Towards a Functional-Structural Model for Macadamia

Dr Russ Stephenson
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FINAL REPORT

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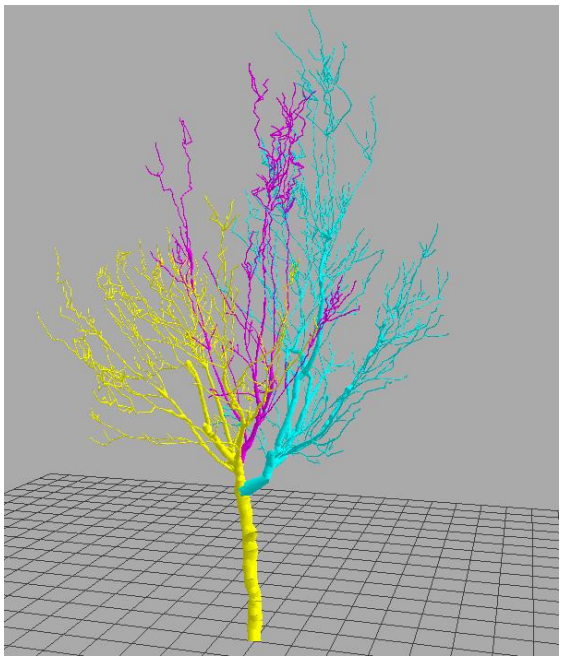
MC 07021

TOWARDS THE DEVELOPMENT OF A FUNCTIONAL-STRUCTURAL MODEL FOR MACADAMIA



Dr Russ Stephenson

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PROJECT DETAILS

Project title:	Towards a Functional-Structural Model for Macadamia
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Purpose of the Report:	To report progress in the PhD programs of Mr S Karimaei and Ms J Conway into factors required for the development of a Functional-Structural model for macadamias.

Acknowledgements: Financial support from the Australian Macadamia Society, Horticulture Australia Limited the University of Queensland is gratefully acknowledged.

Date of Report: 30 April 2013

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Media Summary

The Department of Agriculture, Fisheries and Forestry (DAFF) initiated an internally funded project to develop expertise in functional-structural modelling of tropical and sub-tropical tree fruit crops. Macadamia was chosen as the model crop for these initial studies. The DAFF project used self-organising model to explore canopy management options. The amount of light available for growth was sensed at the leaf level and used to represent vigour, which was then accumulated acropetally. Buds also sensed the light environment but only to provide demand in subsequent redistribution.

Tree models were initiated by reading in an initial structure digitised from a small tree in the field and then allowed to develop (self-organising) for a number of years. Simulated cultural practices such as hedging, topping, removal of the leader and limb removal were investigated. The model provides insight into the impact of these practices on light distribution within the

canopy and to the orchard floor. The lessons learnt from this will be applied to other evergreen, tropical fruit and nut trees.

The Australian Macadamia Society agreed fund, through Horticulture Australia Limited, two part-scholarships for PhD studies to complement the DAFF macadamia modelling work.. One study (by Sadegh Karimaei) focussed on vegetative growth and the influence of carbohydrate allocation on growth and tree architecture. The other study (by Janine Conway) focussed on axillary bud development and implications for reliability of flowering (and ultimately fruit development), an important yield-limiting factor.

Implications of tree architecture and carbohydrate allocation in Macadamia tree development – Sadegh Karimaei

The dense canopy architecture of macadamia causes problems within the tree itself, as well as for macadamia orchard management. Farmers have to use heavy machinery for hedging regularly to improve orchard access. However, hedging may not be the best method for a long-term solution, as it stimulates undesirable vegetative re-growth and increases fruit abscission. This research provides underlying knowledge of macadamia architecture and carbon allocation that will to find alternatives to hedging for canopy management.

Two sets of experiments were carried out in the glasshouse and orchard.

Glasshouse experiments assessed the architecture of small macadamia plants from more than 15 ecotypes of 5 genotypes. A sonic digitizer was used to digitize the small macadamia plants in pots over the time. Detailed information was extracted and their architectural pattern of growth was compared. Photosynthesis of three macadamia genotypes was measured. The effect of rootstocks on micrografted A4 scion was also studied.

Orchard experiments were conducted to determine the relationship between vegetative growth and carbohydrate sources in mature macadamia trees in mid-March and mid-September 2011, coinciding with the two major flushes that are normally produced.

A prototype model of girdled and non-girdled branches has been developed to show the contribution of current photosynthesis (leaf area) and the stored carbohydrate pool to new shoot growth on girdled and non-girdled branches.

Axillary bud behaviour in Macadamia – Janine Conway

Axillary bud behaviour was chosen as the project focus for this study as this is the basis for both the yield-limiting deep shading found in the centre of most commercially managed macadamia trees, and also the basis for inflorescence production. Temperature sequence work showed that warm temperatures followed by cool increased flowering. Cool-followed-by-warm temperatures actually decreased inflorescence emergence. Thus it is temperature sequence and not temperature alone that influences emergence. The same pattern appeared in branching behaviour, but to a lesser extent and in contradiction to some previous research, so it seems the branching response needs more detailed investigation before application. This paper has been submitted for publication.

Branch architecture and location of flowering and branching have been described. Inflorescence numbers per node decrease from older to younger nodes within one growth season, and also from older to younger growth units. Initial analyses indicate that the location of pruning changes the number of inflorescences emerging. Furthermore plant nutrition can change the proportion of growth that forms new branches. Modelling inflorescence and branch point distribution using the data collected will be used to identify further growth patterns These results suggest that axillary buds are predisposed to forming either floral or vegetative axes, but that their fate or behaviour can be modified by environment – be that

inside the tree or outside. In addition, the temperature experiment indicates that flowering is likely to be a two stage process, one of which is microscopic, and may occur many months before any visible sign of flowering develops. This has implications for the timing of management efforts intended to support flowering.

Technical Summary

Implications of tree architecture and carbohydrate allocation in Macadamia tree development – Sadegh Karimaei

The dense canopy architecture of macadamia causes problems within the tree itself, as well as for macadamia orchard management. Farmers have to use heavy machinery for hedging regularly to increase orchard access. But hedging may not be the best method for a long-term solution, as it stimulates undesirable vegetative re-growth and increases fruit abscission. This research provides underlying knowledge of macadamia architecture and carbon allocation that will to find alternatives to hedging for canopy management.

Two sets of experiments were carried out in this study; glasshouse and orchard experiments. Glasshouse experiments were conducted through three architectural assessments with digitizing of small macadamia trees in pots by using a sonic digitizer and Floradig software. Plants were produced from scratch from more than 15 ecotypes of 5 genotypes. They were digitized 2 to 4 times. In digitizing experiments, *M. jansenii* showed the longest internode but smallest number of nodes (nine nodes). *M. ternifolia* showed the shortest internodes and the same number of nodes as others (ten nodes) except *M. jansenii*. Photosynthesis rate of three genotypes of young macadamia plants was measured by a Li-6400 infra-red gas analyser. Another glasshouse experiment was conducted to determine the effects of rootstocks on grafted A4 scion. Micrografting of A4 on different rootstocks was carried out to measure their effect on the growth of scion. Plants were photographed and the new growth of scion was recorded using Photoshop. The smallest amount of growth resulted from the hybrid rootstock but it didn't show a significant difference with *M. ternifolia* at the end. *M. ternifolia* and A4 had a similar growth pattern as well as Beaumont and the hybrid. A4 and *M. ternifolia* initially grew rapidly, apparently due to grafting compatibility. If we consider A4 on A4 100% compatible then *M. ternifolia* should have higher compatibility than Beaumont and the hybrid.

Two orchard experiments were conducted to determine the relationship between vegetative growth and carbohydrate sources in mature macadamia trees coinciding with the major flush periods. Girdling was used to separate a branch with the growing flush from the rest of tree. We considered the leaves on growing flush as providing current photosynthate and the rest of tree providing the pool, or reserve carbohydrate source. Girdled and non-girdled flushes were either defoliated or left intact. Growth length of new flush was fitted in equations to calculate the contributions of carbohydrate sources. Single flush without growing leaves and without girdling (SDng), girdled and defoliated single flush (SDg), non-girdled single flush with growing leaves (SLng) and girdled single flush with growing leaves (SLg). 2 current photosynthate and 2 pool carbohydrates were calculated.

Pool 1 = SDng – SDg = pool – 0 Pool 2 = SLng – SLg = (pool + leaf) – leaf

Leaf 1 = SLng – SDng = (pool + leaf) – pool Leaf 2 = SLg – SDg = leaf – 0

From the results we found that reserve carbohydrate contributes more to vegetative growth of macadamia (75%) than current photosynthate (25%). Data analysis has been finalised for the orchard experiments and a paper of this research will be submitted to Annals of Botany.

A prototype L-systems model of girdled and non-girdled branches has been developed in relation to current photosynthesis (leaf area) and pool (rest of the tree other than the treated branch) from orchard experiments. This prototype model will be parameterised by fitting the data from orchard experiments for girdled and non-girdled branches and used to help determine the contribution of each source of carbohydrate involved in vegetative growth of macadamia. The data from glasshouse experiments will also be used to develop a model for juvenile trees. Then a final model of vegetative growth of macadamia will be developed for the both parts of the life cycle of macadamia trees.

Axillary bud behaviour in Macadamia – Janine Conway

The project began with an investigation of industry needs and definition of research questions that could provide information towards meeting those needs. This appears in the introduction (Chapter 1) of the thesis, which was written up in year one, but will be modified to include new ideas developed from early experimental and survey results, which were incorporated in the design of the latter experiments and surveys. The area of axillary bud behaviour was chosen as the project focus, as this is the basis for both the yield-limiting deep shading found in the centre of most commercially managed macadamia trees, and also the basis for inflorescence production.

Following this stage, a literature review (Chapter 2) was conducted, investigating existing botanical knowledge relevant to the initial research questions. This was submitted as part of the first year review requirements of the University of Queensland, but will also be extended to include new material needed to fully inform later experiments arising from earlier results.

Survey and experimental work is divided into chapters by the nature of the questions addressed by each investigation. Temperature sequence work has been submitted as a journal paper and in this format comprises Chapter 3. The major finding of this experiment was that warm temperatures followed by cool increased flowering over warm-followed-by-warm and cool-followed-by-cool. There was no difference between warm only and cool only treatments. Cool-followed-by-warm decreased inflorescence emergence. Thus it is temperature sequence and not temperature alone that influences emergence. The same pattern appeared in branching behaviour, but to a lesser extent and in contradiction to some previous research, so it seems the branching response needs more detailed investigation before application.

Growth unit architecture and location of flowering and branching investigations make up Chapter 4. The results of these have been analysed and this chapter is written up to the discussion section. Main findings include i) inflorescence numbers per node decrease from older to younger nodes with one growth season, and ii) inflorescence numbers per node decrease from older to younger growth units.

Experiments on the effect of the location of pruning have been finalised and initial data analysis completed, with the next step being full analysis with the aid of a biometrician. This work is written up to the results section, and forms Chapter 5. Initial analyses indicate that the location of pruning does change the number of inflorescences emerging.

Investigations into the effect of nutrition and hydration on branching will form Chapter 6. First layer analysis indicates that changes to plant nutrition does change the proportion of growth that forms new branches.

Investigations into methodology, useful for future research, will form chapter 7.

Modelling inflorescenece and branch point distribution using the data collected will be used to identify further patterns not obvious using statistical analysis. This will entail adding the research results to the basic model developed early in the project. This work will be presented in Chapter 8.

The thesis discussion and conclusion (chapters 9 and 10) will tie together findings in all these areas. The common thread in the above investigations currently seems to point towards axillary buds being predisposed to forming either floral or vegetative axes, but that their fate or behaviour can be modified by environment – be that inside the tree or outside. In addition, the temperature experiment indicates that flowering is likely to be a two stage process, one of which is microscopic, and may occur many months before any visible sign of flowering develops. This has implications for the timing of management efforts intended to support flowering.

Introduction

Background - DAFF modelling project

A significant internal reinvestment by DAFF was funded the initial development of functional-structural modelling capability for tropical fruit and nut trees to enhance the world-leading DAFF field crop modelling group in Toowoomba. A scoping study recommended to the Australian macadamia industry the development of an appropriate physiological model to identify and prioritise the poorly understood but critical physiological processes that affect growth, yield and quality. The benefits of a functional-structural plant modelling approach to crop production are the ability to simplify and gain insights into the system and to explore 'what if' scenarios to optimise the system and compare various management options. Understanding the physiological drivers will underpin successful crop manipulation for improved productivity.

Outcomes of the DAFF funded functional structural modelling project were:

1. Improved systems modelling capabilities for the macadamia industry

This outcome will be delivered by adding 3D orchard modelling capabilities to DAFF – APSRU's existing suite of modelling tools. In this respect, funding from this project was used to appoint a full time systems modeller to compile existing data sets, design experimental protocols, supervise the collection of new data sets, liaise with other national and international 3D modelling experts, and program, in collaboration with APSRU's Software Engineering Group, the required code for model development.

2. Improved viability of macadamia producers

The developed simulation tools will be used to identify improved management strategies (plant arrangements and canopy management) that maximise orchard light interception, which is highly related to kernel yield.

3. Improved adaptation to change i.e. climate variability, market volatility, mitigation poli*cies.*

Future climate projections will be used to simulate the expected trajectories of alternative orchard designs (i.e. cultivars, locations, planting arrangements), orchard management strategies (i.e. canopy architectures and pruning systems), and to evaluate expected impacts from present and future climatic risks on new industry investments.

Currently, the self-organising model (Palubicki et al., 2009) expressed using Lpfg (Karwowski and Prusinkiewicz, 2003) was used as the basis for a model of macadamia (*Macadamia integrifolia* Maiden & Betche) that could be used to explore canopy management options. The model was modified to include three leaves at each node and potentially multiple axial buds at each leaf axil. In the original implementation, sensing of the light environment to guide new growth was done by buds, but this was likely to cause difficulties during subsequent model development as macadamia are thought to have as many as five axillary buds (Bennell, 1984). Instead,

leaves were added, using their midpoint to cause shadowing. The amount of light available for growth was sensed at the leaf level and used to represent vigour, which was then accumulated acropetally.

Buds also sensed the light environment but only to provide demand in the subsequent redistribution phase. Tree models were initiated by reading in an initial structure digitised from a small tree and then allowed to develop for a number of years. Empirical relationships were derived from a set of 24 digitised trees after conversion to multiscale tree graphs (MTG) and analysis with the OpenAlea software library (Godin et al., 1999, Pradal et al., 2008). The ability to write MTG files was embedded within the model so that various tree statistics could be exported for each run of the model. To explore the parameter space, a series of runs was completed using a high-throughput computing platform (HTCondor™). While this was run on a single machine, essentially in batch mode, it could be adapted to large collections of distributively owned computer resources. When combined with MTG generation and analysis with OpenAlea, it provided a convenient way in which hundreds of simulations could be explored.

We allowed the model trees to develop using self-organisation and simulated cultural practices such as hedging, topping, removal of the leader and limb removal. The model provides insight into the impact of these practices on light distribution within the canopy and to the orchard floor by coupling the model with QuasiMC (Cieslak et al., 2008) to simulate the light environment. The lessons learnt from this will be applied to other evergreen, tropical fruit and nut trees.

While the macadamia industry may be the first to benefit, these outcomes will provide a platform for future developments for other tropical and sub tropical fruit and nut industries. The developed modelling tools will be generic; in the sense that these advances will be easy to transfer across a range of industries e.g. avocado, and mango orchards.

The DAFF project budget of over $500,000 (internal funding) was not sufficient to cover all the costs to fill the knowledge gaps needed for model development. Additional funding for this current project was provided to appoint two PhD students as a cost-effective way for collection of additional physiological data. Although the DAFF project was separate from the existing project, some background and early results of that project are included to place the results of this industry project in perspective.

Presentation of this report

Because this project consists of two separate PhD investigations which are each quite complex, they are presented separately to avoid confusion. Although all experimental work has been completed, some aspects of the analysis and writing up are still to be completed.

PhD Project 1. Implications of tree architecture and carbohydrate allocation in Macadamia tree development (Sadegh Karimaei)

Advisors: Jim Hanan, Susanne Schmidt and Russ Stephenson

School of Agriculture and Food Sciences 2009 - 2013

Chapter 1: General Introduction

Macadamia is the only native Australian tree commercially produced for its edible nut. Commercial development of macadamia orchards began in Australia in the early 1960's. Recent trends toward higher density orchards have caused problems with orchard management practices, obstructing machinery access and reducing spray efficiency (Huett et al., 2005). On the other hand, mature macadamia trees are large in size with compact branching pattern, which causes dense canopy architecture.

Although hedging is seen as a viable mechanism for resolving these issues, it stimulates more vegetative re-growth in addition to high labour cost. However there may be more efficient and less costly alternatives. To discover possible alternatives, more knowledge about the balance between carbon allocation and plant architectural development are needed. Efficient canopy management will be achieved by understanding factors that affect macadamia tree canopy development. These factors are implemented at two different time scales; a set of initial choices affecting orchard during its life-span (e.g. type of rootstock and orchard density) and a set of annual procedures (e.g. annual training and pruning practices) (Costes et al., 2006).

To study plant morphogenesis, we need to take observations and measurements of the architecture over the time. There is a consensus that plants are constructed from components with specific morphological characteristics and organized at several scales such as metamers and growth units. Although the term plant architecture refers to the dynamic organization of plant components in space, it can be defined statically by topological and geometrical data. Physical connections between plant components are described by "Topology" and the shape, size, orientation and spatial location of the components are described by "Geometry" (Godin et al., 1999).

To analyze the data taken from architectural measurements and predict what might be observed under different conditions, we can use computational models. Plant growth modelling has become a research tool in the fields of agriculture, horticulture and environmental sciences to aid in understanding how plant growth and development interact with environmental factors. To model these interactions, physiological processes involved in growth such as photosynthesis and carbon allocation should be taken into account (Fourcaud et al., 2008). Plant architecture data can be analyzed and used to create functional models of plants. Functional-structural plant models, FSPMs (Sievanen et al., 2000, Godin and Sinoquet, 2005) or virtual plant models (Hanan, 1997, Room et al., 1996) describe plant morphogenesis over the time as governed by physiological processes that are affected by environmental factors (e.g. temperature). They take into account interaction between plant architecture, function of organs (e.g. leaf photosynthesis) and growth processes. Using architectural variables measured at

different growth stages a virtual plant can be developed to explore effects of different factors driven growth such as light interception and photosynthesis (Fourcaud et al., 2008).

According to model macadamia tree's vegetative growth and to discover the relationship between the architecture and carbohydrate allocation of macadamia tree during vegetative growth three experiments are designed in this research. The architectural development of young macadamia trees is studied on different genotypes of macadamia trees which are produced from cuttings and grafting, as well as biomass allocation and its relation to the architecture of the tree in one experiment. In another experiment, the effect of photosynthesis on vegetative growth in macadamia is studied by measuring the growth rate of new flush/flushes in response to the number of leaves on parent shoots with or without girdling to compare the effect of limited or non-limited (pool) carbohydrate on new vegetative growth. And finally another orchard based experiment is conducted to study the relationships between photosynthesis parameters and non-structural carbohydrate at various orchard densities. All data collected from architectural measurements and carbohydrate allocation experiments (photosynthesis and non-structural carbohydrate measurements) will be used to make a functional structural model for macadamia by using L-system. This research will produce much needed fundamental knowledge of tree morphogenesis for future studies.

Distribution of macadamia

The origin of macadamia is along the fringes of rainforests in coastal northern New South Wales and southern Queensland from 25 to 32°S latitude. Edible nuts are found on *Macadamia integrifolia* Maiden & Betche and *Macadamia tetraphylla* L.A.S. Johnson with smooth and rough shells respectively. Nuts in other species such as *M. jansenii* and *M. ternifolia* are not edible due to a cyanogenic glucoside which imparts a bitter taste to the kernel (Nagao and Hirae, 1992) and makes it inedible, but the trees may have the potential to be used as rootstocks for commercial varieties and/ or future breeding efforts.

According to the Australian Macadamia Society, there are about 6 million trees in Australia, growing on 17,000 hectares. *Macadamia integrifolia* and the hybrids of *Macadamia integrifolia* X *Macadamia tetraphylla* are the commercially preferred species. Australia is leading world production of macadamia with 35,000 tonnes nut in shell (NIS) in 2008 (Australian Macadamia Society website).

Botany of macadamia

Macadamia is a long-lived evergreen medium to large tree in the Proteaceae family. It grows to a height of up to 20m and a spread of up to 15m. It produces several vegetative flushes annually with two peaks of flushing in spring and late summer. Leaves are arranged in whorls of three in *M. integrifolia* and four in *M. tetraphylla*. In the axil of each leaf three buds are arranged longitudinally, therefore multiple branches or flower racemes may develop from every leaf whorl or node. Each pendulous raceme produces approximately 200 creamy to white flowers, most of which fall off in the first 5 to 6 weeks after fertilization resulting in less than 5% of flowers setting nuts (Kiptot et al., 2007).

Commercial cultivars and hybrids of macadamia

In the 1880s macadamia germplasm was introduced to Hawaii from Australia and by 1912 the Hawaii Agricultural Experiment Station (HAES) had begun distributing seedlings for commercial plantings. The first commercial orchards of macadamia were established in the 1920s.

Most commercial cultivars of macadamia in Hawaii and Australia are based on *M. integrifolia*. However *M. tetraphylla* and hybrids of *M. integrifolia* × *M. tetraphylla* are dominant cultivars in temperate areas such as California and Israel. Hybridization between *M. integrifolia* and *M. tetraphylla* occurs either naturally or by controlled crosses and they have provided viable progeny (Walklate et al., 1997). The Australian Macadamia Breeding Program has introduced top five cultivars including 'HAES 814', 'Own Venture', 'A4', and 'HAES 804'. **'**Beaumont**'** as a hybrid cultivar of *M. integrifolia* × *M. tetraphylla* is a popular rootstock in South Africa with advantages of high strike success and vigorous nursery growth. In a breeding program at Hidden Valley Plantations in 1972 **'**A4**'** and 'A16' were the first plants to achieve Plant Breeders Rights (PBR) in Australia (Richards et al., 1994). Bell et al. (1988) found that A4 had the greatest kernel recovery[1], first grade kernel and kernel mass across six countries. Stephenson and Gallagher (2000) described raw kernel visual appearance for kernel quality in macadamia and ranked 'A4' the best (most attractive for customers) in comparison with other cultivars.

Potential dwarfing rootstocks of macadamia

Controlling tree size and vigour is a central interest in the fruit tree industry. More vigorous trees with excessive vegetative growth cause a dense canopy and crowded orchard that need to be hedged and pruned regularly, which will cause even more vegetative regrowth and consequently more costs particularly for labour. Tree vigour is mainly controlled by dwarfing rootstocks, a tool to restrict tree volume and promote earlier flowering and yield (Seleznyova et al., 2008, Atkinson and Else, 2001). Dwarfing rootstocks have the capacity to allow higher levels of mechanization in fruit tree orchards.

There is a lack of information about dwarfing rootstocks in macadamia. There are some species and hybrids with potentially dwarfing effects particularly *M. jansenii* and *M. ternifolia* which are originally occur in central eastern Queensland, and north and south of Queensland respectively and their hybrids with *M. integrifolia*. These are available from the germplasm collection of the NSW Centre for Tropical Horticulture (NSW Department of Primary Industry), Alstonville.

[1] Percentage mass of kernel from a given mass of nuts

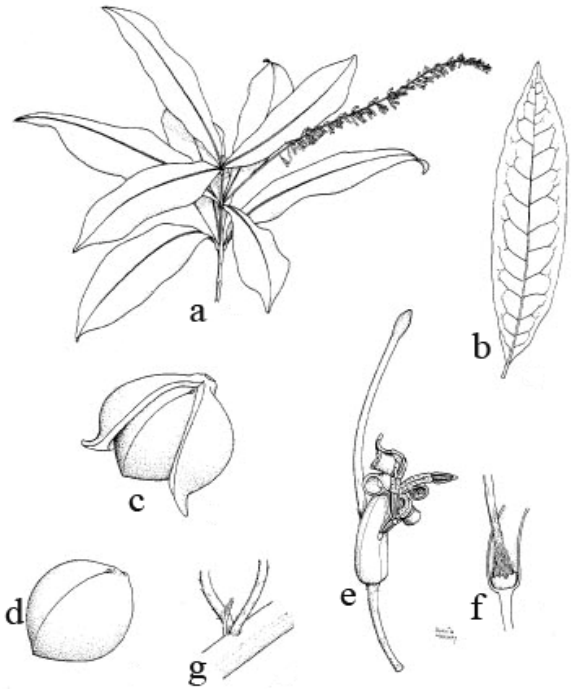


Figure. 1. *Macadamia jansenii* (a) habit; (b) leaf ; (c) fruit; (d) seed; (e) flower; (f) flower section showing ovary and nectary; and (g) bract on rachis at base of flower pair (Gross and Weston, 1992)

M. jansenii also known as the 'Bulberin Nut' was discovered by Ray Jansen from Bundaberg Queensland, in June 1982, growing in a complex notophyll[2] vine forest in State Forest 391 Bulberin, north west of Bundaberg. It is a multi-stemmed tree and smaller than other macadamia species, reaching a height of 6–9m. Adult leaves in whorls of three are 10–17.5 cm long and 2.5–5 cm wide, scarcely glossy above and paler beneath. The leaves are held on a stalk 2–14 mm long, and gradually become hairless as they mature. Bulberin Nut has creamish-brown flowers and small inedible fruit of around 1.5cm diameter (Figure. 1) (Gross and Weston, 1992). Although Bulberin Nut is listed as endangered and a total population of 33 plants was recorded in 1992, it is available in the Alstonville germplasm collection.

M. ternifolia, also known as the Small-fruited Queensland Nut, is a small multi-stemmed tree with 6–8 m height that grows in the north (Coomboorian) and south (Mount Nebo) of Queensland. New growth is pink to red. Leaves are mostly in whorls of three, on petioles (leaf stalks) that are 0.3–1.3 cm long. Leaf blades are obovate to

[2] Notophyll refers to plants with leaf size between 7.5 cm and 12.5 cm long.

elliptic to narrowly elliptic, the apex is acuminate, the base is cuneate to attenuate and leaf margins are coarsely and irregularly serrate. Juvenile leaves are longest and are glabrous. Inflorescences are 4–20 cm long. Fruits are greyish, turning brownish, and are about 1.3–1.7 cm long (Figure. 2) (Stanley and Ross, 1986). Small-fruited Queensland Nut is listed as vulnerable under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and available in the Alstonville germplasm collection as well.

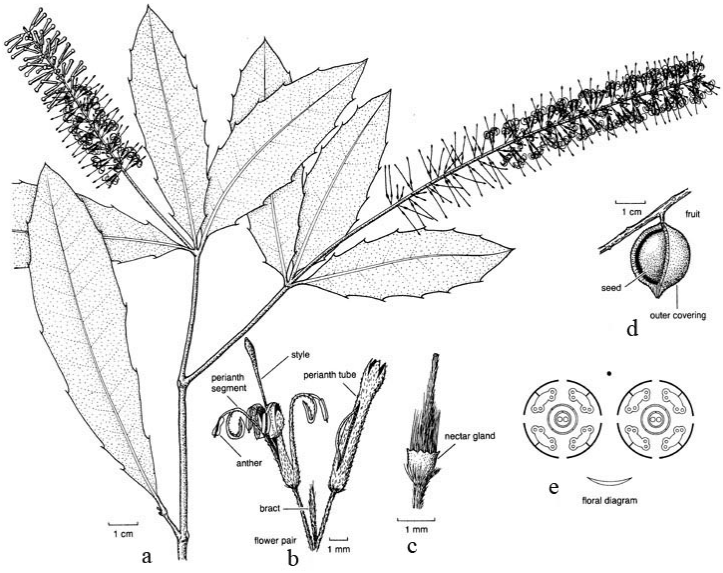


Figure. 2. *Macadamia ternifolia,* (a) Habit; (b) flower; (c) flower section showing ovary and nectary; (d) fruit; and (e) floral diagram (Stanley and Ross, 1986)

Propagation of macadamia

Commercial macadamia trees are grafted onto seedling rootstocks (mostly *M. tetraphylla*) which cause variation in growth habit. There may also be incompatibilities using *M. tetraphylla* as the rootstocks for *M. integrifolia* varieties, for example the scion may grow more than the rootstock and an inverted bottleneck-shaped trunk is developed or vice versa, the rootstock may grow more than the scion. Also, an indentation or crease may appear at the graft union with horizontal cracks in

the bark above and below the union. On the other hand scions of *M. integrifolia* produce nuts with better quality (smooth shell).

To avoid genotypic variation of seedling rootstocks, they may be produced vegetatively by cutting or air-layering. Although rootstocks produced by cuttings may not contain well established roots and are more susceptible to wind, the South African producers believe they can and do produce well established roots in macadamia. In addition clonal rootstocks will provide more uniform orchards for training and maintenance (Stephenson et al., 2003).

Hardner and McConchie (2004) evaluated twelve cultivars of macadamia as scions on own-rooted cuttings, seedling and clonal rootstocks. They found a moderate correlation between the nursery vigour and the strike rate of cultivars. The rate of cutting strike ranged from 23% for 849 to 80% for Beaumont with an average of 57%. Beaumont, A268 and 264 had the highest budding success whereas A16 and 741 had the lowest. The authors found that Beaumont and A268 were the best performing clonal rootstocks with the highest results for strike rate, growth and rootstock budding success (Hardner et al., 2004).

Limitations in the production of macadamia

Australian commercial orchards of macadamia were grown at 10m x 8m spacing (250 trees/ha) in the early 1960's. Subsequently, there has been a trend toward high density orchards, the majority being at 8m x 4 m (312 trees/ha). Some newer orchards are planted at up to 1,000 trees/ha. Many of these high density orchards are now approaching maturity and problems have arisen with orchard management practices, with canopies obstructing machinery access and reducing spray efficiency. Another problem associated with higher orchard density and tall trees is long term reduction in yield due to shading of the orchard floor, which cause lower groundcover growth and leaves the soil bare and prone to erosion and dries out slowly, delaying harvest (Huett, 2004).

Severe topping and hedging of mature macadamia trees may result in a short term reduction in yield by stimulating vegetative regrowth and removing fruiting wood (Huett et al., 2005). Although moderate hedging to maintain orchard access has less effect on yield of macadamia (up to 13%), regular topping and hedging to maintain tree height and width lead to severe internal shading and compromises the renewal of fruiting wood and eventually reduces yield (Huett et al., 2005). The stimulus of regrowth not only results a dense canopy, but also inhibits flowering and compete with nut production for assimilates and light.

Architectural development and the relationship between current photosynthate and non-structural carbohydrate reserves have not been studied in macadamia. It is important to explore the architecture of macadamia tree and the allocation of carbohydrate in different parts of macadamia tree to expand our knowledge for future studies.

Aim of PhD study No.1.

The main aim of this research study is to understand the architecture of the macadamia tree and the relationship between tree architecture and carbohydrate allocation during vegetative growth. Computer modelling was used alongside physiological experiments to simulate the vegetative growth based on the various sources of carbohydrate.

The Australian Macadamia Industry is interested in research related to canopy management of macadamia trees due to the problems that have been arisen from high orchard densities that has affected canopy management practices directly and long term reduction in yield indirectly. Lack of long-term strategy to control the size of macadamia canopy is due to the limited knowledge of the physiological processes governing vegetative and floral shoot and nut development (Huett et al., 2005).

Four approaches comprise this research:

1. **Understanding vegetative growth in macadamia tree**

   Two experiments in autumn and spring were conducted to study the contributions of two carbohydrate sources i.e. current photosynthate and stored carbohydrate in vegetative growth of macadamia.

2. **Study of the architecture and the effects of rootstocks on young macadamia trees**

   Architectural characteristics of young macadamia trees in pots and the growth extent of a scion grafted on various rootstocks (possible dwarfing rootstocks) were studied by measuring architectural parameters such as number of nodes, stem girth, leaf area, and branching angle.

