logman, 1986; Karlsson and Heins, 1994; Prusinkiewicz et al., 1994; Deleuze and Hollier, 1995; Baltunis and Greenwood, 1999; Godin et al., 1999). Some of these studies used modelling as a tool for enhancing the effectiveness of 'conventional' non-linear data analyses (Borchert 1976; Thornley, 1977; Causton, 1985; Thornley and Johnson, 1990) by seeking the

mechanism underlying biological processes of growth. There were also attempts to model the growth of crown development through recording a branching mechanism (Michalewicz, 1997). However, no report was found that would enable the examination of the growth of a complete population of shoots in the crown recorded at the level of individual axillary and apical meristems. In this respect, the growth analysis of the Metrosideros Model parameters is unique and important in both model evaluation and the architectural effects of developmental state and temperature treatments.

Dynamic growth modelling was employed in this chapter to achieve three main objectives. These were:

- To test the suitability of the architectural Metrosideros Model to represent biological growth in general.
- To test the Metrosideros Model and its parameters for the ability to capture quantitatively information about two-dimensional tree structure and its dynamic change during plant growth.
- To model the experimental data with a growth function, estimate growth parameters for each plant, and analyse these growth parameters with respect to the effect of developmental state and temperature treatments.

By analysing the effect of experimental factors on three fundamentally important topological parameters, the hypothesis that tree architecture and/or its rate of change are affected by developmental state was tested. In this respect, it was also of interest to examine how the architecture growth parameters were affected by different temperature treatments. From any identified interactions of temperature with developmental state, an optimal treatment to accelerate the progress of phase change in juvenile plants and plantlets could possibly be deduced.

In order to facilitate the dynamic growth analysis, available mathematical models for plant growth, and statistical methods for their application to the experimental data were examined. To take full advantages of the means of non-linear analysis, the selected parameters of Chapman-Richards function were examined for content of biologically meaningful information. In this respect, estimated values of the intrinsic

growth rate coefficient were compared with the information gained from the conventionally calculated time-dependent calculated relative growth rate for its ability to identify ontogenetic characteristics. The growth rate-related shape parameter *b* of the Chapman-Richards function was also examined for its biological properties, and possible future use in long-term modelling of developmental processes.

4.2 Methods

4.2.1 Dynamic data of architectural parameters

Three different architectural parameters were used for comparative growth analysis of the effects of developmental state and temperature. For the initial evaluation of the experimental data and development of the growth modelling method and statistical analysis of the modelled growth functions, the size-complexity factor was used. This choice was made because it embodied both the tree topological size as well as complexity of the tree connectivity. Thus, it was assumed that if a common growth function could be fitted to the size-complexity data, then the same should be feasible to apply to the other two parameters of size and complexity separately.

The dynamics of each of the three topological parameters were examined using data sets of 11 weekly and 2 fortnightly repeated non-destructive measurements of six plant replications for the juvenile and rejuvenated (plantlets) states, and four replications for the adult state. These data were recorded over the period of four months on plants as described in Chapter 2 (see Section 2.2). The GLM procedure of SAS (SAS, Version 8.0) was used to assess the effect of experimental factors within the factorial design of 4x3 levels of temperature and state factors, respectively.

4.2.2 Topological parameters

4.2.2.1 Topological size

In the Metrosideros Model, topological size was represented by the total number of segments in the tree, as sometimes used in the Dendrite Tree Model (van Pelt and Verwer, 1986; van Pelt et al., 1989). In the Dendrite Tree Model (Verwer and van Pelt, 1983, 1985, 1986, 1990; van Pelt and Verwer, 1983, 1984a,b, 1985, 1986, van Pelt et al., 1986, 1989a, 1989b; Uylings et al., 1989a, 1989b; Verwer et al., 1992), the size parameter referred alternatively to both the number of terminal segments or the total number of segments. This could create some confusion about the parameter topological size. However, for either bifurcating or trifurcating trees, the number of terminal segments is directly related to the total number of segments, and thus the choice of either of these parameters to express topological tree size is arbitrary. Because the experimental data in the current study were normalised for fitting the growth function, the value of the topological size parameter would have been identical whichever segment number had been used, since the ratio between the size parameters remained the same. In the trifurcating tree model of *Metrosideros excelsa,* the total number of segments (T) was calculated from:

$$T = \left(\frac{3n - 1}{2} \right) - 1 \qquad (4.1)$$

where n is number of terminal segments.

4.2.2.2 Topological size-complexity factor

As noted above (see Section 1.2.4.6.) the size-complexity factor was not defined as a topological parameter as such in the original Dendrite Tree Model. However, in this study it was expressed from the sum component of the mean centrifugal order number calculations. The size-complexity factor was thus calculated as sum of the products of the number of segments at an order, multiplied by that order number:

$$\sum_{\gamma=0}^{\gamma_{max}} \gamma s_{\alpha}(\gamma) \qquad (4.2)$$

The size-complexity factor thus accommodated the number of segments (i.e. topological size) weighted by their topological centrifugal order.

4.2.2.3 Topological complexity (Mean Centrifugal Order Number)

The topological complexity was calculated from:

$$\bar{\gamma}_{\alpha}(n) = \frac{1}{\left(\frac{3n-1}{2}\right)-1} \cdot \sum_{\gamma=0}^{\gamma_{max}} \gamma s_{\alpha}(\gamma) \qquad (4.3)$$

which is the size-complexity factor divided by the total number of segments, i.e. topological size parameter.

4.2.3 Visual examination of experimental data

Each of the three topological parameters was initially plotted against time in order to evaluate the shape and variation between individual plants visually. In addition, data were normalised by dividing all measurements by the initial value (one-sided normalisation), and by subtracting one and dividing by the final measurement (both-sided normalisation). Examining the graphs of both-sided normalised data, the upper portion was skewed towards an artificially imposed upper asymptote in data sets that in their raw or one-sided normalised versions showed the growth being still at its linear stage. This could be seen from the graph for topological size of juvenile plants (Figure 3.1). Thus, the upper part of the modelled growth in some treatments would have been less precise than the lower part of the function, had double-normalisation been used. This was in addition to the fact that the last two observations were made at fortnightly as opposed to weekly intervals, and therefore there were fewer points at

the upper end of the growth curve. Despite the advantage that double-sided normalisation graphs showed less variation between replications, these data were not used further. After the preliminary evaluation of the dynamic topological data, including the visual, initial attempts at growth function fitting and growth parameter simulations, it was decided that one-side normalised data would be better than the double-sided for function fitting. This method of data 'normalisation' should not be confused with that of achieving normal data distribution, which is a standard procedure of data examination before statistical analyses.

The one-sided normalised data provided better conditions for a realistic estimate of the upper asymptote. This approach was complemented with the use of growth functions that did not require direct estimation of the upper asymptote parameter during the fitting exercise. Both the modified Richards and Chapman-Richards functions were similar in this respect, and were suitable for fitting the diverse type of data in this experiment. Moreover, the form of the Chapman-Richards function used effectively allowed the time of parameter estimation to be set from 0 to 15, thus fitting the experimental data more reliably in that particular range. Consequently, by the extrapolation of the remaining part of the growth function in data sets where such prediction became necessary, the growth forecasts also became more reliable in comparison to the direct estimate of the upper asymptote.

4.2.4 Screening for a suitable biological growth function

Due to the non-linearity of biological growth, functions are often used in the analysis of experimental data in both animal and plant studies that record growth by repeated measurements (Hunt, 1982; Causton, 1983, Zeide, 1993b). The biological growth expressed in units of size-mass W (e.g. dry weight, length etc.) is mathematically described as a function of time t:

$$W = f(t)$$

The function *f (t)* of biological growth under normal conditions is represented by a logistic, sigmoid, s-shape curve (Thornley and Johnson, 1990; Causton, 1991; Zeide, 1993b). Decreasing growth rate, and thus the shape of the function, is caused by limited substrate availability as the biological unit grows and develops (Hunt, 1982). Theoretically, if substrate availability is unlimited, biological growth may continue in an exponential manner. To some extent, this can be achieved *in vitro* under a regular sub-culturing regime for plant multiplication purposes. The overall decrease in the function relative growth rate is often affected by the so-called intrinsic or inherent growth rate coefficient (see Section 1.2.6). The growth parameter of the 'intrinsic' rate coefficient reflects the overall shape and slope of the growth rate function. In general, therefore, this single parameter exhibits similar features to growth parameters examining both the function shape and relative growth rate. Predominantly, however, it reports on the slope of the linear part of the growth function.

Several mathematical functions have a sigmoid shape and would potentially be suitable for plant growth modelling (Richards, 1959; Thornley and Johnson, 1990; Ratkowsky, 1990; Causton 1991; 1994; Zeide, 1993b). Preliminarily, the experimental data were fitted with four different growth functions of the following general forms:

1) Gompertz

$$W = ce^{-be^{-at}} \tag{4.4}$$

Where the intercept(W_0) and upper asymptote (W_∞) are given by :

$W_0 = ce^{-a}$

$W_\infty = c$

2) Richards (Richards, 1959)

$$W = c\left(1 - de^{-at}\right)^{\frac{1}{b}} \tag{4.5a}$$

Where the intercept (W_0) and upper asymptote (W_0) are given by:

$$W_0 = c\left(1 - de\right)^{\frac{1}{b}}$$
$$W_\infty = c$$

Two modified forms were also tested for fit to non-normalised data

$$W = \left[\left(W_f - W_0\right)\left(1 + W_0 \cdot e^{-at}\right)\right]^{\frac{1}{b}} + W_0 \qquad (4.5b)$$

$$W = A_0 + \left(W_0 - A_0\right)\left(\frac{1 + e^{B}}{1 + e^{B-at}}\right)^{\frac{1}{b}} \qquad (4.5c)$$

3) Chapman-Richards

$$W = c\left(1 - e^{-at}\right)^{\frac{1}{b}} \qquad (4.6a)$$

Where the intercept (W_0) and asymptote (W_∞) are given by :

$$W_0 = 0$$
$$W_\infty = c$$

For data fitting a modified form was used:

$$W = W_{15}\left(\frac{1 - e^{-at}}{1 - e^{15-a}}\right)^{\frac{1}{b}} \qquad (4.6b)$$

4) Schnute's (Korf) equation basic form

$$W = ce^{-bt^{-a}} \qquad (4.7a)$$

Where intercept (W_0) and asymptote (W_∞) are given by :

$$W_0 = 0$$
$$W_\infty = ac$$

For data fitting a difference form was used:

$$W = \left[c^b + \left(d^b - c^b\right)\frac{1 - e^{-a(X-x1)}}{1 - e^{-a(x2-x1)}}\right]^{\frac{1}{b}} \qquad (4.7b)$$

In all equations, the growth rate coefficient or its equivalent is marked *a*, and it is always negative as it determines the rate of growth decay (Richards, 1959). Growth parameter *b* is found in the power part of the Richards and related functions.

There were advantages and disadvantages to each of these functions, and these were examined in order to select a function suitable to represent the experimental data.

The Gompertz function appeared to be flexible enough and convergence with data could be achieved over most treatments, although it did not cope well with data sets that were not approaching the upper asymptote. This was the case for growth of adult plants at the temperature regimes of16/8, 32/24 and 32/8 ^{o}C, plantlets at 24/16 and 32/42 ^{o}C, and juveniles at 24/16 and 32/8 ^{o}C.

The original Richards function had good flexibility. However, it would have required more experimental data approaching the upper asymptote to achieve good convergence at this part of function.

The adjusted Richards equation had good flexibility and would have been suitable overall, since it did not require upper asymptote estimation due to the mathematical re-arrangement of the original Richards equation. However, because of the elimination of the upper asymptote parameter, the function required the estimation of four parameters. Consequently, the high number of evaluated parameters could have compromised the reliability of parameter estimation in data sets where the number of points was of a limited size, as was the case in these experimental data. Therefore, this function was not used.

The Chapman-Richards function had three parameters to estimate and did not require upper asymptote information from the experimental data. It became clear from initial evaluations, that this function would fit data from most individual plants well, and its three parameters could be estimated from the experimental data range with good confidence intervals. The third parameter of this function was the value of the function at a certain time (set to 105 days, i.e. the final measurement in the experimental data set). The first derivative was expressed analytically, relative growth rates calculated and further analysed at different time-points of the function.

Similarly, the coefficient of intrinsic growth rate (a) was estimated and further analysed with respect to the experimental factors and the objectives of this study. Estimated growth parameters of the Chapman-Richards function were used to model the growth for each experimental factor in both absolute and relative forms, and the shape of relative growth further analysed using five points along the modelled curve.

The Schnute's function did not require upper asymptote information either, appeared to be sufficiently flexible, and required an estimate of only two parameters. However, analytical derivatives of this function were not available. The first derivatives were necessary for comparisons of growth rates. While this shortcoming could have been overcome by numerical calculation of the growth rates, it would have led to unnecessarily complicated statistical analyses and this function not further examined.

4.2.5 Growth function fitting

The growth of individual plants was fitted with the Chapman-Richards growth function. The estimated growth parameters were then used to construct modelled growth for each experimental factor, while variation between the estimated growth parameters was used to analyse statistical differences between modelled growth.

Because the considered growth function was capable of modelling a single sigmoid curve, the experimental data were examined for multiple periods of growth. An alternative approach to fitting multiple growth flushes would have been the use of a polynomial function. However, due to the mathematical and statistical complexity of polynomial function parameters that had very little or no biological meaning (Thornley and Johnson, 1990), such an option was not considered for this study.

In the data sets where more than one growth flush was detected, the second flush data were separated at the point of the smallest increase prior to the commencement of the second flush of exponential growth. More than one growth flush was detected in juvenile plants grown at 24/16 ^{o}C and in plantlets at 32/24 ^{o}C. The second flush

growth was further examined. However, the small number of points did not warrant their statistical analysis, and these data were disregarded in further statistical growth analysis.

Using one-sided normalised topological parameter data sets of 11 weekly and two fortnightly repeated measurements for each individual plant, growth data were fitted by the Chapman-Richards equations of the following form:

$$W = W_{15}\left(\frac{1-e^{-at}}{1-e^{-15a}}\right)^{\frac{1}{b}} \tag{4.5b}$$

where W is the parameter value at time t, and a and b are the function growth parameters.

The estimated growth parameters were then use to model a growth function for each experimental factor. The functions of each factor were then standardised, and used for calculation of relative growth as follows:

$$f(t) = \frac{W}{W_{\infty}} = \left(1-e^{-at}\right)^{\frac{1}{b}} \tag{4.8}$$

where W is the function value at time t and W_{∞} is value at time $t = \infty$.

The first derivative of the standardised growth function represented a relative growth rate at a particular time t, and was calculated as follows:

$$f'(t) = \frac{a}{b}\left(1-e^{-at}\right)^{(1/b)-1} e^{-at} \tag{4.9}$$

the time at which the function equals a given value, f, is given by:

$$t = -\frac{1}{a}\ln\left(1-f^{b}\right) \tag{4.10}$$

and the inflexion point of the function occurs:

$$t_{(\inf)} = -\frac{1}{a}\ln b\,. \tag{4.10a}$$

Therefore, using equation (4.10), the time $t_{0.5}$, when the value of the function f is midway between zero and the asymptote is given by:

$$t_{0.5} = -\frac{1}{a}\ln(1-0.5^b)\,. \qquad (4.10b)$$

The time $t_{0.05}$ when function f is at 5% of the value of the upper asymptote indicates the beginning of a rise.

$$t_{0.05} = -\frac{1}{a}\ln\left(1-0.05^b\right) \qquad (4.10c)$$

The time $t_{0.95}$ when the function f is at 95% value of the upper asymptote indicates the beginning of a decline.

$$t_{0.95} = -\frac{1}{a}\ln\left(1-0.95^b\right) \qquad (4.10d)$$

Relative growth rate was calculated combining functions (4.9) and (4.10b). The value of relative growth rate at middle point ($t_{0.5}$) is given by:

$$f'_{0.5} = \frac{a}{b}\left(1-e^{-0.5a}\right)^{\left(\frac{1}{b}-1\right)}e^{-0.5a} \qquad (4.11)$$

The value of relative growth rate at inflection point (from equations 4.9 and 4.10a) is given by:

$$f'_{(\inf)} = a\left(1-b\right)^{\left(\frac{1}{b}-1\right)} \qquad (4.12)$$

4.2.6 Statistical assessments of the growth function's suitability

Preliminary assessments of the data using the four growth functions were made using Model Maker Version 2.0 software (SB Technology Ltd., England). This program used the Marquardt method for non-linear regression modelling (in SB ModelMaker Version 2.0, 1994 manual, quoting Marquardt, 1963). The program was used for its user-friendliness over the SAS Version 6.0 (SAS Institute, Cary, NC) program at that time. It provided identical results to SAS when the Marquardt method was used, while being simpler to use for the initial assessments of the functions and their fit to the experimental data. It also provided optimisation of selected parameters, statistics on the Goodness of Fit, such as weighted sum of squares (WSS), mean square (MS), variance ratio (F-values) and the probability that the model explained the variation

by chance (Q and P-values). Values were provided for R^2, commonly used as an indicator of Goodness of Fit, but is not routinely provided by SAS for non-linear regressions (SAS, Version 8.0). ModelMaker also provided confidence intervals for single or multiple parameters, and an instant view of a graph or table of the fit and the confidence intervals. These features made ModelMaker ideal for the initial assessment of the behaviour of the functions. Once the function was selected, the final analyses were then carried out using SAS, Version 8.0 (SAS Institute, Cary, NC). The SAS program was then used to further analyse the modelled growth function and estimated growth parameters from the experimental data of the three topological parameters.

4.2.7 Analysis of modelled growth

4.2.7.1 Growth function shape

The Chapman-Richards function parameters a, b and W_{15} (function values at 15 weeks) were estimated for each plant. Resulting parameters were than pooled together by experimental factor, and analysed using ANOVA's LSMeans for statistical differences between treatments.

The modelled growth functions for all factors were statistically analysed with respect to the function growth characteristics, such as growth rate coefficient, and at points of distinguishable growth behaviour for relative growth rate, and time of reaching function values. These points of interest were the point of reaching 5% ($t_{0.05}$, Function 4.7b) of the maximum growth (W'_{15}, or W'_7 in analyses excluding GLP, Function 4.3b), the midpoint of the function ($t_{0.5}$, Function 4.7b), the point of inflexion ($t_{(inf)}$, Function 4.7a) and at 95% ($t_{0.95}$, Function 4.7d) of the function's course in the normalised form, i.e. scaled from 0 to 1. The lower (5%) and upper (95%) points covered the exponential growth at the concave beginning, and at growth decline, prior to the approach of the upper asymptote, respectively. They indicated the speed of growth commencement as well as the speed with which growth slowed down before growth cessation. The middle and inflexion points were

expected to yield similar results, representing the growth behaviour within the linear part of the growth function. The GML procedure comparing the LSMean (SAS, Version 8.0) was used for the shape parameter analyses, and for the relative growth rates and growth rate coefficient analyses.

4.2.7.2 Relative growth rate analyses

Relative growth rate is often of interest in analysis of dynamic plant growth. However, significant statistical difference between factors is often not attained in these analyses (Causton, 1991). This is partly due to the effect of size differences between plants and partly due to sampling errors from destructive repeated method of growth recordings (Causton, 1991; 1994). While sampling error due to the non-destructive nature of measurements made in this study were expected to be minimal, size differences between the states existed. The effect of size on calculated relative growth rate is not possible to estimate before the analysis of particular experimental data. Moreover, conclusions from comparisons of the time-dependent relative growth rates, even if differences are significant, are not simple to draw. Consequently, relative growth rate at the middle and inflection points from the estimated growth function were calculated and analysed for the effect of experimental factors. Using this method, the calculated relative growth rate and thus the comparisons between experimental factors were largely independent of time. In this respect, therefore, the results of the two types of growth analysis performed in this study (see next Section) were expected to be similar.

Because of the time dependence of relative growth rate, analysis of the growth function's estimated values of so-called intrinsic (or inherent) growth rate coefficient (Richards, 1959; Hunt, 1981) were also performed. Consequently, the time independent nature of the growth rate coefficient would be more suitable for comparisons between ontogenetic states than the relative growth rate. The latter may be associated in long-term growth analysis with physiological ageing, as reviewed earlier (see Section 1.2.6). The growth rate coefficient affects predominantly the slope of the linear growth, but also the shape of the function (Figure 4.5). In the case

of Chapman–Richards equation, the growth rate coefficient was marked as parameter *a* and, similar to other growth functions, it can be found in the exponential part of the equation. The estimated values of the growth rate coefficient were analysed for the effect of experimental factors using LS Means test after square root transformation.

Because of a high correlation between the growth rate coefficient *a* and the growth parameter *b* in the Chapman-Richards function, analyses of parameter *b* were also performed. Parameter *b* values were transformed by natural logarithm before analysis. However, these analyses assessed only any direct effect of experimental factors on parameter *b*, and did not examine the correlation between *a* and *b* parameters and their effect on function shape.

4.2.7.3 Inclusion and exclusion of the growth lag period

Because dynamic growth analysis is a time dependent process, and because the times of experimental observation did not coincide with that of plant growth commencement, experimental data were analysed in two ways.

Firstly, the growth function was fitted to the data sets from the beginning of the experiment to the last record point of the first growth flush, i.e. overall experimental growth of the first flush that included the growth lag period. The second type of analysis was performed on data sets that did not include the time during which there was no increase in topological parameters values, i.e. the growth lag period was excluded from the fitting procedure. From this type of growth analysis, information on the growth function shape was sought.

4.3 Results

Results are reported for the three topological parameters (size-complexity, size, and complexity) with respect to the estimated intrinsic growth rate coefficient, and the calculated relative growth rate at the middle point and point of inflection. The shape

of the function was examined for time at which 5 %, 50% and 95% of the maximum function value was reached. Analyses of absolute growth at 15 weeks (and 7 weeks in analyses that excluded the growth lag period (-GLP)) were also carried out.

4.3.1 Topological size-complexity factor

The fit of the Chapman-Richards function was very good for the individual plant growth experimental data (R^2>96%) for all analysed plants.

4.3.1.1 Growth analysis including the lag period

Function shape

There were highly significant effects ($P < 0.01$) of plant State and Temperature factors, but not of the interaction between them. Further analyses therefore concentrated on comparisons within these two factors.

According to the model of the first growth flush, there were significant differences ($P \leq 0.05$) between the three plant states in the time to reach the 5% point on the normalised scale. The time to reach this point was shortest for plantlets, followed by juvenile and adult plants. All other points analysed (50% and 95% of growth, and the point of inflection) were reached fastest by plantlets. There were no statistical differences between juvenile seedlings and adult plants at these points (Figure 4.1; Table 4.1).

All points on the growth function were reached fastest in the 24/16 and 32/24 °C temperature treatments, which were not significantly different from each other. The 32/8 °C treatment gave the next fastest growth response, followed by the 16/8 °C treatment. Times to reach each point were not significantly different between these treatments except at the 5% point (Figure 4.2; Table 4.2).

Table 4.1 The effect of ontogenetic state on growth function shape characteristics for topological size-complexity factor with the growth lag period included (GLP) or excluded (-GLP)

State	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth	
									W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	48a	26a	74a	58a	67a	47a	124a	125a	7.6a	5.6a
Plantlets	38b	27a	54b	45a	49b	40a	80b	81b	7.0a	5.1a
Adult	62c	14b	90a	49a	83a	29b	140a	140a	2.2b	2.9b

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.2 The effect of temperature regime on growth function shape characteristics for topological size-complexity factor with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth	
									W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	69a	27a	104a	66a	95a	48a	167a	153a	3.7a	4.7a
24/16	34b	14b	48b	28b	43b	23b	72bc	56b	4.4a	4.1ab
32/24	36b	16b	57b	41b	51b	29b	97bc	97b	7.2b	6.3ac
32/8	58c	31a	81a	70a	75c	54a	123ac	155a	7.0b	2.9ab

Means followed by the same letters within a column are not significantly different (P = 0.05)

Relative growth rates and growth rate coefficient for size-complexity (including GLP)

Overall, State and Temperature had significant effects ($P \leq 0.05$) on the analysed growth rates, but there was no significant interaction between these factors.

Plantlets had the highest relative growth rate at the middle and inflection points, and there was no difference in this growth parameter between juvenile ($P=0.023$) and adult plants ($P=0.030$).

There was no overall statistical difference between states in growth parameter *b*. The only significant effect on *b* was between the temperature treatments 24/16 and 32/24 ^{o}C. Relative growth rates at the mid-point and the inflection point were highest at 24/16 ^{o}C, whereas those for the other temperature treatments were approximately half of those at 24/16 ^{o}C, and did not differ among themselves.

There were highly significant ($P \leq 0.01$) effects of State and Temperature on the growth rate coefficient, and not of their interaction. As for the relative growth rates, plantlets had the highest growth rate coefficient, and those for juvenile and adult plants did not differ (Figure 4.1; Table 4.3). The highest growth rate coefficient was estimated for the temperature 24/16 ^{o}C, which was significantly different ($P \leq 0.0016$) from that at all other temperatures (Table 4.4).

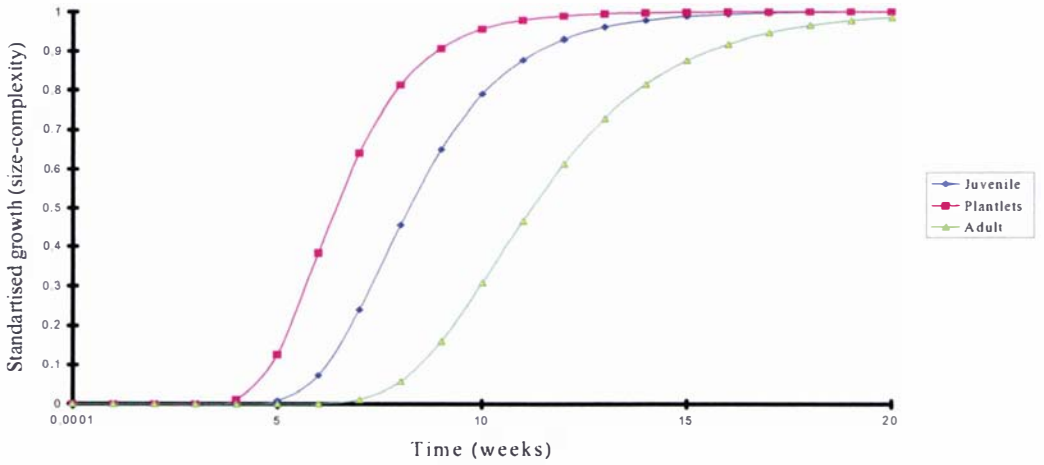

Figure 4.1 Relative growth of plantlets, juvenile and adult plants for topological size-complexity

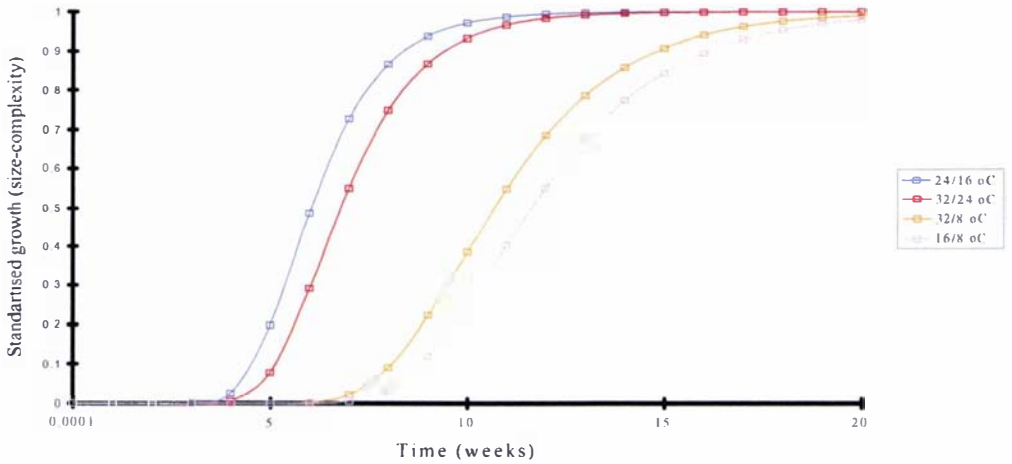

Figure 4.2 Relative growth in four temperature regimes for topological size-complexity

4.3.1.2 Growth analysis for size-complexity excluding the Growth Lag Period

Function shape

Similar to the analyses that included GLP, both experimental factors had significant ($P \leq 0.05$) effects on function shape. Growth during the initial exponential growth (5% point) was fastest in mature plants, and this was significantly different from that of juvenile plants ($P=0.0105$) and plantlets ($P=0.0056$). Adult plants reached the inflection point of growth first. However, time to reach 50% of growth in adult plants did not differ from that for plantlets and seedlings. Although plantlets and seedlings took longer to reach 5% of growth, plantlets reached 95% of growth fastest, whereas seedlings reached this point in the same length of time as adult plants (Table 4.1).

The time of the size-complexity factor to reach any of the analysed points of the fitted function were the same for the temperature treatments 24/16 and 32/24 ^{o}C. Similarly, the times to reach these points in the 16/8 and 32/8 ^{o}C treatments were not statistically different, although these were approximately twice as long as for the former temperatures (Table 4.2).

Relative growth rates and growth rate coefficient for size-complexity (excluding GLP)

As for the analyses when GLP was included, when it was excluded there was no difference in growth rate coefficient or relative growth rate of juvenile and adult plants. Similarly, highest values were for plantlets, although these were not significantly different from those of adult plants. There was also a significant effect of Temperature, with 24/16 ^{o}C having the highest growth rates compared to all other treatments (Table 4.4).

Table 4.3 The effect of ontogenetic state on the topological size-complexity growth rate coefficient and relative growth rate with the growth lag period included (GLP) or excluded (-GLP)

State	Growth rate coefficient, √a		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	0.733a	0.664a	0.196a	0.182a	0.208a	0.197a
Plantlets	0.880b	0.834bc	0.286b	0.269bc	0.304b	0.288bc
Adult	0.710a	0.683ac	0.198a	0.222ac	0.211a	0.247ac

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.4 The effect of temperature regime on the topological size-complexity growth rate coefficient and relative growth rate with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	Growth rate coefficient, √a		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	0.637a	0.582a	0.150a	0.145a	0.159a	0.159a
24/16	0.994b	0.977b	0.370b	0.370b	0.394b	0.401b
32/24	0.744a	0.703a	0.205a	0.202a	0.218a	0.223a
32/8	0.721a	0.645a	0.182a	0.180a	0.193a	0.194a

Means followed by the same letters within a column are not significantly different (P = 0.05)

Maximal absolute growth of size-complexity factor

On the modelled absolute growth scale, juvenile seedlings increased ca. 7.6-fold of the value of their original topological size-complexity parameter during the first flush. This was not significantly different from that of plantlets at a 7-fold increase.

However, both of these states differed significantly from the adult state, which increased by only 2.5-fold (Table 4.1; Figure 4.3).

The highest absolute growth (estimated as upper asymptote values) in size-complexity factor was recorded at the 32/8 and 32/24 °C, while the lowest was at a temperature of 24/16 and 16/8 °C (Table 4.2; Figure 4.4). The two treatments with the highest growth rates differed significantly from the two treatments with lower growth rates. These results should, however, only be viewed as complementary to the results of the standardised growth function since the growth function analyses concentrated on the first flush growth only.

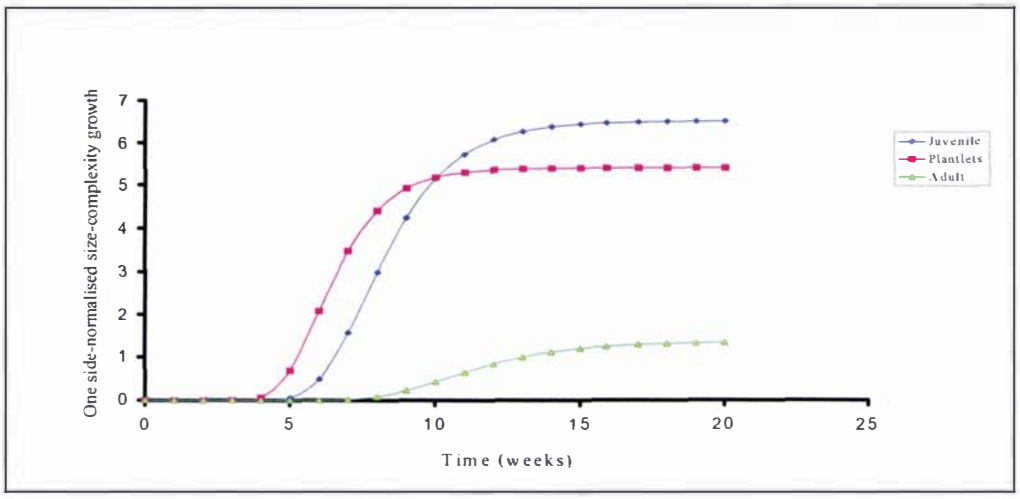

Figure 4.3 Absolute growth of plantlets, juvenile and adult plants for topological size-complexity

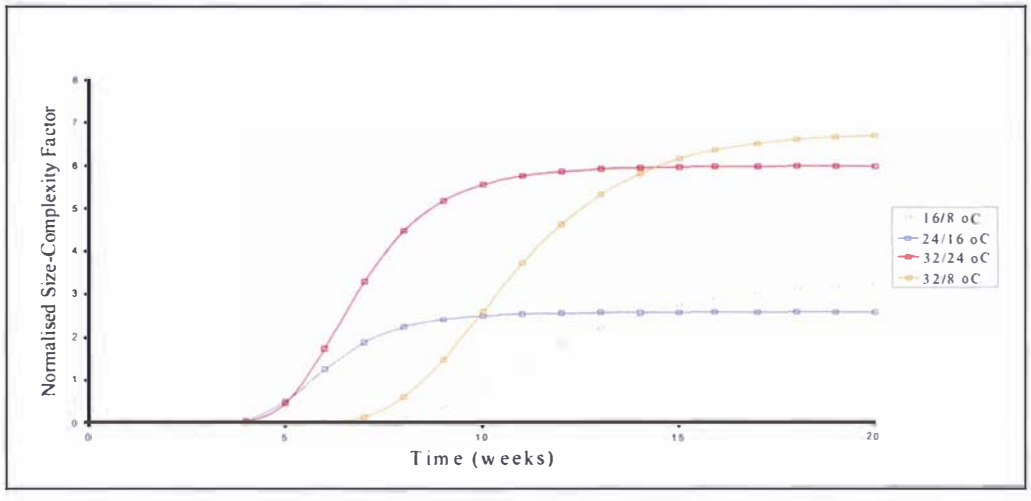

Figure 4.4 Absolute growth in four temperature regimes for topological size-complexity

4.3.2 Topological size

The fit of the Chapman-Richards growth function for the topological size parameter was again very good for individual plants (in the range of R^2 = 98 to 99.8 %).

4.3.2.1 Growth analysis including the Growth Lag Phase

Function shape

Overall analyses of the experimental factors showed highly significant effects ($P<0.01$) of both plant state and temperature treatments, but not of the interaction between them. Further analyses therefore concentrated on comparisons within these two factors.

Comparative statistical analyses using the estimated growth function parameters showed that there were statistically significant differences between the temperature treatments, but not between the states for most examined growth parameters. At the exponential part of the growth function (time to reach 5% of function value), there was a statistically significant difference ($P = 0.044$) between plantlets and adult plants (Table 4.5).

With respect to the temperature effect, all analysed points of the growth function were reached more slowly ($P\leq0.01$) at 16/8 ^{o}C than at 24/16 and 32/24 ^{o}C. The latter reached these points statistically at the same time. There was no significant difference in these growth parameters between temperatures 16/8 and 32/8 ^{o}C (Table 4.6).

Table 4.5 The effect of ontogenetic state on the growth function shape characteristics for topological size with the growth lag period included (GLP) or excluded (-GLP)

State	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	58ab	29a	104a	67a	90a	53a	194a	145a	5.9a	4.7a
Plantlets	38a	26a	54a	46b	50a	40b	81a	85b	4.5b	3.3b
Adult	62b	15b	87a	50b	81a	31b	134a	137b	1.8c	2.3b

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.6 The effect of temperature regime on growth function shape characteristics for topological size with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	82a	30a	137a	77a	120a	57a	242a	181a	2.8a	3.7a
24/16	33b	14b	46b	28b	43b	23b	71b	57b	3.1a	2.9ab
32/24	35bc	16b	53b	39b	48b	28b	88b	92b	5.1b	4.7ac
32/8	61ac	33a	90a	74a	82a	58a	146a	160a	5.2b	2.4ab

Means followed by the same letters within a column are not significantly different (P = 0.05)

Relative growth rates and growth rate coefficient for topological size (including GLP)

Plantlets had the highest growth rate at the inflection and the middle points, and there were no significant differences between juvenile seedlings and adult plants at these points. Similarly, the estimated growth rate coefficient was significantly higher ($P \leq 0.0344$) in plantlets, than that in juvenile and adult plants, which were not significantly different from each other.

All plants had significantly ($P \leq 0.001$) higher relative growth rates and growth rate coefficients at 24/16 ^{o}C than at all other temperatures, which, in general did not differ (Table 4.7).

Table 4.7 The effect of ontogenetic state on growth rate coefficient and relative growth rate for topological size with the growth lag period included (GLP) or excluded (-GLP)

State	Growth rate coefficient, $\sqrt{a}$		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	0.705a	0.620a	0.187a	0.160a	0.200a	0.175a
Plantlets	0.858b	0.808b	0.270b	0.250b	0.287b	0.268b
Adult	0.717a	0.698ab	0.200a	0.228ab	0.212a	0.253ab

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.8 The effect of temperature regime on growth rate coefficient and relative growth rate for topological size with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	Growth rate coefficient, √a		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	0.618a	0.526a	0.146a	0.120a	0.154a	0.132a
24/16	0.953b	0.935b	0.338b	0.339b	0.360b	0.368b
32/24	0.761c	0.737c	0.213a	0.217c	0.228a	0.238c
32/8	0.709ac	0.636ac	0.178a	0.176ac	0.189a	0.190ac

Means followed by the same letters within a column are not significantly different (P = 0.05)

4.3.2.2 Growth analysis for size excluding the Growth Lag Period

Function shape

Plants in the adult state reached the point of 5% growth significantly ($P \leq 0.0089$) earlier than both seedlings and plantlets, which were not significantly different in this respect. Plantlets and adult plants did not differ in the time taken to reach the points of inflection, middle and 95% growth. On the other hand, the growth of seedlings became significantly slower ($P \leq 0.033$) in comparison to the growth of plantlets and adult plants at the middle, inflection and 95% points (Table 4.5).

Plants reached all examined function points earliest in the 24/16 °C temperature regime, but times taken to reach these points were not significantly different from those at 32/24 °C. Growth in the 16/8 and 32/8 °C temperature regimes did not differ from each other, but were significantly ($P \leq 0.03$) slower than those in the other two treatments (Table 4.6).