3. **Study of the relationship between photosynthesis and non-structural carbohydrate in macadamia trees**

   Photosynthesis measurement in young macadamia trees and non-structural carbohydrate measurements in mature macadamia trees with different densities were carried out to study the effect of canopy density on photosynthesis and carbon allocation in macadamia tree.

4. **Modelling study: simulation of vegetative growth of macadamia tree**

   A prototype model was created based on the carbohydrate allocation and the model was calibrated by the data obtained from experiments.

The first approach aimed to determine how the current photosynthate and carbohydrate reserves affect the vegetative growth of young macadamia trees. The growth parameters of the new shoot/shoots in response to the number of leaves (various leaf area) on the parent shoot with combination of girdling treatment on parent shoot (with or without girdling) were measured and a model of various carbohydrate sources contributions was developed for vegetative growth in macadamia tree.The second approach aimed to explore the architecture of macadamia and compared a range of clonal rootstocks to identify those that produce superior tree architecture in one experiment. Clonal rootstocks were produced from the cuttings of 22 genotypes. In another experiment the effects of different rootstocks on the morphology of young macadamia trees were studied. The third approach aimed to

study the effect of canopy density on photosynthesis (current photosynthate) and carbohydrate reserve in macadamia trees grown in orchard. in forth approach a prototype model of macadamia tree's vegetative growth was developed and the data from experiments were used to calibrate this model.

The outcomes of these three experiments will help us to understand macadamia architecture and how we can manipulate it efficiently. The results will also lead us to understand the relationship of macadamia tree architecture with carbohydrate allocation. The achievements of this study will be a first step in introducing rootstocks and commercial varieties that provide homogenous and efficient canopy structure for light capture and minimal pruning requirements as well as lower maturity age (precocity). We might be able to summarise the relationship between carbohydrate allocation and architectural development of macadamia in figure 3.

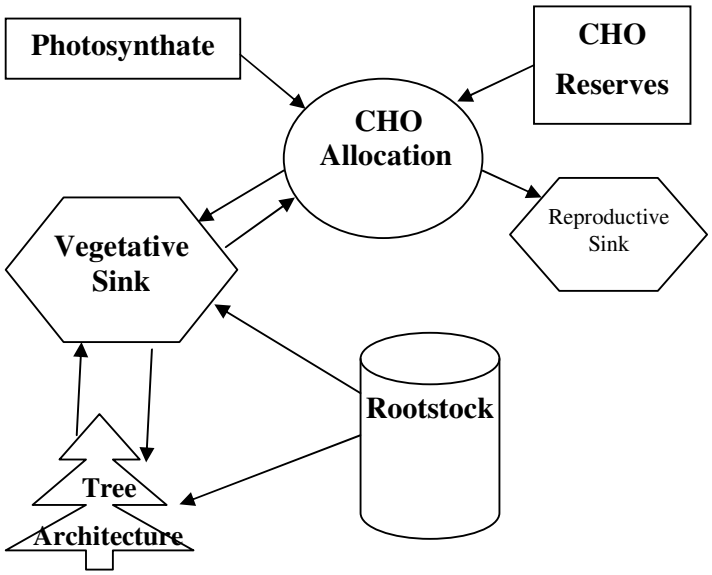


Figure. 3. Schematic relationship between carbohydrate allocation and tree architecture

Table 2. Researches relevant to this study.

Title of Published studies	Species	Key study
Nagao and Hirae (1992)	Macadamia	Cultivation and Physiology
Huett (2004)	Macadamia	Physiology review
Hardner et al. (2009)	Macadamia	Genetic resources
Huett et al. (2005)	Macadamia	Physiological constraints
Lloyd (1991)	Macadamia	Photosynthesis
Olesen et al. (2008)	Macadamia	Carbohydrate allocation
Stephenson and Trochoulias (1994)	Macadamia	Carbohydrate allocation
Yano et al. (2002)	Peach	Carbohydrate allocation
Barthelemy and Caraglio (2007)	Trees	Tree architecture
Costes et al. (2006)	Trees	Tree architecture
Costes and Lauri (1995)	Trees	Tree architecture
Spann et al. (2007)	Pistachio	Tree architecture and rootstock effect
Costes and Garcia-Villanueva (2007)	Apple	Dwarfing rootstocks Effect
Seleznyova et al. (2008)	Apple	Dwarfing rootstocks Effect
Clearwater et al. (2006)	Kiwifruit	Dwarfing rootstocks effect
Godin and Sinoquet (2005)	Plants	Plant modelling
Fourcaud et al. (2008)	Plants	Plant modelling
Prusinkiewiez et al. (2000)	Plants	Virtual plants and L-systems
Hanan 1997	Plants	Virtual plants and L-systems

Chapter 2: Understanding vegetative growth in macadamia

A model to study the relationship between photosynthate and vegetative growth in macadamia trees in relation to CHO resource

Background

Vegetative growth in macadamia

There are three buds (one primary and two secondary) aligned directly below one another within each leaf axil. Although, there are up to nine potential lateral branches at each leaf whorl can grow from these buds (Nagao and Hirae, 1992, Storey et al., 1953), during normal flushing only a single shoot will develop within each leaf axil (Nagao et al., 1994). Flushes emerge from branch apices or from buds in leaf axils.

Carbohydrate in fruit trees

Carbohydrates are quantitatively the most important components of woody plants. They comprise almost 75 percent of the dry weight of woody plants and are the primary organic source of energy for synthesis of other organic compounds (Costes et al., 1999).

Carbohydrate plays an important role in vegetative and reproductive growth of fruit trees, which consequently affects the architecture of the tree, therefore it is crucial to understand macadamia carbohydrate status in the form of carbohydrate allocation. Many different terms have been employed in the literature to describe carbon flow or movement. In this study we will consider terminology proposed by Dickson and Isebrands (1993) for carbon allocation and carbon partitioning; carbon allocation is the process of distribution of carbohydrate within the plant to different parts (i.e., source to "sink"), while partitioning is the process of carbon flow into and among different chemical fractions (i.e., different molecules, different storage and transport pools) (Joyeux-Faure et al., 2003, Dickson and Isebrands, 1993).

Non-structural carbohydrates (NSCs) in woody plants (including fruit trees) are translocated from sources to sinks in all directions. Although most NSC transport is through the phloem, to some extent it can be transported through the xylem sap (Costes et al., 1999). Xylem sap can also supply high concentrations of soluble carbohydrates to tissues during periods of limited photosynthetic CO_2 fixation (Heizmann et al., 2001).

In temperate zone trees the pathway for loading of sucrose from leaves is different than in tropical trees. While in temperate zone trees carbon is loaded to phloem through the apoplast, tropical trees use less efficient symplastic loading mechanisms through the plasmodesmata (Susila and Naus, 2007).

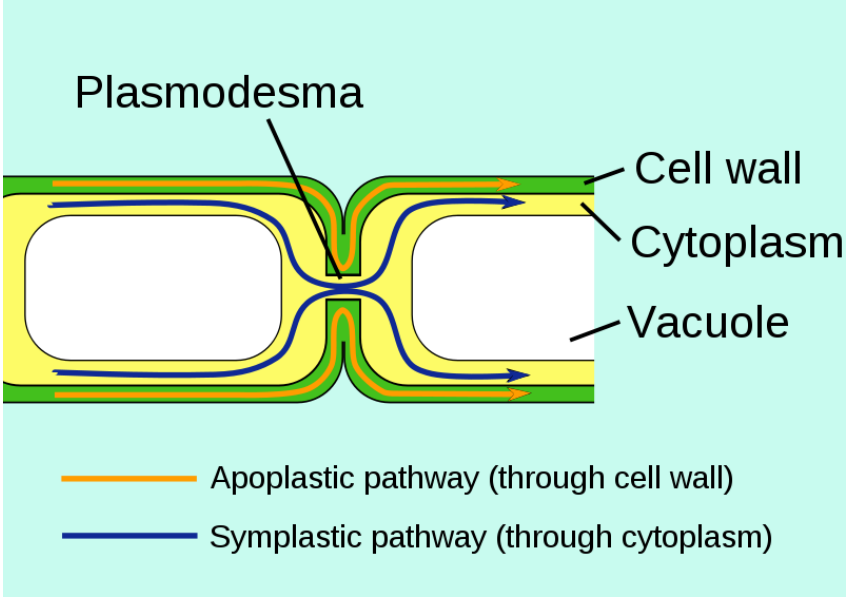
Annual carbohydrate cycle in macadamia

Carbohydrates are quantitatively the most important components of woody plants. They comprise almost 75 percent of the dry weight of woody plants and are the primary organic source of energy (Costes et al., 1999). Woody plants use and accumulate different amounts of carbohydrates annually according to species and genotype. Recurrently flushing species deplete carbohydrates when flushes grow and replenish them between flushes (Susila and Naus, 2007).

In macadamia, carbohydrate accumulation occurs in autumn and winter and depletion in spring (Stephenson et al., 1989b), and major flushes happen in late summer (March) and early spring (September) (Stephenson et al., 1986b, Stephenson et al., 1986a). During the spring, photosynthesis cannot provide sufficient carbohydrate for higher demand of both vegetative flushing and flowering, therefore the plant uses its reserves. Carbohydrate reserve was highly depleted during oil accumulation in macadamia's nut, while in raceme aborted trees (absence of reproductive sink) depletion of CHO was lower (Figure 2) (Stephenson et al., 1989a).

Macadamia generally has a lower photosynthesis rate (7.5-14.5 μmol $CO_2/m^2.s$) than temperate fruit and nut trees (7-20.6 μmol $CO_2/m^2.s$) (Flore and Lakso, 1989). In

temperate zone trees the pathway for loading of sucrose from leaves is different from in tropical trees. While in temperate zone trees carbon is loaded to phloem through the apoplast, tropical trees use less efficient symplastic loading mechanisms through the plasmodesmata (Philippe et al., 1975). On the other hand, macadamia's long-lived leaves have a low light compensation point with a degree of shade tolerance (Demmig-Adams et al., 1997), which can explain its maximal canopy photosynthesis in lower light intensity (in 30–40% of total light) (Flore and Lakso, 1989). However, seasonal differences in environment (i.e. temperature and light) should be considered, as they will affect the amount of assimilates that are produced and modify the proportion of new growth occurring between winter and spring. More knowledge is needed to explain the relationship between current photosynthate and vegetative growth in macadamia.



Carbohydrate in macadamia

Recent soft and immature flush leaves in macadamia are initially net sinks for photosynthate (Huett, 2004). Leaf carbohydrate demand and high temperature contributed to the negative *A* (net photosynthesis) values of emerging macadamia flush leaves. Unlike other tropical species, leaves in macadamia didn't show a decline in A_{max} (light saturation net assimilation rate) as leaves aged over 6 months and were able to maintain relatively constant A_{max} irrespective of leaf age (Kitajima et al., 1997). The optimum temperature range for macadamias is 20–25ºC (Trochoulias and Lahav, 1983). The photosynthetic capacity of macadamia canopies will be depressed at high ambient temperatures, which can occur from late spring to early autumn and may affect carbohydrate supply to the developing nut (Huett, 2004).

Maximal canopy photosynthesis for macadamias occurs with approximately 30–40% of total light. Shaded leaves cannot compensate for the loss of photosynthetic capacity by re-exposure to light (Flore and Lakso, 1989). Macadamias long-lived leaves with a low light compensation point have a degree of shade tolerance (Demmig-Adams et al., 1997).

Although macadamia generally has a lower A_{max} than temperate fruit and nut trees (Flore and Lakso, 1989) (Table 3), macadamia has similar A_{max} values to citrus. Both species have dense outer canopies and pruning is not commonly employed to improve light distribution. Lloyd (1991) recorded A_{max} at 14.5 μmol/m^2.s for fully irradiated macadamia leaves in optimum range of 15–20°C temperature and 62% relative humidity, but this decreased to 9.0 μmol/m^2.s at 30°C.

Table 3. A_{max} (µmol CO_2 /m^2 .s for tree crops. From Flore and Lakso 1989.

Crop	A_{max}
Almond	18.0
Apple	15.7
Apricot	7.0
Avocado	4.8–19.0
Macadamia	**7.5–14.5**[A]
Orange	9.9
Peach	13.3
Pear	20.2
Pecan	14.5
Plum	20.6
Tropical tree spp	10-12

[A]From Lloyd (1991) at 15–20°C.

It seems improvements to yield cannot be achieved in macadamia by increasing photosynthetic capacity of leaves because yield is more sink-limited than source-limited (Huett *et al*. 2003). This means that when the canopy is exposed to high light levels, photosynthetic capacity increases with crop load up to moderate levels (Huett, 2004).

As in other fruit trees, developing fruits of macadamia are dependent on carbohydrate allocations from adjacent irradiated leaves. Trueman and Turnbull (1994) performed an experiment on girdled branches of macadamia, to show that each fruit needs 50 available leaves (Trueman and Turnbull, 1994).

The source of vegetative growth in macadamia – Carbohydrate reserve or current photosynthate?

There are contradicting arguments about the effect of carbohydrate (CHO) reserves versus current photosynthate on the vegetative growth of subtropical evergreen fruit trees. Previous studies claim that the level of CHO reserves is responsible for flush development in evergreen trees including macadamia (Cormack and Bate, 1976), avocado (Liu et al., 1999) and lychee (Menzel et al., 1995). In contrast, Olesen et al. (2008) considered current photosynthates to be the main source of carbohydrate for new growth in macadamia and it was proposed that carbohydrate reserves play a secondary but important buffering role during periods of high carbon demand. Stephenson et al. (1989a) asserted that major vegetative flushing activity in macadamia did not coincide with significant depletion of reserves (Figure 2), although the secondary decline in stored CHO in July may be due to maturing of vegetative

growth that commenced the previous May. However there is not enough evidence for the level of carbohydrate reserves contribution for vegetative growth in macadamia.

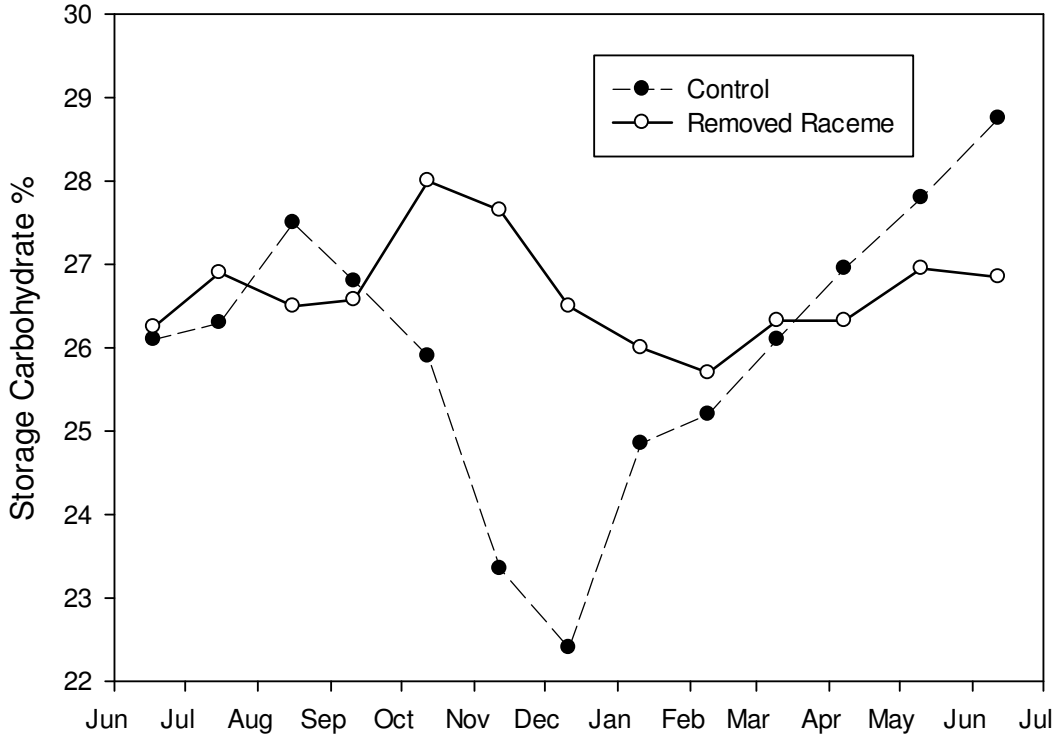


Figure.2. Seasonal pattern of storage carbohydrates in trunk wood tissues of macadamia trees subjected to growth manipulation treatments (RR= removed raceme), Stephenson et al. (1989a) with permission.

Reproductive sink versus vegetative sink

Reproductive sinks have a priority to attract carbohydrates rather than vegetative sinks from limited resources (Soderstrom et al., 1988). Although the results from the Sakai (1981) study in kiwi fruit showed that the vegetative sink had a higher priority, different sink types have different priorities when they are supplied with different levels of carbohydrate ((Saleem and Buxton, 1976, Stephenson et al., 1986c, Lacointe and Minchin, 2008). There are no published studies describing the relationship between sources and sinks in macadamia and we cannot explain sink priority without a detailed experiment having both vegetative and reproductive sinks simultaneously.

Objectives

The vegetative growth of macadamia tree and the effect of CHO sources on vegetative growth (current photosynthate and/or CHO reserves) will be studied in this

experiment. Growth rate of new shoot/shoots on a branch with or without girdling will be measured to produce a model of macadamia vegetative growth in relation to the current photosynthesis and/or carbohydrate reserve.

Leaf area index was also measured to find out the effect of light/photosynthesis on the process.

This study will help to develop a functional-structural model for future carbohydrate allocation studies. Our understanding of the relationship between the current photosynthates and carbohydrate reserves and their impact on vegetative growth and canopy development will contribute to the development of more efficient orchard management.

In order to investigate the relationship between the current photosynthate and carbohydrate reserves and their impact on vegetative growth and canopy development in mature macadamia trees two experiments were designed to study those relationships and the contribution of each carbohydrate (CHO) source. A vegetative sink was created by allowing vegetative regrowth to develop after pruning, and current photosynthate source was adjusted by retaining a different number of mature leaves on each parent branch (Stephenson et al., 1986c). Phloem girdling of half of shoots was implemented to adjust the CHO reserve source. In this study carbohydrate is considered as the only source of energy responsible for vegetative growth in macadamia (Costes et al., 1999) coming from two sources: current photosynthesis by the leaves and CHO reserves (Figure 1). The effect of CHO resources on vegetative growth in macadamia is studied by measuring the growth of new flush/flushes (shoot/shoots) over time in response to the number of leaves left on the parent shoot to compare the effect of number of leaves as the source of current photosynthate. Girdling was applied to compare limited and non-limited CHO resources. This study investigates how the current photosynthate and/or carbohydrate reserves can affect the vegetative growth of macadamia tree and the level of their contributions. We hypothesised that in limited/girdled PSs, current photosynthate from leaves is the only CHO resource responsible for vegetative growth (CHO in PS is neglected) while in non-limited/non-girdled parent shoots, CHO resources are current photosynthate from leaves on parent shoot plus CHO reserves.

Materials and methods

Experiment 1. March

Thirty trees were selected from Fullerton macadamia orchard in Beerwah in Glasshouse Mountain region (26.856447S, 152.919633E) in March 2011.

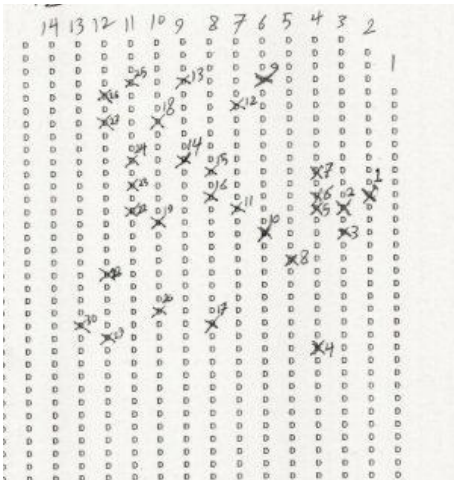


Figure 5. Right: Lindsay Fullerton's macadamia orchard map, crossed trees are selected based on the availability of similarities in girth and length for 3 pair branches on individual trees (each pair branches includes girdled and non-girdled treatment). Left: a photo of orchard, 816 scions grafted on H2 rootstocks and planted in 9 x 4m in 2009 from 2 year-old nursery plants.

Trees were planted in February 2009 from two year-old nursery plants (variety 816s were grafted on H2 rootstocks). Trees and branches were selected in March 2011 and treatments conducted between March 16 and 24, the time when macadamia trees started to accumulate CHO in their tissues. For the experiment design, trees were selected based on the availability of similar branches in girth and length for background treatments and then randomly selected for new growth type treatments. To prevent competition between reproductive growth and vegetative growth, all developing fruits were removed.

Parent shoots treatments:

Three pairs of branches, between 21cm and 48cm long and 3.9 and 5.9 mm girth diameter and without lateral branches were selected on each tree based on their availability. Branches were selected from outer canopy positions at a similar height from the ground (160 to 200 cm) without considering branch orders. Then they were decapitated between 17.5cm and 29cm long from base to stimulate new growth immediately below the cut surface. These branches which were holding the new growth are named hereafter "parent shoot". Two, four or six leaves were retained on each parent shoot and the others were removed. Leaves were considered as the source for current photosynthate. One of the parent shoots of pair branches was girdled and the other pair left intact without girdling (Figure 3). Treatments were applied in 16 March 2011.

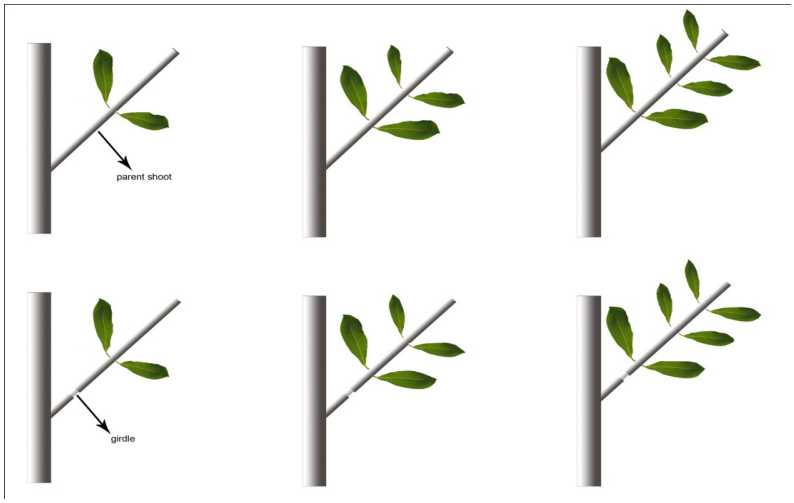


Figure 6. Background treatments on each tree; Six branches (parent shoot) were selected in a tree with 2, 4 or 6 leaves retained on each and with or without girdling.

Figure 6. A) Schematic of girdled and non-girdled branches with different number of leaves, B) Girdled and non-girdled branches

A completely randomized design (CRD) with 10 replications implemented as the experimental design for this study. Each tree with applications of girdling (with and without girdling) and 3 sets of leaf numbers (2, 4 and 6) on each pair of 6 selected branches have been considered as a replication. Trees have been selected randomly for treatments on new growth; "total shoot growth with mature leaves"; all new growth on branches are allowed to grow, "single shoot growth with mature leaves"; only one new growth (new flush) is allowed to grow and the rest are pinched/cut off and the leaves on new growth are allowed to mature, and "single shoot growth with removed maturing leaves"; only one new growth (new flush) is allowed to grow and the rest are pinched/cut off and the leaves on new growth are not allowed to mature, hereafter these 3 treatments will be represented as TSGM, SSGM and SSGR respectively. Ten trees considered for each treatment as replications.

Table . Treatments are selected randomly for each tree (10 reps for each treatment)

tree number	1	2	3	4	5	6	7	8	9	10
treatment	TG3	TG1	TG8	SGR3	TSGM2	SSGM9	TSGM9	TSGM7	SSGM5	SSGR8
tree number	11	12	13	14	15	16	17	18	19	20
treatment	TSGM5	SSGR10	SSGM10	SSGML8	TSGM6	SSGR9	SSGM2	SSGR5	SSGR4	SSGR7
tree number	21	22	23	24	25	26	27	28	29	30
treatment	SSGR1	SSGR6	TSGM4	SSGRL2	SSGM4	SSGM1	SSGM3	TSGM10	SSGM7	SSGM6

TSGM3; Total Single Growth with Mature leaves, SSGM; Single Shoot Growth with Mature leaves and SSGR; Single Shoot Growth with Removed leaves

The length and girth of new growing shoot and the total number and length of internodes were measured. Fresh and dry weight of new growth will be measured at the end of experiment.

New growth type treatments

After selecting the parent shoots and applied the background treatments, treatments of new growth type grown from bud/buds under the cut surface on parent shoots were conducted on each tree.

A completely randomized design (CRD) with 10 replications was implemented as the experimental design. A tree with background treatments was used as a replication. Trees have been selected randomly for treatments based on the new growth type including:

1. Total growth with growing leaves (TG),
2. A single new flush with leaves (SL) and
3. A single new flush without leaves (SD)

In single new flush treatment (SD) additional growth (i.e. growth in addition to the initial new flush arising below the cut surface) was prevented by early pinching the second lot of new growing buds/flush. First measurement of new flush/flushes took place in 5 April and the last measurement took place in 14 July. The growth of the new flush was measured 15 times with one week intervals in early stage and two weeks later, over a 5 month timeframe. Girdling prevents the translocation of CHO from leaves to roots, therefore current photosynthate from leaves and CHO of the parent shoot above the girdled point can be considered as the main sources of energy for vegetative growth, and stored CHO reserves plus current photosynthate for those not girdled. Girdling was applied by removing a 5 mm width strip of bark from the middle of the first internode approximately 5cm from the branch base. Measurements over time included the length and girth of internodes on new growing shoot/shoots and the total number of internodes. After the last measurement of length and girth of growing flushes, branches were harvested in the early morning and put into boxes filled with ice and transferred to the lab and their fresh weights were measured the same day. Parent shoots length and girth was measured at the beginning and their fresh and dry weight at the end of the experiment. Also leaves on the parent shoot were removed and their area was measured through scanning. Then new growths and parent shoots were dried in an oven for two weeks at 60°C and weighed to determine their dry weight.

Figure 3. A schematic of the treatments; girdled and non-girdled branches with different number of leaves (2, 4 and 6). PS: parent shoot, which are girdled and non-girdled, TG: total new growth (the new growths on pruned PARENT SHOOT is not modified), SL: a single new flush with leaves growing, SD: a single new flush without leaves growing.

Statistical analysis

For statistical analysis a linear mixed effects model was established, using R software. Trees were selected randomly to apply shoot type growth treatments) and fixed factors were the independent variables applied, including "girdling", "number of leaves", "leaf area" (LA) and "parent shoot fresh weight" (PSfw), "parent shoot dry weight" (PSdw) and "parent shoot girth" (PSG), and dependent variables (responses) included "growth length" (GLt), "internode girth"(ING), "number of internodes" (INC), "new growth fresh weight" (NGfw) and "new growth dry weight" (NGdw). A linear mixed effects model was established by using "nlme package" (Pereira et al., 2011).

Experiment 2. September

Forty trees were selected in 1st September 2011. The selection of pair branches on each tree is similar to the March experiment. Six branches (3 pairs) were selected on each tree with 2, 4 and 6 leaves were left (the best healthy leaves) on each pair and then half of the branches (each pair) were girdled in 6^{th} and 7^{th} of September. All branches and leaves on parent shoot were removed and during the experiment every sign of new growth (growing buds) were also pinched. In this experiment total growth (TG) was not applied as a treatment of new growth type (NGT).

Forty trees were selected randomly for treatments based on the new growth type (each 20) including:

1. A single new flush with leaves (SL) and
2. A single new flush without leaves (SD)

tree number	1	2	3	4	5	6	7	8	9	10
treatment	D7	L13	L10	L8	D16	D14	L20	L1	L9	D1
tree number	11	12	13	14	15	16	17	18	19	20
treatment	TSGM5	SSGR10	SSGM10	SSGML8	TSGM6	SSGR9	SSGM2	SSGR5	SSGR4	SSGR7
tree number	21	22	23	24	25	26	27	28	29	30
treatment	SSGR1	SSGR6	TSGM4	SSGRL2	SSGM4	SSGM1	SSGM3	TSGM10	SSGM7	SSGM6

Leaf area index and Total leaf area

Leaves were collected from Parent shoots (PS) and labelled and were digitized to measure the leaf area for each treatment (average of replications) with a scanner and the "Adobe Photoshop CS2" software. Light interception or leaf area index (LAI) only measured for second orchard experiment by using a LI-COR instrument on October 2011.

Non-structural carbohydrate analysis

Carbohydrate sampling

For the second experiment 20 samples of whole growth of the single flush resulted from the bud under the cutting point of each replication of each treatment (similar branches to main treatments) were sampled for non-structural carbohydrate analysis. Samples were excised at dawn, weighed and placed in ice in the field and transport to the lab and oven-dried at 60°C and weighed again. The whole growth of the new stem was ground to powder using a cyclonic mill with a 1 mm sieve.

Carbohydrate analysis

Twenty samples of single shoots grown on girdled parent shoots (10 samples) and non-girdled parent shoots (10 samples) were selected out of 240. Total non-structural carbohydrates including water soluble carbohydrates and starch were extracted from the powder. GOPOD (Karkalas, 1985)

The Soluble Carbohydrates test is a dietary proximate analysis and does not measure specific carbohydrates. The procedure calls for a solvent extraction, followed by passing though a C18 cleaning column to remove chlorophyll, tannins and other organic compounds that could interfere with the test, then acid hydrolysis to convert any non-reducing sugars to reducing sugars, then a measure of the reducing capacity of the solution by addition of potassium ferricyanide and measurement on a UV-vis spectrophotometer.

The procedure is calibrated using a glucose standard, and the results given as the glucose-equivalent amount of soluble carbohydrates present in the sample.

In practice, the actual amounts of carbohydrate present in the sample can vary slightly from the determined figure, because not all of the reducing sugars which the carbohydrates might break down into have the same reducing capacity as glucose.

Wood density (Zanne et al., 2009)

Results

Number of leaves on parent shoot (PS) didn't have a significant effect in our statistical models for both experiments, which seems to be due to the variation of areas of individual leaves. Therefore we used leaf area instead of number of leaves on parent shoot as an independent variable. However, we didn't use leaf area on PS to calculate the contributions of CHO sources in this study. Instead, we compared the growth of new flush with or without growing leaves (new growth type; NGT).

Experiment 1 - March

For statistical analysis we used the flush length (FL) and volume (FV) of new single flushes grown on parent shoots in date 25 May of growth measurement period, when they didn't show significant growth afterward. FL was affected by NGT significantly ($P<0.007$) while FV was not affected significantly. Girdling affected FL and FV significantly, $P<0.002$ and $P<0.004$ respectively. The interaction between girdle and NGT was only significant for FL ($P<0.02$).

Parent shoot dry weight (PSdw) had no significant effect on FL and FV but the interaction between girdle and PSdw significantly affected them ($P<0.005$ and $P<0.003$ respectively). Interaction between NGT and PSdw didn't show any significant effect on FL and FV. Parent shoot girth (PSG) had significant effect on FL ($P<0.03$) and FV ($P<0.04$) and its interaction with NGT was only significant for FL. Interaction between NGT, girdle and PSdw, interactions between NGT, girdle and PSG and interaction between NGT, PSdw and PSG had significant effects only on FL ($P<0.04$, $P<0.01$ and $P<0.02$ respectively). However, interaction between girdle, PSdw and PSG significantly affected both FL and FV ($P<0.003$ and $P<0.002$ respectively).
Finally, interaction between all independent variables (NGT, girdle, PSdw and PSG) affected significantly FL ($P<0.02$) but without significant effect on FV.