Relative growth rates and growth rate coefficient for topological size (excluding GLP)

These analyses yielded somewhat different results from those that included GLP. Notably, the growth parameter *b*, which is highly correlated to growth rate coefficient, became significantly affected by developmental state. In general, the growth parameter *b* was affected by the differences between factors associated with the Growth Lag Period rather than by experimental factors themselves. However, no further analyses were carried out to examine the correlation between GLP, parameter *b* and the growth rate coefficient, except function simulations (Figure 4.5).

With respect to the calculated relative growth rates and the growth rate coefficient, similar to the responses when GLP was included, plantlets had the highest growth rates, but these were not significantly different from those of adult plants. Growth rates of juvenile plants did not differ significantly from those of adult plants.

In the 24/16 °C temperature regime plants had significantly highest growth rates, followed by the 32/24 °C regime, and the other two temperatures.

4.3.3 Topological complexity

The fit of the Chapman-Richards growth function for topological complexity of individual plants was good for plantlets and mature plants ($R^2 > 96$ %). However, experimental data for topological complexity of juvenile seedlings did not follow a sigmoid shape, and thus the biological growth function could not be fitted to these data. Because the topological complexity data of juvenile plants in all temperature regimes (see Chapter 3, Figure 3.4) did not follow the basic assumption of biological growth, it was excluded from further growth analyses.

4.3.3.1 Growth analysis including the Growth Lag Period

Function shape

Statistical analysis of the complexity growth parameter revealed that both State and Temperature had significant effects ($P \leq 0.003$) on the shape of the function. Further LSMeans tests revealed a similar response pattern to that for the size-complexity factor. Plantlets reached all points of the function faster than adult plants (Figure 4.9). Similarly, the times to reach 5, 50 and 95% of growth were not significantly different at temperature regimes 32/24 and 24/16 ^{o}C, these being shorter for those at 16/8 and 32/8 ^{o}C (Table 4.10)

Table 4.9 The effect of ontogenetic state on growth function shape characteristics for topological complexity with the growth lag period included (GLP) or excluded (-GLP)

State	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth	
									W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Plantlets	29a	20a	41a	35a	37a	30a	62a	68a	1.57a	1.48a
Adult	59b	9b	80b	40a	74b	18a	116b	127b	1.18b	1.27b

Means followed by the same letters within a column are not significantly different ($P = 0.05$)

Table 4.10 The effect of temperature regime on growth function characteristics for topological complexity with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	5% (Days), $t_{0.05}$		50% (Days), $t_{0.5}$		Inflection (Days), $t_{(inf)}$		95% (Days), $t_{0.95}$		Absolute growth	
									W_{15}	W_7
	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	54a	13a	74a	47a	69a	21ab	111a	147a	1.32a	1.43a
24/16	35b	11a	43b	19b	41b	15b	60b	39c	1.40a	1.40a
32/24	34b	13a	52b	32a	47b	22ab	84c	77bc	1.48b	1.49a
32/8	53a	22a	71a	53a	66a	37a	105ac	130ab	1.30a	1.17b

Means followed by the same letters within a column are not significantly different (P = 0.05)

Relative growth rates and growth rate coefficient for topological complexity (including GLP)

There was a significant effect of both factor Temperature and State ($P \leq 0.04$) on the growth rate coefficient, although their interaction was not significant. On the other hand, overall relative growth rates at the middle and inflection points were significantly affected by temperature regime ($P \leq 0.02$), and not by state ($P \leq 0.08$). The growth rate coefficient for topological complexity was significantly ($P = 0.0433$) higher in plantlets than in adult plants, and at 24/16 °C compared to all other temperatures (Table 4.10).

The relative growth rates for topological complexity calculated at the middle and inflection points was higher in the plantlets than in the adult plants, but these were not statistically significant effects ($P = 0.0758$). The highest relative growth rate in plant complexity occurred in the 24/16 °C temperature regime (Table 4.12).

4.3.3.2 Growth analysis for complexity excluding the Growth Lag Period

Function shape

Plantlets reached 5% of the growth function more slowly than adult plants ($P \leq 0.018$), yet reached the 95% point faster (Table 4.9). Other growth function shape indicators were not significantly affected by state. Temperature had little effect on the growth function shape, except at the 95% point which was reached more quickly at 24/16 ^{o}C (Table 4.10).

Relative growth rates and growth rate coefficient

The growth rate coefficient for topological complexity was significantly ($P = 0.011$) higher in plantlets than in adult plants. However, the calculated relative growth rates at the middle and inflection points were not significantly different (Table 4.11).

In general, the growth rate coefficient and relative growth rate at the middle point were significantly ($P \leq 0.04$) higher in plants grown at the 24/16 ^{o}C temperature regime (Table 4.12).

Table 4.11 The effect of ontogenetic state on growth rate coefficient and relative growth rate for topological complexity with the growth lag period included (GLP) or excluded (-GLP)

State	Growth rate coefficient, √a		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	NA	NA	NA	NA	NA	NA
Plantlets	1.017a	1.016a	0.391a	0.407a	0.416a	0.438a
Adult	0.821b	0.683b	0.263a	0.239a	0.279a	0.286a

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.12 The effect of temperature regime on growth rate coefficient and relative growth rate for topological complexity with the growth lag period included (GLP) or excluded (-GLP)

Temperature regime (°C)	Growth rate coefficient, √a		Relative growth rate – middle point, $f'_{0.5}$		Relative growth rate – inflection point, $f'_{(inf)}$	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	0.770a	0.615a	0.228a	0.196a	0.243a	0.244a
24/16	1.193b	1.64b	0.527b	0.517b	0.562b	0.565b
32/24	0.872a	0.890ab	0.283a	0.323a	0.301a	0.356a
32/8	0.842a	0.717a	0.267a	0.243a	0.284a	0.267a

Means followed by the same letters within a column are not significantly different (P = 0.05)

4.3.4 Analysis of growth parameter *b*

Overall, State affected growth parameter *b* only when the GLP was excluded from analysis, while Temperature had no significant effect on this growth parameter (Tables 4.13 and 4.14).

Table 4.13 Effect of ontogenetic state on growth parameter *b*

State	Parameter b (negative ln b values)					
	Size-complexity		Size		Complexity	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
Juvenile	4.6a	2.4a	4.3a	2.1a	NA	NA
Plantlets	5.2a	3.5b	5.0a	3.3b	5.7a	4.0a
Adult	5.7a	1.5a	5.7a	1.6a	7.0a	1.1b

Means followed by the same letters within a column are not significantly different (P = 0.05)

Table 4.14 Effect of temperature regimes on growth parameter *b*

Temperature regime	Parameter b (in negative ln b values)					
	Size-complexity		Size		Complexity	
	GLP	-GLP	GLP	-GLP	GLP	-GLP
16/8	5.0a	2.2a	4.9a	1.9a	5.6a	1.7a
24/16	6.2ab	3.0a	5.8ab	2.7a	8.6ab	3.3a
32/24	3.8ac	2.1a	3.9ac	2.2a	4.2ac	2.5a
32/8	5.6ae	2.7a	5.4a	2.6a	7.1a	2.9a

Means followed by the same letters within a column are not significantly different (P = 0.05)

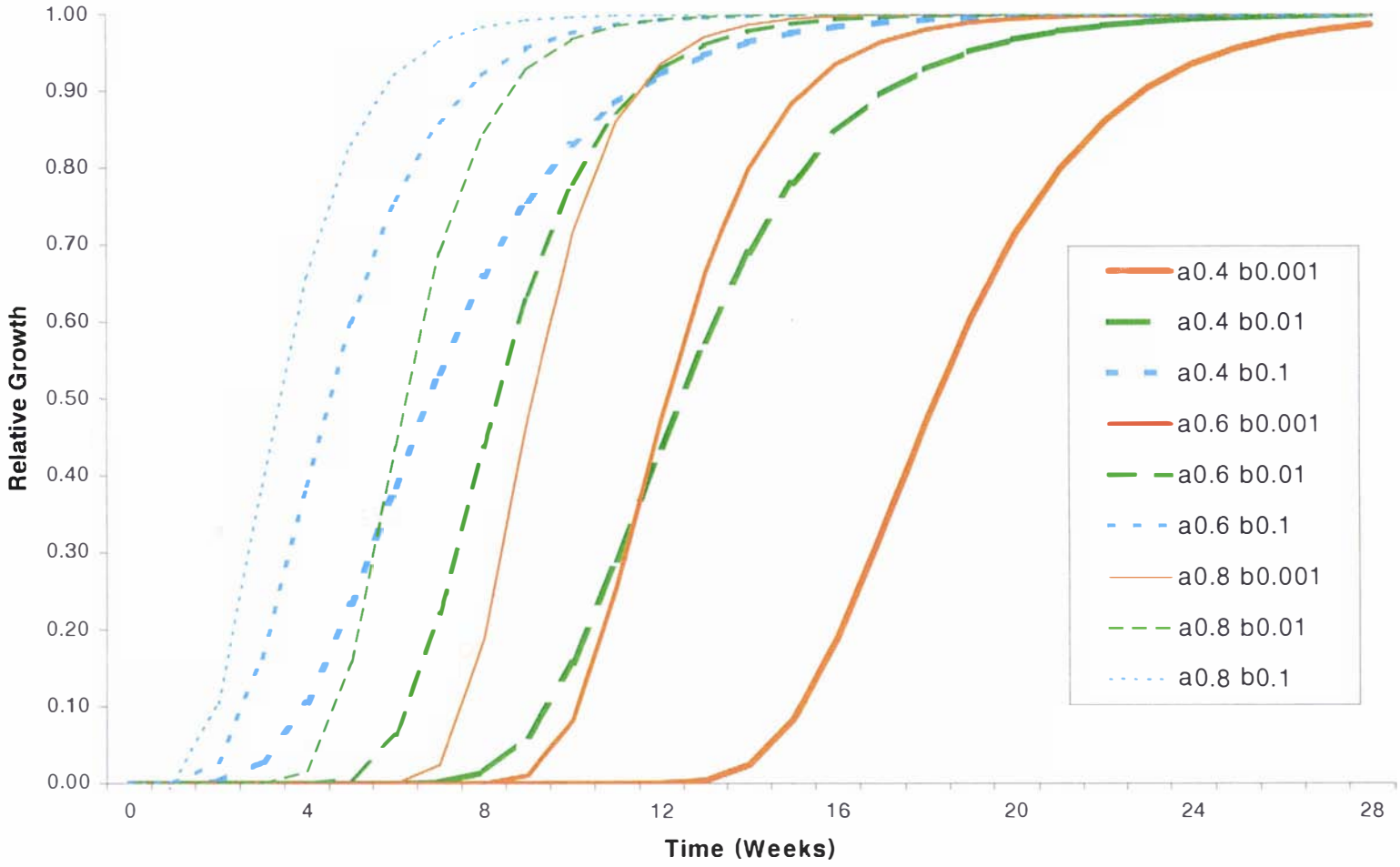

Figure 4.5 Simulations of Chapman-Richards relative growth function showing the relationship between inherent growth rate *a* and growth parameter *b*.

4.4 Discussion

Dynamic plant growth modelling is still not widely used for analysing plant responses to experimental factors (Thornley and Johnson, 1990; Zeide, 1993b). In this chapter, the newly developed Metrosideros Model was used to capture and analyse dynamic growth of the three topological parameters of size, size-complexity factor and complexity (see Chapter 3, and Section 4.3.2. in this chapter). Because of this, and because of the novel methods used in this study, the effectiveness of these methods and the usefulness of the derived information are first discussed. This is followed by a discussion of the biological significance of the experimental results.

4.4.1 Method of growth analysis

Individual plant growth fitting

The decision to fit the growth function to data for individual trees was made based on a preliminary evaluation of the data sets and simulated behaviour of the growth functions (see Section 4.2.5). Recent reports also show a preference for fitting growth functions to individual shoots (Deleuze and Houllier, 1995; Baltunis and Greenwood, 1999), as opposed to the set of replications conventionally used for analysis of linear data sets.

This approach had a number of advantages. Applying the function to each plant separately resolved the significant problem of differences in the time at which individual plants started to grow, since plants did not initiate growth at the same time. However, the real time sequence of the treatment effect would have been broken if the first growth increase had been taken as time zero plus one (T_{0+1}) for each plant. Since growth modelling is a process that examines data in a time-dependent manner, it was crucial that a real time sequence were upheld. However, identification of time zero with respect to the beginning of modelled growth was not a simple issue. Theoretically, time zero should be set to the point at which the growth

function begins its fit, i.e. the last point of the non-increasing GLP immediately before the growth value increases.

It would have been feasible to eliminate the GLP if all replications had begun to grow at the same time. Equally, averaging the GLP across all plants in treatment would have broken the real time sequence, and the phenological information in each parameter would have been lost (M. Kimberley and R. Shula, pers. comm.). Moreover, setting time zero to the beginning of the experiment and fitting the growth function to all the replications would have been unsatisfactory because growth parameter estimates would have been affected by the number of points in the GLP. By fitting the function to data for individual plants, a good statistical fit was achieved, similarly to those reported by Karlsson and Heins (1994), Hawke (1998), and Baltunis and Greenwood (1999). Due to this and to the number of recording points, the fitted function represented the experimental data in a meaningful manner. It was not a good fit by chance (Ratkowsky, 1990; Thornley and Johnson, 1990; SB ModelMaker, 1994). It was reasonable to expect that the three-parameter growth function would yield reliable value estimates (Richards, 1959; Ratkowsky, 1990). Because the growth data were fitted separately for each plant, there were 4 - 6 plant replications for each of the experimental factors, which ensured statistical reliability of further analyses.

Shape parameters and two types of growth analysis

By fitting experimental data sets with the Chapman-Richards function, single growth flushes were modelled in real time. This was a desirable feature of the growth analysis in order to examine the effects of experimental factors on growth. However, for the analysis of the function shape across treatments, it was necessary to measure the time taken to reach certain growth points (5, 50, and 95% of growth) from a common point of time.

The two types of analyses (with and without GLP) also enabled a crosscheck of the effect of the GLP on the modelled growth analyses with respect to the estimated growth rates, as well as the statistical suitability of the fitted function. By yielding

very similar statistical results for growth rates, it further indicated that the growth function explained the experimental data on statistically sound basis, as opposed to pure chance of the function fitting to the experimental data (Ratkowsky, 1990). This was important because the examination of subsequently calculated growth rates of tree size and/or complexity and their correlation to developmental phase change (Hackett, 1985; Haffner et al., 1991; Hackett and Murray, 1993; Poethig, 1993; Greenwood, 1995) was one of the main emphases of this study.

Intrinsic growth rate coefficient

Overall, the intrinsic growth rate coefficient corresponded well with the calculated values of relative growth rate, and as such could reliably be used to assess relative growth rate over the linear section of examined growth. This allowed relative growth rate to be assessed independently of time. Hence, the coefficient could possibly be more suitable for ontogenetic studies, the growth rate being associated with a particular developmental state instead of varying in time as is relative growth rate.

In order to distinguish the effect of experimental parameters on intrinsic growth rate in the modelled relative growth (Figures 4.1 and 4.2), parameter *b* was set to a constant value of 0.01708 (ln 0.01708 = -4.70). Apart from the properties of parameter *b* described above, such an approach was also justified statistically. Analysis showed that parameter *b* was not affected statistically by Temperature or State when the parameter of topological size was analysed excluding the GLP.

Growth Lag Period

The whole first growth flush of the experimental data set for each plant was used for fitting the Chapman-Richards growth function. As mentioned earlier, the non-increasing data of the Growth Lag Period were not entirely compatible mathematically with the theoretical values of the lower asymptote of the growth function, which had a slow but increasing trend for time t>0. However, this was of

theoretical importance only, and did not present a significant problem for obtaining a good fit of the Richards-Chapman function.

Information regarding the GLP was found to be integrated in both *a* and *b* growth parameters as shown from growth simulations (Figure 4.5). Consequently, if parameter *a* were to be set to a constant value, or averaged for a particular experimental data set, parameter *b* would contain significant information regarding the GLP itself. This characteristic of parameter *b* has not been recognised in the growth modelling literature (see Section 1.2.6). The properties of parameter *b* could be used in future research for longer term growth modelling and analysis, where the phenological information would undoubtedly lead to greater accuracy of growth predictions.

Parameter *b*

In the original paper by Richards (1959) and later literature, parameter *b* has been considered as having no biological meaning (Ziede, 1993). A high correlation between growth parameters *a* and *b* was found in this study. The two parameters mutually affect the time at which exponential growth commences, while the slope of linear growth is reflected by parameter *a* (Richards, 1959, Ratkowsky, 1990; Zeide, 1993b) independently of parameter *b* (see Figure 4.5). This suggests that the intrinsic correlation between the two growth parameters, for which the Richards function was criticised (Ratkowsky, 1990), may under some circumstances be used to express phenological information of a growth cycle in relation to growth rate coefficient. In this respect, it was interesting to note that growth rate and growth shoot phenology were previously shown to be correlated by Baltunis and Greenwood (1999). Indications from the current study are that the relationship between growth rate and shoot phenology may be expressed by using the intrinsic growth rate coefficient (*a*) and growth parameter *b* correlation. Moreover, parameter *b* also affected the slope of the growth function at the exponential section of growth, and possibly the slope of decline at the upper asymptote. These growth characteristics are also an important

indication of shoot growth synchronisation when whole crown growth is examined as in this study.

Normalisation

Normalisation of the data was important for a number of reasons. Normalisation simplified variation between individual plants for graphical display, while no information was lost (Thornley and Johnson, 1990). The topological parameter data sets also became relative through normalisation. Because plants of the three developmental states differed in absolute values of their original topological parameters, the normalised values of topological parameters, in particular those for size, made initial evaluations and comparative analyses feasible. In this respect Karlsson and Heins (1994) also transformed their data by normalisation to a 0 to 1 scale before function fitting, in order to eliminate variation associated with absolute growth.

Normalisation also aided the fitting of the growth function. Normalised data allowed the estimation of the function initial value to be eliminated. The initial values became common to all replications, and thus growth analyses could concentrate solely on the growth function dynamics. Moreover, it also allowed for relatively precise fitting around the initial growth period (including the GLP), since one-side normalised experimental data were indeed very close to the real numerical values of the lower asymptote of the Chapman-Richards function. That was further aided by subtracting one from the experimental data and hence levelling the original values to zero, which is the real value of the Chapman-Richards function at time zero. Unlike in the growth study by Karlsson and Heins (1994), it was not possible to use double normalised data in this current study, because in some of the plants, growth data did not approach the upper asymptote. Double-normalisation would have distorted these incomplete growth data by enforcing an artificial approach of the upper asymptote. This was clear from initial evaluation of the plotted double-normalised graphs (see Chapter 3, Figure 3.1-3).

The decision to use normalised data for individual plant fitting influenced the final choice of growth function by affecting the number of growth parameters that needed to be estimated (Ratkowsky, 1990). For example, due to the differences in initial and/or final size of the plants for which the growth function would have to be estimated, the number of growth parameters would have increased by two for non-normalised data sets. This would have significantly increased the confidence interval of the fitted function. On the other hand, a two-parameter function would not have had the flexibility to model the complex responses of the 3x4 factorial experiment, and would have resulted in non-convergence with the experimental data. This was consistent with published reports (Ratkowsky, 1990; Thornley and Johnson, 1990; Zeide, 1993b), and confirmed through preliminary fitting of growth functions.

Although all examined functions were able to represent growth of some of the plants, the possibility of using one common function was sought in this study in order to use estimated growth parameters for comparative analysis. At the same time, it was ensured that the rules of correct fitting procedures (Thornley and Johnson, 1990; Zeide, 1993b) were not compromised in terms of the number of observations, coverage of the growth period and the corresponding number of estimated growth parameters.