P-values of different treatments' effects on FL and FV in March and September experiments resulted from statistical models

	March		September	
Treatments	(FL)	(FV)	(FL)	(FV)
NGT*	0.0063^{**}	0.5028^{ns}	0.0258^{*}	0.0963^{ns}
girdle	0.0016^{**}	0.0032^{**}	$<.0001^{***}$	0.0002^{***}
PSdw	0.2107^{ns}	0.4676^{ns}	0.1909^{ns}	0.6404^{ns}

PSG	0.0256*	0.0392*	0.0192**	0.1136ns
NGT:girdle	0.0109**	0.2561ns	0.0001***	0.0290*
NGT:PSdw	0.0562ns	0.7250ns	0.3645ns	0.8133ns
NGT:PSG	0.0010**	0.3393ns	0.0183**	0.0816ns
girdle:PSdw	0.0046*	0.0023**	0.0561ns	0.3250ns
girdle:PSG	0.0014**	0.0037**	0.0003***	0.0002***
PSdw:PSG	0.1007ns	0.2335ns	0.1821ns	0.6984ns
NGT:girdle:PSdw	0.0303*	0.4797ns	0.0477*	0.8122ns
NGT:girdle:PSG	0.0074**	0.1951ns	0.0007***	0.1266ns
NGT:PSdw:PSG	0.0176**	0.9464ns	0.2029ns	0.5831ns
girdle:PSdw:PSG	0.0026**	0.0017**	0.9961ns	0.1504ns
NGT:girdle:PSdw:PSG	0.0139**	0.3509ns		

*NGT: new growth type, girdle: girdling, PSdw: parent shoot dry weight, PSG: parent shoot girth

The growth patterns of single defoliated flush (SD) and single flush with growing leaves (SL) grown on parent shoots with or without girdling were almost similar except for SD non-girdled. However, their growth rate was significantly different for instance in day 70. SD non-girdled showed a slightly increase in growth 70 days after when treatments were applied.

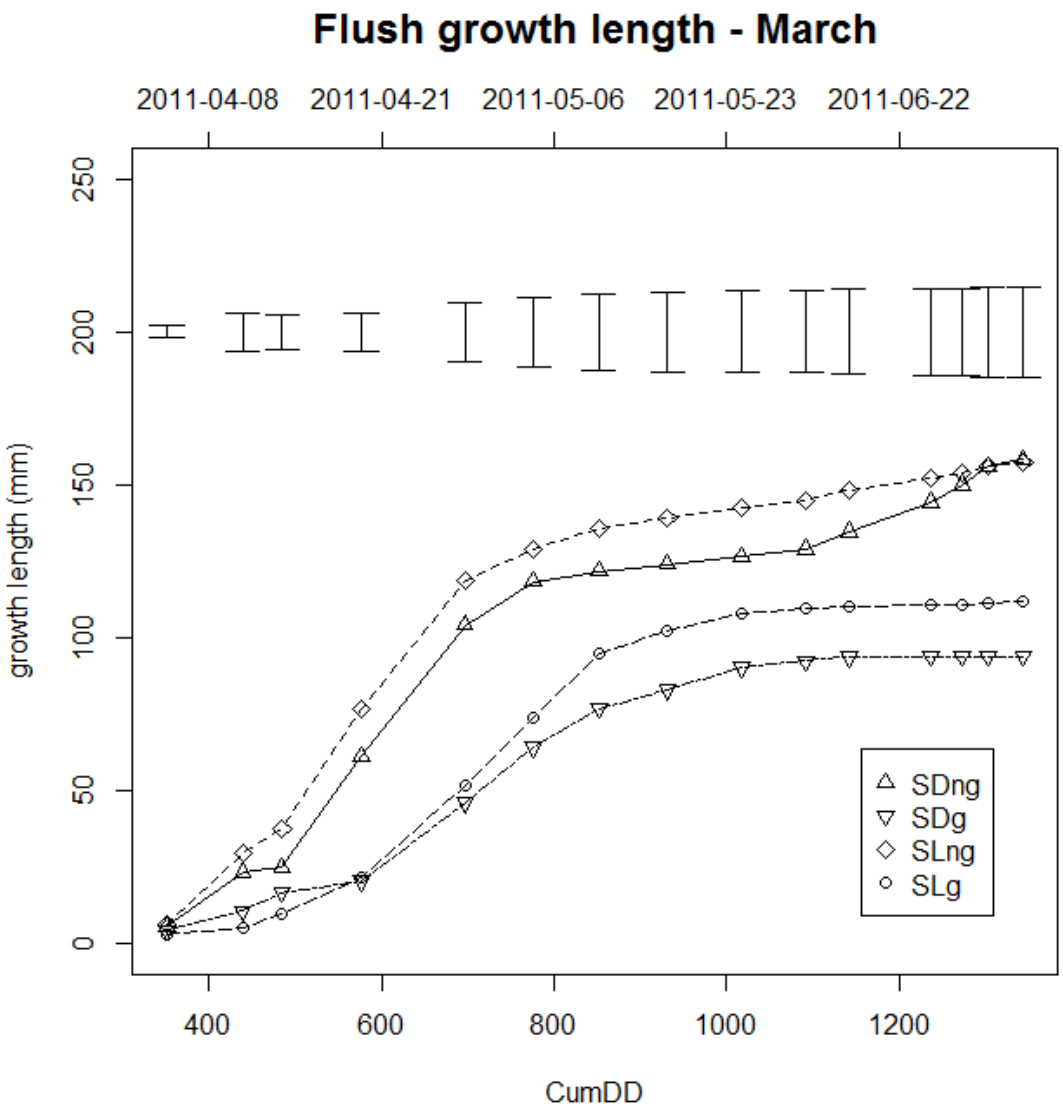


Figure 5. Growth pattern of new single flush length (FL) of different shoot types on girdled and non-girdled PSs for dates of measurement (upper x scale) and for cumulative degree day (lower x scale). Vertical bars represent least significant differences (LSD) ($p \leq 0.05$) between NGT×girdle treatments for the FL in different times of measurements.

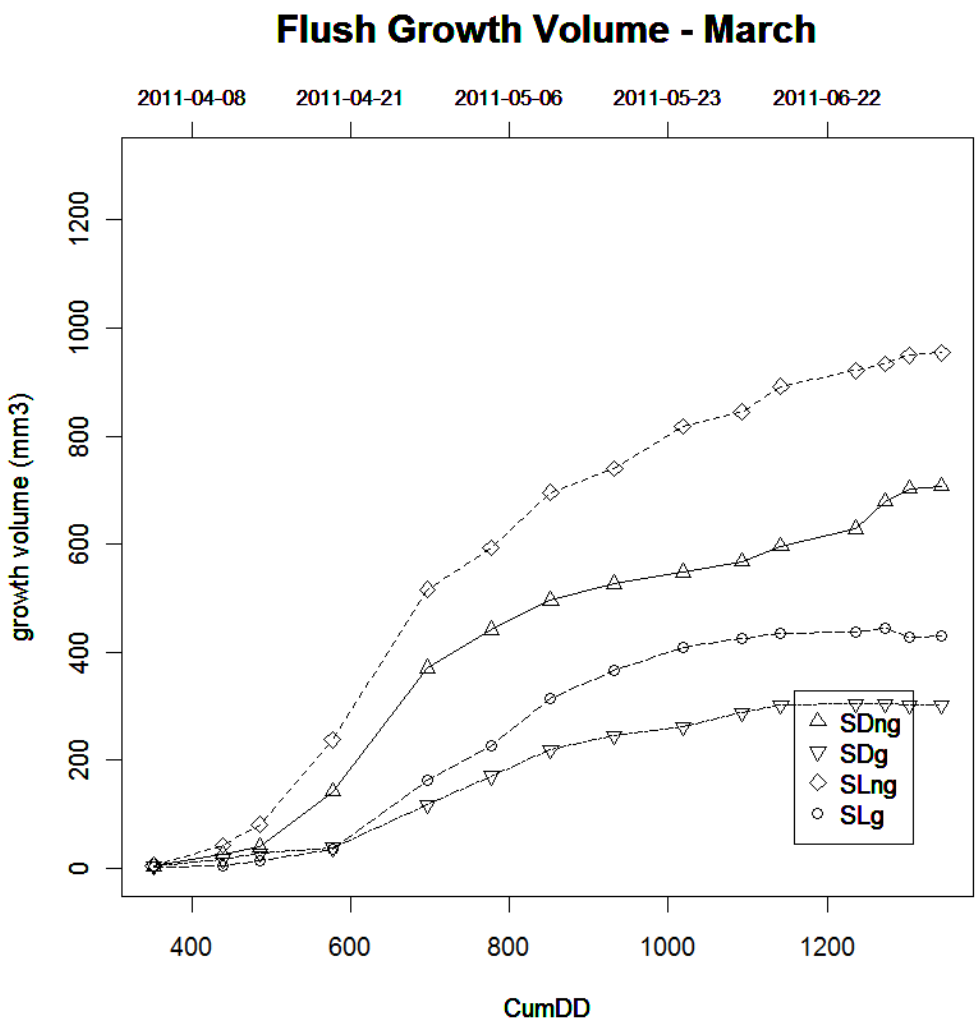


Figure 6. Growth pattern of a new single flush volume (FV) of different shoot types on girdled and non-girdled PSs for cumulative degree day

Experiment 2 – September

For statistical analysis, we used growth data in date 28 September. FL and FV of new single flush grown on PS were also considered for this experiment in our models. NGT had only significant effect in FL ($P<0.03$). Girdling had strong effect either on FL ($P<0.0001$) or FV ($P<0.0003$). PSdw affected neither FL nor FV. PSG only affected FL significantly ($P<0.02$). The interaction between NGT and girdle significantly affected FL ($P<0.0002$) and FV ($P<0.03$). Only FL was affected significantly by the interaction between NGT and PSG ($P<0.02$). Interaction between girdle and PSG strongly affected either FL ($P<0.0004$) or FV ($P<0.0003$). Three way interactions between NGT, girdle and PSdw and between NGT, girdle and PSG significantly affected only FL respectively $P<0.05$ and $P<0.0008$.

Unlike March experiment, the growth patterns of single new flushes of defoliated (SD) and with growing leaves (SL) on non-girdled PSs are similar. This similarity is even more on girdled PSs as is shown in Figure 7. This level of similarity might be

due to the double number of samples in September experiment, which means more accuracy with higher R-squared values in comparison to March experiment. R-squared of 0.69 and 0.66 for September experiment models vs. 0.28 and 0.45 for March experiment models.

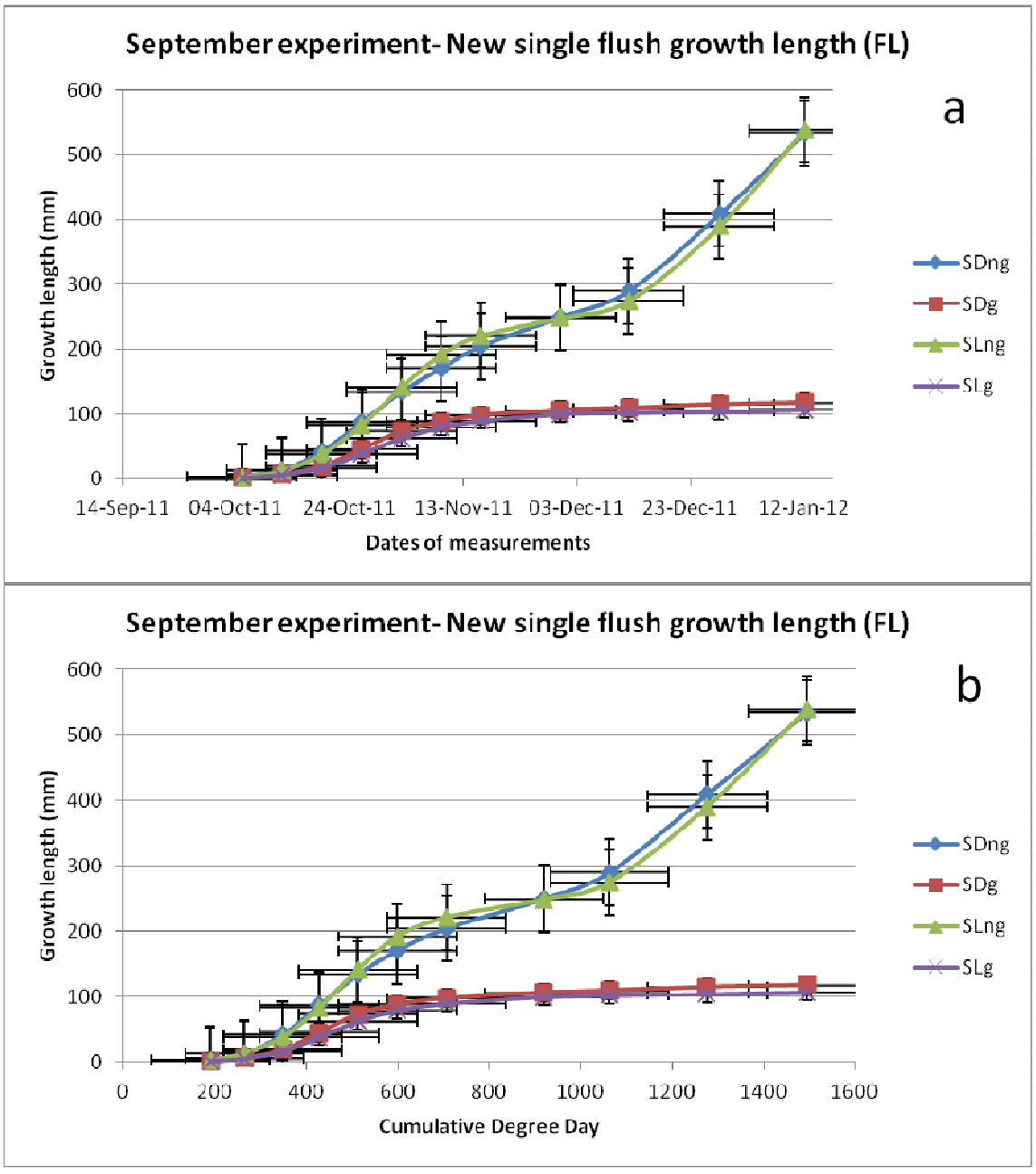


Figure 7. Growth pattern of new single flush volume (FV) of different shoot types on girdled and non-girdled PSs for dates of measurement (a) and for cumulative degree day (b)

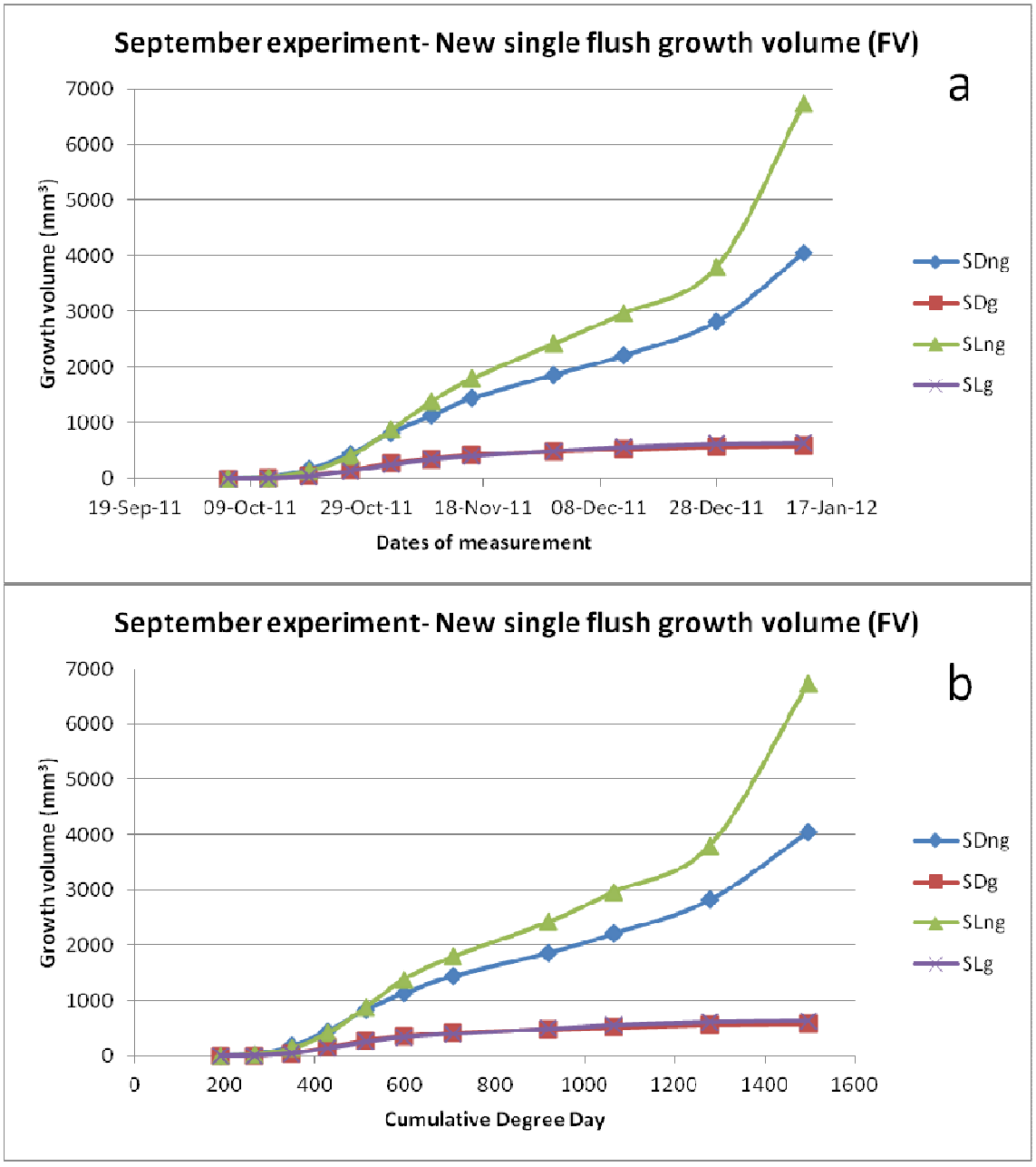

September experiment- New single flush growth volume (FV)
a
Growth volume (mm³)
7000
6000
5000
4000
3000
2000
1000
0
19-Sep-11
09-Oct-11
29-Oct-11
18-Nov-11
08-Dec-11
28-Dec-11
17-Jan-12
Dates of measurement
SDng
SDg
SLng
SLg
September experiment- New single flush growth volume (FV)
b
Growth volume (mm³)
7000
6000
5000
4000
3000
2000
1000
0
0
200
400
600
800
1000
1200
1400
1600
Cumulative Degree Day
SDng
SDg
SLng
SLg

Figure 4 Significant difference between SDng and SDg flushes in September

Discussion

The contribution of a CHO source in vegetative growth is actually equal to the amount of CHO level is spent by a specific source or depleted by the growing flush for a certain amount of growth.

Flush length (FL) and new growth volume (FV) were the variables in our study that their responses to girdling and independent variable of new growth type (NGT) were considered to evaluate the level of CHO contribution of two CHO sources (reserved CHO and current photosynthate) responsible for vegetative growth in macadamia trees. Growth length and girth of single flushes grown on girdled or non-girdled PS with or without growing leaves was measured during the growing period. Also, their fresh and dry weight was measured once only after harvest.

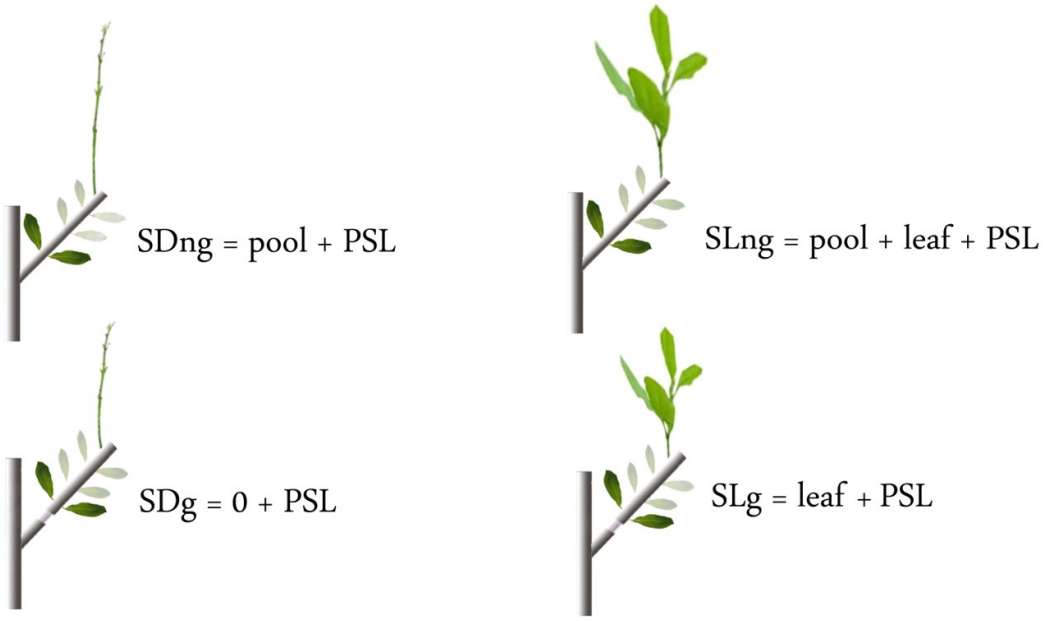
Equations are developed from the relationships between girdled and non-girdled SD and SL growth types for different growth traits. Contributions of CHO sources in FL and FV are determined during the growth period based on these equations as well as FL and FV values at specific dates alongside with fresh and dry weight of new growth at the end of growth period. Graphs of their contributions in growth are produced during the growth period. Proportions of CHO sources contributions were also determined for specific dates of growth and fresh and dry weight of growth at the end of growth period.

We considered two growth types with girdling treatments as below (Figure 8):

- *SDng*: single shoot without growing leaves (defoliated) and non-girdled
- *SDg*: single shoot without growing leaves (defoliated) and girdled
- *SLng*: single shoot with growing leaves and non-girdled
- *SLg*: single shoot with growing leaves and girdled

Then we considered different sources of energy (probably non-structural carbohydrate and/or current photosynthate) for growth of these new shoot types on PSs.

Following equations are made for each single shoot with or without girdling and with or without having growing leaves on new growth (new flush):



We did not consider the number of leaves (2, 4 or 6) on PSs in our assumptions, as PSL exists in all equations. Therefore, flush length (FL) and other growth traits of new single flush on PSs with different treatments are summed up for PSLs and PSL effect can be omitted in our sources' CHO contribution determination.

We define pool as an energy source (CHO reserve) from whole tree.

Through the following equations the sources of energy derived from reserves (pool) or current photosynthate (leaf), responsible for the growth of the new shoot were calculate.

1. **Pool 1** = SDng – SDg = pool – 0
2. **Leaf 1** = SLng – SDng = (pool + leaf) – pool
3. **Leaf 2** = SLg – SDg = leaf – 0
4. **Pool 2** = SLng – SLg = (pool + leaf) – leaf

We used the above equations to calculate two sources' contribution including reserve (Pool) and current photosynthate (leaf). Each CHO source contribution was calculated for two instances (Pool 1, Pool 2, Leaf 1 and Leaf 2). Values of flush length (FL) of new single flush over time (Figures) were used to produce vegetative growth contribution of each source.

We also used FL, FV, NGfw and NGdw values at the end of experiment to calculate the percentage of sources' contributions for these growth attributes in both experiments in March and September 2011 (Table 3).

Experiment 1. March

The above equations were used to calculate the contribution of each source in vegetative growth of macadamia in two instances for each; Pool1 and Pool2 for reserved CHO and Leaf1 and Leaf2 for current photosynthate. Four curves related to these sources are shown in Figure 8.

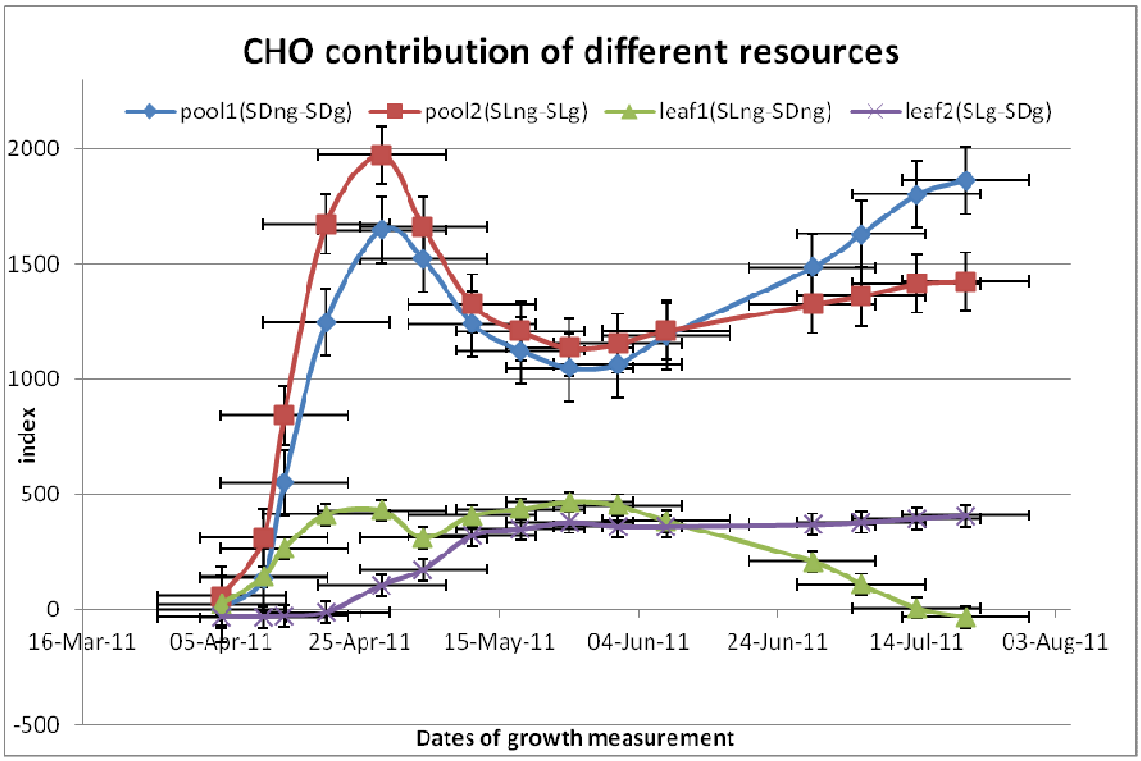


Figure 8. Contribution of different CHO sources (pool or leaf) in growth length of new shoot over the time

Different pool contributions (pool 1 and pool 2) and different leaf contributions (leaf 1 and leaf 2) which were used to calculate the growth length of new shoot showed similar patterns (Figure 8). These similar curves for each CHO source contribution increase the confidence of our assumptions. Therefore, an average of two instances used for each CHO source contribution is made to produce the final graph (Figure 9).

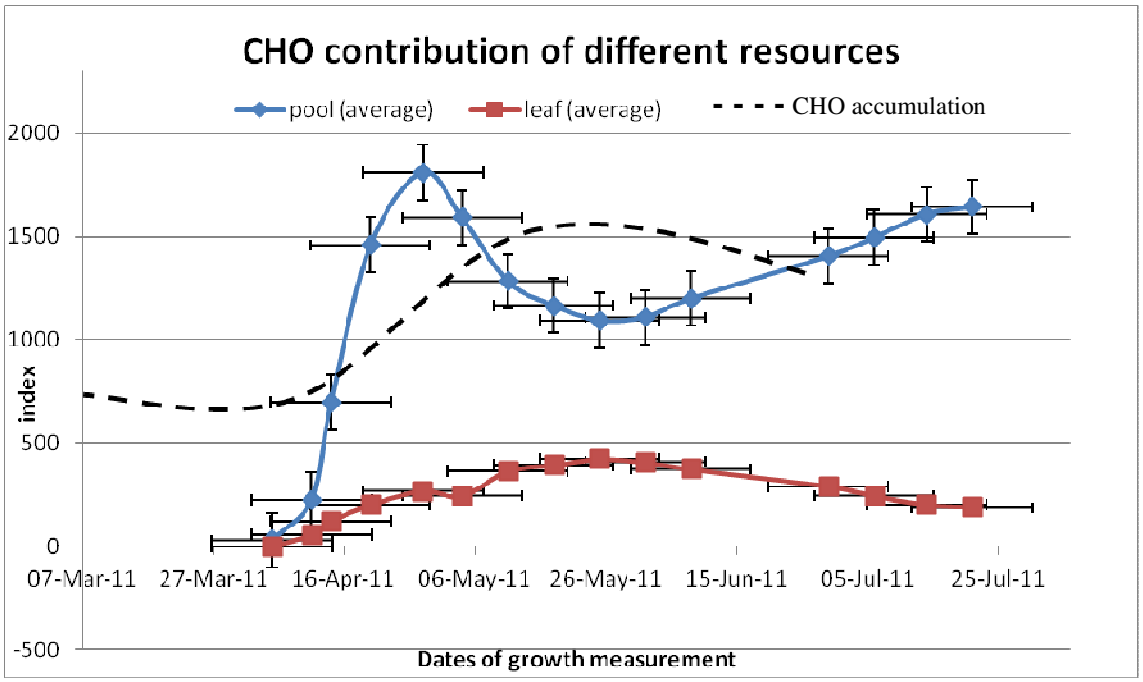
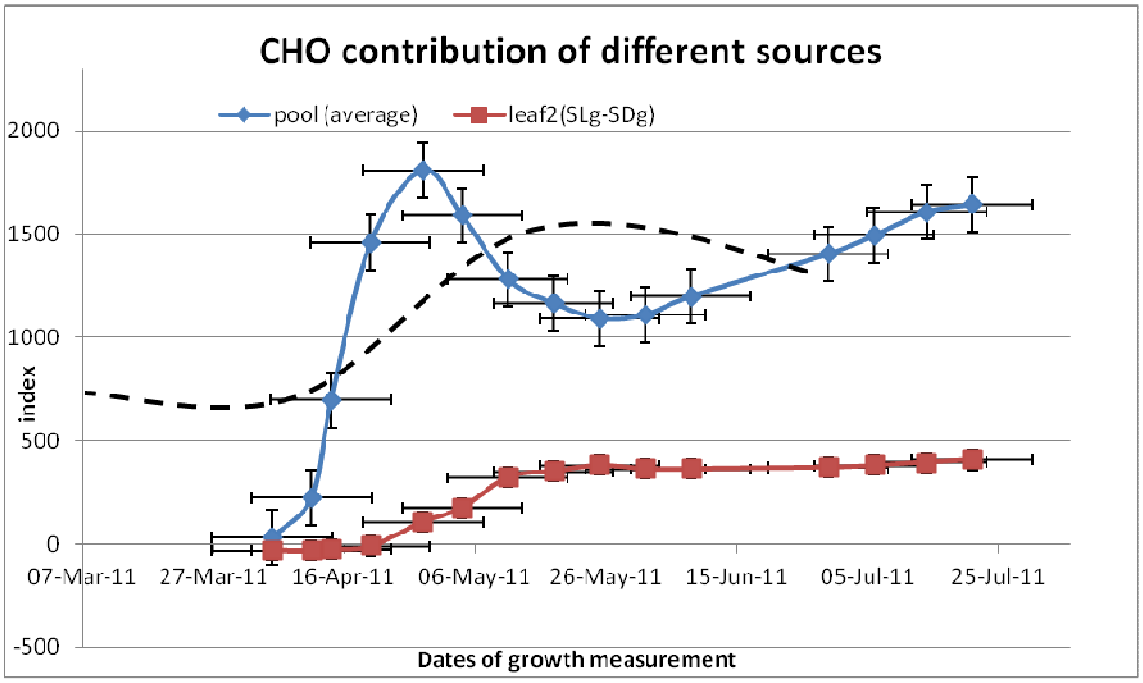


Figure 9. Reserved CHO (pool) and current photosynthate (leaf) contributions in vegetative growth of macadamia (growth length of a single flush; FL). Dashed curve shows carbohydrate accumulation in macadamia tree (trunk wood tissues) in absence of reproductive sink, this curve is extracted from the graph produced by Stephenson et al. (1989a) for the same period of time of current study.