4.4.2 Testing the Metrosideros Model

Architectural information

The difference in statistical results of the effects of State and Temperature between the three analysed topological parameters of size, size-complexity and complexity suggested that the Metrosideros Model and its parameters captured differences between the architectural vectors of size and complexity. However, since the two-dimensional definition of the growth parameters is new in botanical tree modelling studies, comparisons with other reports are difficult to make.

Clearly, size had some effect on the size-complexity factor because of the calculating procedure. Such an effect was also acknowledged by van Pelt et al. (1989) (see

Section 1.2.4.8) in the Dendrite Tree Model. However, the complexity parameter (Mean Centrifugal Order Number) was able to separate the information for size from that for structural complexity in a powerful manner. This was shown by the different function governing the complexity growth in juvenile seedlings (Figure 3.3; Chapter 3). Moreover, statistically significant differences were quantified for the complexity parameter between plantlets and adult plants. In these two states, complexity growth was otherwise governed by a sigmoid function similar to that for the size parameter. Architectural differences in the growth of ontogenetic states were previously ascertained by descriptive methods as concluded by Borchert (1976). Poethig (1990) and Snowball (1989) pointed out the inadequacy of descriptive methods. However, methods for quantification of plant architecture parameters are still not available (Buck-Sorlin and Bell, 2000; Pearcy and Valladares, 2000), despite numerous attempts, some of which also dealt with the issue in association with plant ontogeny. For example, Ford (1985) and Ramphrey and Davidson (1991) examined branching ratio, McGowran (1998) developed the so-called Discriminant Score in order to distinguish between ontogenetic states, and other attempts at architecture quantification in relation to phase change were made by Tomlinson (1978), Atkinson (1992), Kelly (1994) and Day and Gould (1997) (see Section 1.2.2. for details).

Capture of growth dynamics

The Metrosideros Model was demonstrated to be suitable for capturing dynamic growth of *Metrosideros excelsa* trees in a sound mathematical manner. The growth function fitted to experimental data obtained from the Metrosideros Model had characteristics to be expected for biological growth in general (Richards, 1959; Hunt, 1982; Causton, 1985; Thornley and Johnson, 1990; Ratkowsky, 1990; Zeide, 1993). Growth measured architecturally was also similar to those reported in studies using metric expressions of tree growth (Karlsson and Heins, 1994; Baltunis and Greenwood, 1999; Sabatier and Barthelemy, 1999). These authors measured and modelled single shoot elongation in trees and herbaceous plants, and growth followed a similar function to that of the whole crown in this project. In addition,

plant growth modelled in forestry for yield forecast from measurements of stem diameter generally generate sigmoid types of function (Madgwick, 1994; Hawke, 1998; Richie, 1999; Methol, 2001) similar to that of Chapman-Richards applied to topological parameters in this study.

The middle and inflection points were found to be positioned close to each other on the modelled growth function. Causton (1986), claimed that growth curves for higher plants differ from those of microbiological colonies in the position of the inflection point. Causton (1986) believed that while the inflection point for microbiological colonies is in the middle of a sigmoid function, that for higher plants is usually elsewhere (Causton, 1986). From the results obtained in this study, growth expressed topologically in plants of *M. excelsa* was represented in a manner similar to that of microorganisms (Causton, 1986). Thus, by using a nodal approach to measure plant growth, as proposed by Room et al. (1994) and others (Prusinkiewicz et al., 1994; Snowball et al., 1994a,b; Godin et al., 1997; Honda et al., 1997; Ramphrey and Prusinkiewicz, 1997; Yokozawa et al., 1998; Sachs, 1999), a common growth pattern across unrelated organisms is indicated, as suggested by Crawford and Young (1990).

Overall, there was limited availability of previous comparable information on the dynamic growth of whole two-dimensional crown structures due to unavailability of architecture methods (Pearcy and Valladares, 1998). In this sense, the current study provides a first step towards the modelling of seasonal growth, and growth over the life span plant. The developed Metrosideros Model thus presents a new tool for the study of phase change phenomena by expressing plant architectural parameters. These plant architectural parameters and their dynamic change were then successfully examined with respect to phase change, as proposed earlier by Borchert (1976).

4.4.3 Experimental factors

Ontogenetic states

Quantitative expression and examination of possible differences in dynamics growth patterns between plants of different ontogenetic state as suggested previously by Borchert (1976), Borchert and Tomlinson (1984) and others (Tomlinson, 1978; Atkinson, 1992; Kelly, 1994; Day and Gould, 1997; McGowran, 1998) has been the main goal of this chapter. In addition, response to temperature allowed a preliminary estimate to be made of a temperature optimal for hastening the progress of phase change.

Other plant growth studies rarely examined the modelled growth in such detail as that used in this study. This was due to a number of differences in modelling approach, including the omission of growth phenology, the method used for measuring tree growth, or due to long-term growth examinations where phenological information would compromise the feasibility of modelling. Commonly, experimental growth data in most studies concentrated on re-construction of plant growth by fitting growth function in order to calculate relative growth rates (Richards, 1959; Causton, 1991; 1994; Magnussen, 1995). This enabled these authors to examine growth against a common relative time, as opposed to real time sequences that were used in this study.

Alternatively, analyses of growth dynamics were carried out by other authors under arbitrarily set conditions that also enabled the examination of growth functions under common relative time. For example, although Baltunis and Greenwood (1999) conducted statistical analyses similar to those in this study, they used arbitrarily defined points of growth evaluation, such as growth initiation set to the point of 20 mm of shoot length. Consequently, this obscured the real time sequences of shoot growth. Moreover, growth data were often fitted from an arbitrarily defined time zero (Baltunis and Greenwood, 1999), or using relative time (Karlsson and Heins, 1994), instead of capturing real time sequences and, thus, shoot phenology. In this respect, direct comparison of the current study with other growth modelling studies is

difficult. This is in addition to the use of two-dimensional parameters for whole crowns of shoots in comparison to reports of single shoot growth (e.g. Karlsson and Heins, 1994; Baltunis and Greenwood, 1999). Moreover, in these studies growth was not examined in relation to phase change. Although developmental processes such as flowering (Karlsson and Heins, 1994) have been studied by growth analysis, reports of growth analysis with respect to phase change have not appeared in the literature. Borchert (1976) and Pearson et al. (1994) noted this inadequacy and a poor understanding of phase change itself as result.

Examining the growth of the plants in the three developmental states with respect to the position of point of inflection, i.e. point of highest relative growth rate, the growth model for plantlets was approaching almost exact symmetry. The growth of juvenile plants approached the point of inflection earlier than did plantlets, but still relatively close to the middle point of growth. On the other hand, adult plants reached maximal growth rate significantly earlier than both juvenile plants and plantlets (Table 4.1; Table 4.11). This was likely to be due to a high degree of shoot growth synchronisation. This conclusion was also be supported by the initial Growth Lag Period analyses (Chapter 3, Table 3.4), showing high synchronisation between adult tree replications. High synchronisation of shoots growth was correlated previously with the adult state by Borchert and Tomlinson (1984) and Borchert (1972, 1973, 1975, 1983), while juveniles showed asynchronous shoot growth initiation (Borchert and Tomlinson, 1984). However, the similarities in growth rates for topological size between plants in the juvenile and adult states were somewhat surprising. This is in view of a general acceptance that slower growth rates (reduced vigour) are associated with the adult state (Corderso et al., 1985; Hackett, 1985; Westwood, 1987; Greenwood, 1995), rather than associated with physiological ageing or changes in growth phenology.

A number of explanations for these results can be considered. For example, growth rate evaluation from one growth period may not necessarily be indicative of seasonal or long-term growth rate. However, if additional growth information is considered, such as the short GLP for rejuvenated plantlets (Chapter 3, Table 3.4) and the tendency for multiple growth flushes as observed in the 24/26 and 32/24^{o}C temperature regimes, then a seasonally-adjusted growth rate for plantlets would be

even higher. Moreover, Causton (1991; 1994) examined a number of studies of relative growth rates, and reported that the effect of experimental factors on relative growth rate was generally not statistically significant. The highly statistically significant differences detected for relative growth rates, particularly for topological size between plants of different state were remarkable. Snowball (1989) and Snowball et al. (1994a,b) who investigated phase change in relation to plant size (i.e. height) noted that hypotheses that assumed a slower growth rate in the adult phase were often established intuitively. In this respect, even in forestry, where tree size and growth modelling can be considered to be well established, Madgwick (1994) disputed the merits of tree size biometrics in many, if not most, studies. Considering this, and the results of the current study, it is suggested that a loss of 'vigour' defined as plant maximum relative growth rate (Magnussen, 1995) in the adult state (Borchert, 1976; Hackett, 1985) is not correlated with phase change. Rather, the decline in relative growth rate in adult plants is a result of the overall long-term decline in the number of flushes or the length of each growth period. Borchert and Tomlinson (1984) have made a similar suggestion. Consequently, in the long-term examination of growth carried out by other authors, it is not possible to distinguish between the effects of physiological ageing and of maturation on plant growth rate. Such uncertainty about the effect on plant growth associated with either with maturation and/or physiological ageing still exists (Peer and Greenwood, 2001).

It is suggested that the use of the intrinsic growth rate coefficient in ontogenetic studies may be more appropriate than the use of relative growth rate. This is due to the fact that in growth studies the mathematically time dependent relative growth rates are commonly analysed in a manner that effectively eliminates real time sequences (Richards, 1959; M. Kimberley, pers. comm.). This factor could lead to the situation in which relative growth rates do not show statistically significant effects of experimental factors (Causton, 1991; 1994). This may be because relative growth rates from different growth stages are compared or calculated from long-term growth data that combine growth periods with non-growth periods. Therefore, reported lower relative growth rates associated with maturation in long-term studies, such as that of Menzies and Klomp (1988), should be considered as seasonal growth increment rather than growth rate, because it combines information from a number of growth flushes (M. Kimberley pers. comm.).

With respect to plant complexity, the growth modelling results indicated that topological parameters contain information about tree structure, and thus should be able to distinguish quantitatively between developmental states or their strategies for building crown complexity. Of particular importance was the result that the underlying growth function for the complexity parameter in the juvenile plants was different from that for the plantlets and adult plants, which followed a general sigmoid shape. In addition, there were differences between plantlets and adult plants in growth rates for topological size and complexity. These results suggest that the three topological parameters indeed accommodated different architectural information that may have the ability to distinguish between the juvenile state, plantlets and adult state. Consequently, detailed analyses of tree architecture using the Metrosideros Model were warranted. These are described later (Chapter 5).

By focusing on modular growth expression (Room et al., 1994), such as meristem pool size and its complexity, rather than on measuring growth in metric units, a new concept of correlation between plant size-complexity and ontogenetic process was presented. Based on these results and literature reports (Snowball, 1994b; Greenwood, 1995; Sachs, 1999), it would appear that it is not only the distance (topological or metric) from the root system examined by Snowball (1989) that is instrumental in the attainment of phase change. Importantly, a particular balance of meristem distribution (complexity) and their distance (size) from the root of the plant may be the determining factors of phase change.

Moreover, it is hypothesised that the spatial dynamics of growth in crown meristems may be instrumental in both the determination of phase change, and the later determination of particular meristems to reproductive growth in the adult plant. Such a hypothesis bridges the gap between the two otherwise separate developmental events of phase change and the reproductive determination of the apical meristem. This approach is similar to a hypothesis proposed recently by Sachs (1999) that also takes into account the distance of meristems from the root (equivalent to centrifugal topological distance) and the cumulative memory of the number of nodes produced by the apex. Despite the similarities between these hypotheses, Sachs (1999) considered architectural complexity only indirectly. As pointed out earlier, plant complexity was disregarded in the literature owing to the non-existence of suitable

biometric methods (Pearcy and Vallanderes, 2000). In this respect, the successfully tested Metrosideros Model and its parameters in growth analysis, as described in this chapter, presents a new and useful experimental tool.

Temperature effect

Overall, all *M. excelsa* plants progressed through a growth flush in the shortest time at the temperature regimes 24/16 and 32/24 °C, while achieving the highest growth rates at the 24/16 °C (Table 4.4). This indicated a wide range of optimal day temperature in *M. excelsa*. A wide range of optimal temperatures or a lack of a growth response to optimal thermoperiodicity was also reported by Malek et al. (1992). In contrast, Starrett et al. (1993) found an optimal day/night temperature for growth of rhododendron seedlings to be 26/22 °C, which the authors believed, could also increase the rate of development of seedlings. In this respect, Morrison and Lawlor (1999) believed that temperature has an essential effect on the rate of ontogenetic development.

The effect on most analysed growth parameters of 32/8 and 16/8 °C temperature regimes did not differ significantly when GLP was eliminated from analysis. Hence, the results are indicating an importance of night temperature rather than the day temperature. The importance of the effect of night temperature on plant growth and elongation was found earlier by Hellmers and Rook (1973) for *Pinus radiata* seedlings, and reported by Kozlowski and Pallardy (1997) for plant growth in general.

4.5 Conclusion

It can be concluded that the Metrosideros Model and its architecture parameters based on the topological method (van Pelt et al., 1989) are suitable for the quantitative expression of the dynamic growth in tree architecture in a biologically meaningful way. Repeated measurements of topological parameters enabled separate

analyses of plant size and structural complexity. The Metrosideros Model was thus able to capture plant size and complexity and quantitatively distinguish these two dimensions of topological information in trifurcating *Metrosideros excelsa* trees.

All three developmental states examined followed the same basic sigmoid function that represented growth in terms of the crown topological size and size-complexity. However, in juvenile plants a function other than a sigmoid curve governed the growth of topological complexity. Moreover, non-linear analyses of the effects of experimental factors revealed that developmental states were distinguished by quantification of growth characteristics within a common growth function.

Plantlets consistently showed the highest relative growth rates for size. Initially, growth of complexity in plantlets was also higher than that in adult plants. However, their growth became similar later. The 24/16 ^{o}C temperature regime promoted optimal architectural growth in this study and thus development of juvenile plants and plantlets most effectively. However, this should be regarded as a preliminary result, as an optimal temperature regime would need to be pinpointed in future research. A combination of temperature regimes may be a more effective way of promoting phase change through the differential requirement of *Metrosideros excelsa* for stimulation of tree size or complexity growth.

The Chapman-Richards function growth parameter b, its character and possible biological meaning in correlation to the inherent growth rate a and growth phenology were partially assessed. The use of these parameters for growth function examination and longer-term growth and development modelling could be possible, although further mathematical and statistical exploration would be needed.

CHAPTER FIVE

Detailed analysis of crown architecture using the Metrosideros Model

5.1 Introduction

In this chapter, the architecture of crown structure in *Metrosideros excelsa* is examined and analysed in detail. In this detailed analysis of crown topology, the structure was broken down to the scale of individual branches and meristems in centrifugal order. Architecture parameters and methods of architecture classification and analysis further to those described in Chapter 3 were explored. Metric properties of the plant crown were also analysed in relation to the topological structure. The effects of temperature and developmental state on these parameters of crown structure were examined.

The principal objective in this chapter was to test the Size-Complexity Hypothesis using the topological parameters from the Metrosideros Model, which was successfully tested by the non-linear analyses described in Chapter 4. In addition, detailed architectural analyses were carried out to test further the suitability of the Metrosideros Model. In order to achieve these two main objectives, a number of mathematical and statistical methods were explored that could facilitate detailed architectural comparisons between trees. These methods were then used to analyse statistically the effects of State and Temperature on the architecture of experimental trees. Tree architecture and experimental factor effects were examined and compared using parameters and methods that considered the whole crown structure. This was in addition to those that examined architectural information within the canopy.

5.2 Methods for the calculation of advanced architectural parameters

The advanced software described in Chapter 3 was used to calculate the advanced architecture parameters. Apart from calculating the defined architectural parameters, this program also provided tables of model units broken down according to order, branch and node.

A number of advanced plant architecture parameters were derived from the Metrosideros Model. Some of these parameters, in their bifurcating form, had previously been examined for their ability to represent and distinguish between specific tree architecture structures. This had been done through mathematical or statistical analyses, simulations and application to experimental data and had been extensively reported (van Pelt et al., 1989; van Pelt et al. 1992; van Pelt 1997). Because *M. excelsa* is inherently trifurcating, modifications to the calculations for advanced parameters were required in the Metrosideros Model.

5.2.1 Topological orders analyses

For each tree, a list of results was calculated giving information on the numbers of nodes, branching points, terminal segments (meristems), internal segments, apices, and branch ends, and the probability of branching for each order. These results were tabulated by temperature regime treatments (1-4), developmental state (1-3), replication (1-6) and time (1-2). Data in this form were subjected to ANOVA using the SAS procedure GLM.

The distributions of nodes, branching points and apices within the crown were examined, and the variance between experimental factors analysed at the beginning and end of the experiment. Similarly, the patterns of branching probability per order were estimated for each separate plant, and the parameters statistically analysed and further assessed in terms of the Q-S branching model (van Pelt et al., 1989), as described below.

5.2.1.1 Distribution of units

Firstly, the distribution of all model units was examined in each centrifugal order. Effectively, when Metrosideros Model units were connected within an order, they represented a metamer (Room et al., 1994), which in botanical terms consists of a node and an internode. However, for the purposes of the Metrosideros Model, this conglomeration of model units is called a topological node (node).

The calculation of the number of nodes was based on the trifurcating assumption. Therefore, in any order, a node comprised either two terminal segments (axillary meristems) and an axis, or three terminal segments (axillary and apex meristems). In order to quantify the distribution of nodes, the order with the most nodes was identified, and that order number was divided by the highest order number of the tree. In this way, the relative topological distance that contained the most nodes was expressed as a *Percentage of the highest topological distance,* and thus comparisons between trees of different size were possible. When trees with two regions of high node concentration were found, only the order of highest node number was used and statistically analysed.

5.2.1.2 Distribution of apices

Similarly, a parameter expressing the distribution of apices within the topological distance of the crown was quantified as a *Percentage of the highest topological distance.*

5.2.1.3 Distribution of branching points

Again, the same method of relative topological distance (the *Percentage of the highest topological distance*) was used to quantify and analyse branching point distribution.

The position (order) of the highest branching point distribution differed from the point of highest branching probability (described below), because the branching probability calculations depended on the number of potential branching points as well as realised branching.

5.2.1.4 Branching probabilities

In order to quantify different branching patterns in dendrite trees van Pelt and associates (van Pelt et al., 1984; 1989) developed a parameter that expressed the probability of branching events at two separate levels, which were then combined in the Q-mode and S-mode values. The equivalent parameter was developed for the Metrosideros Model. However, significant differences in the assumptions and the definitions of topological units existed between the Q-S model for dendrite trees (van Pelt et al., 1989) and the Metrosideros Model. These were addressed in the following way.

Firstly, in the Metrosideros Model, the potential for branching to occur was at the predetermined sites of the terminal segments (meristems). In contrast, the Dendrite Tree Model did not have predetermined sites of branching, and the branching position was equally random from both internal and terminal segments. Consequently, for the Metrosideros Model, the branching probabilities were dependent on centrifugal order only. The expression of the Q branching mode could have been considered only for adventitious branching in botanical trees, which was disregarded by assumption in the Metrosideros Model. Hence, the quantification of the Q branching mode was not further considered in this study, and Q was set to a constant value of zero.

Secondly, unlike in the Dendrite Tree Model, in the Metrosideros Model, the probability of branching could be calculated directly from experimental data at each order. This was done using the number of possible branching sites (terminal segments or meristems) and the amount of realised branching, i.e. the number of internal segments originating at a particular point. These calculations were carried out as follows:

$$P_i(\gamma) = \frac{N_i}{N_t + N_i} \qquad (5.1)$$

where $P_i(\gamma)$ is the probability of a terminal segment branching at the order γ, N_t is the number of terminal segments in that order, and N_i is the number of internal segments in that order. The probability of branching for each order was calculated, as well as the mean branching probability ± SD for the whole crown. The overall crown branching probability was calculated in two different ways. These were done by averaging the probabilities from each order, and by using the total number of terminal and internal segments of the whole crown. The overall branching probability resulting from the use of these two methods differed only marginally, and these differences were not further analysed.