Pool 1 and pool 2 show more similarity than the contributions from leaf 1 and leaf 2. Source contribution from leaf 1 shows a consistent pattern rather than leaf 2. This is due to the equations we used for their calculations. Leaf 1 is calculated from the difference between girdled branches while leaf 2 is calculated from the difference between non-girdled branches. Non-girdled branches are affected by pool while girdled branches are not. Girdled branches are completely separated from pool in girdling point. On the other hand we cannot consider the leaf contribution in shoot growth from the beginning of experiment (day zero) until 4 May, 49 days after treatments were applied. At this time leaves on SL flushes were fully expanded and mature enough to be productive (Figure 9). Therefore, for our concluded graph of

CHO contributions of two sources responsible for vegetative growth of macadamia in March experiment, we have considered the average of pool 1 and pool 2 for CHO reserve, and only leaf 2 for current photosynthate (Figure 10).



Dashed curve in figures 9 and 10 is released from graph 2 for the non-structural carbohydrate storage in macadamia tree between March and July, when our study was occurred. It shows consistency with our graph, which shows depletion of source of energy (Figure 11).

In the first 23 days of vegetative growth in macadamia the main source of energy (carbohydrate) is from reserves (pool) (94.5%), then it is dropped down when leaves (current photosynthate) activity is increased until day 50 (74.2%). From day 50 until the end of growth period, reserves contribution to the vegetative growth in macadamia is increased (89.71% at the end of experiment). Leaves or current photosynthate showed a different and constant effect on the growth of the new shoot when they were matured and fully productive after day 36.

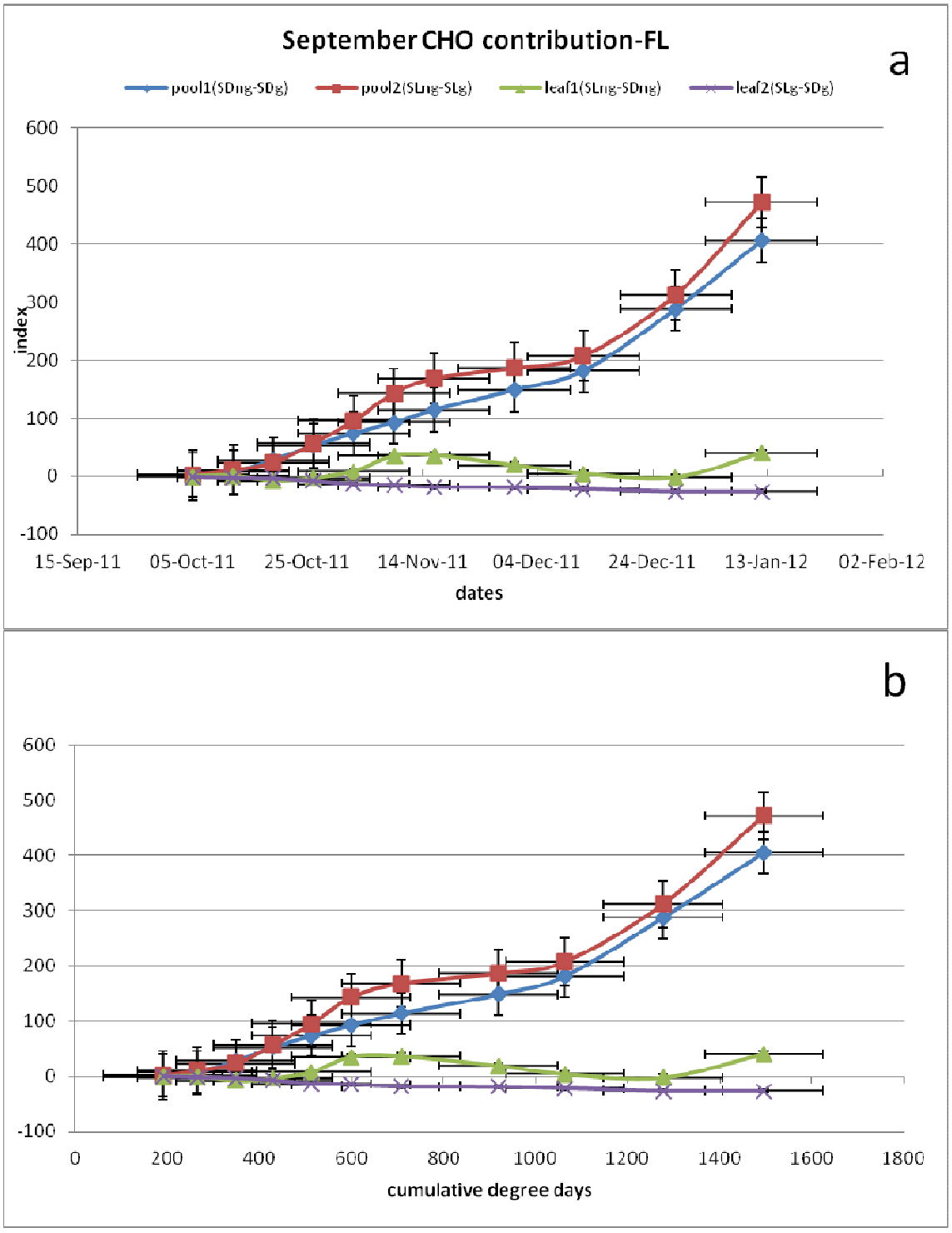
Table 3. Contribution of each CHO source for different growth traits

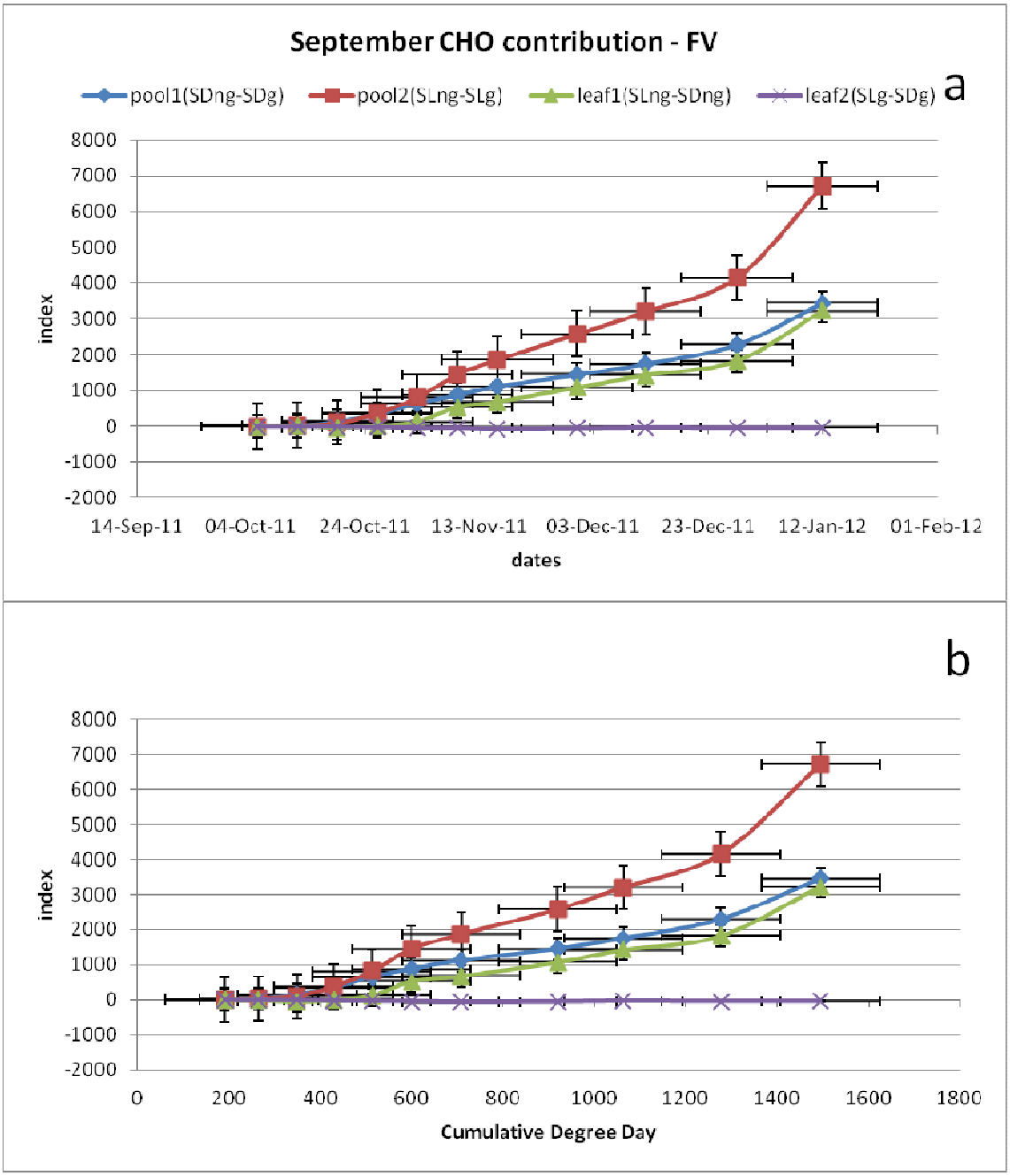
	pool%	leaf%	pool1%	leaf2%
NGfw	73.07567	26.92433	85.93195	14.06805
NGdw	56.34074	43.65926	62.2534	37.7466
FL - 18May2011	74.75946	25.24054	76.18725	23.81275
FL -1Jun2011	73.15303	26.84697	74.61431	25.38569
FL - 8Jun2011	76.25556	23.74444	76.64516	23.35484
FL-21Jul11	89.7107	10.2893	82.03435	17.96565
FV-21Jul11	74.32897	25.67103	80.5437	19.4563
FL-sum	82.53504	17.46496	83.35241	16.64759
FV-sum	71.60399	28.39601	79.14322	20.85678
Total average	74.64035	25.35965	77.85619	22.14381

We can generalise the level of contributions of two sources of energy in macadamia for vegetative growth. According to the figure 8, both pool 1 and pool 2, and leaf 1 and leaf 2 crossovers in day 64, so we can consider this point for their contributions (76.26% for reserve and 23.74% for current photosynthate). On the other hand "new growth fresh weight" as we discussed before, is the variable with the highest R-square that we can rely for our assumption of the sources' contributions in vegetative growth of macadamia (73.08% for reserves and 26.92% for current photosynthate) (table 3). So we can say generally that between 73% and 76% of the energy needed for vegetative growth is from reserved CHO and 24% to 27% from current photosynthate. However there is a constant effect from current photosynthate on the vegetative growth of macadamia when the leaves are matured, whereas the effect of reserved CHO fluctuates during the season.

Experiment 2. September

We used the same definitions, equations and calculations for CHO sources contribution like the first experiment in March.


September CHO contribution-FL
a
pool1(SDng-SDg)
pool2(SLng-SLg)
leaf1(SLng-SDng)
leaf2(SLg-SDg)
600
500
400
300
200
100
0
-100
index
15-Sep-11
05-Oct-11
25-Oct-11
14-Nov-11
04-Dec-11
24-Dec-11
13-Jan-12
02-Feb-12
dates
b
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000
1200
1400
1600
1800
cumulative degree days



Conclusion

The lower photosynthesis rate in macadamia than temperate fruit and nut trees (Flore and Lakso, 1989) is due to a slow and less efficient symplastic loading of photosynthate mechanism through the plasmodesmata can describe the lower contribution of current photosynthate source in vegetative growth of macadamia. Loading of photosynthate from leaves to petiole

On the other hand, macadamia's long-lived leaves have a low light compensation point with a degree of shade tolerance (Demmig-Adams et al., 1997), which can

explain its maximal canopy photosynthesis in lower light intensity (in 30–40% of total light) (Flore and Lakso, 1989).

Chapter 3: Architectural study of young macadamia trees

Introduction

Plant architecture is a term applied to the organization of plant components in space which can change with time. At a given time, plant architecture can be defined by topological and geometric information. Topology deals with the physical connections between plant components, while geometry includes the shape, size, orientation and spatial location of the components. At a given scale, topology expresses succession and branching relationships between plant components. (Godin et al., 1999).

There is a lack of uniformity in macadamia orchards in Australia due to use of seedling rootstocks rather than clonal rootstocks. Genetic variation in seedling rootstocks results in uneven canopy development with more vigorous growth, which makes orchard management more difficult. Using clonal rootstocks without genetic variation results a uniform orchard canopy and the possibility of using dwarfing rootstocks. Little is known about macadamia architecture and carbohydrate allocation. Knowledge of the tree canopy architecture will help us to manipulate it efficiently by horticultural practices to a desirable structure, for example an open canopy to enhance light penetration and smaller trees for high density orchards.

The physiology of subtropical evergreen fruit trees is not as well understood as that of deciduous species which have been extensively researched. Many subtropical evergreen fruit trees (e.g. avocado, lychee and mango) grow by recurrent flushes (Whiley et al., 1989; Olesen et al., 2002; Olesen, 2005); can flower profusely but set relatively few fruit (Blumenfeld and Gazzit, 1974; McConchie and Batten 1991; Anila and Radha, 2003); and are large trees with a high leaf area to canopy surface area ratio (Possingham, 1986; Menzel et al., 2000). There have been relatively few studies on the response of subtropical evergreen fruit trees to pruning, in contrast to the abundance of studies for deciduous species (Mika, 1986). Macadamia (Macadamia integrifolia, M. integrifolia × tetraphylla) is fairly typical of subtropical evergreen fruit trees. It flushes throughout the year but with peaks in spring and late summer (Stephenson et al., 1986). Flower racemes are initiated in leaf axils and anthesis occurs in early spring (Moncur et al., 1985). Mature trees may produce .10 000 racemes, each consisting of 100–300 flowers but, typically, only around 0.3% of flowers develop into mature fruit (Urata, 1954; Ito, 1980). Canopy leaf density is high, at .400 leaves per cubic metre in the upper canopy (McFadyen et al., 2010), and trees potentially attain a height of 18 m and a width of 15 m (Cull, 1983) unless managed by pruning (Costes and Guedon, 2002).

Fruit tree architecture

Plants are modular organisms that develop by the repetition of elementary botanical entities (growth units). A stem can be considered as a succession of entities composed of a node, its associated leaf (or leaves) and axillary bud(s) plus the subtending internode, representing the basic structural unit of the plant body commonly called the

metamer. During growth, the superposition and repetition of this elementary entity builds up the leafy axis (Barthelemy and Caraglio, 2007).

Applications developed in horticulture have focused mainly on two within-tree scales: (1) organ arrangement, including both vegetative and floral organs, and their relative equilibrium, and (2) fruiting branches and whole tree behaviour. These two scales constitute a basic framework that is then used to interpret the effect of agronomical practices at the tree and orchard scales (Gross and Weston, 1992).

Preformation and neoformation

The identification of architectural models in a given species first requires identifying the categories of shoots that are observed within the tree structure on the basis of morphological criteria. As with many other species, fruit trees exhibit a polymorphic development of axes. Usually, two main shoot categories, i.e., short and long shoots, are distinguished by a simple visual observation.

In the case of rhythmic growth, all the metamers and organs of the future elongated shoot may be present at an embryonic stage in a bud before the elongation of the shoot deriving from it; in this case the shoot is referred to as 'preformed' and its constitutive organs as 'preformed organs' or 'preformation'. In preformed growth, leaves are formed in a bud during one season, but the bud does not expand until the following season (Barthelemy and Caraglio, 2007). The number of leaves in a preformed bud depends on things such as the species, the position of the bud in the canopy, the size of the shoot that the bud is formed on, environmental conditions and other factors (Steiger, 2003). In contrast, neoformed growth has leaf development and expansion occurring in the same season, without the formation of a bud. The duration of preformed organs at an embryonic stage in a bud may vary from several days or weeks to several years. In other cases, more organs than those included at an embryonic stage in the bud are elongated: these supplementary, non-preformed elements are referred to as 'neoformed organs'. As a consequence stems or shoots may comprise only preformed metamers or, more rarely, may be entirely neoformed. In many cases, a preformed part can be followed by a neoformed part and thus give rise to a mixed shoot (Barthelemy and Caraglio, 2007).

Spann et al. (2003) observed that on mature pistachio trees, neoformed growth is generally found only in the top of the canopy, suggesting that light may be an important factor in the development of neoformed growth. However, most annual extension growth in mature tree crowns was preformed, contrasting with the relatively high rate of neoformation found in young tree crowns. Large amounts of neoformed growth in young trees may allow the trees to become established quickly and secure resources, whereas predominantly preformed growth in mature trees may allow for continued crown expansion without outgrowing available resources (Steiger, 2003).

Reiteration and proleptic and sylleptic shoots

Oldeman (1974) defined 'reiteration' as a morphogenetic process through which the organism replicates its own elementary architecture (architectural unit). Although some plants conform to their architectural unit during their whole life span, most

plants repeat their architectural unit during their development, late in ontogeny, or under particular conditions. Reiteration encompasses several aspects (sprouts, root-suckers, etc.). Reiteration process may involve the expression of the total architectural unit from axis 1 to the most differentiated axis category ('complete' or 'total' reiteration), or the expression of part of the developmental sequence duplicating only part of the species' architectural unit ('partial reiteration') (Barthelemy and Caraglio, 2007, Oldeman, 1974).

Reiterated complexes may originate from dormant meristems and reiteration in this case is called 'proleptic' or 'delayed'. By contrast, reiteration may result from a shift in the functioning of the apical meristem of a growing shoot that will finally produce a 'less differentiated structure', i.e. a branch apex that after some time of functioning gives rise to a 'supernumerary trunk'. In this case, the reiteration is described as 'sylleptic' (or better 'immediate') or 'reiteration by dedifferentiation'. Either of these two types of reiterations may be qualified as total or partial (Barthelemy and Caraglio, 2007).

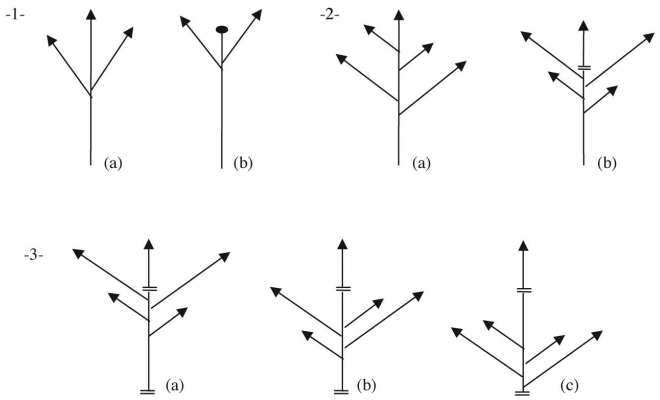


Figure.4. Axillary shoot positions and associated terminology regarding branching: 1 (a) monopodial, (b) sympodial; 2 (a) sylleptic, (b) proleptic; 3 (a) acrotonic, (b) mesotonic, (c) basitonic (Source: Caraglio and Barthélémy 1997).

Distinction between proleptic and sylleptic shoots

Sylleptic shoots mostly appear during the early developmental years and are known as "feathers" in the nursery, and are naturally located in a median position in fruit trees.

Because of the immediate extension of lateral organs, sylleptic shoots generally lack proximal cataphylls and present a relatively long most proximal internode termed a hypopodium. In contrast, proleptic shoots (delayed branches) present very short internodes and one or several cataphylls in their proximal portion, close to the point of insertion. In delayed branching it has been shown that the duration of the delay may be of several weeks to a year and even several years. In the case of proleptic or delayed shoots, axillary buds after having passed the dormant period develop along

the previously expanded shoot, and the axillary shoot development strongly depends on the bud position along the bearer shoot (Figure. 4) (Gross and Weston, 1992).

In this research architecture such as proleptic or sylleptic branching, and preformation and neoformation and will compare the effects of different rootstocks on morphological characters of young trees.

Figure . Sylleptic shoots in *M. jansenii*

Objectives

The aim of this research was to study the architectural development during vegetative growth in young macadamia trees. Allometric relationships between shoot length, number of leaves, and leaf area of young macadamia trees were studied to reconstruct 3D architecture from sampling of 3D coordinates. The architectural development was measured over the time by a sonic digitizer with the relative 3D picture of plants at every stage in one experiment and the growth of different scions was measured periodically in another.

146 young macadamia plants of different genotypes and ecotypes were digitized for twice and then 8 genotypes were set in a Latin Square design for the main experiment, which are being digitized 3 to 4 times. For the second experiment A4 scion was grafted on these 4 genotypes and then the growth of scions were measured over the time to explore the effects of those genotypes as rootstocks.

Hypotheses

Different macadamia genotypes have various growth habits. Vegetative vigour characteristics including length and girth of new growth, number of nodes and length of internodes, leaf area, leaf angle, branching angle, new flushes growth during time (the growth rate).

Different rootstocks affect the architecture of tree with the mentioned characteristics above and carbohydrate allocation on the scion in young macadamia trees.

In young macadamia trees, different genotypes have different architectural development patterns and different rootstocks affect the architectural development of the same scion.

Experiment 3 Production of young macadamia plants (cutting and grafting)

Introduction

Commercial cultivars of macadamia in Hawaii and Australia are based on *M. integrifolia* and seedlings of *M. tetraphylla* are predominantly used for rootstocks. Macadamia trees for orchards in Australia are generally propagated by grafting onto seedling rootstocks and using clonal rootstocks or own rooted cuttings are less common (Stephenson 1990a; Nagao and Hirae 1992; Trochoulias 1992; Bell 1996; Hardner and McConchie 2006). Genetic variation of seedlings growth rate in nursery has been reported by Hardner (2004) and Hardner and McConchie (2006). Uniform orchards would be achieved by using clonal propagation of rootstock with control of genetic variation (Howard 1987) with advantages of effective canopy management.

While *M. tetraphylla* seedlings grow faster and are more uniform (Phiri 1985; Hamilton 1988; Nagao and Hirae 1992; Trochoulias 1992) enabling grafting to occur six months earlier than expected with *M. integrifolia* rootstocks (Hamilton 1988), using *M. tetraphylla* seedling rootstocks results incompatibilities with scions (*M. integrifolia)* and even produces lower nuts quality (Nagao and Hirae 1992). Recently

Australian macadamia orchards (since 1990's) are established from open pollinated seeds from *M. integrifolia*, cultivar H2.

Genetic variation resulting from seedling rootstocks may have negative impact on an efficient orchard management. Rootstocks can be produced vegetatively by clonal propagation from cutting or air-layering to avoid it. Although, rootstocks produced by cuttings may not contain well established roots and are more susceptible to wind, the uniformity of vegetatively propagated rootstocks could provide more uniform orchards for training and maintenance (Stephenson et al., 2003).

There is a lack of information about rootstocks and their clonal propagation in macadamia. Previously macadamia cuttings strike rate is studied by Hardner and McConchie (2004). They found best strike result for Beaumont with 80% and lowest for 849 with 23%. A268 and Beaumont produced most vigorous plants. They also found a moderate correlation between the nursery vigour and the strike rate of cultivars. They suggested Beaumont and A268 as the best performing clonal rootstocks with the highest results for strike rate, growth and rootstock budding success (Hardner et al., 2004). The aim of this study was to compare strike rate and quality of cuttings of different macadamia genotypes.

Materials and Methods

Cuttings

269 Cuttings of eighteen genotypes were collected in November 5, 2009 from vigorously growing branches of *M. integrifolia*, *M.tetraphylla*, *M. jansenii*, *M. ternifolya*, A4, Beaumont, *M. integrifolia* × *M. ternifolia* (660) and a wild hybrid from NSW Centre for Tropical Horticulture (NSW Department of Primary Industry), Alstonville. Cuttings were taken from the apical tip of branches which were vigorous, actively growing, healthy and straight and had at least 0.5m in length of current season growth semi-hard wood, with 2-3 nodes, and 3 to 6mm in diameter. Cuttings were immersed in rooting gel immediately and planted into individual pots filled with rooting mixture (2:1 sand/coir) and sprayed with water to keep them wet and cool. Then they were transferred into the mist system in CSIRO, Queensland Bioscience Precinct the next day. The Mist system was sat up to 10 second mist every 8 minutes for daytime and 6 second mist every hour for night time, and the temperature 25±1°C. They started producing roots after 2 months.

Rooted cuttings were transferred to the UQ glasshouse on 27 April 2010. They were re-potted to 8L pots with pot mixture. Glasshouse area was disinfected with using bleach the day before. Plants were misted twice a day up to 1 minute for not being suffered from dry environment.

For each cutting, numbers roots, root's length, extent of callus formed, number of proteoid roots and number of new growth (terminal or axillary flushes) on the cutting stem were recorded. Total leaf area of each plant was measured in 26 July 2010 (after 3 months growth) by photographs taken from above and then analysed with Photoshop software.

Strike rate and rooting traits of cuttings were studied.

A4 is introduced with the best nut quality (Bell et al.1988 and, Stephenson and Gallagher 2000) and Beaumont is a popular rootstock in South Africa due to its high strike success and vigorous nursery growth. *M. jansenii* is a multi-stemmed tree smaller than other macadamia species, reaching a height of 6–9m (Gross and Weston, 1992). *M. jansenii* is listed as endangered and a total population of 33 plants was recorded in 1992. *M. ternifolia* is also a small multi-stemmed tree that grows to 6–8 m. (Stanley and Ross, 1986). It is listed as vulnerable under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).

Cuttings started to producing root after 2 months and then all cuttings pulled out from rooting medium on 27 April 2010 and rooting characteristics recorded. When the cuttings were removed from the small pots, roots were washed with tap water to disperse soil residues. For each cutting, roots numbers, roots length, extent of callus formed, number of proteoid roots and number of new growth (terminal or axillary flushes) on the cutting stem were recorded.

Figure.9. Macadamia cuttings under mist system in CSIRO, Queensland Bioscience Precinct

Results

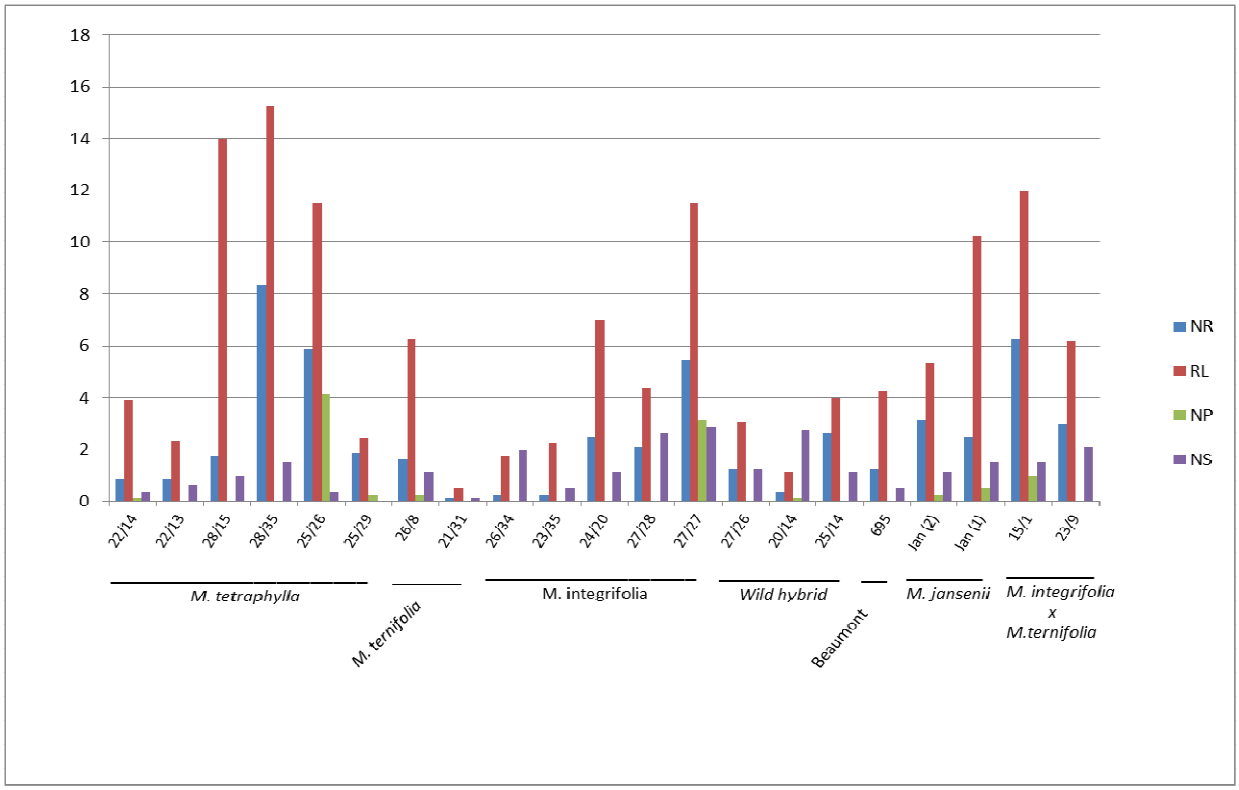
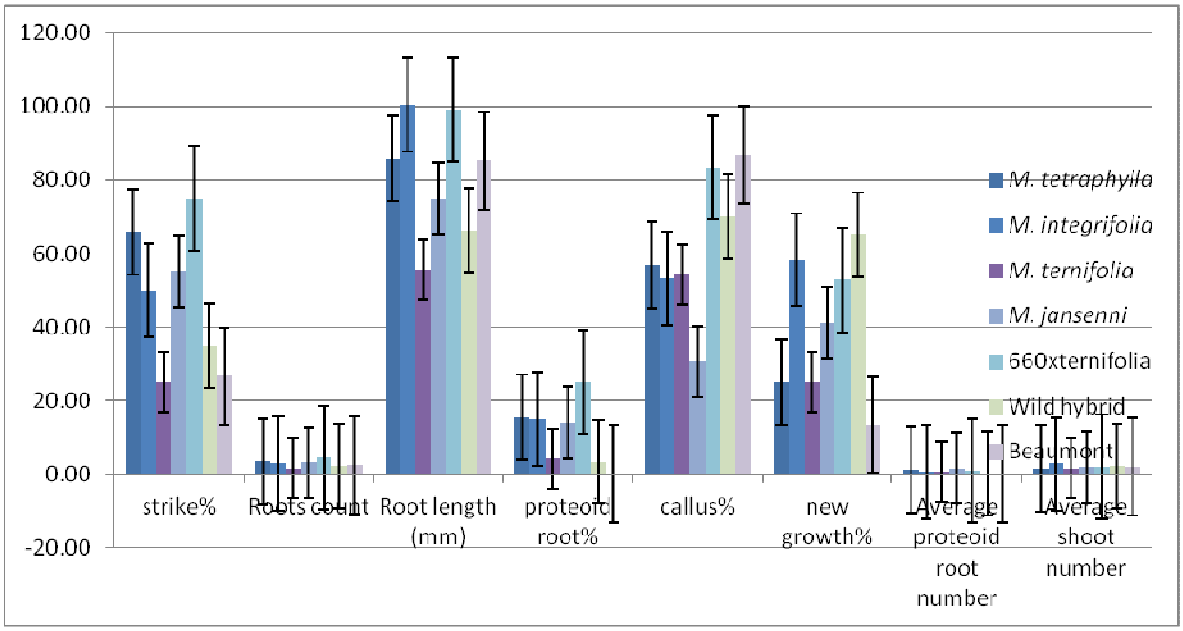


Figure 10. Number of roots (NR), root length (RL), number of proteoid roots (NP) and number of new growing shoots (NS) of cuttings of macadamia genotypes recorded when they were re-potted to 8L pots.



Grafting

A4 was grafted on Beaumont, *M. ternifolia,* Hybrid (*M. ternifolia* X *M. integrifolia*) and on its own.