The patterns of the branching probabilities were examined visually. Branching probability decreased non-linearly with order number up to the 6th order (Appendix II.). However, the trend from order 6 and higher was of a linear nature and was analysed using linear regression. The function fitted to the branching probability data per order was as follow:

Branching probability = $a + b$. *order number*

where a was an intercept parameter and b the slope of the function.

The linear function was fitted in a separate regression for each plant, and thus the two parameters estimated. ANOVA was then used to test for the effects of state, time and temperature treatments on the a and b parameters generated from the single plant

regressions. The parameter *b* represents the values of the S-branching mode in Q-S branching model.

5.2.2 Branch analyses

From the results generated by the Metrosideros Model processing program, a list of data organised for each branch in ascending order was used for the following analyses.

Tables of the individual branch-related results were organised similarly to the topological order related data, i.e. by temperature treatment (1-4), state (1-3), time (beginning and end of experiment) and tree replications (1-6). The data in the table were then subjected to ANOVA in order to analyse correlation between the length of branches and their topological position and the effect of experimental factors. In addition, the total number of leaves, total number of branches, and total length of branches were analysed with respect to the experimental factors.

5.2.2.1 Length-order analyses

The length of the internodes with respect to increasing order number was analysed in order to ascertain the correlation between these two architectural parameters in accordance with Horton's Second Law (Horton, 1945; Leopold, 1971; Park, 1985). After fitting the data with a line for preliminary function assessment (Appendix III.), a separate regression for each plant was fitted with the function of the following form:

$$Length = a.\ ((starting\ order+1)^{-b}).number\ of\ internodes$$

from which the intercept *a* (internode length in order 1) and slope *b* (changes in internodes length in subsequent orders) was estimated for each plant, and these parameters then analysed using ANOVA.

5.2.2.2 Total number of branches

The data were transformed by natural logarithm before analysis in order to achieve a normalised data distribution. Moreover, significant differences in total number of branches between developmental states existed at the beginning of the experiment. Therefore, analyses of covariance were performed in order to determine the effect of the initial number of branches. Analyses of the effects on temperature and developmental state were carried out separately at the end of the experiment.

5.2.2.3 Total length of branches

Total length of crown branches data were transformed by natural logarithm transformation before the statistical analyses. The overall length of all branches at the beginning and at the end of the experiment was also analysed using analysis of covariance.

5.2.2.4 Total number of leaves

Total number of leaves data were also transformed by natural logarithm before statistical analysis in order to improve distribution of analysed data.

Analyses of the total number of leaves were performed in order to obtain two types of result. Firstly, analyses of covariance using a LSMeans method was performed in order to analyse the effect of experimental factors, taking into account the effect of initial leaf number difference between developmental states. This was similar to the analysis for total number of branches.

Secondly, non-transformed mean number of leaves was calculated in order to combine these results with the mean leaf area derived from separate measurements.

5.2.2.5 Mean leaf area

Leaf area experimental data were transformed by natural logarithm. Separate analyses were performed for data of leaf area prior to the experiment and at the end of the experimental growth. Detailed LSMeans tests were performed to evaluate the effect of experimental factors. Calculated mean leaf area results were recalculated from the transformed statistical results.

Data of leaf area were recorded at the end of experiment by destructive measurement using a LI-3000 leaf area meter (LiCor Instruments, Lincoln, NE). The mean leaf area was multiplied by total mean number of leaves for each developmental state. These results were not analysed statistically.

5.2.2.6 Mean number of nodes per branch

The data were transformed by natural logarithm prior to the analyses. LSMeans test was used to analyse data separately at the beginning and the end of the experiment.

5.2.3 Whole structure analysis

Analysis and comparison of whole two-dimensional botanical tree structures was a field for which no suitable method existed (Buck-Sorlin and Bell, 2000). In the current study three methods that could to some extent be considered as whole tree analyses were adjusted and applied to the experimental data. These were related to Horton's Second law (Horton, 1945), comparisons based on tree types quantified by Mean Centrifugal Order Number within one particular tree size (van Pelt et al., 1989) and the Tree Asymmetry Index (van Pelt, 1997).

The three methods were used to examine their suitability for the Metrosideros Model topological parameter comparisons. In addition, attempts were made to develop new methods that would allow for architectural comparisons across a range of tree sizes.

5.2.3.1 Analysis of asymmetry

The main advantages of the partition asymmetry parameter were summarised by van Pelt et al. (1992). These were: 1) a single number suitable for characterisation of sets of trees irrespective of their size, 2) its value of asymmetry being highly sensitive to the developmental history of the tree and, 3) its ability to be used to statistically compare different sets of trees. Since these were the features of an architectural parameter needed in this study, the possibility presented itself to develop a parameter based on foundations similar to those of the Tree Asymmetry Index (TAI) (Pelt et al., 1992).

For the calculations in the current study, it was assumed that if the total number of terminal segments (T) of any particular trifurcating branching partition was equal to the number of all three parts of this partition:

$$T = a + b + c \tag{5.2}$$

then the highest possible partition asymmetry D between any two portions of the trifurcating partition was equal to:

$$D = T - 3 \tag{5.3}$$

Consequently, the asymmetry of the trifurcating partition was expressed as a mean of the mutual partition asymmetries, calculated as:

$$\begin{aligned} A_{rs} &= \frac{|a-b|}{T-3} \\ A_{rp} &= \frac{|b-d|}{T-3} \\ A_{sp} &= \frac{|d-a|}{T-3} \end{aligned} \tag{5.4}$$

$$A_n = \frac{1}{3}\sum A_{(prs)} \qquad (5.5)$$

where the mean of the three sub-partitions $A_{(prs)}$ was the partition asymmetry A_n. As was the case for bifurcating trees (van Pelt et al., 1992; van Pelt, 1997), the mean of all the partition asymmetries within the tree as calculated for each branching point was the TAI.

The range of values that TAI could achieve in trifurcating trees lay between zero (fully symmetric tree) and a value approaching 0.6667 (a fully asymmetric tree), compared with values in the bifurcating Dendrite Tree Model, which were from zero to approaching unity (van Pelt, 1997; see Figure 1.9, Chapter 1). The calculated TAI values in the current study were standardised to a range from zero to unity so that the results would to some extent be comparable with those of van Pelt (1997).

Similarly to the analyses of parameters relating to order and branches, the values for tree asymmetry were organised in tables by treatment, state, time and replication. Linear regression was used to estimate the intercept parameter a and slope b of the function for each plant, using:

$$\textit{Tree Asymmetry Index} = a + b \,.\, \textit{time}$$

Where *time* was the time sequence of repeated measurements.

These parameters were then analysed for the effect of experimental factors by ANOVA using the GLM procedure and LSMeans for detailed analyses.

Because the Tree Asymmetry Index (TAI) was calculated as the mean of all crown sub-trees, the standard deviation of the TAI was also analysed. The same linear function of each plant was used to analyse the standard deviation, and further LSMeans analyses were carried out to evaluate the effect of each experimental factor.

5.2.3.2 Comparison of Mean Centrifugal Order Number with respect to Size

A method for comparing the tree types within a particular size was proposed by van Pelt et al. (1989). The suitability of such a method was examined for the topological parameters calculated from the Metrosideros Model.

5.2.3.3 Comparisons of Mean Centrifugal Order Number with respect to the Highest Order Number

This method was based on a new approach in principle to the comparison of the tree structures across sizes.

Mean Centrifugal Order Number was calculated using values of the highest centrifugal order for the branching extremes of trifurcating *Thin* and *Compact Trees.* The Mean Centrifugal Order Number of trees of the same highest order number can be compared for their relative position on the plane delimited by these two branching extremes regardless of their topological size.

The plane was be calculated for the trifurcating tree from the expression of Mean Centrifugal Order Number of the *Compact* and *Thin Trees.* These parameters were derived for the Metrosideros Model in Chapter 3 as follow:

$$\bar{\gamma}_{compact} = \frac{2}{9^{\gamma_{max}} - 3} \cdot \sum_{\gamma=0}^{\gamma_{max}} \gamma . 3^{\gamma} \qquad (5.6)$$

$$\bar{\gamma}_{thin} = \frac{3\{\gamma_{max} \cdot (\gamma_{max} + 1)\}}{2} \cdot \frac{1}{3\gamma_{max} + 1} \qquad (5.7)$$

Experimental data for Mean Centrifugal Order Number were plotted on this plane (Figure 5.1).

The results of weekly repeated measurements of Mean Centrifugal Order Number were analysed with respect to the corresponding highest order number. From

preliminary evaluations of the data and their distribution (Figure 5.2) it was decided to use regression analysis to fit a linear function of the following form:

Mean Centrifugal Order Number = a + b . *Highest Order Number*

Where a was the intercept, and b the slope of the function. These parameters of the linear regression were estimated for each plant separately ($R^2 > 57\%$), and then analysed using ANOVA to evaluate the effect of experimental factors.

5.2.3.4 Comparisons of branching points Mean Centrifugal Order Number with respect to the Highest Order Number – The relative mean branching ratio

In order to improve the power of comparative statistical analysis of the architectural differences across tree sizes, a new method was explored and developed.

As in the previous section, this method also exploited the concept of relative comparison made between the limits of the two branching extremes of *Thin* and *Compact Trees* against highest order number. However, the parameter of Mean Centrifugal Order Number was calculated with respect to the structure of branching points, as opposed to model segments.

Maximal number of branching points in the *Compact Tree* ($Br\text{-}p_{Compact}$) in the i^{th} order could be expressed as equal to:

$$Br\text{-}p_{Compact} = 2 \,.\, 3^{(i-1)} \qquad (5.8)$$

Further, based on the calculation of the Mean Centrifugal Order Number in the trifurcating tree model (Chapter 3, Section 3.2.2.) but using the number of branching points in each order, the branching points Mean Centrifugal Order Number for any tree can be expressed as:

$$\bar{\gamma}_{Br-p} = \frac{\sum_{i}^{\gamma_{max}} i \cdot s_{\gamma_i}}{\sum_{i}^{\gamma_{max}} s_{\gamma_i}} \tag{5.9}$$

Similarly, the Mean Centrifugal Order Number with respect to branching points for a *Compact Tree* was then expressed as follows:

$$\bar{\gamma}_{Br-p.Compact} = \frac{\sum_{i=1}^{\gamma_{max}} i \cdot 2 \cdot 3^{(i-1)}}{\sum 2 \cdot 3^{(i-1)}} \tag{5.10}$$

Hence, for direct comparisons of the branching complexity of trees of different size within the plane of the *Thin Tree* extremes ($\bar{\gamma}_{Br-pThin} = 0$) and the *Compact Tree* extremes, a relative ratio of each examined tree was calculated as follow:

$$Ratio = \frac{\bar{\gamma}_{Br-p}}{\bar{\gamma}_{Br-pCompact}} \tag{5.11}$$

This *relative mean branching ratio* or the percentage of maximal branching expressed the position of any particular tree as percentage of branching between the *Thin Tree* (0 %) and the *Compact Tree* (100 %) in the Mean Centrifugal Order Number of branching points and Highest Order Number plane. Using values of *relative mean branching ratio,* comparisons of the architectural complexity factor across different tree sizes were made.

Calculated experimental values of the complexity *relative mean branching ratio* parameter were further analysed by ANOVA using GLM procedure LSMeans for the effect of experimental factors. Because of the high distinguishing power of this method and the relatively narrow range of experimental values of highest order number, only values before the experiment (highest order number range 14 to 47)

and at the end (highest order number range 19 to 58) of the experiment were analysed.

5.3 Results

5.3.1 Topological order analyses

5.3.1.1 Distribution of nodes

The distribution of nodes in relation to the topological position within the crown showed a highly significant effect of State and Time ($P < 0.001$), but no effect of Temperature. The interaction between State and Time was also significant for this architectural parameter.

Detailed LSMeans analyses showed that at the beginning of the experiment there were significant differences between all developmental states, with the greatest number of nodes occurring at increasingly higher orders in the crown of juvenile, plantlets and adult plants, respectively (Table 5.1). However, while the position in the crown at which the greatest number of nodes was found increased in juvenile plants and plantlets, that for adult plants did not differ at the two times. Distributions of nodes for plantlets and adult plants were the same at the end of the experiment.

Table 5.1 The effect of State and Time on the order containing the most nodes, expressed as a mean percentage of the highest order number of each respective tree at the start (T_1) and the end (T_2) of the experiment

State	T_1	T_2	Mean
Juvenile	19a a	37b b	28a
Plantlets	36b a	62c d	49b
Adult	60c a	67c a	63c
Mean	37a	55b	

Means followed by the same letter are not significantly different (P=0.05); the first letter applies to differences between states, and the second letter to differences between times.

5.3.1.2 Distribution of apices

The apex distribution was affected significantly (P≤0.05) by both State and Temperature. There was also a significant interaction between the factors of Time and State.

There was a significant difference only between temperature 16/8 and 32/24 °C with respect to distribution of apices. Treatment 16/8 °C had the highest order at which most apices occurred (75% of topological distance from the root to the highest order), while in other temperature treatments, most apices occurred at lower levels of topological distance (60 to 70%).

At the start of the experiment, the distribution of apices was significantly lower in rejuvenated plantlets than in both juvenile and adult plants. In juveniles, however, the apex distribution was not significantly different from that in adult plants, but it became significantly different during the experiment. In contrast, the apex distribution parameter of plantlets was not statistically different from that of adult plants at the end of the experiment (Table 5.2).

Table 5.2 The means of relative position (%) of highest number of apices within tree centrifugal order structure scaled from 1 to 100.

State	T_1	T_2	Mean
Juvenile	74a a	56a b	65a
Plantlets	57b a	66b a	61a
Adult	75a a	76b a	76b
Mean	69a	66 a	

Means followed by the same letter are not significantly different (P=0.05); the first set of letters applies to differences between states, and second letter to differences between times.

5.3.1.3 Branching points distribution

Temperature did not have a significant effect on the distribution of branching points. Developmental State, Time and their interaction had significant effects (P<0.001) on branching position. Seedlings, plantlets and adult plants in that order branched most at increasing topological distance (Table 5.3).

Table 5.3 The means of relative position (%) of highest number of branching points within tree centrifugal order structure scaled from 1 to 100.

State	T_1	T_2	Mean
Juvenile	9a a	12a a	11a
Plantlets	12a a	42b b	27b
Adults	47b a	47b a	47c
Mean	23a	34b	

Means followed by the same letter are not significantly different (P=0.05); the first letter applies to differences between states, and the second letter to differences between times.

At the start of the experiment, seedlings and plantlets were not significantly different (P<0.001) in their position of highest branching point concentration, but were both significantly different (P<0.001) from adult plants. On the other hand, at the end of the experiment, plantlets did not differ significantly from adult plants, but both these

states differed significantly (P<0.001) from seedlings in this parameter. The values for adult plants did not change significantly during the experiment.

5.3.1.4 Branching probability distribution

Developmental State, but not Temperature, affected the slope parameter *b* significantly (P= 0.0046). The slope parameter *b* was negative and thus the probability of branching decreased with increasing order number. The rate of decrease in the probability of branching with increasing order was not significantly different between juveniles and plantlets (-0.00895 and –0.00743, respectively). However, both these states differed significantly from adult plants, where the probability of branching decreased less sharply with increasing order (-0.00424). The intercept parameter *a* (0.451) was not affected significantly by any of the experimental factors.

5.3.2 Branch analyses

5.3.2.1 Internode length-order analyses

Analyses showed that the intercept parameter *a*, viz. the branch length at the lowest (first) order number, was significantly affected by State (P=0.014) but only marginally by Temperature (P=0.059). Seedlings and plantlets were not significantly different from each other (internode lengths of 17.2 and 18.7 mm, respectively), but both were longer than in the adult state (13.8 mm).

On the other hand, the rate parameter *b* was affected significantly by both experimental factors, Temperature (P=0.021) and developmental State (P=0.035). Also, for parameter *b* also the interaction between the two factors was significant (P=0.05) (Table 5.4).

Table 5.4 The rate (parameter *b*) of decline in internode length with increasing centrifugal order as affected by Temperature

State Temperature regime	16/8 °C	24/16 °C	32/24 °C	32/8 °C	Mean
Juvenile	-0.39a a	-0.13a b	-0.38a a	-0.10a c	-0.19a
Plantlets	-0.35a a	-0.30a a	-0.32a a	-0.31b a	-0.32b
Adults	-0.25a a	-0.18a a	-0.16a a	-0.13a a	-0.18a
Mean	-0.33a	-0.21a	-0.29a	-0.11b	

Means followed by the same letter are not significantly different (P=0.05); the first letter applies to differences between states, and the second letter to differences between temperature regimes.

The overall negative rate of parameter *b* showed a tendency of decreasing branch length with increasing order number. There was no significant difference between the states at any temperature except in the 32/8 °C regime, when internode length decreased significantly more rapidly in plantlets than in both juvenile and adult plants. The last two did not differ significantly in the slope of branch length decrease.

The rate of the branch length decrease with order was not affected significantly by Temperature in the adult plants or plantlets. In the seedlings, on the other hand, the slope parameter did not differ significantly between temperatures 16/8 and 32/24 °C, while in all other temperatures the parameter differed significantly ($P \leq 0.024$).

5.3.2.2 Total number of branches

Analysis of covariance with respect to the initial and final time of recording showed an overall significant effect of the initial number of branches, and effects of Temperature, developmental State and their interaction ($P<0.01$).

Further LSMeans tests showed significant difference between temperature 16/8 °C and 32/24 °C as well as 32/24 and 32/8 °C. The highest number of branches was produced at 32/24 °C.

Significant differences existed in the number of branches at the end of the experiment, adjusted for the initial time, between seedling and plantlets but not between other compared states.

Plantlets grew the highest mean number of branches, while adult plants the lowest mean number of branches, relative to the original size. Adult plants had approximately ten times more branches than juvenile plants or plantlets at the start of the experiment. Total number of branches in adult plants was not affected by Temperature, whereas that of juvenile plants and plantlets generally increased 2-3 fold (Table 5.5).

Table 5.5 Mean total number of branches at the end of experiment adjusted for the effect of initial total number of branches

State Temperature regime	16/8 °C	24/16 °C	32/24 °C	32/8 °C	Mean
Juvenile	38a a	40a a	84a b	13a c	36a
Plantlets	31a a	44a a	64a b	83b b	52c
Adult	35a a	34a a	25b a	42b a	33ac
Mean	34a	39a	51b	36a	

Means followed by the same letter are not significantly different (P=0.05); the first letter applies to differences between states, and the second letter to differences between temperature regimes.

5.3.2.3 Total length of branches

Analysis of transformed data showed a highly significant overall effect of the all three examined factors Temperature, State, and Time as well as their interactions.

Detailed LSMeans tests were performed and showed that the total branch length differed significantly between all states at the beginning as well as at the end of the experiment. Analysis of covariance showed significant effects of the initial branch length, Temperature (both P<0.0001), developmental State (P=0.042) and the interaction of these two factors (P<0.001)(Table 5.6).

Table 5.6 Mean total length of branches at the end of experiment adjusted for the effect of initial total length (mm) of branches

State/Temperature regime	16/8 °C	24/16 °C	32/24 °C	32/8 °C	Mean
Juvenile	1598a a	3376a b	2529a b	944a c	1883a
Plantlets	1639a a	2516a ad	4018bc c	2209b ad	2459b
Adult	2695a a	2846a a	2565ac a	2735b a	2709a
Mean	1917a	2892b	2965b	1773a	

Means followed by the same letter are not significantly different (P=0.05), the first letter applies to differences between states, and the second letter to differences between temperature regimes.