Micro-grafting was conducted in 23 May 2011 on 20 rooted cuttings of each genotype in Alstonville and kept under mist system. They were transferred to UQ in 31 August 2011 and 6 of each arranged in a 4X6 RCBD design and have being photographed every week (ongoing). Their growth will be analysed with Photoshop at the end of this experiment. Length, girth and Number of internodes of A4 scions on rootstocks will be measured over the time in response to the effect of rootstocks (Beaumont, *M. jansenii*, *M. ternifolia* and the hybrid). Other measurements such as leaf area and photosynthesis will be measured if possible.

Rootstocks

A second batch of cuttings for the second part of the Architectural experiment (micrografting) was collected on 14/05/2010 in small pots from NSW CTH, 5 trays of Beaumont, *M. jansenii*, *M. ternifolia* and a hybrid (*M. integrifolia X M. ternifolia*) as rootstocks. Each tray contained 54 cuttings. Media for pots comprised of ½ peat moss, ¼ coir and ¼ sand.

Beaumont a popular rootstock in South Africa, *M. ternifolia* the smallest macadamia tree as dwarfing rootstock, and a hybrid, *M. integrifolia* (660) x *M. ternifolia* were used as rootstocks for the second study (Effects of rootstocks on young macadamia tree architecture and carbohydrate allocation). Beaumont has been used as a clonal rootstock in South Africa due to its high cutting strike success (80%), vigorous nursery growth, early flowering (precocious), and medium kernel recovery (35.9%).

Scion

A4 was used as the scion for all rootstocks. A4 is a hybrid between *M.integrifolia* x *M. tetraphylla* with characteristics of small canopy width (4.2 m), without abnormal vertical growth, large nut (7.1g), average fruit set per raceme 1.6, high kernel recovery (41.9%) and nut in shell production of 12kg NIS/year. A4 is a superior cultivar due to early flowering and precocity, higher kernel recovery (41.9%) and higher kernel quality (97% first grade kernel and 3.2g kernel mass) (Hardner et al., 2009)

Grafting method

Young grafted macadamia trees for this experiment were produced by micrografting (a procedure that introduced by Cameron McConchie at CSIRO, Queensland Bioscience Precinct) on rootstocks in early stage of growth. Rootstocks are provided from cuttings of the mentioned species and varieties and scions are only taken from the cultivar A4.

If the grafting materials are free from contamination the success rate of grafting should be 90%. This drops to 40% graft success rate when scion material is collected from the orchard. The drop in success has been attributed to mould infection which

cannot be controlled using common fungicides. Rootstocks with diameter between 2 and 3 mm at the point of grafting have proven to be most successful. Also the texture of the wood is important; it should not be too rubbery or too firm. When choosing scion wood the growth should be new but not still in fresh stage and it should have started to harden. The size and firmness of scion wood should match as closely as possible the stem of rootstock. Once rootstocks are selected, their stem should be cleaned with sterile water and then be cut with secateurs above the first or second set of leaves, leaving as much stem as possible. Then a 45º angle bevel cut in the stem of rootstock should be created by using a scalpel blade. A grafting clip which is pointed up should be slid over the cut end of the rootstock stem in the next step. To prepare scion material, all but small amount of leaf area should be cut off. As with the rootstock, the longer the amount of stem, the better the graft clip will fit. When scion is cut, it should have one or two leaves. Similar to rootstock preparation a 45 º bevel cut in stem of scion should be created. When bevel surface of scion stem matches the size and shape of rootstock stem, it should be slid into the graft clip and making sure that the bevel's edges are connected. This completes the grafting process.

After grafting

Grafts were promptly moved into a healing chamber with high relative humidity (RH: 85-95%), low light (shade/dark) and temperature between 22-25ºC for 2-5 days (dark and high RH condition). Once the plants returned to normal turgor pressure for a couple of days they were exposed to indirect light and slowly decreasing humidity by increasing the periods of misting intervals to be exposed to normal RH and temperature and finally we moved grafts to a sunlit glasshouse. High humidity (85-95%) was achieved by covering plants with sealed plastic bag and excess water in the bottom. Grafted plants were left in high humidity until the scions produced new growth. Once the plant has produced a number of leaves the grafting clip was removed. The grafting process takes 3-4 weeks but it takes a further 6 weeks to produce enough growth for digitizing.

Plant growth media

Young micrografted plants were grown in 8L pots filled with pot mix available at UQ glasshouses. Plants were grown in a naturally lit glasshouse with 60% full sunlight and maximum temperature of 28ºC.

Preliminary Architectural measurements

Thirty pot plants of *M. jansenii*, Beaumont and A4 (Figure. 7), which were created from cuttings by Trever Olesen at NSW Centre for Tropical Horticulture (NSW Department of Primary Industry), Alstonville on November 5, 2009 (almost three month old), were transferred to a glasshouse at CSIRO (Long Pocket) and subsequently moved to UQ glasshouse no.2 on 27/01/2010 and re-potted in 8L pots with Green Finger potting media (address: 71 Lawrence Drive, Nerang Queensland). The age of plants at the time of measurements was almost 6 months.

Figure.7. Three batch of plants grown from cuttings of A4, Beaumont and *M. jansenii*.

CV: A4 Rep: 1 Date 11.02		
node	length(cm)	diameter (mm)
Mn1	5	6.7
Mn2	5.5	5.4
Mn3	2.8	5.2
S1n1	2.5	9.5
S1n2	10	1.9
S1n3	4	1.6
S2n1	10	1.8
S2n2	5.5	1.45
S3n	3	1.4
S4n1	3	5.4
S4n2	3	4.1
S4n3	2	4.4
S4n4	1.5	4.3
S4n5	3	4.1
S4n6	4	3.6
S4n7	4	3.7
S4n8	5	3.2
S4n9	2.5	2.8
S4n10	1.8	2.5
S4n11	2.5	2
S4n12	1.5	1.6

Architectural parameters which were measured comprised: branch distance from the ground level, length, number of nodes, diameter of branch at base, middle and tip of the main stem and lateral branches, number of lateral shoots on the parent branch and branching angle. I tried to do the measurements by hand (using a ruler for height, calliper for diameter and protractor for angles) (Table 3), photographic analysis by Photoshop software and finally using digitizer.

In conclusion, using a digitizer was more convenient but the only problem is girth or diameter measurement that I have to use a calliper later or at the same time.

Table 3. Architectural measurements of two macadamia cultivars A4 by hand to compare with other architectural measurement procedures

	Variety: A4 Rep 1			Date: 15/02/2010		
	distance from the ground (cm)	length (cm)	number of nodes	number of lateral shoot	angle	other
M*	13	13	3	3		
S1**	5	23	3	0	30	
S2	5	15.5	2	0	30	
S3	10.5	3	1	0	10	
S4	13.5	36	12	0	35	new flush on nodes 9-12
	Variety: A4 Rep 2			**Date: 15/02/2010**		
M	18	16	4			
S1	18	34	9	0	40	new flush on nodes 7-9

*M represents the main stem

**S represents the lateral branch and the number represents the order of branch

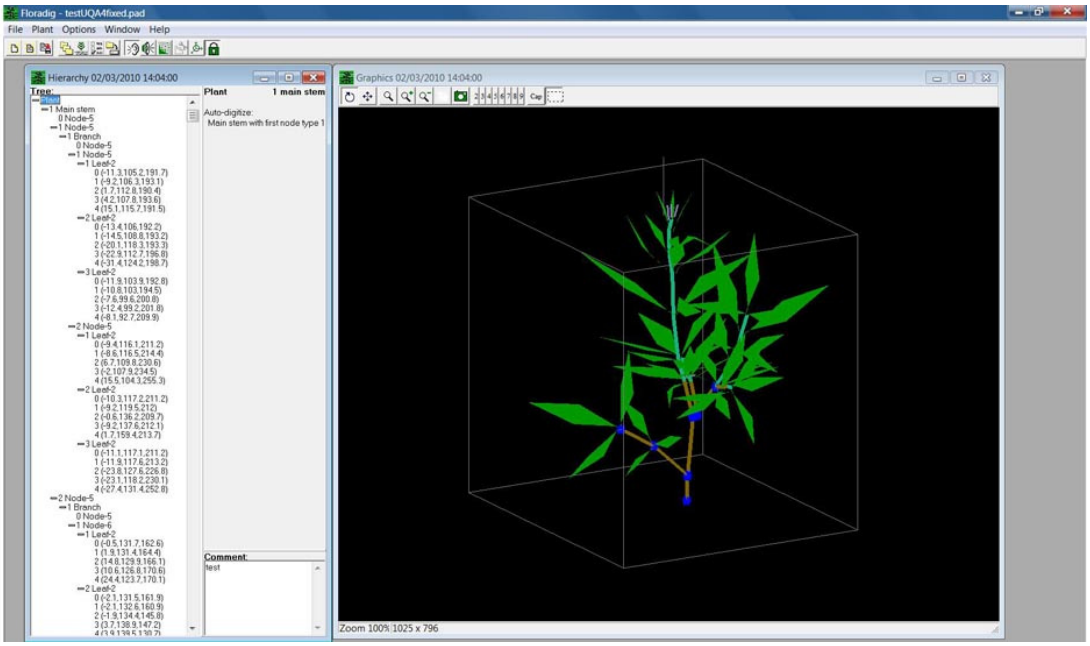


Figure.8. A figure of macadamia A4 from Floradig software created by data derived from a digitizer with magnetic field and triggers to the nodes and leaves points.

Experiment 4. Architectural study 1: Non-grafted young macadamia trees

All of 146 survived plants from out of 269 cuttings of 18 genotypes were digitized for once and then eight of them were selected and were arranged in a Latin Square design and digitized for 3 times.

In this experiment the architectural characteristics of macadamia comprised eight genotypes of four species (*M.tetraphylla, M. integrifolia, M. jansenii and M. ternifolia*), an unknown wild hybrid, 660(Keaau)×*M. ternifolia*, Beaumont and A4 were studied in a 8×8 Latin Square design (LS). The reason of applying Latin square design was that greenhouse trials in which the experimental pots are arranged in a straight line perpendicular to the glass walls, such that the differences among rows of pots and distance from the glass wall are expected to be the major sources of variability.

Material and methods

Two macadamia hybrids of A4 and Beaumont with *M. jansenii* species (one year-old) were set up in a randomized complete block (RCBD) design on 3 benches in UQ glasshouse (8 block) and were digitized for three times from 10/08/2010 to 24/08/2010. The data then transferred to spreadsheets for statistical analysis. Internode length, pitch, girth and number of leaves for every node and leaf length and width released.

Architectural analysis of different genotypes

A sonic digitizer was used to measure the following architectural traits of young macadamia trees over the time (Figures 2 and 3)::

1. The number of nodes and
2. Length of internodes of all terminal and lateral shoots and their node position along the parent shoot.
3. Stem diameter (trunk, branches and shank girth diameters of each internode during growing period)
4. leaf area
5. New shoots (flushes) growth rate by measuring their length.

Figure 2. The set up of the digitizer: hardware, software, triangle with microphones on each corner and the pointer with trigger. When the pointer is triggered the spatial position of its tip is recorded.

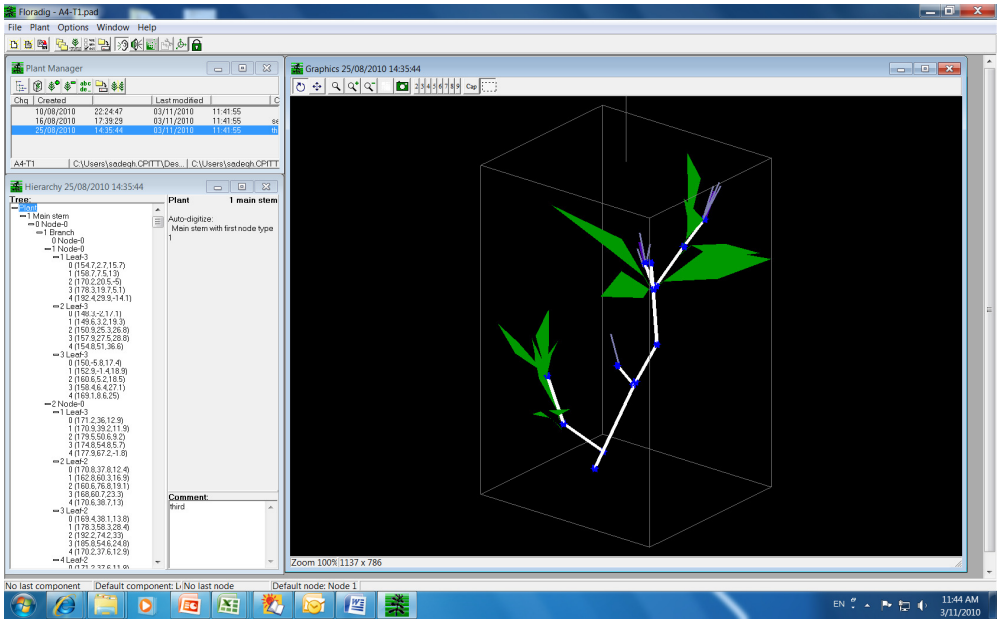


Figure 3. A 3D picture of macadamia A4 plant as digitized with a sonic digitizer + Floradig. To measure architectural attributes of a plant, digitizing is more reliable, easy to use and provides huge amount of data from number of nodes to hierarchy of the branches collected in a spreadsheet for analysis.

Results

There were only significant difference (<0.01) for pitch and number of leaves (Figure 4), however *M. jansenii* showed different number of nodes and internode length from proximal to distal with A4 and Beaumont on the main stem (Figure 5). Unlike A4, Beaumont and *M. jansenii* showed descending angle for first order branches from proximal to distal (Figure 6).

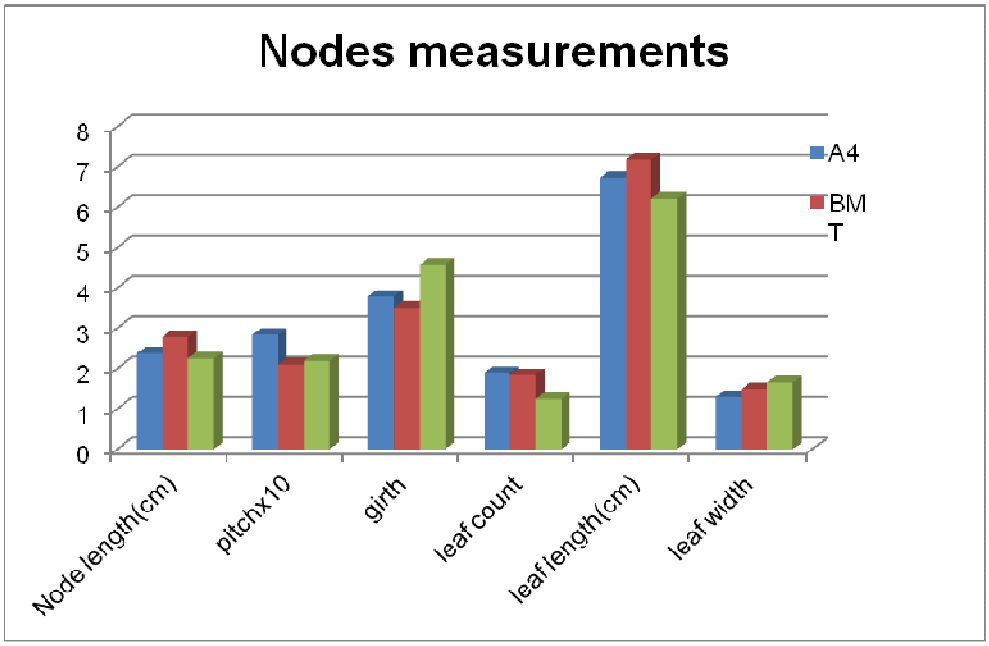


Figure 4. The averages of internode node length, pitch, girth and number of leaves and leaf length and wide without considering the node level

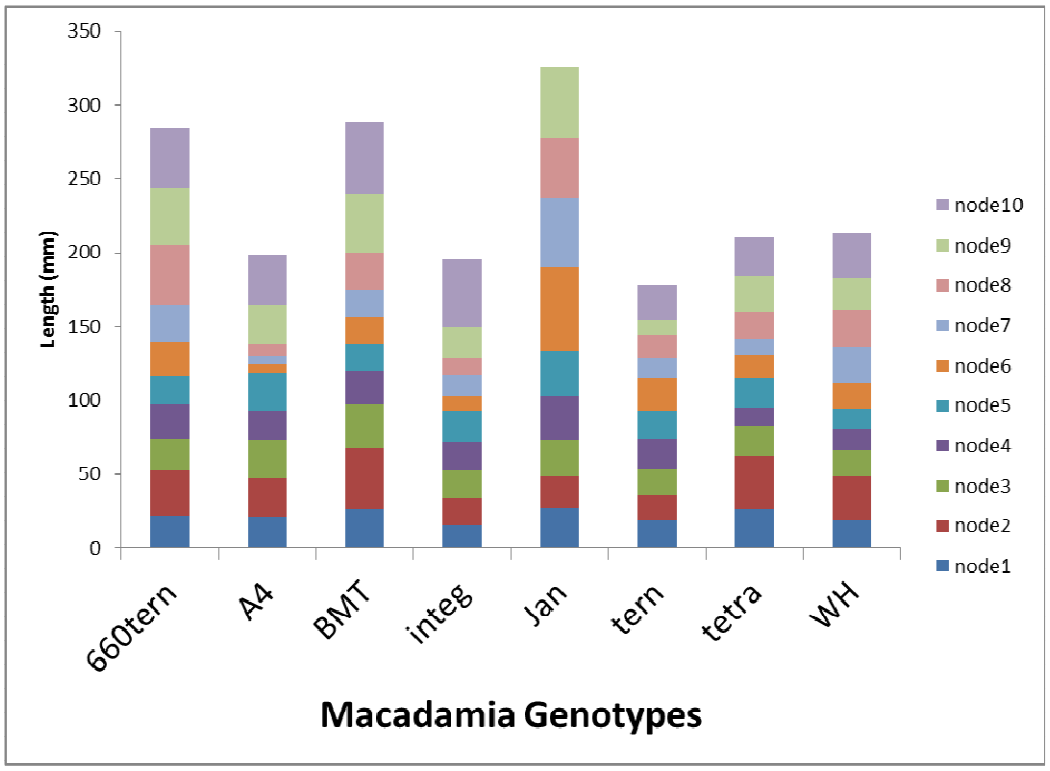


Figure 5. Node distribution and internode length *in M. jansenii*, A4 and Beaumont

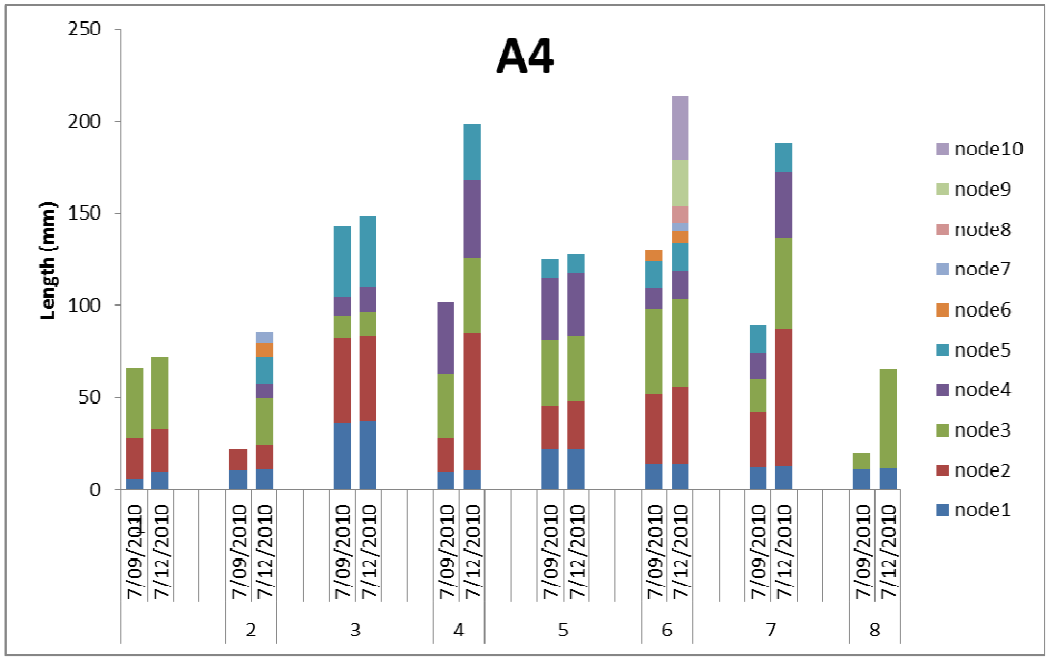


Figure 6. Digitizing result of 64 young macadamia plants (order 0/ main stem) in a Latin square design. 8 replications (lower numbers) of digitized plants for 2 dates (upper numbers)

Conclusion

Smaller leaf and wider branching angle of *M. jansenii* are dwarfing characteristics, which suggest further exploration of this species in future work. With planned work in this study we hope to discover more dwarfing effects of different genotypes.

Experiment 5. Architectural study 2: Grafted young macadamia trees

Introduction

Rootstocks are widely used by horticulturists to control the size and vigour of perennial fruit trees (Berninger et al., 2000) since dwarfing rootstocks cause trees to be more compact, manageable and to bear fruit at a younger age. Generally dwarfing rootstocks reduce the amount of scion dry weight and vegetative growth. They also reduce total leaf area and change leaf orientation. These factors may contribute to the reduction in tree size (Atkinson and Else, 2001) and change the architectural growth pattern of the tree. Tree architecture is a result of equilibrium between endogenous growth processes and exogenous constraints exerted by the environment (Phenotype = Genotype + Environment). Architectural analysis can be used to identify and separate the effects by means of observation and experimentation. Precise morphological observations and simulation programmes have greatly increased our understanding of plant structure and development and have led to the establishment of a conceptual and methodological framework for plant form analysis and understanding (Barthelemy and Caraglio, 2007).

There are numerous studies about the effects of rootstock on the growth of fruit trees. A revolution in the apple fruit industry happened when East Malling dwarfing rootstocks were introduced. Dwarfing rootstocks limit excessive growth, enhance the cropping efficiency (yield per tree size) and reduce the time required for a tree to come into cropping (precocity) (Atkinson and Else, 2001).

There are no dwarfing rootstocks for most subtropical evergreen fruit tree species and many of these crops are pruned with mechanical hedging equipment to control tree size for efficient orchard management (Possingham, 1986).

Rootstocks have been used to control fruit tree size in many species. Tree vigour is mainly controlled by dwarfing rootstocks which are widely employed in intensive orchards to restrict tree volume and promote earlier flowering. Apple (Costes and Lauri, 1995) and peach cultivars (Weibel et al., 2003) when grafted on dwarfing rootstocks, have reduced growing period compare to non-dwarfing rootstocks. Also it has been demonstrated that some rootstocks reduce the internode length of fruiting branches in apple trees (Fuks and Godin, 2004, Godin, 2004).

Costes and Garcia-Villanueva (2007) studied the effect of rootstock on branching pattern in apple (Figure. 5). They showed that rootstock affected the percentage of axillary budbreak on extension growth units. Number of nodes developed during the previous year affected the number of axillary annual shoots per branch (Costes et al., 2001). They concluded that the effect of rootstock on aerial growth is cumulative and superimposed year after year.

Clearwater et al. (2003) described effects of eight clonal rootstocks of kiwifruit on the patterns of shoot development and the production of different types of shoots and scion leaf area index (LAI). They found the apex of long shoots had continuous growth and produced more nodes throughout the growing season whereas short and medium shoots produced a restricted number of preformed leaves before the shoot apex ceased growth and aborted, resulting in a 'terminated' shoot. Slower-growing shoots were more likely to terminate. Rootstock influenced the process of shoot termination independently of its effect on final leaf size. Spann et al. (2003) found that growth differences among trees on different rootstocks were associated with greater stimulation of neoformed growth in trees on the more vigorous rootstocks.

Rootstocks may have major effects on scion's growth. Generally dwarfing rootstocks reduce the amount of scion dry weight by a reduction in growth rate of shoots and of the time period over which they grow. Compared to a vigorous rootstock that produces more vegetative growth, a dwarfing rootstock directs a greater proportion of dry weight into fruit production rather than into vegetative growth. Scions on dwarfing rootstocks are expected to have less leaf area than those on vigorous rootstocks. The effects of a reduction in leaf area means scion shoots on dwarfing rootstocks may produce less assimilate relative to scions on vigorous rootstocks. Differences in the way leaves are orientated on the tree may also influence their ability to intercept solar radiation and convert this into fruit production via photosynthesis. Factors such as these may contribute to the observed reduction in tree size associated with dwarfing rootstocks, but they do not necessarily explain differences in the way dry weight is allocated between tree growth and cropping (Atkinson and Else, 2001).

In fruit trees, when growing conditions are appropriate, the first annual growth of the stem developing from grafted bud is the longest in the tree and bears the limbs which will later make up the tree structure. This lead us to believe that it might be possible to evaluate tree growth and branching habits by analysing the branching pattern of the first annual shoot of the trunk (Costes and Guedon, 2002). The effects of rootstock on fruit tree architecture

Using dwarfing rootstocks may decrease the growth rate and final size of trees. In this research we will compare the effects of different rootstocks on the growth rate of macadamia trees (Huett, 2004).

Materials and methods

Experimental design:

A randomized complete block design (RCBD) was used to arrange small grafted plants on two benches in Long pocket glasshouse no. 3 (Figure 6).

Macadamia A4 was grafted on Beaumont, *M. ternifolia*, a wild hybrid (*M. ternifolia* X *M. integrifolia)* and on its own.

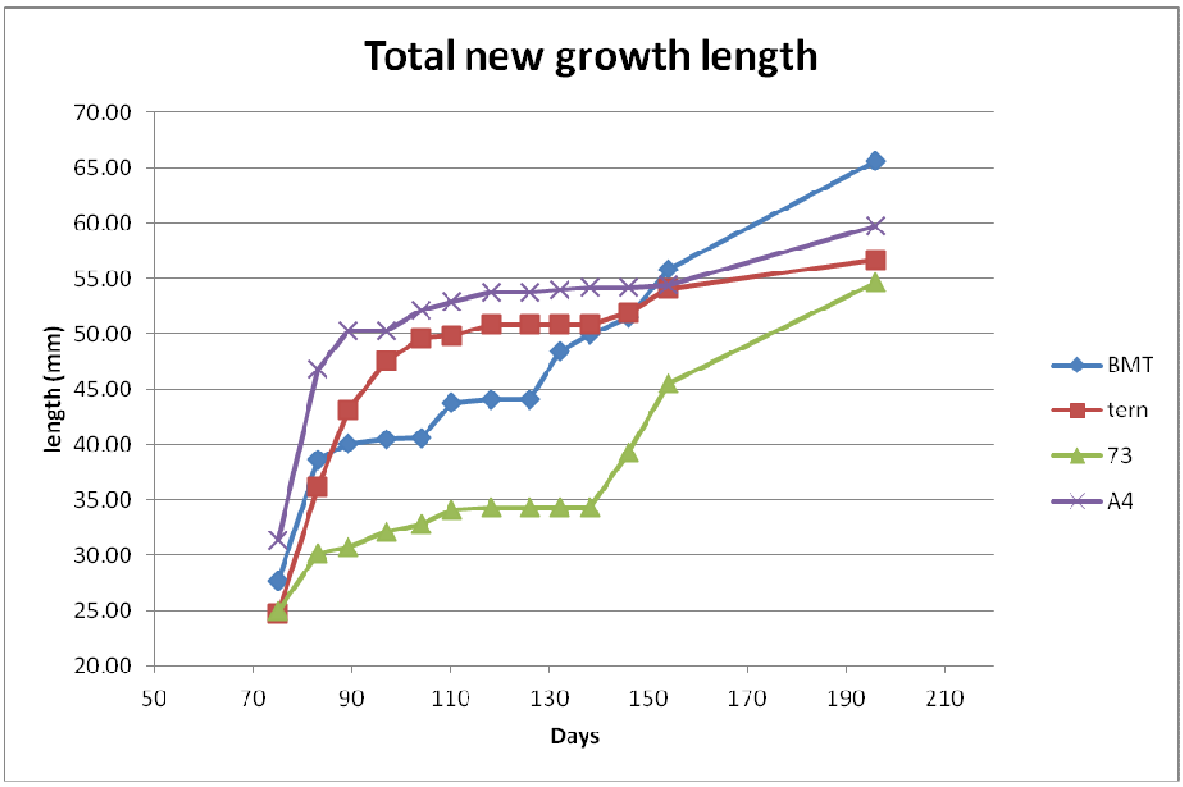
BMT: A4/Beaumont ***tern***: A4/*M. ternifolia* ***H***: A4/hybrid ***A***: A4/A4

Using 6 blocks, each block consists of 4 rootstocks mentioned above. Total plants are 24.

Block1	Block2	Block3	Block4	Block5	Block6
H 1	A 2	tern 3	H 4	A 5	A 6
A1	BMT 2	A 3	BMT 4	Tern 5	BMT 6
Tern 1	Tern 2	BMT 3	A 4	H 5	Tern 6
BMT 1	H2	H3	Tern 4	BMT 5	H6

Figure. 6. Diagram of 4 rootstocks arranged in 6 blocks. Rootstocks used for this trial comprises Beaumont (**BMT**), *M. ternifolia* (**tern**), wild hybrid (*M. ternifolia* X *M. integrifolia)* (**H**) and A4 (**A**). The heavy lines in the upper left-hand corner highlight one block of the randomized complete block design, the entire planting contained 20 blocks.

Results



Chapter 4. Photosynthesis and Carbohydrate accumulation in Macadamia

Photosynthesis

Photosynthesis was measured on 2 fully expanded mature leaves of each vegetatively propagated macadamia plants of A4, Beaumont and *M. jansenii* (the same plants that I used for architectural preliminary measurements). Number of stomata was counted on 2 leaves of each type as well. Measurements carried out in 01/03/2010 and 02/03/2010 in UQ glasshouse no. 2 under the temperature of 22°C (cloudy and rainy days). Measurements were made with a portable open gas exchange LI-6400, Li-Cor (Figure.9). Maximum photosynthetic rate (Amax) was measured at 1500, 800, 700, 600, 500, 400 and 50 µmol m^{-2} s^{-1} PPFD, ambient leaf temperature (Tleaf), and a constant CO_2 concentration of 380 µmol mol^{-1}.