Branch length was unaffected by Temperature in adult plants. In juvenile plants and plantlets, longest branch lengths tended to occur at 24/16 and 32/24 °C. There was no difference in branch lengths between states at 16/8 and 24/16 °C.

5.3.2.4 Total number of leaves

Analysis of covariance of the leaf numbers at the end of the experiment showed highly significant ($P<0.0001$) effects of all factors, i.e. initial number of leaves, temperature, state and the interaction of the last two factors.

There was no significant difference in total leaf number in plants grown at 16/8 and 32/8 ^{o}C, and at 24/26 and 32/24 ^{o}C.

Plantlets had the highest total number of leaves and that was significantly ($P=0.003$) higher than in seedlings and adult plants. Total number of leaves in adult plants was not affected by Temperature. In general, that of juvenile plants and plantlets increased with temperature (Table 5.7).

Table 5.7 Mean total number of leaves at the end of experiment adjusted for the effect of initial total number of leaves

State Temperature regime	16/8 ^{o}C	24/16 ^{o}C	32/24 ^{o}C	32/8 ^{o}C	Mean
Juvenile	364a a	590a b	713a c	160a d	395a
Plantlets	365a a	577a bd	910a c	762bd cd	618c
Adult	383a a	352a a	312b a	414ad a	364a
Mean	371a	493b	587b	369a	

Means followed by the same letter are not significantly different ($P=0.05$), the first letter applies to differences between states, and the second letter to differences between temperature regimes.

5.3.2.5 Mean leaf area

Mean leaf area at the start of the experiment was highest in seedlings (861 mm^2) and was significantly different (P=0.017) from that in plantlets (612 mm^2), but not from that in adult plants (661 mm^2).

Analysis of the mean leaf area changes during the experiment showed significant (P<0.0001) main effects of both developmental State and Temperature, and an interaction between these factors (P=0.03) (Table 5.8). The highest mean leaf area (P<0.001) was in seedlings (901 mm^2) followed by plantlets and adult plants (432 mm^2 and 319 mm^2, respectively), which were also significantly different (P=0.015).

All four temperature regimes differed significantly (P≤0.0058). Mean leaf area decreased as Temperature increased (942, 667, 225 and 98 mm^2 at temperatures 16/8 °C, 24/16 and 32/24 and 32/8 °C, respectively). Mean leaf area declined steadily with increasing temperature, although that in juvenile plants decreased most rapidly.

Table 5.8 Mean leaf area (mm^2)

State / Temperature regime	16/8 °C	24/16 °C	32/24 °C	32/8 °C	Mean
Juvenile	1447a a	1027a b	230a c	Missing	901*
Plantlets	860b a	515b b	243a c	108a d	432a (539*)
Adult	518c a	468b a	202a b	88a c	319b (396*)
Mean	942a	670b	225c	98d	

Means followed by the same letter are not significantly different (P=0.05), the first letter applies to differences between states, and the second letter to differences between temperature regimes.

* Means calculated from first three temperature regimes only

5.3.2.6 Total mean leaf area

Despite a significantly lower number of leaves in comparison to plantlets the total mean leaf area was the highest in juvenile seedlings. Adult plants grew the lowest number of leaves as well as overall mean leaf area during the experiment (Table 5.9).

Table 5.9 Total number and mean leaf area at the end of experimental growth

State	Total # of leaves	Mean leaf area (mm^2)	Total mean leaf area (mm^2)
Juvenile	395	901*	355895*
Plantlets	618	432 (539*)	266976 (333102*)
Adult	364	319 (396*)	116116 (144144*)

*Means calculated for three temperature regimes only

5.3.2.7 Mean number of nodes per branch

There were significant ($P \leq 0.001$) differences between all three states in the number of nodes per branch at the start of experiment, but not at the end of the experiment. At the start of the experiment, the mean number of nodes per branch was 10.5, 7.7 and 6.6 in seedlings, plantlets and adult plants, respectively.

At the end of the experiment, State had no significant effect on number of nodes per branch, but temperature had a significant effect ($P = 0.008$) on this parameter. Plants had the highest number of nodes per branch (7.3) in a temperatures 24/16 $^{\bullet}C$, which was significantly ($P \leq 0.037$) higher than in other temperatures (5.2 – 5.9), which were not significantly different.

5.3.3 Whole structure analysis

5.3.3.1 Asymmetry analysis

Tree Asymmetry Index

Only plant State had a significant effect on the intercept *a* (P=0.027) and the slope *b* (P=0.035) when the linear regression fitted to the Tree Asymmetry Index values was analysed for effects of experimental factors. The value of the intercept *a* reflected tree asymmetry at the beginning of the experiment, i.e. for crowns that had developed under unknown conditions.

The highest intercept value was recorded for adult plants (0.675) and the lowest for plantlets (0.562). These states differed significantly (P=0.0085), but neither differed from juvenile plants, which had an intermediate value (0.631).

On the other hand, the slope of the function reflected the changes in the Tree Asymmetry Index during the experiment. Asymmetry of crowns in both seedlings and plantlets increased during the experiment, but the rate of asymmetry increase was not significantly different between these two states (seedlings b =0.0084, plantlets b = 0.0070). The adult state differed significantly (P≤0.05) from both seedlings and plantlets because its asymmetry changed by decreasing slightly during the experiment (b=-0.000035). Because the value of slope decrease was close to zero, the asymmetry of adult plants during the experiment could be considered as unchanging (Appendix IV.).

Standard deviation of Tree Asymmetry Index

The ANOVA of the standard deviation of TAI values revealed that there were no significant effects of either developmental State or Temperature treatments.

5.3.3.2 Comparisons of Mean Centrifugal Order Number with respect to Size

Due to the trifurcating branching in trees of *Metrosideros excelsa,* the number of tree types for each size family was substantially higher than in the bifurcating dendrite trees. Moreover, the botanical trees tended to be many-fold larger, especially when detailed levels of structure were examined, i.e. using meristems as a basic units of the model. Consequently, the method of tree comparison within the same size was ineffective in the current study. The difficulty was apparent from plots in which the Size-Mean Centrifugal Order Number plane was not limited by the *Compact Tree-Thin Tree* extremes (Figure 5.1).

No further statistical evaluations were carried out, especially due to the large differences in tree sizes between adult plants and both juveniles and plantlets.

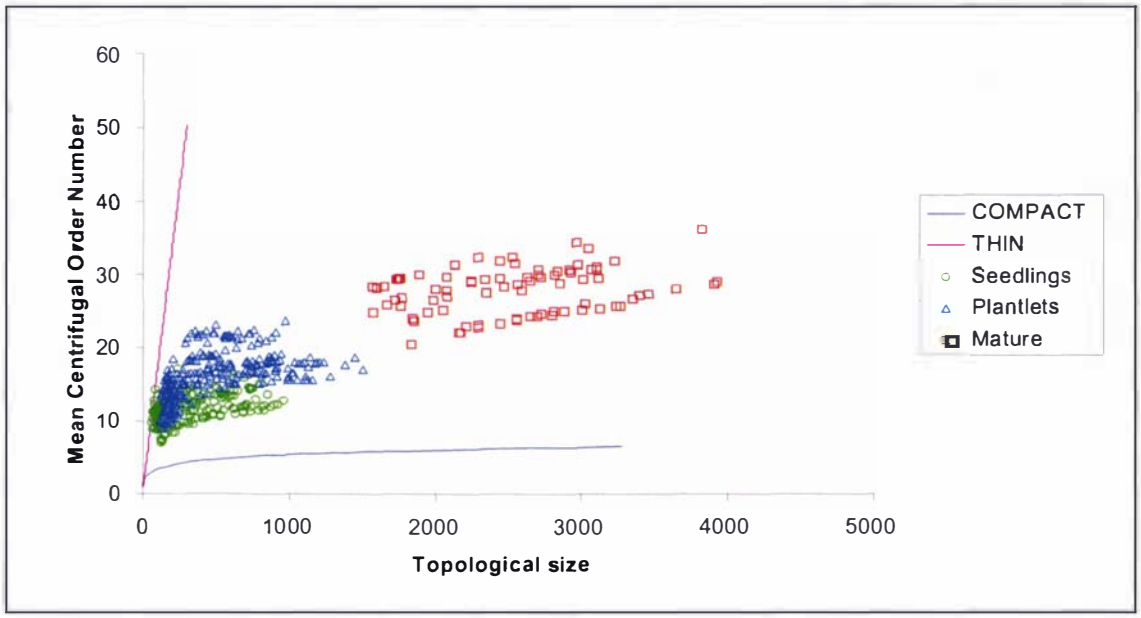

Figure 5.1 The relationship Mean Centrifugal Order Number and topological size for adult, juvenile plant and plantlets within the boundaries of the *Compact Tree – Thin Tree* structural extremes.

5.3.3.3 Comparisons of Mean Centrifugal Order Number with respect to the Highest Order Number

In the linear regression of Mean Centrifugal Order Number against Highest Order Number, analysis of variance of the intercept *a* showed that only developmental State had a significant effect (P=0.018). The intercept values were 1.4, -4.2 and 2.6 for juveniles, plantlets and adult plants, respectively.

The slope parameter *b* also showed a significant effect (P=0.034) of developmental State, but not of Temperature. The slopes for all states were positive, and thus there was a tendency towards increasing Mean Centrifugal Order Number with increasing topological order in all states. The rate of increase was highest in plantlets (*b* = 0.715), which was significantly (P=0.008) greater than the rate of increase in juvenile plants (*b* = 0.416). However, the rate of increase in adult plants (*b* = 0.526) was not significantly different from either juveniles or plantlets.

The amount and position of branching in the trees of different states were examined in comparison with the slope values at the borders of the *Thin Tree* (*b* = 0.5) - *Compact Tree* (*b* = 1) plane (Figure 5.2). Plantlets branched predominantly in higher topological positions of the crown. On the other hand, juvenile plants grew similarly to the *Thin Tree* with occasional branching at low orders that resulted in Mean Centrifugal Order Number values under the lower limit of the *Thin Tree*. The slope for adult plants remained almost parallel to the lower (*Thin Tree*) value of the plane.

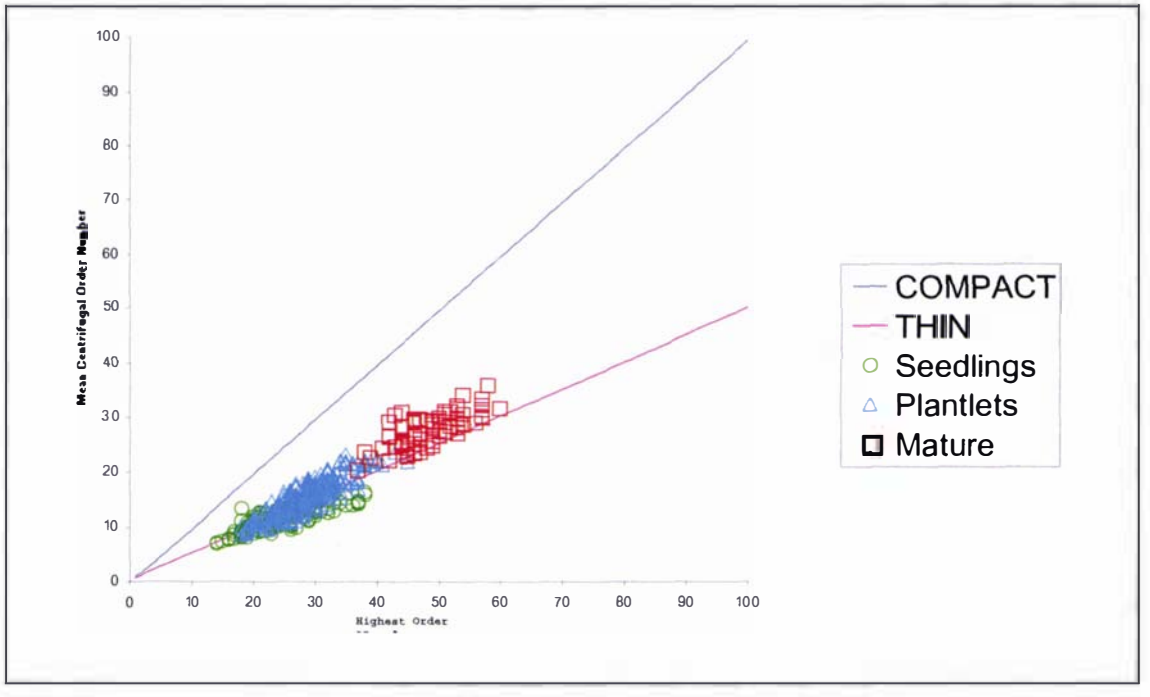

Figure 5.2 The relationship between Mean Centrifugal Order Number and Highest Order Number in the plane defined by the *Compact* and *Thin Tree* extremes with experimental plant values plotted for juvenile and adult plants and plantlets

5.3.3.4 Comparisons of branching points Mean Centrifugal Order Number with respect to the Highest Order – relative mean branching ratio

In this analysis, the values of Mean Centrifugal Order Number of branching points was expressed as a ratio of the upper limit of a *Compact Tree* Mean Centrifugal Order Number branching points against the highest order number (*relative mean branching ratio).* In this method, the lower limit of *Thin Tree* Mean Centrifugal Order Number equalled zero for any tree size represented by its highest order number. Thus the values of *relative mean branching ratio* represented the relative positions of tree structures between the non-branching *Thin Tree* (0.0) and the fully branched *Compact Tree* (1.0) (Figure 5.3).

The analysis showed an overall significant effect of ontogenetic state ($P<0.0001$) and time ($P<0.0001$), as well as the interaction of time and state ($P<0.0001$). No significant effect of temperature on this parameter was detected. Overall, there were significant ($P<0.0001$) differences between all states. The highest *relative mean branching ratio* was in adult plants (0.496), followed by plantlets (0.330) and seedlings (0.206).

Both seedlings and plantlets changed significantly ($P<0.0001$) in this branching parameter during the course of the experiment, increasing approximately three- (from 0.116 to 0.297) and two-fold (from 0.232 to 0.427), respectively. The differences between seedlings and plantlets in this parameter before the experiment started were also significant ($P<0.0001$). On the other hand, adult plants did not change significantly (from 0.483 to 0.508) in their branching ratio but remained in the vicinity of halfway between the *Thin* and *Compact Trees* during the experiment.

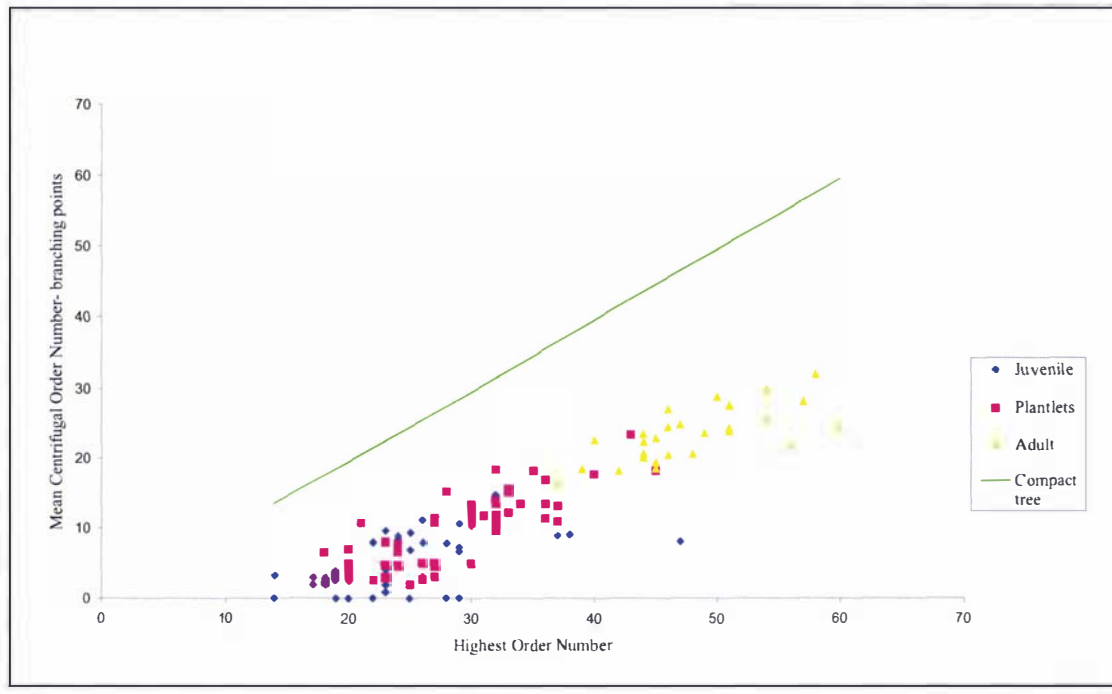

Figure 5.3 The relationship between Mean Centrifugal Order Number of branching points and Highest Order Number in the plane defined by the *Compact* and *Thin Tree* extremes with experimental plant values plotted for juvenile and adult plants and plantlets

5.4 Discussion

5.4.1 Architectural analysis

Two-dimensional architectural parameters are by their nature difficult to analyse, and only a limited number of mathematical methods for such analyses exist (Buck-Sorlin and Bell, 2000; Pearcy and Valladares, 2000). This is due to the need to separate the dimension of structural complexity from that of size in order to enable statistical analysis between different tree sizes. Yet, structural complexity is inherently connected to the parameter of size. In this respect, van Pelt and associates (van Pelt et al., 1989; 1992; and van Pelt, 1997) proposed three main approaches, such as Mean Centrifugal Order Number comparisons within a particular topological size, Q-S model of branching probabilities, and Tree Asymmetry Index. In the Metrosideros

Model a number of other architectural parameters and comparative methods were explored and applied to analyse experimental data. The results of these detailed architectural analyses are discussed as follows.

5.4.2 Topological structure - order analyses

The results for node, branching point and apex distribution within the tree crown suggested these were strongly developmentally determined. In general, the absence of temperature effects on plant structural properties with respect to ontogenesis was somewhat unexpected in view of previous reports. For example, Starrett et al. (1993) and Malek et al. (1992a,b) examined the effect of temperature on growth and development in Rosebay rhododendron seedlings, and believed that an optimal growth temperature could be identified that would hasten maturation. Similarly, Snowball et al. (1994a,b) found that *Citrus* seedlings grown at a temperature that would promote rapid growth produced a higher numbers of nodes and attained the ability to flower earlier than plants treated otherwise. Westwood (1978) also assumed that higher temperatures would hasten growth and thereby shorten the time juvenile plants take to flower. Nevertheless, none of these previous studies examined plant structural complexity, owing to the unavailability of architectural tools (Buck-Sorlin and Bell, 2000; Pearcy and Valladares, 2000), and thus predominantly size was being examined rather than structural organisation.

Although the distribution of apices had a lower discrimination power owing to a lesser number of apices in any particular order (2-5), this important parameter yielded similar results to those of the other two parameters. The difference between results for node and branching point distributions of juvenile plants at the start of the experiment could, however, have been due to insufficient numbers of apices in juvenile plants. Alternatively, the occurrence of most apices in high centrifugal orders at the start of the experiment in juvenile plants was an indication of the presence of long sylleptic shoots typically associated with plants in this state (Borchert, 1976).

The distribution of apices in higher topological orders in plantlets at the end of the experiment was an indication that phase change was occurring in these plants, with the distribution becoming similar to that found in adult plants. On the other hand, due to the reasons given above, the same values of the parameter at the start of the experiment may not necessarily indicate the approach of phase change in the much smaller juvenile seedlings. This example clearly showed the caution that needs to be taken when interpreting architecturally related results.

Despite these considerations, the position of the highest number of units generally tended to increase as the plantlets and juvenile plants grew during the experiment. Values of unit distributions remained stable in the adult plants throughout the experiment, while values for plantlets were closely approaching those for adult plants at the end of the experiment. In this respect, therefore, the increasing values of the parameters quantified phase change in plantlets during the course of experiment. In general, distribution of units in juvenile seedlings also increased in order, but remained far below the values of adult plants. With respect to the unit distribution parameters, however, it was not possible to identify any particular temperature that hastened phase change.

The probability of branching decreased more rapidly (non-linearly) in approximately the first six orders than in the rest of the crown where the decrease was linear. The non-linear decrease in the lowest orders was presumably related to the growth habit of the *M. excelsa* trees, which are reported to produce few strong main branches close to the root base (Salmon, 1989). On the other hand, the analysis of linear changes in branching probability separated branching of the adult state from that of both juvenile plants and plantlets.

Applying the results of the branching probabilities analysis to the Q-S branching model (van Pelt et al., 1989), the trifurcating *M. excelsa* adult tree can be denoted as QS (0,- 0.004), the juvenile plant as QS (0, -0.0089), and a plantlet as QS (0,-0.0079) at the end of the experiment. Consequently, this architecture parameter alone demonstrated that plantlets were similar to juvenile seedlings, and thus fully rejuvenated. This was in contrast to the topological units distribution analyses that showed plantlets and adult plants to be not statistically different at the end of the

experiment. While contrasting results from different architectural parameters may appear contradictory, they support the hypothesis of a multilevel determination of ontogenetic state and thus phase change (Romberg, 1976; Hackett, 1985; Poethig, 1993; Greenwood, 1995) by showing that partial reversion of adult characteristics (Greenwood 1995; Huang et al., 1995) affected only some levels of architectural structure. Also, while branching probabilities and units distributions are likely to be related architecture features, they may not necessarily be equivalent. Detecting these subtle architectural differences thus showed effectiveness of the Metrosideros Model.

5.4.3 Branch analyses

In terms of changes in internode length with respect to their centrifugal distribution the Metrosideros Method allowed an assessment to be made of the effect of state and temperature in terms of the Horton's Second Law (Horton, 1945). In general, consistency with Horton's Second Law was demonstrated by a decline in internode lengths occurring with increasing distance from the base. However, this decline fitted best a power decline function (see Zeide, 1993b), rather than exponential decay in length as assumed by Horton (1945). This was consistent with examinations of this parameter in botanical trees made by Barker et al. (1973) and Park (1985). The results also confirmed the hypothesis that differences in structural parameters exist between ontogenetic states (Borchert, 1976). Moreover, the differences between plantlets and both juvenile and adult plants were observed and expressed quantitatively.

In contrast to other complexity-related parameters such unit distributions, the pattern of internode elongation was influenced by the temperature treatments. That was not unexpected since numerous earlier studies reported an effect of temperature on shoot internode elongation (e.g. Erwin et al., 1994; Myster and Moe, 1995). However, in this study, the detailed analysis revealed that the temperature regimes affected only the growth in juvenile seedlings, but not in adult plants, suggesting an ontogenetic effect. The rate of internode length decrease in plantlets and adult plants was independent of temperature in this study. Thus, the parameter again showed the

structural growth stability in adult plants, and suggested only limited rejuvenation (Poethig, 1990) with respect to this parameter in plantlets. Stability of adult characteristics was reported, for example, by Haffner et al. (1991), Brand and Lineberger (1992a,b) and Greenwood (1995).

Importantly, from the point of view of the validation of the architectural model, the fact that the experimental results followed Horton's Second Law (Horton, 1945; Leopold, 1971) showed that the ascending centrifugal ordering (Weibull, 1963) employed in the Metrosideros Model has a biological relevance to botanical trees. The botanical relevance of ascending centrifugal ordering had previously been suggested by Atkinson (1992) and Kelly (1994). Patterns consistent with Horton's Second Law were also observed in simulations of length-order relationship in dendrite trees (van Veen and van Pelt, 1993). Moreover, a pattern of decreasing path length with topological distance was reported by Cheetham et al. (1980) in branching structures of arborescent animals of cheilostome bryozoan. The results for the length-order relationship suggest a possible common mechanism in structural organisation in biological organisms, as proposed by Crawford and Young (1990).

From the analysis of the number of internodes per branch, it had been expected that the growth of sylleptic shoots would be associated with the juvenile state (Borchert, 1976; Borchert and Tomlinson, 1984). That was the case at the start of the experiment when plant states differed in the number of internodes per branch. Seedlings had the highest number of internodes per branch, followed by plantlets and adult plants with the lowest number of nodes. However, at the end of the experiment, the number of internodes per branch was not affected by ontogenetic state. Overall, it appeared that the weight of the number of non-sylleptic shoots in the bigger tree crown that grew during the experiment obscured the early effect of sylleptic growth in this parameter. Consequently, the parameter of number of nodes per shoot did perform well as a quantitative marker of ontogenetic state.

As part of the detailed architectural analysis, the total number of tree structure parts was also analysed. These parameters hold architectural information, and are widely used (e.g. Godin et al., 1999; Buck-Sorlin and Bell, 2000). However, they express

predominantly only the vector of tree size and contain little information about structure-complexity.

The analysis showed that juvenile plants and plantlets grew a high number of branches at 32/24 ^{o}C. At 32/8 ^{o}C, plantlets also grew a high number of branches while juvenile plants grew the lowest. It is possible, however, that the health status of juvenile plants was adversely affected in the latter temperature regime.

The highest total lengths of branches occurred at 32/24 and 24/16 ^{o}C. Thus, the effect of temperature on the total number and length of branches did not show a similar response pattern despite the fact that both parameters represent tree size. That, however, was not an unexpected result because an optimal temperature for bud break (Chuine et al., 1999), which affects number of branches, would be likely to differ from an optimal temperature for shoot elongation (Erwin et al., 1994; Karlsson and Heins, 1994).

Because the juvenile and adult states were not separated by the size-related parameters, it appears that a size increment expressed by these parameters was not associated with ontogenetic development. Rather, the result suggests that the size increment of trees is strongly genetically encoded for the species, and thus the difference recorded for plantlets could possibly be a carry-over effect of *in vitro* treatment, rather than an effect associated with reversion of phase change. Moreover, these parameters need to be considered as conveying predominantly size information, and because the original size of the adult plants was much greater than those of other plants, comparisons of developmental states are difficult to interpret. Because of the single-dimension size expression and statistical uncertainties, the results of total number parameters were considered as indicative only.

However, what clearly distinguished plants of differing state was the lack of response of size parameters to temperature for adult plants. This was a feature similar to the temperature effect on parameters associated with structural complexity. These differences of experimental responses between juvenile and adult states highlight the need for observations carried under different, well-defined conditions as proposed by

Borchert (1976) for ontogenetic studies to enable more general conclusions to be made.

An optimal temperature for growth of juvenile plants of *M. excelsa* may not necessarily be consistent with that for hastening phase change. Moreover, even for size growth, the optimum appeared to be within a wide range of temperatures, and varied between different size parameters. The dilemma of determining optimal growth temperatures with respect to ontogenetic development was discussed previously by Starrett et al. (1993) and Malek et al. (1992). On the other hand, e.g. Hackett (1985), Hutchison et al. (1990) and Sachs (1999) believed that in general, growth stimulating treatments hasten phase change. However, based on the results from the current study, it is argued that none of these studies examined size and complexity separately. These two groups of architecture parameters responded to environmental stimuli in different ways. Moreover, structural complexity rather than size parameters was associated with ontogenetic states.

5.4.4 Whole structure analyses

Tree Asymmetry Index and Mean Centrifugal Order Number belong to the structural complexity parameters that analyse the tree structure as a whole, rather than at certain level as the distribution of units within the centrifugal ordering.

Before the Tree Asymmetry Index was analysed, the method of its calculation was scrutinised by examining variation of the standard deviation from the sub-tree Partial Tree Asymmetry calculations. These results showed that the Tree Asymmetry Index values were not a mean resulting from polarised extremes. Thus, the calculation method of the Tree Asymmetry Index as a mean of sub-tree asymmetries did not adversely affect the overall architectural information that the Tree Asymmetry Index provided. In other words, the distribution of the sub-trees partition asymmetry values was homogeneous in each tree, and over all experimental factors. In accordance with the definition of architectural parameters used by Thornley and Johnson (1990), the method of Tree Asymmetry Index calculation enabled tree architecture data to be

downscaled without loss of architectural information. This was an important conclusion since the Tree Asymmetry Index was considered to be largely independent of tree size, and therefore suitable for comparing trees of different size statistically (Verwer and van Pelt, 1986; van Pelt et al., 1992; van Veen and van Pelt, 1992; van Pelt, 1997).

From the results of the Tree Asymmetry Index analysis, adult plants exhibited a stability and consistency in the way in which crown architecture was built, relatively independently of a wide range of temperature regimes. This was in contrast to architectural development in both juvenile plants and plantlets, for which Tree Asymmetry Index values were steadily increasing during the experiment. These findings were consistent with results from the other structural-complexity parameters in this study, i.e. distribution of units, length-order analysis. That no effect of temperature on Tree Asymmetry Index values was detected, suggested that the overall development of the structural properties of the crown were strongly genetically dependent, whilst being strongly related to ontogenetic state. As such, Tree Asymmetry Index was a suitable quantitative marker of the ontogenetic plant state.

The Mean Centrifugal Order Number was calculated with respect to segments or branching points, and analysed against topological Size and Highest Order Number. The method of comparing Mean Centrifugal Order Number with respect to size was proposed by van Pelt and associates (Verwer and van Pelt, 1986; van Pelt et al., 1989; van Pelt et al., 1992; van Veen and van Pelt, 1992) in the Dendrite Tree Model. However, when it was used for the experimental data of the trifurcating Metrosideros Model, comparisons between distant tree sizes were not feasible. The plot of experimental data within the extreme limits (Figure 5.1) best illustrated this. It showed that for trees of bigger topological size, the Mean Centrifugal Order Number-Size plane became almost identical to the upper and lower limits of the *Thin-Compact Trees* plane that are needed for relative comparisons. The values of Mean Centrifugal Order Number in *Compact Trees* for sizes bigger than about 120 segments approached ∞. Because of the limitation of this method of relative comparison analysis, it was not pursued further.

The newly developed method of tree comparison using Mean Centrifugal Order Number with respect to Highest Order Number was examined successfully. However, theoretical issues presented by the method could complicate analysis of the results. In analytical examination of the mathematical functions, it was noted that some tree structures could reach values of Mean Centrifugal Order Number lower than the *Thin Tree* limit of the Mean Centrifugal Order Number-Highest Order Number plane. This was caused by the weight of low order branching in the otherwise topologically elevated *Thin Tree*. Although, these 'beyond the lower limit' extreme values remained in the close vicinity of the *Thin Tree* line (Figure 5.2), they presented a mathematical obstacle to the comparison of trees. Due to this undesirable feature of the method at the lower limit, and to the fact that the experimental data for juvenile plants contained some of these extreme values, the relative comparison analysis could not be performed as initially intended. Rather, analyses of complexity rate changes were performed. Similar to other structure-complexity parameters, the rates of change of complexity were independent of temperature. The rate of complexity change separated plantlets from both juvenile and adult plants. Also consistently with other results, the analysis showed the stability of the rate parameter in plants in the adult state, which followed the lower limit rate ($b = 0.5$). Also in this respect, juvenile plants were stable in the rate of change of complexity. However, that result was probably influenced by the extreme lower values observed in juvenile plants.

The second newly developed method, which compared Mean Centrifugal Order Number of branching points with respect to Highest Order Number was most convenient for across-tree size comparisons. This parameter showed directly the position of any tree on the scale from zero (*Thin Tree*) to 1 (*Compact* tree), thereby allowing for comparisons of structural complexity completely independently of size (Figure 5.3). For example, about 70,000 different sizes and their corresponding tree types (an estimated amount averaging at about 10^{150} types within each size) could be compared within the Highest Order Number of 40. That was a significant improvement on the method of van Pelt et al. (1989), which was able to compare within a single size of 500 basic segments in the bifurcating tree, about 10^{192} corresponding tree types (van Pelt et al., 1989).

Analysis of this parameter enabled the quantification of the differences between plants of the three ontogenetic states with respect to their patterns of crown building during the experiment. Moreover, juvenile plants and plantlets were shown to have trends of increasing branch complexity towards the value of adult plants. Plantlets built structures that were more complex than those of juveniles, but neither of the two states reached those of the adult state at the end of experiment. In adult plants, this structure-complexity parameter was again stable throughout the experiment in all treatments, and remained in the vicinity of the 50% line between the *Thin* and *Compact Trees* of branching extremes. Moreover, as for other examined structure-complexity parameters, the branching pattern within the tree crown expressed by this parameter was strongly linked to state, while temperature regimes had little effect on the final phenotypic expression of tree structure. This suggested that manipulation of structure-complexity related architecture parameters through environmental conditions might be less effective than, for example, pruning. In this respect, for example, pruning of non-branching leaders at high centrifugal orders would significantly increase the *relative mean branching ratio* value. Whether that would affect the parameters themselves alone, or whether there is biological meaning behind the behaviour of this parameter remains to be confirmed by future research and/or model simulations. The results so far suggest that there was biological meaning to the *relative mean branching ratio* parameter. For example, with respect to pruning of the leader, it is know that apical dominance, or a lack of it, is associated with phase change in general (Borchert, 1976), and in *M. excelsa* in particular, apical abortion is a characteristic of adult plants (Dawson, 1968a). These architecture-affecting characteristics would have an effect on the value of *relative mean branching ratio*. Moreover, the importance of the distance of apical meristems from the root system as determinant of phase change, stressed by Borchert (1976), Snowball (1989) and Sachs (1999), also support the use of the highest topological order (topological distance from root). This gives solid biological meaning to the newly developed method for architectural comparisons.

5.5 Conclusion

With respect to the evaluation of the Metrosideros Model, it was concluded that centrifugal ordering of model units represented tree structures in a biologically meaningful manner. Since the units of the Metrosideros Model also have direct botanical meaning and have defined connection to the branching mechanism, it was also concluded that the Metrosideros Model represented the botanical tree structures in a mechanistic manner (Thornley and Johnson, 1990). This was a significant improvement on the current status of tree architecture modelling.

The Metrosideros Model was tested through its application to experimental data collection and analysis. This confirmed the ability of the to capture, quantify and analyse architecture parameters of botanical trees. The newly developed method was based on the Mean Centrifugal Order Number and Highest Order Number principle, but had precisely expressed extreme limits, and thus was suitable for relative comparisons. To achieve this, a branching points (link-vertex) system was used (MacDonald, 1984) as opposed to a segments (links recording) system to calculate the parameter corresponding to the Mean Centrifugal Order Number. This method not only has good potential as a research tool for quantification of tree architecture, but would also have a practical use in size/complexity quantification of nursery tree production. This is due to its relative simplicity in terms of data collection, expression of parameters and feasibility of comparative analysis across sizes, branching patterns and species. Moreover, automated data collection similar to that reported by Godin et al. (1999) and flexible parameter calculations according to the branching pattern of a species should be possible to achieve in the near future.

With respect to the quantification of architectural differences between ontogenetic states, it was concluded that many complexity parameters were strongly associated with a particular ontogenetic state in *Metrosideros excelsa*. Relative increase in size parameters, however, was most affected by temperature, and these parameters generally did not distinguish between ontogenetic states. Therefore it is concluded that the aspect of the Size/Complexity Hypothesis relating to size is rejected. However, the complexity aspect of the Hypothesis cannot be rejected by the results

of the current research. At least for *Metrosideros excelsa*, plants undergo phase change when they attain a certain complexity, but not a certain size.

The experimental results showed the need and importance of separate evaluations of architecture parameters, such as size and complexity, notably in developmental studies. Therefore, in future, the quantified complexity parameters could allow for observation of the progress of phase change, and thus aid in the elucidation of the ontogenetic process. The Metrosideros Model could be used for simulation of crown development leading to the specification of optimal complexity growth and perhaps a related minimal size threshold. Optimal complexity could be achieved by other means, in particular through pruning, since environmental manipulation by temperature might not be as effective in changing tree complexity in terms of hastening the process of phase change.

CHAPTER SIX

General Discussion

Numerous attempts are reported in the literature to identify markers of phase change. Markers are reported at various levels, from the molecular, biochemical, cellular, and morphological to that of the whole plant (see Section 1.2.1). However, often these markers were not quantifiable, they were relevant only to the particular species being studied, or yielded inconsistent results (see Section 1.2.2). Yet, the dynamics of phase change cannot be examined without unambiguous quantitative markers (Telfer et al., 1997). Consequently, processes at three different levels of observation were identified, and hypotheses of the relationship of these processes to phase change formulated. Methods that would allow for quantification of these processes were explored or developed to facilitate testing of the hypotheses.

In many respects, the results of analyses at these three levels of observation were mutually supportive. For example, changes in leaf morphology detected in plantlets, and later in juvenile plants, were concomitant with increases in $\delta^{13}C$, which were themselves consistent with a presumed increase in resistance to gas exchange (Bauer and Bauer, 1980). These changes were also consistent with the bulk of studies in the published literature, albeit the latter being mainly of plants growing in the field (Donovan and Ehleringer, 1994). The results supported the hypothesis that changes in *M. excelsa* leaf morphology would occur in association with change in carbon isotope discrimination. The return of plantlets to the adult state earlier than juvenile plants was in agreement with other literature reports concerning rejuvenation (Durzan, 1990; Greenwood, 1995). These morphological and gas exchange-related observations of phase change were also paralleled by some results at the third, the architectural, level of observation. The architectural parameters of structural complexity and branching pattern showed both plantlets and juvenile plants undergoing phase change, with the former developing more quickly. Results for leaf morphology, carbon isotope discrimination and architecture also consistently showed that adult plants remained stable at all temperatures. Thus, the objectives of this

study, to explore and identify markers that would enable the quantification of phase change, were achieved.

However, there were exceptions to this correspondence between results from the three levels of observation. The divergence of $\delta^{13}C$ in leaves of plantlets and juvenile plants away from that leaves of adult plants seemed anomalous. Explanations for this phenomenon have been advanced (Chapter 2). More concerning was the inability of the carbon isotope discrimination method to discriminate between the distinct ontogenetic states of juvenile and adult plants at the start of the experimental period. Therefore, while this marker of phase change provided an insight into the functioning of leaf gas exchange, and might be useful within a clearly defined experimental environment, it does not measure an inherent attribute of plant state.

It was all the more rewarding, therefore, to find that the architecture parameters of structural complexity and branching pattern were not only able to distinguish juvenile plants, plantlets and adult plants, but were also independent of the effects of temperature. These parameters appear to measure expression of an attribute that is inherent to each state. They also successfully tracked the progress of phase change.

Architecture modelling showed that phase change occurred in plantlets and juvenile plants (the latter less quickly) independently of temperature. This would appear to contradict the results for changes that occurred in leaf morphology and carbon isotope discrimination, because the latter were promoted at 24/16 ^{o}C relative to the other temperature regimes. It can be concluded from this that while all three levels of observation (leaf morphology, carbon isotope discrimination and crown architecture) track phase change, each relates to a program of change that might occur largely independent of another. In this sense, the results of this study support the proposition of Telfer et al. (1997) that there are intertwined yet independent programs of ontogenetic development of vegetative phase change in *Arabidopsis thaliana* and other plants. It can be envisaged that these several programs of vegetative development each then contribute, at different rates and with different levels of expression, to the phenomenon Goebel (1900) described as homoblastic phase change.