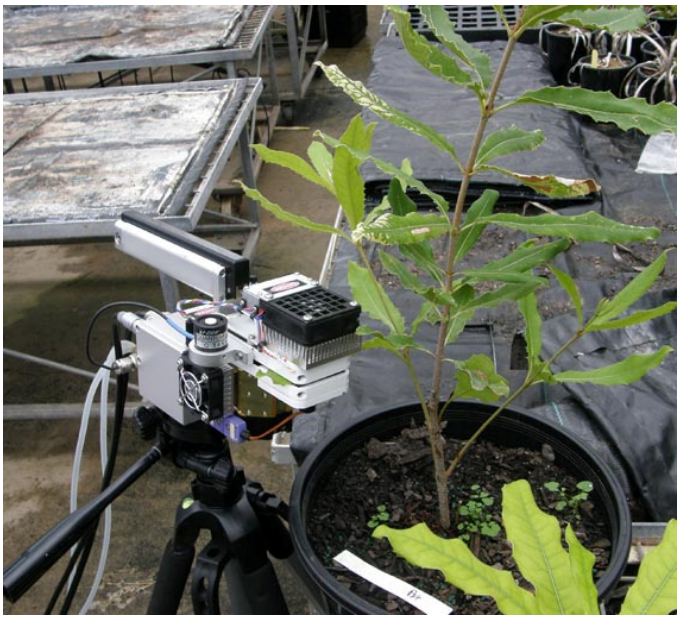


Figure.12 A mature macadamia A4 leaf is sealed within the head chamber of LI-6400 to measure the photosynthesis rate (µmol CO_2. m^{-2}·s^{-1})

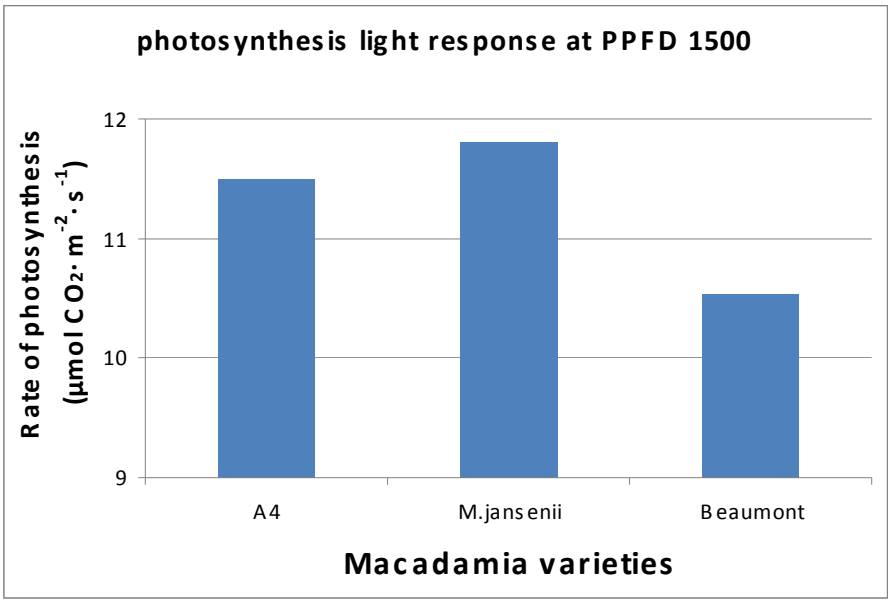


Figure.13. Photosynthesis rate at PPFD (irradiance) 1500 µmol photons·m-2·s-1

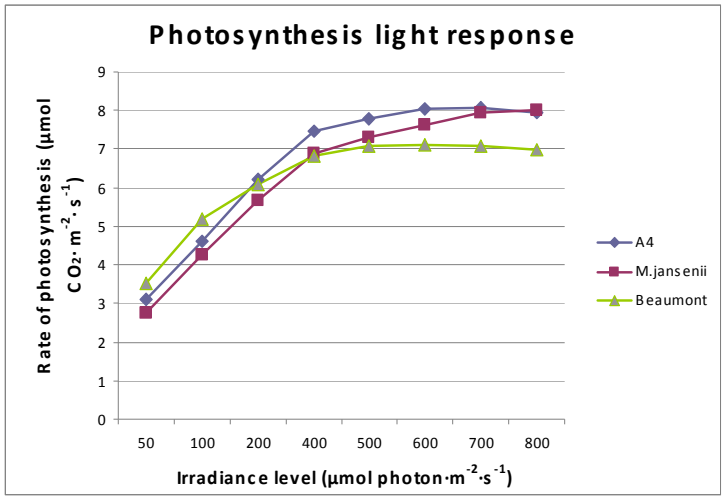


Figure.14. Photosynthesis rate in macadamia leaves at different level of irradiance

The photosynthesis rate of A4, *M. jansenii* and Beaumont are compared in figures 13 and 14. Beaumont shows a significant (<0.1) lower photosynthesis rate at irradiance of 1500 µmol photon·m^{-2}·s^{-1} (Table 7). There wasn't significant difference between A4, *M. jansenii* and Beaumont in photosynthesis rate at light intensities of 800 µmol·m^{-2}·s^{-1} and lower. But the difference between Beaumont and others was significant. Beaumont had a lower photosynthesis rate than *M. jansenii* and A4.

While the rates were not significantly different at light intensities 800 and lower with this sampling regime, trends in the data indicate that Beaumont's photosynthesis rate increased at lower light intensities (50 and 100 m-2 s-1) in comparison with *M.jansenii* and A4, but at light intensity 200 µmol·m-2·s-1 turned to lower photosynthesis rate than A4 and at 440 µmol·m-2·s-1 turned to lower photosynthesis rate than *M.jansenii*. The peak light density to increase photosynthesis rate was lower for Beaumont than A4 and *M.jansenii*. The rate of photosynthesis was similar at 800 µmol·m-2·s-1 for A4 and *M. jansenii*. The lower rate of photosynthesis of Beaumont (Figure. 13) at light intensity of 1500 µmol·m^{-2}·s^{-1} could affect the vegetative growth of this species of macadamia.

Table 7. Average photosynthesis rate of macadamia leaves at different light intensities

	PPFD (µmol·m^{-2}·s^{-1})								
	1500	**800**	**700**	**600**	**500**	**400**	**200**	**100**	**50**
A4	11.49±0.2*	7.96±0.21	8.07±0.17	8.02±0.2	7.77±0.23	7.45±0.24	6.23±0.31	4.6±0.39	3.1±0.66
Jan	11.8±0.39	8±0.48	7.93±0.5	7.63±0.56	7.3±0.64	6.87±0.7	5.66±0.68	4.25±0.35	2.76±0.62
BMT	10.54±0.47**	6.99±0.93	7.28±0.8	7.29±0.8	7.09±0.94	7.01±0.78	6.16±0.66	5.19±0.45	3.52±0.63

* Standard Error

** Significantly different to A4 and *M. jansenii* at 0.06

References:

Atkinson C, Else M. 2001. Understanding how rootstocks dwarf fruit trees. *Compact Fruit Tree,* 34: 46-49.

Barthelemy D, Caraglio Y. 2007. Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany,* 99: 375-407.

Bell HFD, Bell MA, Bell DJD. 1988. Macadamia integrifolia X tetraphylla. *Plant Varieties Journal,* 1: 7-12.

Berninger F, Nikinmaa E, Sievanen R, Nygren P. 2000. Modelling of reserve carbohydrate dynamics, regrowth and nodulation in a N-2-fixing tree managed by periodic prunings. *Plant Cell and Environment,* 23: 1025-1040.

Cormack DB, Bate GC. 1976. Seasonal fluctuation of total Nonstructural carbohydrate levels within the macadamia-integrifolia cultivar Kakea and its relation to shoot extension. *Rhodesian Journal of Agricultural Research,* 14: 39-45.

Costes E, Garcia-Villanueva E. 2007. Clarifying the effects of dwarfing rootstock on vegetative and reproductive growth during tree development: A study on apple trees. *Annals of Botany,* 100: 347-357.

Costes E, Guedon Y. 2002. Modelling branching patterns on 1-year-old trunks of six apple cultivars. *Annals of Botany,* 89: 513-524.

Costes E, Lauri PE. 1995. Growth process relative to sylleptic branching and blooming of the apple tree. *Architecture of Forest and Fruit Trees*: 41-50

Costes E, Lauri PE, Regnard JL. 2006. Analyzing fruit tree architecture: implications for tree management and fruit production. *Horticultural Reviews,* 32: 1-61.

Costes E, Salles JC, Garcia G. 2001. Growth and branching pattern along the main axis of two apple cultivars grafted on two different rootstocks. *Proceedings of the Seventh International Symposium on Orchard and Plantation Systems*: 131-138

Costes E, Sinoquet H, Godin C, Kelner JJ. 1999. 3D digitizing based on tree topology: Application to study the variability of apple quality within the canopy. *Proceedings of the Fifth International Symposium on Computer Modelling in Fruit Research and Orchard Management*: 271-280

Demmig-Adams B, Adams WW, III, Grace SC. 1997. Physiology of light tolerance in plants. *Horticultural Reviews,* 18: 215-246.

Dickson RE, Isebrands JG. 1993. Carbon Allocation Terminology: Should It Be More Rational? *Bulletin of the Ecological Society of America,* 74: 175-177.

Firth DJ, Whalley RDB, Johns GG. 2003. Distribution and density of the root system of macadamia on krasnozem soil and some effects of legume groundcovers on fibrous root density. *Australian Journal of Experimental Agriculture,* 43: 503-514.

Flore JA, Lakso AN. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *Horticultural Reviews,* 11: 111-157.

Fourcaud T, Zhang X, Stokes A, Lambers H, Korner C. 2008. Plant growth modelling and applications: The increasing importance of plant architecture in growth models. *Annals of Botany,* 101: 1053-1063.

Godin C, Costes E, Sinoquet H. 1999. A method for describing plant architecture which integrates topology and geometry. *Annals of Botany,* 84: 343-357.

Godin C, Sinoquet H. 2005. Functional-structural plant modelling. *New Phytologist,* 166: 705-708.

Gross CL, Weston PH. 1992. Macadamia jansenii, new species (Proteaceae), a new species from central Queensland. *Australian Systematic Botany,* 5: 725-728.

Hanan J. 1997. Virtual plants - integrating architectural and physiological models. *Environmental Modelling & Software,* 12: 35-42.

Hardner C, Wilkie J, McConchie C. 2004. The world Macadamia industry and research activities. *NUCIS Newsletter*: 36-39.

Hardner CM, Peace C, Lowe AJ, Neal J, Pisanu P. 2009. Genetic resources and domestication of Macadamia. *Horticultural Reviews,* 35: 1-125.

Heizmann U, Kreuzwieser J, Schnitzler JP, Bruggemann N, Rennenberg H. 2001. Assimilate transport in the xylem sap of pedunculate oak (Quercus robur) saplings. *Plant Biology,* 3: 132-138.

Huett DO. 2004. Macadamia physiology review: a canopy light response study and literature review. *Australian Journal of Agricultural Research,* 55: 609-624.

Huett DO, McFadyen LM, Olesen T. 2005. Physiological constraints to macadamia (Macadamia integrifolia) production. *Proceedings of the International Symposium on Harnessing the Potential of Horticulture in the Asian-Pacific Region*: 243-246

Joyeux-Faure M, Arnaud C, Godin-Ribuot D, Ribuot C. 2003. Heat stress preconditioning and delayed myocardial protection: what is new? *Cardiovascular Research,* 60: 469-477.

Karkalas J. 1985. An improved enzymic method for the determination of native and modified starch. *Journal of the Science of Food and Agriculture,* 36: 1019-1027.

Kiptot E, Hebinck P, Franzel S, Richards P. 2007. Adopters, testers or pseudo-adopters? Dynamics of the use of improved tree fallows by farmers in western Kenya. *Agricultural Systems,* 94: 509-519.

Kitajima K, Mulkey S, Wright S. 1997. Decline of photosynthetic capacity with leaf age in relation to leaf longevities for five tropical canopy tree species. *Am. J. Bot.,* 84: 702.

Lacointe A, Minchin PEH. 2008. Modelling phloem and xylem transport within a complex architecture. *Functional Plant Biology,* 35: 772-780.

Lindenmayer A. 1968. Mathematical models of cellular interaction in development, Parts I and II. *Journal of Theoretical Biology,* 18: 280-315.

Liu X, Robinson PW, Madore MA, Witney GW, Arpaia ML. 1999. 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. *Journal of the American Society for Horticultural Science,* 124: 671-675.

Lloyd J. 1991. Modeling Stomatal Responses to Environment in Macadamia-Integrifolia. *Australian Journal of Plant Physiology,* 18: 649-660.

Menzel CM, Rasmussen TS, Simpson DR. 1995. Carbohydrate Reserves in Lychee Trees (Litchi-Chinensis Sonn). *Journal of Horticultural Science,* 70: 245-255.

Nagao MA, Hirae HH. 1992. Macadamia - Cultivation and Physiology. *Critical Reviews in Plant Sciences,* 10: 441-470.

Nagao MA, Hoa EB, Yoshimoto JM, Yoshimura ER, Notley E, Fuchigami LH. 1994. Relationship between Vegetative Flushing and Flowering of Macadamia-Integrifolia in Hawaii. *Scientia Horticulturae,* 60: 9-16.

Oldeman RAA. 1974. The architecture of the forest of French Guiana. *Memoires ORSTOM*: 204 pp.

Olesen T, Robertson D, Muldoon S, Meyer R. 2008. The role of carbohydrate reserves in evergreen tree development, with particular reference to macadamia. *Scientia Horticulturae,* 117: 73-77.

Pereira CS, de Siqueira DL, Salomao LCC, Cecon PR, dos Santos D. 2011. Fruit Production and Carbohydrate Contents in the Leaves of Acid Lime Tree 'Tahiti' Girdled and Treated with Gibberellic Acid. *Revista Brasileira De Fruticultura,* 33: 706-712.

Philippe M, Fournet B, Schrevel J. 1975. Macromolecular Carbohydrate Composition in Vegetative and Sexual Stages of Parasitic Protozoan Gregarina-Blaberae and of Host Hemolymph Blaberus-Craniifer. *Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences Serie D,* 280: 1801-1804.

Prusinkiewiez P, Hanan J, Mech R. 2000. An L-system-based plant modeling language. *Applications of Graph Transformations with Industrial Relevance, Proceedings,* 1779: 395-410.

Renton M, Kaitaniemi P, Hanan J. 2005. Functional-structural plant modelling using a combination of architectural analysis, L-systems and a canonical model of function. *Ecological Modelling,* 184: 277-298.

Richards DE, Brown WF, Ruegsegger G, Bates DB. 1994. Replacement Value of Tree Legumes for Concentrates in Forage-Based Diets .2. Replacement Value of Leucaena-Leucocephala and Gliricidia-Sepium for Lactating Goats. *Animal Feed Science and Technology,* 46: 53-65.

Room PM, Hanan JS, Prusinkiewicz P. 1996. Virtual plants: New perspectives for ecologists, pathologists and agricultural scientists. *Trends in Plant Science,* 1: 33-38.

Sakai S. 1981. Preservability and Carbohydrate Reserve of Tea Vegetative Organs in a Long-Term Storage. *Jarq-Japan Agricultural Research Quarterly,* 15: 39-45.

Saleem MB, Buxton DR. 1976. Carbohydrate Status of Narrow Row Cotton as Related to Vegetative and Fruit Development. *Crop Science,* 16: 523-526.

Seleznyova AN, Tustin DS, Thorp TG. 2008. Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: Precocious transition to flowering affects the composition and vigour of annual shoots. *Annals of Botany,* 101: 679-687.

Sievanen R, Nikinmaa E, Nygren P, Ozier-Lafontaine H, Perttunen J, Hakula H. 2000. Components of functional-structural tree models. *Annals of Forest Science,* 57: 399-412.

Soderstrom B, Finlay RD, Read DJ. 1988. The Structure and Function of the Vegetative Mycelium of Ectomycorrhizal Plants .4. Qualitative-Analysis of Carbohydrate Contents of Mycelium Interconnecting Host Plants. *New Phytologist,* 109: 163-166.

Stanley TD, Ross EM. 1986. *Flora of south-eastern Queensland*, Queensland Department of Primary Industries, Brisbane.

Steiger DL. 2003. Genetic relationships of macadamia cultivars and species revealed by AFLP markers (vol 132, pg 269, 2003). *Euphytica,* 133: 377-377.

Stephenson RA, Cull BW, Mayer DG, Price G, Stock J. 1986a. Seasonal Patterns of Macadamia Leaf Nutrient Levels in South East Queensland. *Scientia Horticulturae,* 30: 63-71.

Stephenson RA, Cull BW, Stock J. 1986b. Vegetative Flushing Patterns of Macadamia Trees in South East Queensland. *Scientia Horticulturae,* 30: 53-62.

Stephenson RA, Cull BW, Stock J. 1986c. Vegetative flushing patterns of macadamia trees in south east Queensland. *Scientia Horticulturae,* 30: 53-62.

Stephenson RA, Gallagher EC, Doogan VJ. 2003. Macadamia responses to mild water stress at different phenological stages. *Australian Journal of Agricultural Research,* 54: 67-75.

Stephenson RA, Gallagher EC, Rasmussen TS. 1989a. Effects of Growth Manipulation on Carbohydrate Reserves of Macadamia Trees. *Scientia Horticulturae,* 40: 227-235.

Stephenson RA, Rasmussen TS, Gallagher EC. 1989b. Timing of nitrogen application to macadamias 2. Storage carbohydrates. *Australian Journal of Experimental Agriculture,* 29: 575 - 579.

Storey WB, Hamilton RA, Fukunaga ET. 1953. The Relationship of Nodal Structure to Training Macadamia Trees. *Proceedings of the American Society for Horticultural Science,* 61: 317-323.

Susila P, Naus J. 2007. A Monte Carlo study of the chlorophyll fluorescence emission and its effect on the leaf spectral reflectance and transmittance under various conditions. *Photochemical & Photobiological Sciences,* 6: 894-902.

Trochoulias T, Lahav E. 1983. The effect of temperature on growth and dry-matter production of macadamia. *Scientia Horticulturae,* 19: 167-176.

Trueman SJ, Turnbull CGN. 1994. Fruit-Set, Abscission and Dry-Matter Accumulation on Girdled Branches of Macadamia. *Annals of Botany,* 74: 667-674.

Walklate PJ, Richardson GM, Baker DE, Richards PA, Cross JV. 1997. Short-range LIDAR measurement of top fruit tree canopies for pesticide applications research in the UK. *Advances in Laser Remote Sensing for Terrestrial and Oceanographic Applications,* 3059: 143-151

Weibel A, Johnson RS, DeJong TM. 2003. Comparative vegetative growth responses of two peach cultivars grown on size-controlling versus standard rootstocks. *Journal of the American Society for Horticultural Science,* 128: 463-471.

Zanne AE, Lopez-Gonzalez G, Coomes DA, Ilic J, Jansen S, Lewis SL, Miller RB, Swenson NG, Wiemann MC, Chave J. 2009. Data from: Towards a worldwide wood economics spectrum. Dryad Data Repository.

PhD Project 2. Axillary bud behaviour in Macadamia - Janine Conway

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School of Agriculture and Food Sciences 2009 - 2013

Introduction

Axillary buds of macadamia trees produce the plant's inflorescences as well as new branches. Many buds however are never released from dormancy. The likelihood of release appears to be linked to the bud's position along the row of several in each axil, and to the position of the node on the growth flush. The nature of a new shoot has been linked to its age or position in the tree's architecture at time of release, and to the temperature at this time. In another layer of control, if a released bud is florally evoked it soon undergoes a second dormancy, and release from this seems to be dependent upon temperature at the time of subsequent growth flushes.

This project will refine understandings of location of inflorescence and branch production, and investigate environmental and physiological triggers of dormancy release and floral induction. Part of this will involve microscopic studies of growth of new axes before they become visible to the naked eye. The project will also investigate interactions between evocation and release from dormancy.

The results of the study will feed into a functional – structural model aiding macadamia canopy management. It is hoped this knowledge will contribute to management of light distribution within the canopy, and of branch growth into orchard alleys, to extending the flowering life of branch segments, and to optimising the ratio of vegetative to floral shoot production.

Chapter 1. Literature review

Background

Macadamia nuts have been part of the human diet since before written history in Australia (Australian National Botanic Gardens 2000). They are produced by the trees *Macadamia integrifolia* and *M. tetraphylla* and hybrids and grafts of the two. Other species in the genus also produce nuts but they are less palatable or toxic (Nagao and Hirae 1992). *M. integrifolia* and *M. tetraphylla* are native to the subtropical coastal ranges of eastern Australia (Stephenson and Trochoulias 1994), where they have been cultivated commercially since the 1880s. As the most widely commercialised Australian native food plant to date, in 2003-4 29 kT were produced, worth 102 million dollars (Department of Agriculture Food and Fisheries 2005). As a source of protein that can be produced with less water and energy input than meat, and less erosion and carbon dioxide output, tree nuts may be consumed in greater amounts as

pressures facing natural resources grow with changing population numbers and lifestyles.

Mature macadamias are large trees that, in cultivation, are usually maintained in hedgerows. Hedging maintains access to the top of the canopy for pest and disease control, and lets some light onto the orchard floor. The latter keeps ground cover alive and so reduces erosion while maximising traction for orchard machinery (Huett 2004). However, growth from numerous axillary buds after hedging results in new branch formation, which creates a thick outer layer of the canopy. This shades the interior of the canopy, reducing productivity of inner leaves, new axis growth, and subsequently nut yields (Huett and Smith 2008b). Greater understanding of the branching process and how the tree controls this may aid in balancing the need for alleys between hedges and sustaining production levels by reducing internal shading. An attractive possibility to industry is a reduction in hedging combined with greater penetration of light into the canopy.

Figure 1. Macadamia trees in hedged orchard

Figure 2. New branch emergence from axil of abscised leaf

Similar to branch production, inflorescences are formed from axillary buds, and both number and location are important to successful crop production. The number ultimately restricts the possible nut crop size. There are approximately 2,500 to 10,000 inflorescences on a mature (175 yr old) tree, each carrying 100-300 flowers (Moncur *et al.* 1985; Nagao and Hirae 1992), although only 0.3% of flowers ultimately produce a mature nut (Stephenson and Trochoulias 1994). Inflorescences can be located just inside the canopy edge or somewhat deeper (O'Hare *et al.* 2004). The depth effects how much of the flowering wood is lost in hedging.

Figure 3. Inflorescence emerging between petiole and peduncle of older inflorescence (top of branch)

This study will investigate the control of axillary bud fate in *M. integrifolia a*nd *M. tetraphylla*. It aims to understand how dormancy in these buds is controlled and how outgrowing buds are determined to be vegetative or floral. It will also attempt to develop methods for manipulating bud fate with a long-term view to improvements in nut production, via management of branch and inflorescence number and location.

Macadamia biology and shoot growth

Macadamias are members of the Proteaceae family of plants and as such are considered to have evolved in the tropics or sub-tropics (Johnson and Briggs 1963). Their closest well known relatives are the genera of Grevillia and Banksia. The Australian species of macadamia are found on the edges of sub-tropical rainforest, and possess features such as sclerified leaves and proteoid roots that are more common in plants from dry areas and low nutrient soils than in classic rainforest plants. However macadamias are evergreen, and stem and leaf production occurs around the year, in flushes of rapid stem elongation and leaf production interspersed by periods of rest. In sub-tropical areas, growth occurs mostly when monthly mean minimum temperatures are higher than 10ºC (Stephenson and Cull 1986). Flushing, when many branches of a tree are going through rapid growth spurts simultaneously, usually occurs two or three times a year – in spring and summer. When grown in more equatorial regions, flushing is fairly evenly dispersed throughout the year (Nagao and Hirae 1992).

M. integrifolia and *M. tetraphylla* form whorls of three and four leaves respectively, leaves being 10 to 15 cm long and nodes 3 to 5 cm apart [?(Nagao and Hirae 1992)]. Each leaf axil houses three to five buds in a vertical row (Bennel 1984). It appears usual for only the top one to three of the buds at a node to develop into new shoots (Bennel 1984; Storey 1985; Nagao and Hirae 1992), and only one of them to grow out in any flush period (Nagao and Hirae 1992). Axillary buds are generally regarded as undifferentiated at time of formation and thus are able to form either new branches or inflorescences, however no physiological or microscopic work has been done to determine when the trees are induced, the meristems determined or the first floral primordia initiated.

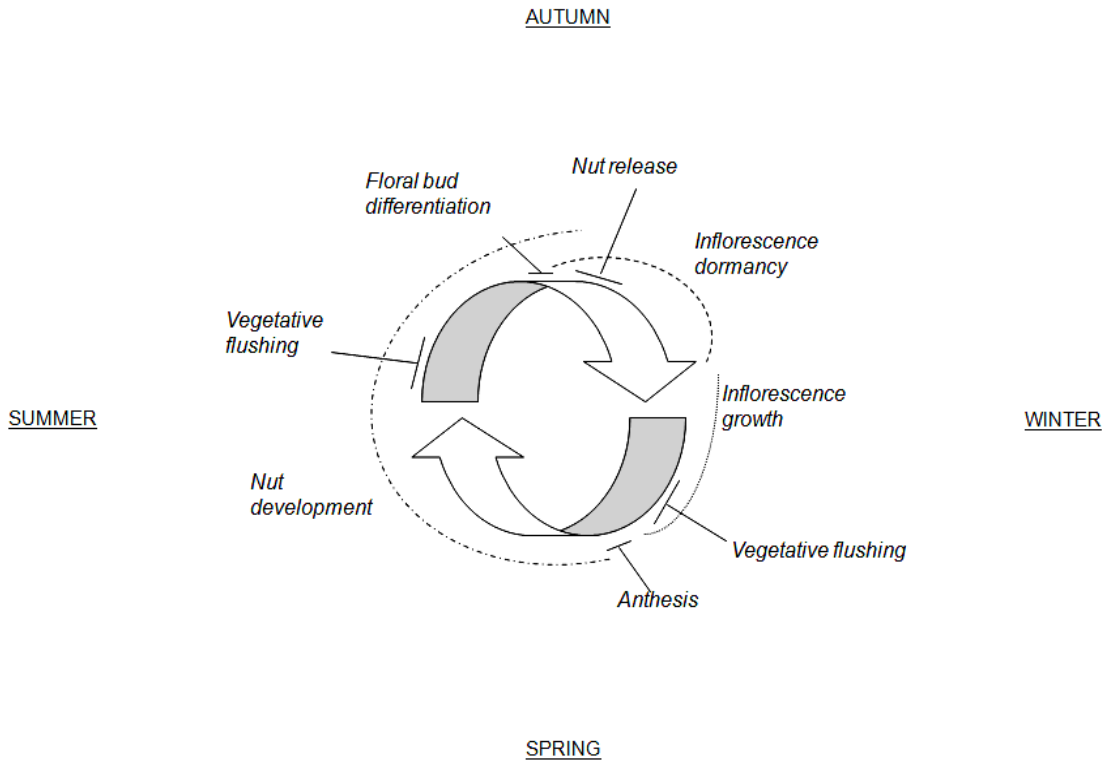

Figure 4. Phenological cycle of macadamia

Vegetative shoot growth

Bennel (1984) reported the vegetative growth of (un-pruned) macadamia trees is usually from the terminal bud of a branch or from axillary buds on fresh growth near it. Production of new branches from axillary buds can be regarded as a multi-step process, requiring first the release of axillary buds from dormancy, and then the elongation of the new branch by the now-apical meristem (Stafstrom and Sussex 1988; Cline 1991).

Environmental conditions including temperature, light intensity, and availability of nutrients including water, have been shown to affect the rate and form of macadamia vegetative growth. Extensive flushing usually occurs when temperatures are between 20 and 30ºC (Nagao and Hirae 1992). Leaf and stem growth is greatest between temperatures of 20 and 25ºC (Stephenson and Trochoulias 1994). The cultivar Keauhou , studied in constant temperature chambers, accumulated dry matter more slowly at 15ºC than at 20 or 25ºC, and not at all at 10ºC (Trochoulias and Lahav 1983). This growth declined from 25ºC to 30ºC and again from 30ºC to 35ºC. Stems formed at 35ºC were thin and, as did tissues formed at 30, they necrosed and desiccated prematurely (Trochoulias and Lahav 1983). [After hedging, "cooler weather" in the autumn and winter temperatures of northern NSW resulted in growth of longer branches (McFadyen *et al.* 2008).]

Temperature also effects leaf health and thus will effect growth indirectly. Constant exposure of mature leaves to 35ºC results in their death, and 30ºC will induce chlorosis. In the field, chlorosis is seen on the NW side of trees at hot sites (Stephenson and Trochoulias 1994).

Above 35ºC the *number* of new branches produced is greater (Trochoulias and Lahav 1983). "Warmer" temperatures also result in the production of more branches after hedging (McFadyen *et al.* 2008), although the actual temperatures were not given [find on BOM site].

These relationships between temperature and vegetative growth explain and are explained by some aspects of photosynthetic performance. Carbon assimilation in macadamias is highest at 25ºC - around 9 umol/m^2/s - and drops quickly below 15ºC and above 26ºC (Nagao and Hirae 1992; Stephenson and Trochoulias 1994; Huett 2004). Chlorosis, occurring as a result of continued exposure to high temperatures, would probably reduce photosynthetic capacity. However growth rates and photosynthetic capacity don't always trend the same way: younger leaves are growing quickly but are carbohydrate sinks until around the time of hardening (Huett 2004).

Macadamia vegetative growth can be limited by lack of water (Stephenson and Trochoulias 1994). Stomata close in order to prevent water loss at leaf water potentials of 2.0 MPa , but potentials can fall below this and permanently damage the leaf. Leaf tip necrosis occurs at water potentials of -1.5 to -3.0 MPa, and leaf abscission after below -5.0MPa (Stephenson *et al.* 1989). When water is plentiful, it is suspected that macadamia growth responds positively – Stephenson and Cull (1986) concluded that the proportion of the canopy flushing at any one time is related (among other factors) to the amount of recent rainfall[, although other factors clouded the relationship]. Macadamias produce well with 1000mm rain per year and even better with 2000mm/yr [](Stephenson and Trochoulias 1994).

(Stephenson and Cull 1986) considered that nitrogen applied to the soil in the two months prior to the beginning of a flush may increase the proportion of the canopy that subsequently flushed, but the relationship was not clear due to other strong influences such as rainfall. If nitrogen is applied as 1.5kg of urea in April or early November a significant increase in trunk girth occurs in the next season[/year], although this is not the case for applications at other times of the year or applications spread evenly throughout the year (Stephenson and Gallagher 1983).

Shoot growth has also been related to light availability. In Broomhall's (pers. com. 2009) study, greater than 5 % of incident light was required for branch elongation. The number of growing tips and the increase in length are both reduced at 10% of incidental levels or less. Growth will stop completely before light is reduced to around 1% of incidental levels – in this depth of shade the shoot will die. Most macadamias produce dense canopies and some internal shading is the norm even when trees are widely spaced. This shading often reduces light levels to below the 50 PAR threshold for leaf survival (Huett and Smith 2008a) and a leafless zone develops in the interior of the canopy. This is a concern for industry as it may reduce yield through both lower total photosynthesis and through production of fewer inflorescences.

Mechanical pruning or hedging of macadamias is a common part of the commercial orchard environment that affects the form of macadamia growth. The random location of cuts along the stems releases buds from multiple leaf axils to produce many new shoots in the next flush, and sometimes multiple buds in an axil (Nagao and Hirae 1992). (This is opposed to the usual extension growth and limited side branching (Bennel 1984)). The flushing of more branches of a tree can be synchronised by hedging (Olesen 2005). After hedging, flushes come two or three months apart – two months apart in the warmest areas or seasons and three in cooler. Pruning a shoot immediately above its junction with other shoots directed the growth of the next flush into elongating those remaining shoots (Huett and Smith 2008b).

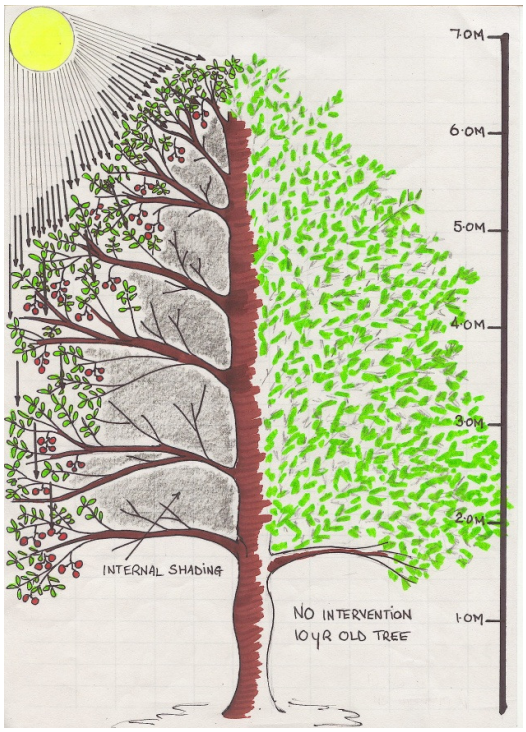

Figure 5. Internal shading shifts leaf and fruit production to the top and outside of the canopy. From Huett and Smith (2007)

> **new: Bennel states that usually only the two most distal buds in the axillary series grow out, but it seems inflorescences often arise from the most proximal, when more distal buds in the series appear to be dormant.

This authors observation also casts nasturshiums on the theory that several buds in a series are evoked but the outgrowth of one suppresses that of the lower ones, by apical dominance.

Floral shoot growth

Sequence and timing

Under Australian conditions, new inflorescences become visible between March and May, when white apices can just be seen behind bud bracts (Bennel 1984; Moncur *et al.* 1985)(Stephenson, pers.com). When they reach this stage they do not grow further for 50 to 96 days (Moncur *et al.* 1985). They resume growth during winter, and elongate for around two months to reach 10 – 15 cm before anthesis in late winter to mid spring. The time of anthesis varies between locations but also within trees. The growth of the macadamia inflorescence before it becomes visible to the naked eyes is not well understood. There are no published studies of when the new shoot is florally determined, or how it grows from that point until it pushes open the bract.

Elongation of inflorescences to a macroscopic scale when they can be seen emerging from behind the bract may be linked to a developmental gate of activation of floret meristems.

***(Nagao and Sakai 1990) intro says in Hawaii lull in flowering in Nov-Dec. Climates stats say night temp higher than 23celcius flowering min around August... could indicate only three months between evocation and emergence in warm / hawain climate - no cool temp slow-down to inf development, no synchronisation by mid-winter conditions... would imply late summer evocation in aus the three months folllowing any spring / early summer evocation would be warm enough to enable the same sort of development and thus February flowering.

Location

Knowledge of the where inflorescences are produced within trees, stems or nodes assists canopy management and manipulation of flowering. The locations of inflorescences of a number of nut and fruit trees are known to form predictable patterns (Costes *et al.* 2006). It is known that buds can become less sensitive to hormonal signals as they age (Cline 1991), which could account for some of the patterns of inflorescence location. However for macadamia predictions of inflorescence and nut location are relatively imprecise.

Inflorescences usually develop from buds on hardened wood (Stephenson and Trochoulias 1994). The age and canopy depth of fruitwood varies with species and cultivar, aspect, and branch height (Salter and McConchie 2005). Inflorescences are deeper in the canopy on the East of the tree, and deeper on the higher branches.

Wilkie (pers. com. 2009) found that macadamias are more likely to develop inflorescences on short stems (unbranched sections of a limb) consisting of only one or two flush lengths formed in the previous two years, than on longer stems, or stems consisting of more or older flush lengths. There was however some variation between the four cultivars he studied (A4, 660, 695, and A38). McFadyen *et al.* (2008) also found that longer stems were less likely to bear racemes than shorter ones. These last two studies have used pruning to trigger release of floral buds, and in interpreting results it should be remembered that behaviour of buds on decapitated shoots may be different to that of intact shoots (Dun *et al.* 2006). [However shoot length is known to

at least partially determine flowering in pecans -Malestrom& McMeans 82 – also pruning?]

Low vigour in fruit tree branches has been associated with floral evocation by ... which may be connected to these findings of inflorescences being located on branches of lesser growth

***- girdling increased cumulative infs at 27 weeks post trtmnt (Nagao and Sakai 1990) : n infs restricted by amount sugar accessible?

Axis type

Macadamia shoot axillary buds have three possible fates at any point in their life – to remain dormant, to grow into a branch, or to grow into an inflorescence. If a bud breaks dormancy, the nature of the new shoot grown is controlled by the presence or absence of biochemical signals to turn on floral development genes. In the absence of such signals – dubbed florigin, despite it remaining unidentified to date - the bud continues its development as vegetative tissue. The meristem producing new metaemers of internodes, leave, and more axillary buds, which elongate to form a branch. Commitment to production of inflorescences involves a different pathway of four steps; i) induction of the tree, ii) evocation of the meristem, iii) initiation of the first inflorescence primordia, iv) determination/commitment of the meristem (Henderson and Lawrence 1989; Sedgley and Griffin 1989; Haghavan 1992; Taiz and Zeiger 2006; Scott 2008). The definitions used most consistently in the floral physiology literature (Sedgley and Griffin 1989; Haghavan 1992) are the ones that will be used here, even where they differ from those used by some of authors whose work is discussed. Induction of the tree is the process by which it senses cues to begin the flowering process, and in response manufactures signals and release them into the branch system to inform buds of their floral fate. Tropical trees are generally induced to flower through environmental cues (Wilkie *et al.* 2008). Evocation is the change in genetic and biochemical activity of the meristem in response to perceiving this signal. Initiation of inflorescence or floral primordia, respectively, is the beginnings of the rachis or floral organs such as petals and stamens. Determination / commitment is the point at which removal of the stimulus which evoked the meristem to engage it's floral development genes will not result in reversion to vegetative growth.

Evocation

The only published study using microscopic examination to investigate the control of flowering processes in macadamia was conducted by Sakai *et al.*(Sakai *et al.* 1982), measuring the time to meristem evocation of *M. integrifolia* trees at different temperatures. Evocation was diagnosed by observation of the shapes of the upper surfaces of meristems. The same group had previously found peaked meristems to be associated with floral growth, and gently domed meristems with vegetative growth. Dormant meristems were found to be flat. They found that at 12 ºC the first meristems were evoked by 26 days, at 18 ºC by 38 days, at 21 ºC by 44 days, and above 21 ºC no evocation was detected (in the three month study). In the same study, the time from evocation until emergence of inflorescences also varied with temperature. At 12 ºC it was 5 weeks, and at 18 and 21 ºC it was 10 weeks.

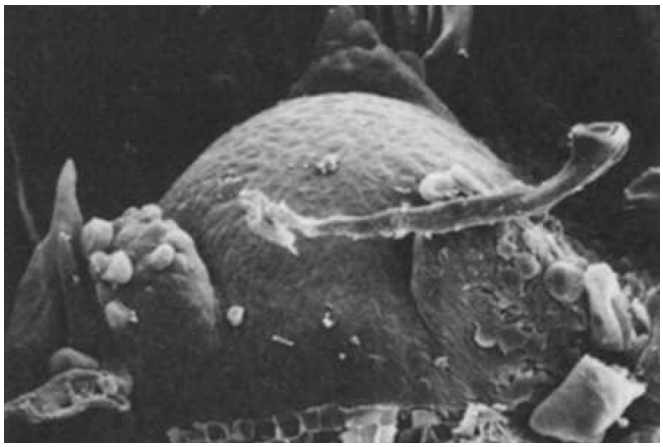

Figure 6. Peaked apical meristem, newly evoked. From Moncur et al. (1985)

The flowering of *Banksia coccinea* and *B. menziesii*, classified in the same sub-family as macadamia, was studied microscopically by Fuss and Sedgley (1990). Both species have similar times of macroscopic inflorescence appearance to macadamia – February and May. The banksia species initiated the first inflorescence primordia (involucral bracts) in late spring (November). Initiation of floral bracts did not occur until mid-summer (January), and floret parts were initiated as late as mid autumn (April).

**** Dupee ++ waratahs may have two periods of evocation... check definition of initiation as used by them

Induction to emergence

All other studies on control of flowering in macadamia are based on macroscopic events. Thus their findings pertain to the sum of the processes of induction, evocation, initiation, and the period of growth before the inflorescence becomes visible. Some extrapolation of macroscopic events to determine the timing of microscopic events has been attempted in the macadamia literature, which apparently has occurred often in the study of floral control, but this approach has been assessed to be very misleading (Sedgley and Griffin 1989). Meristems can be evoked and primordia initiated well before macroscopically visible, and the period of time between the microscopic events of flower production and those observable with the naked eye may vary. The length of time between floret initiation and macroscopic appearance of the inflorescence in *Banksia coccinea*, closely related to macadamia, was observed to range from 1 to 9 months (Fuss and Sedgley 1990). Of course when the stage of inflorescence development has been reached, the fate of the bud has already been determined.

Temperature

In subtropical and tropical trees, a reduction in temperature commonly promotes flowering (Sedgley and Griffin 1989; Wilkie *et al.* 2008), as opposed to cool temperate crops where higher temperatures do the same. Citrus, being classed as a

tropical group, require temperatures of between 10 and 20ºC to promote floral initiation (Sedgley and Griffin 1989) – low in that climate. Olives, warm temperate plants and thus between the zones of sub-tropical and cool temperate, require chilling in order to flower [Hackett and Hartman 64]. Despite occurring in a subtropical zone, it is probable that macadamia flowering is influenced by its genetic origins as a tropical plant.

The number of inflorescences emerging in macadamia varies with temperature. In 1982 Sakai *et al.* reported that more inflorescences emerged from macadamia trees kept at 18 ºC during night than from trees kept at 15 ºC, and even fewer emerged from trees kept at 12 ºC overnight. Almost none emerged from trees kept at 21 ºC. All trees were exposed to ambient temperatures and light during the day These regimes were maintained for 31 weeks, and so probably encompassed both induction / evocation as well as inflorescence development.

At the end of the trial, after 31 weeks, new inflorescences were still emerging from the 18ºC treatment but not the 12 ºC. The authors concluded that the development of inflorescences was slower at this temperature. Alternative explanations could be that at 18 ºC either new inflorescences continued to be initiated for longer, or that there is a chilling requirement in the macadamia flowering process that was satisfied earlier at 12 ºC. A combination of these mechanisms may also be occurring.

Macroscopic growth rates of macadamia inflorescences do not appear to relate to average temperatures in the range of 11 to 20ºC – inflorescences developing at average temps of 20 and 15 ºC both resulted in faster growth than those at 17.5 ºC, the latter rate being very similar to that of 12.5 ºC (Stephenson and Gallagher 1986).

Stephenson and Gallager (Stephenson and Gallagher 1986) found very few inflorescences emerged from macadamias kept at the night temperatures of 5, 10, 15 and 20 ºC for 12 weeks. However when all the trees were transferred to ambient temperatures averaging 11 ºC at night, large numbers of inflorescences emerged from the trees formerly in the 20 ºC treatment by 20 weeks (190/tree, with 250/tree by 21 weeks). A small number if inflorescences continued to emerge from the other treatments (around 25/tree and 35/tree at 20 and 21 weeks). There are two possible explanations for this result. The first is that the trees were induced or meristems evoked or inflorescences initiated at 20ºC, but not at the other temps. Following this, the differences became visible when inflorescences were cued to emerge by the drop in temperature to ambient conditions. The second explanation is that a sudden drop in temperature induced the trees to flower. Night temperatures in the heavily flowering group fell around 10 degrees. Night temperatures in the other groups fell around 15 or 5 ºC, or rose 5 ºC. Day temperatures of all groups went from 25 ºC to an average of 21 ºC. (In all scenarios, the small number of inflorescences emerging from trees in all treatments before return to ambient conditions, and from trees in the three groups from the cooler treatments after return to ambient conditions, (around 20/tree in all cases,) would have been initiated before the experiment began.)

In the experiment conducted by Sakai et al (Sakai *et al.* 1982) inflorescences became visible earlier at lower temperatures. The first inflorescences emerged from the plants at 12 ºC around week 9, as opposed to week 18 in the 18 & 21 ºC treatments. By 20 weeks the number visible was greatest in the 18 ºC treatments, suggesting that

initiation is greater at 18 ºC but rate of development greater at 12. This fits with the first explanation of the results from Stephenson and Gallager's work (Stephenson and Gallagher 1986).

A field study about temperature and flowering in macadamia utilised pruning at weekly intervals from mid-February to early March (Olesen 2005). The pruning reset flushing cycles, so that axillary buds were released in groups approximately a week apart. From mid-April to late October the regular release of bud cohorts was monitored to determine the number of inflorescences produced. Trees pruned from mid- to late-February completed two vegetative flushes before flowering well from buds that swelled mid-July to early August. Trees pruned in early April flushed once vegetatively and then flowered well – that is, produced racemes on a large proportion of pruned branches - from buds that swelled in mid-July. Trees pruned around mid-March did not flower at all, undergoing three vegetative flush cycles, with buds swelling in one of those cycles from late May to mid June (with the subsequent swellings in early October). Trees pruned between the dates giving good flowering and no flowering had a poor flowering, with buds swelling late June / early July or mid-September. It was concluded that when cool temperatures – an average minimum for the month of 6.3 ºC, in this location in late July or early August - coincide with early bud growth, flowering is abundant. Otherwise it is poor. It was noted that heavily flowering trees had bud swell around the time of the winter solstice, but no comment on a link between day length and flowering was made by the authors, possibly because flowering did start, albeit weakly, in buds swelling a month before that date.

Moncur (1985) noted inflorescences just visible behind bracts resumed growth at the same time as a rise in temperature. Growth resumed earlier in cooler areas, again suggesting a possible accumulated chill trigger.

Diurnal range of temperature can affect rate of inflorescence development in olive (Badr and Hartmann 1971), the rate increasing with wider range. Drops in night temperature which effect macadamia flowering may be doing the same, if day temperatures are steady.

Light

Light is another environmental parameter that cues many plants to begin the flowering process. Both daylength and light intensity are known to influence this (Taiz and Zeiger 2006; Scott 2008). Daylength is often at least partially responsible for the start of flowering in perrenials, but in woody perennials photoperiodic control of flowering not common (Sedgley and Griffin 1989). It is known to occur within the Proteaceae family, in *Leucospermum patersonii*, which has been found to flower in response to a drop in either temperature or day length. There have been no controlled studies in macadamia on involvement of photoperiod in induction (Storey 1985), but floral induction seems to have been achieved at both short and long daylengths of 10 h 45 m and 14 h of natural light (Sakai *et al.* 1982; Stephenson and Gallagher 1986). Story (1985) judges there is no apparent effect of day length on macadamia induction in nature.

Light intensity may also play a part in control of flowering in many species, including apple and olive trees (Wilkie *et al.* 2008). However induction effects can be confused with effect on local carbohydrate levels. No studies have been conducted exploring this relationship in macadamia. Low light intensity in the centre of macadamia trees is known to lead to the loss of leaves and flowering activity, but as this loss is confined to that part of the canopy in heavy shade it would not be via floral induction effects. Probably this it is a result of lack of local production of photosynthate []. Shading of individual branches for seven months did not reduce flowering on those branches (Stephenson unpublished). In Leucospermum, Wallerstein found light intensity played a role in floral development, but not bud fate (Wallerstein 1989).

Nutrients

Stepehenson and Gallager (Stephenson and Gallagher 1989) found that low levels of nitrogen in macadamia trees resulted in fewer visible inflorescences. This could be due to reduced development of inflorescences post dormancy [(Taylor 1969)]

Water

Water stress can promote flowering in tropical and sub-tropical trees such as lychee and citrus (Sedgley and Griffin 1989). In macadamia it has been reported that inflorescences resumed growth following rain and a rise in temperature (Moncur *et al.* 1985), but no work has been published on water availability and floral induction. If induction involves bud release from dormancy, it may be that water is involved via its basic role enabling growth and even metabolic processes in quiescent cells. In other tropical / sub tropical species including citrus, lychee and mango , slowing of growth or a period of ecodormancy, possibly water stress induced, can be a prerequisite to floral initiation (Sedgley and Griffin 1989).

Control of flowering in Arabidopsis is known to be through a network of biochemical pathways that integrates a number of environmental and internal factors (Wilkie *et al.* 2008). While temperature currently appears to be the only environmental factor effecting macadamia induction, it would seem logical that a network which can take into account other factors would be beneficial and thus likely to be present in macadamia.

A final factor which may contribute to determining new axis identity is not environmental (or not directly). (Crabbe 1984) proposed that floral buds are those that break from dormancy slowly, whereas those that have a greater rate of growth at that time will be vegetative. However it is not clear whether this is a merely a correlation or if experimental studies have determined growth rate to be the cause and axis type to be the effect (not *visa versa*). It is also possible that the two are both determined by a third factor. It is known that some released buds break from dormancy more slowly than others, probably depending on stage of the division cycle the cell was in when dominated, and how sensitive to auxin that bud is [cline, stafstrom?]. It would be important to differentiate between this aspect of growth speed and general vigour when evaluating the above theory.

Axillary bud dormancy

Many plants posses so many axillary buds that simultaneous and equal development of all would divide the available energy and nutrients into amounts per branch too low to sustain health. Thus many axillary buds remain dormant after being formed, and are only re/activated when needed. The need for bud re/activation may be to fill a void left by stem elongation or damage, or to form determinate organs such as thorns and flowers. The lifespan of dormant axillary buds varies, but many will die before being activated (Stafstrom 1995).

Lang's (1987) definition of dormancy and terminology surrounding it seems to have been adopted by the field. Thus here dormancy will likewise be used to mean a temporary suspension of macroscopic growth of predominantly meristematic structures. Importantly, it does not imply lack of activity on a microscopic scale – microscopic - biochemical activities are often continuous in dormant structures (Lang 1987). Dormancy may be brought on by a growth limiting environment – termed ecodormancy - or by factors within the plant. It can be controlled by the dormant organ itself – endodormancy - or can be controlled by another part of the plant – paradormancy. As noted by (Olsen 2003), there is often overlap between these different types of dormancy – when a growing apex is transformed into a bud at the onset of winter, it is the environment that causes changes within the apex that trigger dormancy i.e. this would be a case of ecodormancy effected via endodormancy.

There is a network of different types of control over dormancy, including apical dominance, drought dormancy, and winter dormancy, that probably interact to a certain extent (McSteen and Leyser 2005). It is thought that not only may the strengths of these factors imposing dormancy vary, but that the sensitivities of buds to these triggers may also differ.

Types of dormancy

Phenologically regulated

Apical dominance

Apical dominance is "the control exerted by apical portions of the shoot over outgrowth of lateral buds"(Cline 1991). Apical dominance is thus the apex keeping buds in a type of paradormancy. Many plants exhibit partial bud inhibition due to apical dominance – in these very slow outgrowth of axillary buds can be detected (Cline 1997). It is important to note that "outgrowth" includes only the initial growth of the bud when it is released from dormancy, and not latter growth which is mere elongation of an already independent young branch (Cline and Harrington 2007). The apex does still influence growth once a bud becomes a branch, but it is termed apical control not apical dominance.

The mechanism by which apical dominance is achieved has been at least partly uncovered. It is clear that auxins produced in the apex play a major role in inhibiting buds located further down the stems of most plants studied, including woody species (Cline and Harrington 2007). Cytokinins produced mainly in the roots promote breaking of axillary bud dormancy. The ratio of auxin to cytokinins appears to be important in determining bud dormancy or activity [?(Klee, 1991)]. Thus the term

apical dominance is perhaps slightly misleading as it disregards the balancing role of the roots.

Roles for other hormones - ethylene, giberrellic acid, abcisic acid – in apical dominance have been investigated but it seems unlikely they are significant (Cline 1991). Recent work had found that the effect of auxin on buds is via a second hormone, strigolactone, which travels up stems from junctions with branches in which auxin is travelling down (Brewer *et al.* 2009). This filled a gap in the process known for some time, namely that auxin from the apical meristem can not enter buds (McSteen and Leyser 2005). Another fact not explained by the basic auxin:cytokin system above is that the presence of auxin does not always effect apical dominance. An explanation of this by [] is that it is movement of auxin through the [vascular cambium (polar auxin transport stream)] that is a part of the communication chain in apical dominance.

***- canalisation

Some theories about mechanisms behind apical dominance still cannot account for all the observed facts. On example of this is the finding by [? Morris *et al.* (2005)] that after decapitation bud outgrowth can be detected before changes in auxin concentrations or transport occur. Such gaps and apparent contradictions in the understanding of apical dominance may be due to differences in methodology used by different researchers. Many studies on apical dominance have used removal of the apex as a methods of studying it's effect. Dun *et al.*(Dun *et al.* 2006) however pointed out that decapitation effects may be different to the effects of auxin reduction in intact plants. Thus genetic modification of plants to alter the endogenous concentration of hormones has become an important tool in investigation of apical dominance of intact plants .

The strength of apical dominance can vary within a plant due to environmental conditions. Reduced light levels increase its strength, and far-red light, the wavelengths transmitted by green leaves, has the same effect. Nutrient availability also effects apical dominance (Cline and Harrington 2007). The strength of AD also varies with internal factors of the tree: florally competent trees have weaker apical dominance than non-reproductive ones, and older trees have weaker apical dominance than younger. Position of buds within a tree and a stem can also influence the effect of apical dominance - larger buds can be quicker to grow out of auxin sensitivity than smaller ones and size of buds can vary with position along a branch

***- substantial variation between ages and between species (Cline and Harrington 2007), *so could expect the same between woody and herbaceous and between temperate and tropicsl*

Information on apical dominance in macadamias is a result of studies on pruning. The timing of axillary bud release from dormancy after pruning is dependent on the maturity of branches and leaves produced in the previous flush: release times were faster when the recent flush was younger, and therefore had a slower?? growth rate (Olesen *et al.* 2006).

Bennel (1984), in a study of vegetative and floral growth of macadamia, noted only the top two buds in a series develop. Dominance of buds in a series over each other has been observed in the tree *Cercis candensis* (Fabaceae) (Owens and Ewers 1997), and may be simply due to apical dominance. Whether inflorescences, as modified branches, can dominate, and by what mechanism, is yet to be investigated.

Fruit

Tamas *et al.* (1979) found that the presence of fruit on pea plants could hold axillary buds dormant. They investigated increased levels of ABA that ocurred at the same time, but this regulator of established branch growth had no effect on number of buds released.

Carbohydrates

Research by [] suggests that the extent of flowering may also be subject to availability of carbohydrate resources within the tree. This is most likely to act through development of inflorescences and flowers to a macroscopic size in times of ample reserves, and abortion of some floral organs when less energy is available.

Environmentally dependent

Ecodormancy probably also effect macadamia axillary buds. The presence of nutrients, including water, may also affect dormancy and outgrowth of axillary buds. This may interact with auxin as a controller of nutrient transport. Changes in orientation of a branch can result in shoot production from buds that otherwise would have stayed dormant – this is thought to be detected as a change in the direction of the force of gravit but could also be related to shadingy. Light is also known to influence the breaking of axillary bud dormancy. In grasses, it has been shown that shade reduces the number of axillary buds breaking dormancy. This is probably mediated by phytochrome B (Kebrom *et al.* 2010). No clear results have been published about the affect of light on woody dicots. (Broomhall 2009) found that fewer macadamia buds were released from dormancy to form branches when light was reduced to 10% incident sunlight.

Bud activity during dormancy and release

Biochemical studies have shown that the absence of macroscopic growth during dormancy is not necessarily accompanied by an absence of sub-cellular activity. In peas, dormant axillary buds are just as metabolically active as transitioning and growing buds [(Stafstrom and Sussex 1988)]. Thus it is possible that they can perceive induction signals before outgrowth. Some of the proteins formed in dormancy were unique to that stage of the meristems existence, while others were the same as those produced during periods of visible growth.

Stafstrom and Sussex (1988) also established the sequence of events that occur following decapitation of pea shoots, before growth is visible. Within minutes of decapitation detectable biochemical changes in the cell membrane include accumulation of potassium, starch accumulation and greater adenine-triphophotase activity . Increases in mRNA coding for ribosome components were detected within

an hour. New types of proteins are expressed within 3 hrs. Also within hours, DNA synthesis begins. Growth is microscopically detectable within 6hrs. By 8 hrs the types of protein manufactured have changed again, perhaps indicating a second stage to the transition between dormancy and independent branch-hood. By 24 h the suite of proteins expressed is the same as mature shoots. Cell division takes 12 hours to around 1 day. After 24 hours pea buds have doubled in length (from (0.1-1.7mm to 0.2-3.4mm). This growth may not be visible without removal of the bud bract. Within 72hrs the largest (of the four in each node) has inhibited the smaller ones and the latter have reverted to dormancy. These buds can be dominated then released the re-dominated many times over, each time growing just a little (Stafstrom and Sussex 1988).

(Batten and McConchie 1995) lychee and mango apical buds exhibiting macroscopic growth are still able to be induced – some forming completely normal inflorescences (check!). some forming partly leafy inflorescences –generally those that were larger at time of exposure to induction stimulus – thus floral stimulants do not have to be present upon release from dormancy, or at transition (biochemical preparation for growth and microscopic growth).

(Batten and McConchie 1995) also found that some dormant (apical) buds formed inflorescences and some formed branches when exposed to induction stimulus at the same time as being released by pruning. This may be because 'dormant' includes all those not visibly growing, but some may have already been released and committed to vegetative growth but were not yet large enough to be seen outside of the bracts (due to very recent determination)

Summary and implications for this project (conclusion)

To understand the control of dormancy and evocation could enable manipulation of both branching and flowering. Key components of this understanding remain vague for *Macadamia integrifolia* and *M. tetraphylla* :

- Evocation of axillary buds, and thus is probably induction of the tree, appears to be affected by temperature, but no microscopic studies of this have been carried out to separate evocation form inflorescence development.

- Dormancy of axillary buds seems partially linked to the flushing cycle, but also to location on the flush length.

- Year-round microscopic examination is required to observe when evocation occurs in nature, but this will be highly inefficient unless the location of inflorescence production can be predicted. Current knowledge of flowering location does not enable this.

- While dormancy refers to lack of visible growth, axillary buds of trees in sub-tropical climates are likely to be biochemically active throughout their existence.

The relationship between axillary bud dormancy and evocation is unclear. It seems logical that in the transition out of dormancy that all buds of all species must go through biochemical changes before visible growth, and so it is possible that evocation can occur during dormancy or transition out of it, or possibly immediately after

transition. If bud can only be evoked when released from dormancy, this provides an explanation as to why all axillary buds do not form inflorescences. However the distribution of inflorescences seems unrelated to the distribution of branches, so even if this were the case, the control of location still involves more layers. Another possibility is that evocation a different type of release from dormancy altogether – not merely the product of generic release plus evocation/commitment.

Chapter 2. Effects of order of exposure to temperature on flowering and branching in macadamia

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Abstract

Effects of the sequence of exposure to warm and cool temperature regimes on macadamia axillary bud behaviour were investigated, to increase understanding of this tree's flowering and branching processes. Three-year-old trees were exposed to sequences of day / night regimes of 25 / 19 ºC ("warm") and 19 / 11.5 ºC ("cool") in controlled environment chambers, beginning after the spring flowering. Each exposure period was two months long, and four sequences of two periods each were investigated: cool-then-cool, cool-then-warm, warm-then-cool, and warm-then-warm. Tip pruning was used to increase number of shoots. After each exposure period the numbers of inflorescences and vegetative stems emerging were counted, and the proportion of trees branching and flowering was calculated. Over the whole four months, there was no difference between warm-then-warm and cool-then-cool treatments in the numbers of inflorescences emerging, but more emerged from the warm-then-cool sequence than the cool-then-warm sequence. These results suggest that two stages may exist in inflorescence development, the first favoured by warm temperatures and the second favoured by cool, supporting the hypothesis of Sakai *et al*. (1982) that macadamia floral evocation is maximised by warmer night temperatures and emergence by cooler ones. Trees in the warm-then-cool sequence also produced more new stems than those in the cool-then-warm sequence, although there was no accompanying difference in proportion of trees branching. More buds becoming inflorescences in a treatment group did not mean fewer buds becoming stems, which suggests that different buds become inflorescences to those that become stems.

Introduction

Commercial orchards of macadamia nut trees (*Macadamia integrifolia* Maiden & Betche and *M. tetraphylla,* and hybrids of the two) are planted densely in order to increase profits early in the life of the orchard. These densities necessitate pruning later in the life of the orchard (hedging, topping and skirting, carried out mechanically) to enable machinery access. As a result of the pruning, many axillary buds are released from apical dominance, growing out to form a thick outer layer of regrowth, shading the tree's interior and eventually reducing flowering and yield (Olesen *et al.* 2011). If bud outgrowth from dormancy could be better understood, self shading or inhibition of flowering may be mitigated, and the macadamia industry and consumers would benefit from more efficient management of orchards for optimum sustainable nut production.

In their natural geographical distribution - the sub-tropical coastal hinterland of eastern Australia - macadamia trees grow mostly during synchronised bursts of tip elongation and axillary bud release, termed flushes. Similar to lychee (*Litchi chenensis)* and avocado (*Persea americana*) (other sub-tropical perennials), macadamia flushes occur at regular periods, several times a year (Stephenson and Trochoulias 1994; Oleson *et al.* 2002; Olesen 2005). The trees are evergreen and there is no phase of whole-plant dormancy enforced by conditions prohibitive to growth, such as freezing or dehydrating.

Both vegetative and floral shoots arise directly from axillary buds. The term shoot is here used to describe the growth arising from just one axillary bud, and not from the secondary axillary buds found along its length. Vegetative shoots are termed stems and floral shoots are the backbone of an inflorescence. The number of new shoots (either vegetative or floral) forming in macadamia during any one flush has been shown to be affected by temperature. At mean temperatures of 16 ºC to 26 ºC, the number of stems emerging per day increased with temperature in orchard macadamias (2009). This is similar to poinsettia (*Euphorbia pulcherrima*) and beans (*Phaseolus vulgaris*), which respond to increased temperature with increased branching (Hagen and Moe 1982; Kigel *et al.* 1991). In other species the relationships are less clear - in chrysanthemums (*Dendranthema grandiflorum*) and apple trees (*Malus domestica*) no consistent trend relating branching to temperature was found (Abbas *et al.* 1980; Schoellhorn *et al.* 1996).

It is not clear when macadamia buds are first evoked, i.e. set on the biochemical path to forming floral growth. However immature inflorescences become visible as the bud bracts open in autumn or winter (Bennel 1984; Moncur *et al.* 1985). In mid- to late winter they emerge from behind these bracts and elongate to 15 cm or more, before anthesis in late winter or early spring. Cool temperatures will result in emergence of inflorescences from macadamia buds if they coincide with the phase of the phenological cycle when flushing is beginning (Olesen 2005).

Other sub-tropical species are known to use temperature as a cue for the beginning of flowering. In lychee, mango (*Mangifera indica*) and avocado, cooler temperatures of the annual range in which they are grown are needed for inflorescences to be produced (Buttrose and Alexander 1978; Shu 1987; Menzel and Simpson 1988; 1995). In citrus, "Washington Navel" variety oranges produce more inflorescences at lower temperatures, within the range 15/8 ºC to 30/25 ºC days/nights, but day length seems also to influence flowering time (Lenz 1969; Moss 1969; Garcia-Luis *et al.* 1992). In lemon, temperature seems to have only a limited effect on flowering and water stress seems the main trigger for floral growth (Chaikiattiyos *et al.* 1994).

Glasshouse studies of the relationship between temperature and flowering in macadamia suggest that temperature affects inflorescence formation in more than one way. Sakai *et al.* (1982) investigated the effect of temperature on the number of inflorescences and the speed of their production. They treated macadamia trees with night temperatures of 12, 15, 18 or 21 ºC, while exposing all to ambient temperatures of around 28 ºC during the day. No statistical analysis of their data was published, but they noted a number of interesting trends. They reported that inflorescences emerged sooner at lower night temperatures, but that the total number of inflorescences emerging in the 31-week-long experiment appeared to increase with night temperature

up to 18 ºC. Very few inflorescences were produced at 21 ºC, and microscopic observations found no floral differentiation of buds in trees at night temperatures of over 21 ºC. The authors put forth two linked hypotheses; i) warmer night temperatures, up to 18 - 21 ºC, favoured evocation, while ii) cooler night temperatures favoured emergence.

Stephenson and Gallagher (1986) found that macadamia trees exposed to night temperatures of 5, 10, 15 or 20 ºC did not differ in numbers of inflorescences emerging over 10 weeks. However, at the end of the experiment the trees were transferred outside to night temperatures of around 11 ºC, and subsequently trees in the group that had been at 20 ºC produced around 5 times the numbers of inflorescences than those of other treatments. The difference was obvious within seven weeks after transfer. These findings fit well with Sakai *et al.*'s (1982) theory (above).

This study set out to make a direct comparison of the effects on macadamia flowering of warm temperatures, cool temperatures and different sequences of the two, following up on Sakai *et al.'s* 1982 theory that warm temperatures favour evocation and cool temperatures favour emergence. This comparison was made by exposing trees to warm or cool temperatures or both, over two exposure periods each two months long. As emergence must be preceded by evocation, if these two processes do have different optimum temperatures, then the sequence in which trees experience warm and cool temperatures should affect the number of inflorescences produced – the hypothesis is that a different order of exposure to the temperature regimes will result in different numbers of inflorescences emerging. Our study aimed to investigate whether temperature regime sequences affect the number of axillary buds forming new stems. As axillary buds that form inflorescences are no longer available to form stems, it may be that conditions resulting in trees forming a greater number of inflorescences would also result in fewer new stems. Clarification of these relationships between temperature and macadamia growth and flowering would assist commercial growers and managers of threatened wild populations to balance vegetative and floral growth for most efficient nut or seed production, as well informing where and how to adapt to climate change.