Jones (1997) advises that the term homoblastic is obsolete, and the phenomenon it describes is merely another example of heteroblasty. However, Jones and other authors who insist that plants, such as *A. thaliana*, generally exhibit heteroblasty, may not be fully aware of the high frequency with which woody plants in the New Zealand flora exhibit extreme differentiation between the juvenile and adult vegetative phases (Kelly, 1994). These heteroblastic (Goebel, 1900) transitions can involve abrupt changes in both leaf morphology and crown architecture. The questions are posed: Does heteroblasty (abrupt vegetative phase change) occur in plants when the putative independent programs of vegetative phase change are in synchrony? Then, homoblasty, in the sense of Goebel (1900), occurs when plants have these programs starting, finishing and giving expression to plant morphology and architecture across a less synchronous time-frame.

An important focus of the research work in this thesis was the testing of the Size/Complexity Hypothesis, i.e. that phase change occurs once a threshold size and/or complexity is attained. Such a hypothesis is somewhat connected to the hypothesis of Telfer et al. (1997), that relative growth rate, or its maximal value expressed as plant vigour (Maughausen, 1995), declines during phase change and remains lower in the adult phase. It was because the definitions of plant size were not unified (Madgwick, 1994), and methods for recording architecture had not been developed (Buck-Sorlin and Bell 2000, Pearcy and Valladares, 2000), that the development of the architectural Metrosideros Model was undertaken. The Size/Complexity Hypothesis was supported for plant canopy complexity, but not in relation to certain plant size or size relative growth rate. While this fulfilled a prime objective of the current study, the development of the Metrosideros Model with its ability to separate size and complexity was a considerable achievement in itself. The ability of the Model to capture two-dimensional architectural information in a mechanistic manner was a significant improvement on the current status of plant architecture modelling, in which issues are generally addressed empirically (Thornley and Johnson, 1990; Day and Gould, 1997; de Reffye and Houllier, 1997; Grace et al., 1998).

Two growth function parameters were examined in a novel way (Causton, 1991; 1994; Radkowsky, 1990) for content of biological information, with emphasis on the

implication of relative growth rate in phase change. Both the intrinsic growth rate coefficient, *a*, and the growth parameter *b* were shown to hold important information for plant ontogeny, a fact that had previously been neglected (Richards, 1959). Contrary to common claims, the hypothesis that phase change is associated with a decline in the relative growth rate (or more suitably, time-independent inherent growth rate) was not confirmed by the results of this study. This conclusion is similar to that of Telfer et al. (1997), who also found phase change to be unrelated to growth rate or plant size in *A. thaliana*. It had already been pointed out by Durzan (1990) that a decline in relative growth rate is associated with physiological ageing rather than with ontogenesis. The confusion between these two concepts still persists in the literature (Peer and Greenwood, 2001).

Apart from statistical differences between the modelled growth functions for size, size-complexity and complexity, an important qualitative difference was uncovered in the growth of topological complexity of juvenile plants. Complexity, unlike size and size-complexity, did not show sigmoidal growth. Therefore, these results suggest that juvenile plants possess a different strategy for building their architecture. This was in agreement with the expectation of Borchert (1976) and Kelly (1994) that juvenile and adult plants could be distinguished architecturally. Moreover, this result showed the ability of the Metrosideros Model parameters to distinguish between the growth of size and the growth of complexity. That such growth differences can exist confirmed the usefulness of dynamic growth modelling as a research tool in general (Zeide, 1993b), and in studies of plant ontogeny in particular (Borchert, 1976).

An effect of temperature on plant size growth was shown, and the temperature of 24/16 ^{o}C identified as being close to a possible optimal temperature or range of optimal temperatures for hastening phase change. This temperature was highlighted in the results for leaf morphology, the $\delta^{13}C$ marker and the growth rates for the topological size parameters. On the other hand, no effect of temperature on plant structural complexity was detected despite the demonstrated association of complexity and phase change.

However, there was a strong effect of temperature on the growth lag period. This is common to the growth of all architecture parameters, and was shown to be associated

with phase change in this study and by Borchert and Tomlinson (1984). In this respect, the incorporation of phenological information into the modelling of plant growth and development was shown to be critical in efforts to increase the precision of growth modelling. In addition, differences in the growth lag period between individual trees suggested a relationship between the increasing synchronisation of shoot growth within the plant canopy and the attainment of the adult ontogenetic state.

The Tree Asymmetry Index was a convenient parameter to use for comparative analyses between trees and across tree sizes. This was also the conclusion of van Pelt (1997), who used this parameter to simulate the effect of pruning on dendrite tree structures under various branching Q-S modes (van Pelt et al., 1989; van Pelt 1997; Cannon et al., 1999). The method developed in the current study based on the principle of comparisons within the highest order number was also shown to be an effective tool for making comparisons across a wide range of sizes, an idea that recently appeared in the literature of comparative neurology (Cannon et al., 1999). This principle has distinct biological meaning in ontogenetic studies because of the believed importance of the distance between the root and apical meristems for phase change to occur. Moreover, the combination of this principle with the new mathematical expression of the upper and lower limits of branching extremes (*Thin* and *Compact Trees,* 0 to 100% of branching probability), gives this new method the ability to provide relative comparisons as a percentage of tree structural complexity. This method provides architectural information complementary to that of the Tree Asymmetry Index, over which it has the advantage of being able to provide a percentage value for branching complexity. These advantages as well as the relative ease (in comparison to TAI) of data collection and calculation, recommend this new method as an analytical research tool. Moreover, it is likely that the calculations could be adjusted for different branching systems, and thus for other species.

It is suggested that future research would also be fruitful in a number of other areas. Work is needed to conclusively elucidate the causes for the similarity that clearly can occur in carbon isotope discrimination between plants in the juvenile and adult states. Such work could include the study of the divergence of $\delta^{13}C$ in plantlets away from that in adult plants. Considering the results for the analysis of architectural

parameters in this study, and the view that size and/or complexity may be related to the acquisition of reproductive competence in adult plants (Hackett, 1985), it is hypothesised that a relationship between plant architecture and the determination of a meristem to become reproductive may exist. Consequently, future research in this direction would be warranted.

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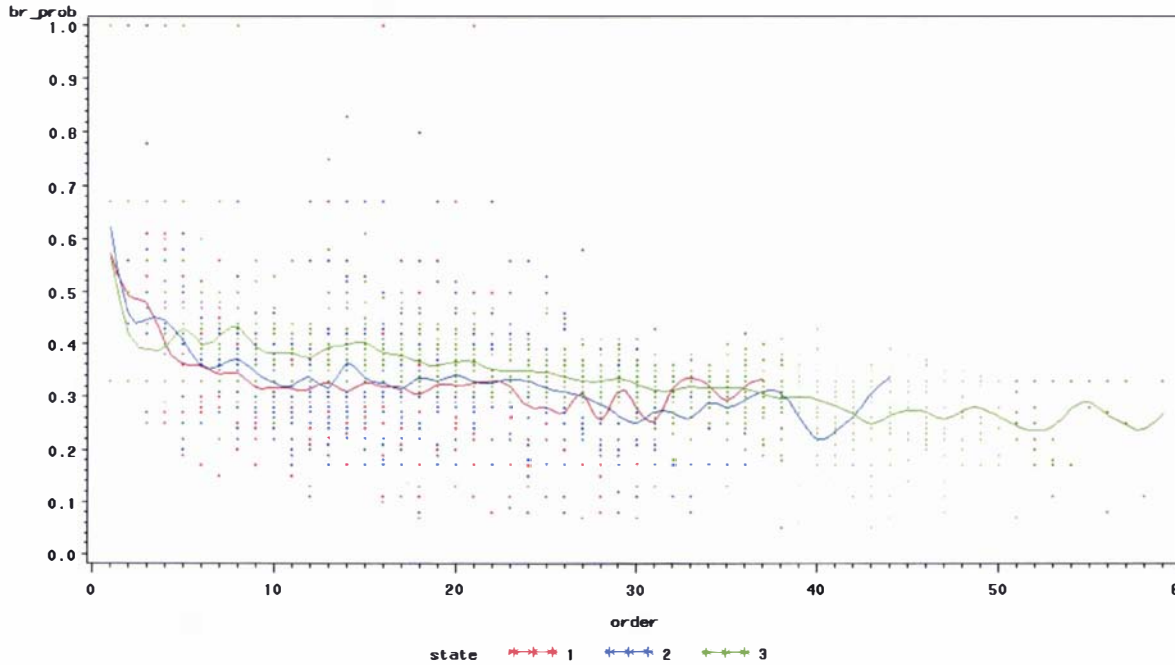
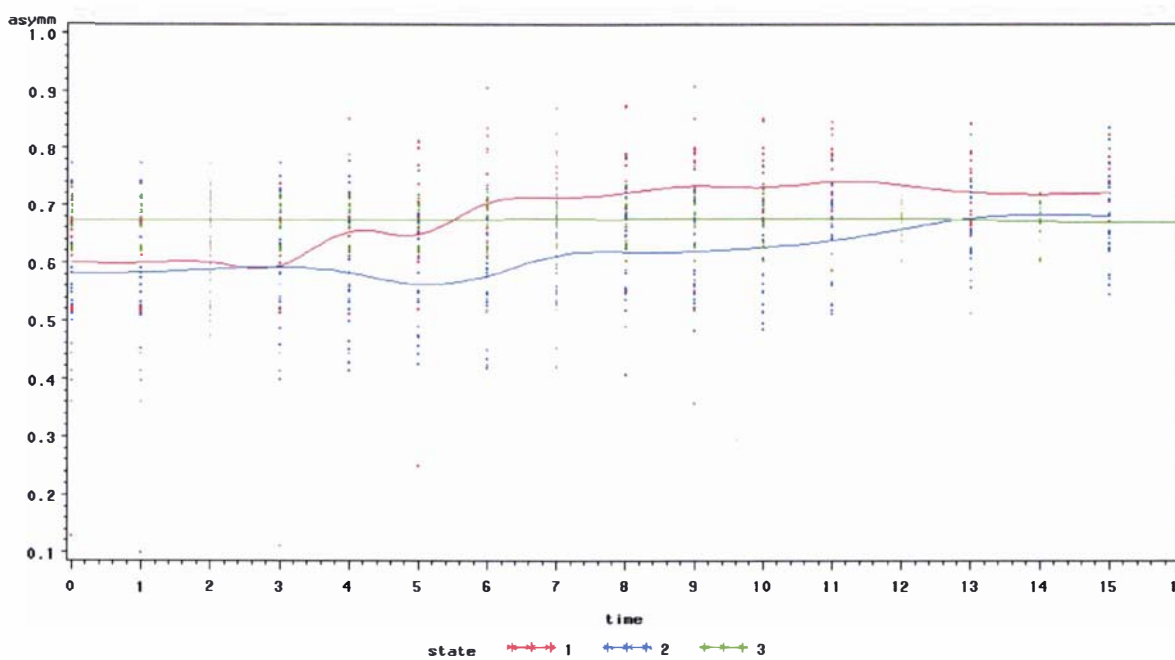
Appendix i. Effect of temperature regime (day/night) on image analysis attributes (mean +/- se) of the abaxial surfaces of leaves from adult, rejuvenated and juvenile plants of *M. excelsa* `Scarlet Pimpernel' harvested at the start and finish of the experiment described in Chapter 2.

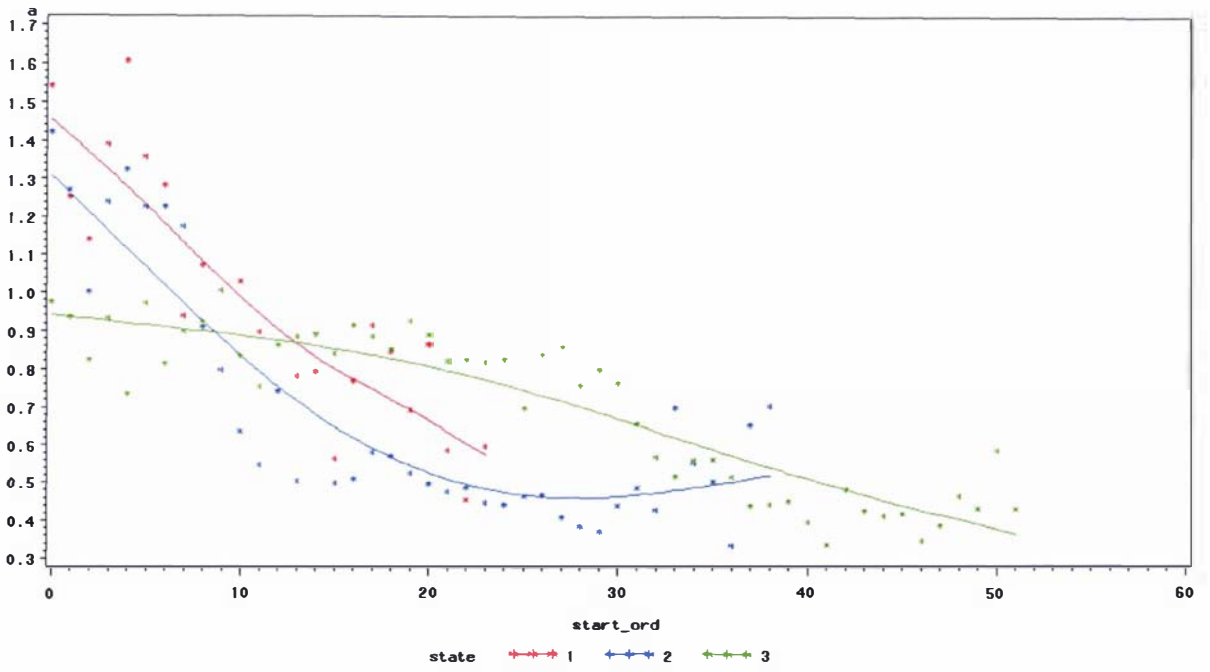
Temp (°C)	State	Time	n	Hue	Saturation (%)	Lightness (%)	Length (mm)	Width (mm)	Area (mm^2)	Perimeter (mm)	Length/width	Roundness
32/24	Adult	Start	6	30.31 ± 2.31	5.54 ± 0.29	49.34 ± 2.14	35.12 ± 3.80	22.91 ± 0.89	593.5 ± 73.6	100.85 ± 6.52	1.54 ± 0.16	17.47 ± 0.57
32/24	Adult	Finish	13	30.23 ± 0.91	7.84 ± 0.34	52.33 ± 0.69	23.92 ± 0.95	16.09 ± 1.08	276.5 ± 22.6	69.67 ± 2.51	1.57 ± 0.11	18.25 ± 0.59
32/24	Rejuvenated	Start	6	48.05 ± 0.65	21.57 ± 0.45	31.48 ± 0.35	44.68 ± 1.28	22.74 ± 0.73	712.2 ± 39.9	115.90 ± 3.28	1.97 ± 0.05	18.95 ± 0.40
32/24	Rejuvenated	Finish	10	48.68 ± 0.23	21.98 ± 0.45	34.29 ± 0.42	31.62 ± 1.49	19.04 ± 0.86	433.7 ± 32.2	87.57 ± 3.55	1.68 ± 0.09	17.87 ± 0.43
32/24	Juvenile	Start	3	46.33 ± 0.27	22.08 ± 0.57	32.95 ± 0.25	62.39 ± 2.36	31.67 ± 1.56	1385.7 ± 121.1	162.27 ± 6.88	1.97 ± 0.02	19.09 ± 0.10
32/24	Juvenile	Finish	3	46.36 ± 1.54	19.80 ± 0.46	35.66 ± 1.54	41.47 ± 2.57	18.22 ± 2.47	516.0 ± 101.1	105.37 ± 7.92	2.32 ± 0.21	22.13 ± 1.17
24/16	Adult	Start	6	27.56 ± 0.65	6.38 ± 0.31	53.61 ± 0.35	27.60 ± 1.18	24.65 ± 0.68	523.8 ± 33.2	90.52 ± 3.28	1.12 ± 0.03	15.71 ± 0.20
24/16	Adult	Finish	6	28.48 ± 0.65	6.94 ± 0.39	52.46 ± 0.54	25.81 ± 2.02	20.13 ± 1.78	395.8 ± 57.5	78.90 ± 5.54	1.31 ± 0.12	16.18 ± 0.29
24/16	Rejuvenated	Start	6	48.31 ± 0.93	22.07 ± 1.06	31.36 ± 0.34	43.12 ± 2.24	21.77 ± 1.63	662.7 ± 89.6	110.02 ± 6.99	2.00 ± 0.06	18.65 ± 0.20
24/16	Rejuvenated	Finish	7	36.99 ± 0.72	9.99 ± 0.69	42.62 ± 1.14	25.79 ± 0.86	24.22 ± 1.35	483.9 ± 42.5	86.69 ± 4.01	1.08 ± 0.04	15.72 ± 0.20
24/16	Juvenile	Start	6	48.58 ± 0.51	20.70 ± 0.55	31.20 ± 0.30	44.69 ± 2.09	21.10 ± 1.34	669.5 ± 71.7	112.15 ± 5.60	2.13 ± 0.08	19.09 ± 0.36
24/16	Juvenile	Finish	10	45.11 ± 0.90	13.45 ± 0.54	36.48 ± 0.76	33.70 ± 2.14	18.21 ± 0.85	443.0 ± 46.9	89.47 ± 4.80	1.86 ± 0.10	18.51 ± 0.29
16/8	Adult	Start	6	36.57 ± 0.62	12.29 ± 0.96	53.53 ± 1.37	42.44 ± 1.63	23.15 ± 0.79	713.0 ± 46.2	113.05 ± 3.57	1.84 ± 0.07	18.04 ± 0.38
16/8	Adult	Finish	6	32.38 ± 0.76	10.66 ± 0.35	55.47 ± 0.58	44.27 ± 2.69	24.72 ± 1.34	819.7 ± 94.3	118.87 ± 6.70	1.79 ± 0.06	17.56 ± 0.47
16/8	Rejuvenated	Start	6	45.51 ± 0.59	19.76 ± 0.32	37.97 ± 0.58	47.65 ± 1.59	23.33 ± 1.11	767.2 ± 57.2	119.38 ± 3.83	2.05 ± 0.07	18.75 ± 0.40
16/8	Rejuvenated	Finish	8	41.52 ± 0.83	18.21 ± 1.02	42.75 ± 0.39	48.71 ± 2.35	30.45 ± 2.22	1061.3 ± 92.4	134.69 ± 5.64	1.65 ± 0.12	17.36 ± 0.24
16/8	Juvenile	Start	6	44.17 ± 0.44	20.78 ± 0.95	38.94 ± 1.13	51.25 ± 1.12	22.32 ± 1.49	808.0 ± 69.4	129.28 ± 4.75	2.33 ± 0.11	20.95 ± 0.36
16/8	Juvenile	Finish	4	42.87 ± 0.44	21.86 ± 0.95	42.47 ± 1.13	53.32 ± 1.12	24.18 ± 1.49	954.0 ± 69.4	134.70 ± 4.75	2.22 ± 0.11	19.69 ± 0.36



Appendix ii. The relationship between branching probability and order number. Smoothing spline function for branching probabilities (br_prob) changes within centrifugal orders, using experimental data for juvenile (state 1), plantlets (state 2) and adults (state 3)



Appendix iii. The relationship between Tree Asymmetry Index and time Smoothing spline function for asymmetry (asymm) changes during the 16 weeks of the experiment (time), using experimental data for juvenile (state 1), plantlets (state 2) and adults (state 3)



Appendix iv. The relationship between length of internodes and order number. Smoothing spline function for length of internodes (a) with increasing centrifugal order (start_ord), using experimental data for juvenile (state 1), plantlets (state 2) and adults (state 3)