Materials and Methods

Fifty-six three-year-old *Macadamia integrifolia x tetraphylla* trees, grown from cuttings for three years in an outdoor nursery in south-east Queensland, were transplanted into pots, 33 cm diameter and 35 cm depth, of sandy-loam pot media in spring 2009. Half of the trees were cultivar A38 and half were cultivar A4. The trees were about 150 cm tall. At the end of the flowering season in early spring, they were moved into a controlled environment facility with day / night temperatures of 26 / 23 ºC to prevent floral evocation (Sakai *et al.* 1982). Three walls of each controlled environment chamber and their roofs were made of translucent plastic, and together with overhead shade-cloth these resulted in the trees receiving light at 60% of outdoor levels. Inflorescences and young fruit were removed, to prevent their inhibition of any new growth. The trees were kept at this temperature for two months and the few new inflorescences emerging during this time were removed.

"Night" temperatures in the glasshouse were set for eight hours during dark, "day" temperatures were set for eight hours during light, and transitions from day to night temperatures and back again were gradual over the intervening four hours. They received one litre of water – sufficient to saturate the media - up to three times per week, when the media surface and saucer were both dry. Each tree received 20 g of low-phosphate slow-release fertiliser every three months.

After the two-month pre-treatment, trees were exposed to one of four treatments, each a sequence of temperature regimes; cool-then-warm (C-W), warm-then-warm (W-W), cool-then-cool (C-C), and warm-then-cool (W-C). This was achieved by exposing half of the trees to warm temperatures and half to cool temperatures for two months, then half of each of these groups stayed under their initial temperature regime and the other half were exposed to the other temperature regime for two months. Four months was chosen as the total experiment duration as this was sufficient for effects of temperature on flowering to be seen in previous experiments (Sakai *et al.* 1982; Stephenson and Gallagher 1986).

At the beginning of the first two-month exposure period an equal number of trees of each cultivar was randomly allocated to either the cool (C) temperature regime - 19.0 / 11.5 ºC - or warm (W) regime - 25.0 / 19.0 ºC. Initial sizes were approximated by summing the length in centimetres of the most central (leader) axis and the secondary axis arising from this. Differences were examined using a Mann-Whitney test. A4 trees had greater initial stem lengths than A38 trees, with means of 645 and 421 cm respectively. This difference in size was highly statistically significant ($P < 0.001$). Each regime was maintained in its own chamber of the building. Trees and temperature settings were swapped between chambers every two weeks to ensure even exposure of treatments to any undetected differences between the apparently identical chambers. At these times, positions of trees within each chamber were re-randomised.

Trees were pruned at week five of the first exposure period, to encourage growth of more new shoots (either stems or inflorescences) to increase statistical clarity (Batten and McConchie 1995). The leader and every second first-order branch was pruned, one growth unit back from the tip, where a growth unit is the portion of a stem created by one continuous period of tissue production by the apical meristem, usually during a spring or summer flush. Typically it consists of about four nodes. This pruning removed any green stem and new leaves towards the end of the parent shoot as well as the apical bud, all of which contribute auxin to maintain apical dominance, which prevents the outgrowth of axillary buds (Warner and Gitlin 1971; Cline 1991).

At the end of the first two-month period, the number of new shoots emerging from each tree was counted. Every inflorescence on each tree was counted, while only stems on the main leader and the first-order branches were counted (around 1/3 of the tree). Small shoots counted at the end of this first period were marked to ensure they were not also counted at the end of the second period. Any tree from which one or more inflorescences emerged was classified as "flowering". Any tree from which one or more new stems emerged was classified as "branching".

The trees were then exposed to the second two-month period of temperature treatment. The leader and every first-order branch were pruned, again at five weeks into the treatment period, one growth unit back from the tip.

Glasshouse chambers' temperatures were each monitored with a pair of "Tiny Tag" brand electronic temperature recorders, each accurate to 0.5 ºC and programmed to record every 15 min. Eight instances of departure from set temperatures reduced the temperature differences between treatments by 2 - 5 ºC, for periods of up to eight hours. None of these departures occurred on consecutive nights and most occurred more than a week apart.

At the end of the second exposure period a large cohort of swollen buds, not yet identifiable (by eye) as either vegetative or floral, was noticed. No such cohort of swollen buds was detected at the end of the first exposure period. The trees were kept outside for six weeks, where temperatures averaged 24.6 / 14.7 ºC, until shoots could be identified as stems or inflorescences, at which time the new shoots were counted and classified as in the first count detailed above. This second count included only shoots that had emerged since the end of the first period.

The effect of temperature regime sequence on both the proportion of trees flowering and the number of inflorescences emerging was analysed. Also, the effect of temperature regime sequence on both the proportion of trees branching (forming new stems) and number of new stems emerging was analysed. Normality of datasets was investigated using Anderson-Darling tests, with non-normal data being transformed (see regression below) or analysed with non-parametric methods.

Differences in the proportion of trees producing new shoots under different treatments were analysed using Pearson chi-squared tests. Differences in new shoot numbers between cultivars were analysed with Mann-Whitney tests. Differences in second period new shoot numbers between either first or second period temperatures were analysed using Mann-Whitney tests. Differences in total new shoot numbers between temperature sequence treatments were examined using ordinal logistic regression, with initial tree size included as a predictor. For inflorescences, regression analyses used a complementary log-log link function, while for stems a probit link function was used. Correlations between stem numbers and inflorescence numbers were investigated using Pearson's coefficient. Effect of temperature on new shoot numbers within cultivars or treatments were examined using Mann-Whitney tests (for comparisons between two groups) or Kruskal-Wallis tests (for comparisons between more than two groups). $P < 0.05$ was used as the indicator of a significant relationship in all analyses. (Fowler *et al.* 1998; Minitab 2007)

Results

Inflorescences

First period: During the first two-month period, more trees flowered in the warm (W) treatment than in the cool (C) treatment (Figure 7, Table 1). There was also a greater number of inflorescences per tree emerging from trees under the W regime. There was no difference between cultivars in the number of inflorescences, and no correlation between tree size and number of inflorescences (data not shown).

Second period: The proportion of trees that flowered in this period (Figure 7) depended on the previous period's temperature, as well as the current period's

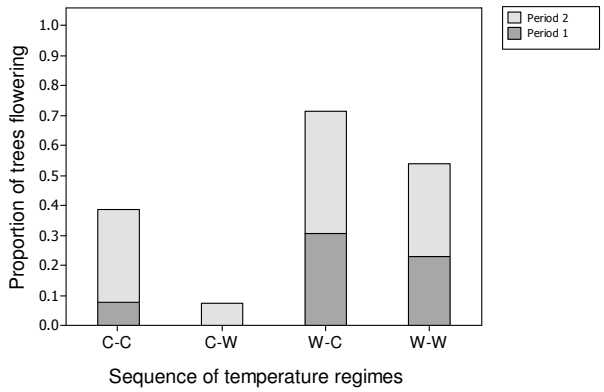

Figure 7. Cumulative effect of temperature regime sequence on proportion of trees flowering over both exposure periods.

Table 1. Effect of the first period of exposure to either cool or warm temperatures on the number of new shoots and proportion of trees shooting in that period.

Temperature regime	**Trees flowering**	**Trees branching**	**Inflorescences emerging per tree**	**Stems emerging per tree**
	(proportion)		(median, *25th-75th percentile*)	
C	0.04	0.78	0 *0 – 0*	7 *1 – 10*
W	0.28	0.88	0 *0 – 1*	8 *3.75 – 18*
P of difference between C and W	0.018	0.010	0.019	0.093

More trees flowered in this second period after being exposed to warm temperatures in the first period (W-C together with W-W) than after being exposed to cool (C-W with C-C). Second period temperatures had the opposite effect, with more trees flowering under cool temperatures in this period (W-C with C-C) than those under warm temperatures (W-W with C-W).

Table 2. P values of effects of first and second period exposure to either warm or cool temperature on second period growth.

Period of exposure to cool or warm	***P* values of effect of temperature on second period growth**			
	Trees flowering	Trees branching	Inflorescences emerging per tree	Stems emerging per tree
First	0.007	0.700	0.027	1.000
Second	0.032	0.186	0.044	0.085

Table 3. *P* values of differences between temperature sequences in proportion of trees branching or flowering and number of inflorescences or stems per tree, over the combination of both periods

Treatments compared	**Trees flowering**	**Trees branching**	**Inflorescences emerging per tree**	**Stems emerging per tree**
C-C and W-W	0.561	1.000	0.800	0.280
C-W and W-C	0.000	[Insufficient variation to test]	0.000	0.001

The number of inflorescences emerging during the second period (Figure 9) was different between every pair of temperature sequences except W-W and C-C

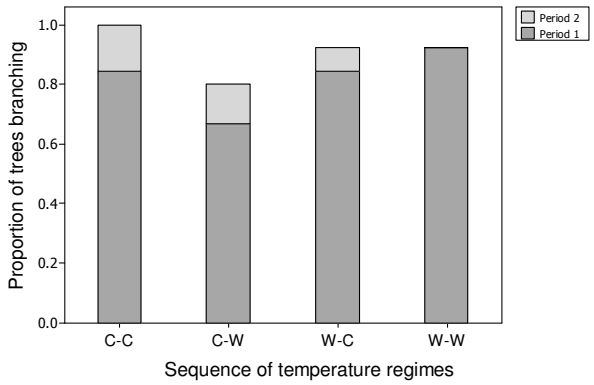

Figure 8. Cumulative effect of temperature regime sequence on proportion of trees branching over both exposure periods.

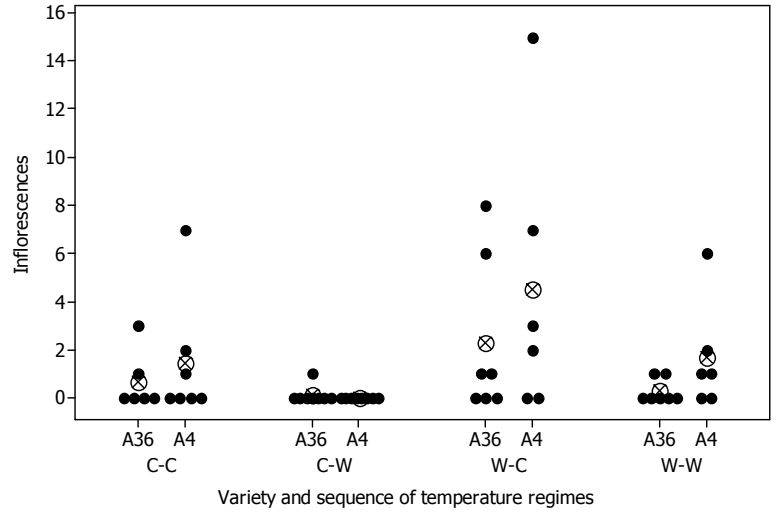

Figure 9. Effect of temperature regime sequence and variety on the number of inflorescences emerging during the second exposure period.
Dots are individual values (trees), crossed circles are means.

More emerged from trees exposed to the warm regime during period one (W-C and W-W) than trees exposed to the cool (C-C and C-W). More emerged from trees exposed to the cool temperatures in the second period (C-C and W-C) than trees exposed to the warm (C-W and W-W).

There was no correlation between plant size and inflorescence number, either overall or within temperature regimes (data not shown). There was no difference between cultivars in numbers of inflorescences emerging during the second period (Figure 9).

Combination of periods: The proportion of trees flowering in total i.e. over the combination of both periods (Figure 7), did differ between temperature sequences.

The number of inflorescences emerging in total (Figure 10) was also different for every pair of regimes except C-C and W-W (

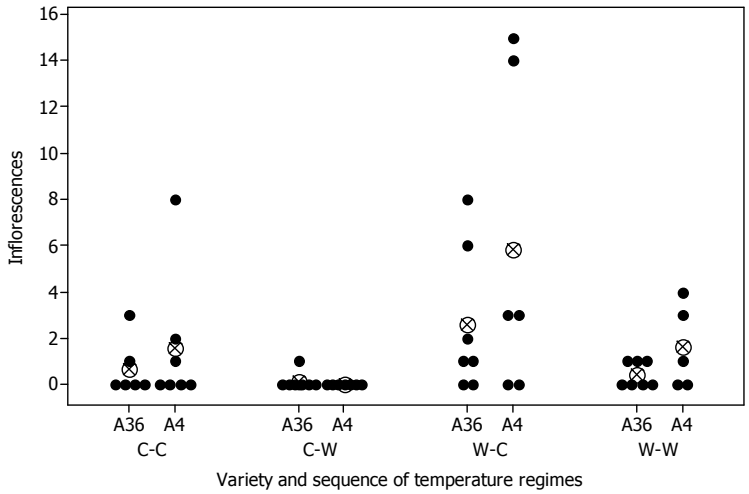

Figure 10. Effect of temperature regime sequence and variety on number of inflorescences emerging in total over both exposure periods. *Dots are individual values (trees), crossed circles are means.*

There was a positive correlation (P=0.009) between tree size and number of inflorescences emerging in the W-W treatment (data not shown), but not in any other treatment. There was no difference between cultivars in the number of inflorescences emerging (Figure 4).

Relationships between first and second period inflorescence emergence: A correlation between the number of inflorescences emerging from a tree in the first period and that emerging in the second period was not detected, over all treatments or in any one treatment.

Stems

First period: After the first period, there was no difference between treatments in the proportion of trees branching (Figure 8, Table 1). There was also no difference in the number of new stems emerging per tree. There was no correlation between the number of stems emerging and the initial size of the trees, either overall or within treatments. Cultivar made no significant difference to the numbers of new stems produced.

Second period: The proportion of trees branching in the second period (Figure 8) was not dependent on temperatures experienced in the previous period, nor was it dependent on temperatures experienced in the second period. The number of new stems (Figure 11) was also similar between trees with different first period temperature regimes, as well as between trees with different second period regimes.

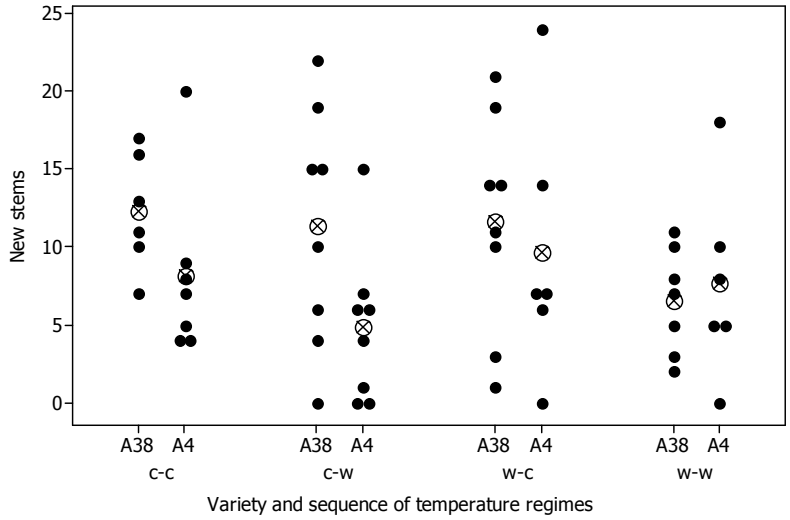

Figure 11. Effect of temperature regime sequence and variety on number of new stems emerging during the second period. *Dots are individual values (trees), crossed circles are means.*

There was a difference between numbers of stems emerging in this period from trees of different cultivars (P= 0.040), with a median of 10 emerging from A38 and of 6 emerging from A4.

Combination of periods: When flowering data for both periods was combined there was little difference between temperature sequences in the proportion of trees branching (Figure 8).

There was a positive correlation between tree size and number of new stems emerging in group W-C (P= 0.021), and a negative correlation between size and number of stems emerging in C-W (P= 0.005) (Figure 13). Temperature sequence and size interacted to effect the number of new stems over the combination of periods (*P*= 0.004) (Figure 12, Figure 13). Fewer stems emerged from C-W than from W-C. The W-W stem emergence was no different to that of C-C.

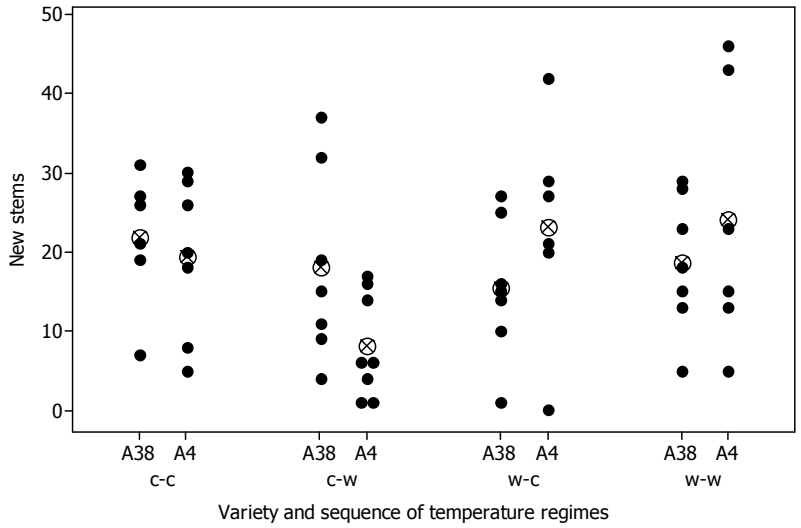

Figure 12**.** Effect of temperature regime sequence and variety on number of stems emerging in total over both periods. *Dots are individual values (trees), crossed circles are means.*

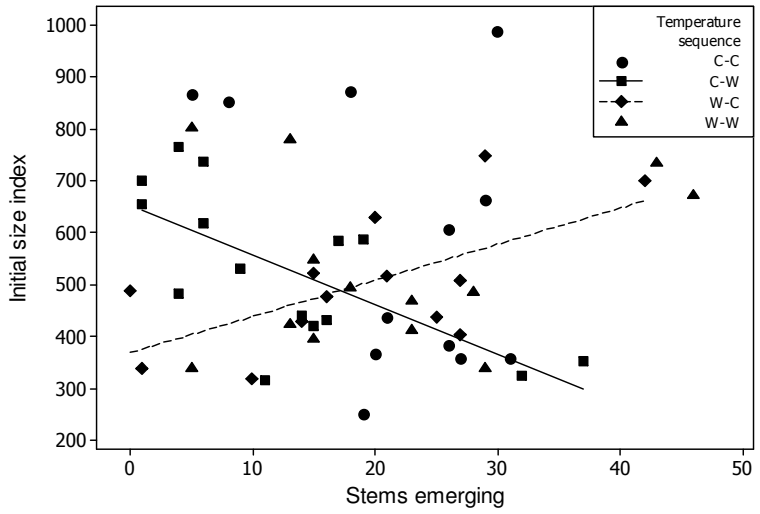

Figure 13. Effect of temperature and size on total stems emerging over both periods. *Initial sizes were approximated by summing the length in centimetres of the most central (leader) axis and the secondary axes arising from this.*

There was no significant difference between the numbers of stems emerging from trees of different cultivars (Figure 12).

Relationships between first and second period stem emergence: The number of stems emerging in the first period from a tree under any temperature sequence treatment was not correlated with the number in the second period, over all treatments or in any one treatment.

Relationship between vegetative and floral growth

There was no correlation between the number of emerging stems and numbers of emerging inflorescences within either period or overall. Neither was there any correlation within any of the treatment groups.

There was no overall correlation between numbers of stems emerging in the first period and the number of inflorescences emerging in the second period, or vice versa. Only trees in the C-C group had a correlation (P= 0.032) between new stem numbers in the first period and inflorescence numbers in the second (data not shown).

Discussion

Flowering

The order of exposure to warm and cool temperature regimes affected flowering, as over the combination of treatment periods a greater number of inflorescences emerged from trees in the W-C sequence than C-W, and a greater proportion of trees flowered in W-C than C-W. This is despite the W-C treatment and the C-W treatment receiving the same total degree days, over the same duration of exposure to the warm regime and the cool regime. Some trees in the W-C treatment did not flower at all. This is possibly because they were still immature and flowering-incompetent, as some of the trees in other treatments may also have been. As a result, the variation as a proportion of the mean for the CW treatment is about twice that of the other treatments.

The number of inflorescences emerging during the second period was greater when the trees had been exposed to warm temperatures in the first period. Also, cooler temperatures in the second period resulted in more inflorescences emerging during this time. These two observations added together logically support the overall difference between W-C and C-W.

The difference between C-W and W-C fits with the hypothesis that an early stage of inflorescence formation is favoured by warm temperatures and a later stage by cool temperatures. As suggested by Sakai *et al.* (1982), the early stage could be evocation and the later stage growth and subsequent emergence from behind the bracts. Dormancy between evocation or initial floral development and emergence have been well documented in Eastern Redbud trees (*Cercis canadensis)* and roses (*Rosa spp*.) (Zamski *et al.* 1985; Owens and Ewers 1997).

If Sakai's theory is correct, when cool temperatures preceded warm in this experiment, there were few buds evoked in the first period and thus few able to emerge as inflorescences during the second period. Then warm temperatures in the second period would result in a small proportion of these few evoked buds growing to become visible. This would explain the low numbers of inflorescences seen in the C-W treatment. It would also explain the high numbers seen in the W-C treatment; warm

temperatures favouring evocation would start many buds along the first, microscopic and hidden stages of floral development, and subsequent cool temperatures would result in a large proportion of those many evoked buds emerging.

An alternative mechanism that could explain these results is that trees build more reserves during warm temperatures than cool, and cool temperatures promote evocation (with emergence following without a temperature trigger). This evocation could occur in more buds of W-C trees than of C-C trees, due to the former having more energy to fuel any sort of growth that occurs. However this scenario would probably have resulted in a linear relationship between initial temperature and number of inflorescences in the experiment of Stephenson and Gallagher (1986), where as only the warmest treatment produced a sizeable number and the rest produced close to none. To determine which mechanism is behind the behaviour of macadamia axillary buds, further work investigating biochemical or microscopic morphological changes to the bud accompanying changes in temperature regime would be useful.

Trees in the W-W treatment did not produce more inflorescences overall than those in the C-C treatment. In contrast, after only two months of exposure to either a warm or a cool temperature regime, more inflorescences had emerged from the warm regime. In the second period, more inflorescences emerged from the cool regime, thus cancelling out the first period results and resulting in no difference overall. This may be due to weak evocation of the cool regime being more limiting over two months than weak emergence in the warm regime, with the evocation catching up over the second two months.

Branching

The number of stems emerging over the combination of the two periods was also dependent on the sequence of temperature regimes: more stems emerged from W-C trees than C-W. However as the magnitude of the difference is small, it may not have a substantial biological impact. The lack of difference in proportion of trees branching over the combination of periods supports further the cautious use of these results as preliminary. If a substantial difference in temperature relationships between emergence and number of trees flowering is accepted, it could be due to temperature acting in tandem with other factors to control stem outgrowth. Release of axillary buds from dormancy may have occurred in most of the trees studied here simply through tip-pruning, removing the tissues imposing apical dominance (Cline 1991). The effect of temperature may not have been strong enough to modify the response of whole trees to pruning, only the response of some axillary buds within responding trees. Such types of interaction between a number of dormancy controls are regarded as common (McSteen and Leyser 2005). In macadamia, the first flush may be cued by temperature (Stephenson and Cull 1986), but latter flushes are triggered internally and are related to the growth rate of the previous flush (Olesen *et al.* 2006). Water supply is thought to affect the number of buds emerging from macadamia, but has not been observed to control the timing of release from dormancy (Stephenson and Trochoulias 1994). Low light levels reduced the number of flushing stems in macadamia (Broomhall 2009; Olesen *et al.* 2011). In lychee and mango, nutrient supply is known to affect release of axillary buds from dormancy (Li *et al.* 2000; Kotur and Murthy 2010). In avocado increased starch concentrations were found immediately before spring flushes and may be part of the axillary bud release mechanism (Robinson

2002). However these factors have been found to play minor or negligible roles in triggering flushing of macadamia (Stephenson and Cull 1986; Olesen *et al.* 2006).

The finding that there was no difference in overall stem emergence or proportion of trees branching between trees exposed to the W-W temperature regime sequence and trees exposed to the C-C sequence (or between W and C) is surprising, given Wilkie *et al.*'s (2009) results that temperature does effect stem emergence. It is possible that the response of vegetative growth to temperature varied between the two studies due to differences between tree maturity, time of year, cultivar, pruning history, and / or orchard versus pot conditions. In particular the second tip-pruning of these plants may have removed many of the nodes most likely to produce new stems – those of the previous growth flush. The subsequent reduced proportion of stems available to count may have reduced the magnitude of difference between these treatments to the point where it was not statistically detectable. In Wilkie *et al.*'s work each stem was only pruned once, and so the stems emerging from the first flush were included in their count. Repetition of this work with a less severe pruning regime would be useful in resolving this issue.

Relationship between vegetative and floral fates

No consistent relationship was found between growth in the first period - either floral or vegetative - and growth in the second period. Logically it could be expected that trees with higher vigour than others or larger trees (with more energy stores and buds, and greater photosynthetic capacity) would produce more growth in both first and second periods, either vegetative or floral. If the trees were using up stored energy to grow, as observed by Karimaei (2012), it could be expected there would be an inverse relationship between first and second period growth, as there would then be less fuel for second period growth. One possible explanation is that trees used stores for first period growth, but second period growth was not less as the new leaves of the first period were fuelling the trees in the second period.

No consistent relationship was detected between the number of inflorescences and the number of stems emerging in either period or the combination of periods. More buds becoming inflorescences did not mean fewer buds becoming stems in the same growth period. This can be read as an indication that different buds become inflorescences to those that become stems.

Methodology

At the end of the second period of this experiment, trees were moved out of the chambers to an outside site (to await differentiation of swollen buds) where the ambient temperatures were in between those of the treatment temperatures. This meant that those trees moved out of the glasshouse from the warm regime experienced a decrease in temperature (of 0.4 / 3.3 ºC), while those moved from the cool regime experienced an increase in temperature (of 5.4 / 3.2 ºC). The effect of this difference in direction of temperature change on bud development, despite all trees being held at the same absolute temperatures, needs to be considered. If this difference in change direction did affect the numbers of emerging shoots, it would reduce or negate the differences between the groups moved from warm treatments and the groups moved from cool treatments. As there were still highly significant differences between these

treatments, it seems that either the effect of the different direction of temperature change at this point was negligible, or that the treatment effect was even larger before storage. Comparing time to bud differentiation in this study and the studies of Stephenson and Gallagher (1986) and Sakai *et al.* (1982), it is clear that the time taken for shoot emergence following a temperature trigger can vary, no doubt with factors such as plant health, light levels, and water availability.

Implications

The relationships between temperature and the extent of flowering in *Macadamia integrifolia x tetraphylla* orchard trees, and wild plants of their ancestor species, will clearly have implications as global climate changes. The detail of such effects cannot be reliably predicted without further study. One area of investigation would need to be the relationship between cool temperatures and the speed and extent of emergence. Warming may decrease or delay emergence, more so at the warmer end of the geographical range of macadamia.

If a two-step flowering process is confirmed, more knowledge of the timing of evocation, especially whether it is a continuous phenomenon or something that occurs in a narrow window, will also be important to forecasts of change. In cooler regions of the range increased temperatures may increase evocation. In warmer regions, increasing temperatures may decrease evocation, where the upper temperature limit of evocation (23 ºC) is approached or exceeded. Alternatively, evocation may occur at different times of the year. The sum of such changes to evocation and emergence is flowering intensity. This may increase or not change in cooler regions, but may decrease in warmer regions. This may be compensated for by longer durations of flowering. Where emergence times are altered, differences in response between varieties may mean those varieties previously matching in flowering time may now not overlap, interrupting cross-pollination in some multi-variety orchards.

Acknowledgments

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Chapter 3. Architecture of macadamia and location of inflorescences

Introduction

Macadamia inflorescence evocation occurs over a range of night temperatures but is known from glasshouse experiments to be relatively high at around 19ºC nights or 25/19 day/night. In SE Queensland this corresponds with the temperatures of December and February. However no swelling of buds in field trees is visible until

March to May. Bud dissection and microscopic investigation of buds throughout this time would help understand the apparent discrepancy between these dates and the mechanisms at work in control of flowering in macadamia. This in turn would help optimise sustainable yield for commercial growers and consumers.

However only a tiny proportion of macadamia buds produce flowers each year, and so random sampling would result in much time spent preparing for microscopy but would result in very few inflorescences to study. Insufficient inflorescences would render any data statistically useless. Knowing at which bud inflorescences are most likely to be forming would make a useful microscopic study practically feasible by reducing the number of buds sampled to a manageable number.

The inflorescences of a number of flowering trees are known to be formed in predictable patterns within the canopy structure (Costes *et al.* 2006). A number of patterns in the location of macadamia inflorescences are already known. More inflorescences are formed in the outer canopy than the inner (Salter and McConchie 2005), and 'short' stems (unbranched lengths) bear more inflorescences than long ones (McFadyen *et al.* 2008; Wilkie 2009). New axes usually emerge only from the top two buds in a node (out of as many as five) (Bennel 1984). These patterns may come about because of apical dominance (Olesen *et al.* 2006) and nodal dominance (Owens and Ewers 1997) [, the latter perhaps being simply another form of apical dominance]. The author has observed that macadamia branches baring relatively large numbers of inflorescences appear to be stunted in comparison with average branches.

Two hypotheses will be addressed in this exercise: i) inflorescences are distributed evenly between branches, and ii) inflorescences are distributed evenly along branches.

Method

Two varieties of macadamia, 741 and 842, growing in commercial orchards near Glasshouse Mountains Township in SE Queensland, were surveyed to ascertain aspects of their architecture and the distribution of their inflorescences throughout the trees. Five-year-old 741s were surveyed in August 2010, 12-year-old 741s were surveyed in August 2011, and 13-year-old 842s were surveyed in August 2012.

Four trees were used for the survey of five-year-olds. Every second stem arising from the leader or trunk was included in the survey, so that an even representation of all branch ages and positions was obtained. Every second stem on the surveyed branches was then surveyed, then every second stem on these stems and so on. 397 stems consisting of 828 growth units and 4,953 nodes of the six-year-olds were surveyed. For every node the following attributes were recorded: the number of inflorescences, position along the growth unit, the position of the growth unit along the stem, the number of nodes on the growth unit, and the number of growth units along the stem. Also recorded was the stem diameter where it joined its parent stem, and the parent stem diameter at this join.

Six trees each were used for the surveys of mature trees (12-year-old 741s and 13-year-olds 842s). Two main stems arising from the leader or trunk on each tree were surveyed, one from between 150 and 200cm from the ground, growing within a 90º

arc around the eastern side of the tree (along the row), and another from between 250 and 350cm from the ground, growing within a 90º arc around the north side of the tree (into the alley, above the skirting height). Every stem arising from these main stems with clearly defined nodes was surveyed, unless the growing tip had been damaged. Older stems with thick, stained or mossy bark which prevented identification of all nodes were omitted from the survey. 1,120 stems consisting of 2,095 growth units and 10,708 nodes were surveyed from the 12-year-olds. 1,277 stems consisting of 2,250 growth units and 10,216 nodes were used from the 13-year-olds. For every node the following attributes were recorded: the number of inflorescences, position along the growth unit, the position of the growth unit along the stem, the number of nodes on the growth unit, and the number of growth units along the stem.

Results

Architecture

The median number of nodes per growth unit was 4 or 5 depending on the tree age / cultivar group (Table 4). The number of nodes per growth unit did not follow a normal distribution, even when divided into trees and position of node along stem, as it was skewed by the lower limit of 0 for number of nodes per unit.

Table 4. Relationships between architectural units in macadamia

Variety	Age	Nodes per growth unit				Growth units per stem			
		median	IQR	*Outliers*	*% outliers*	median	IQR	*Outliers*	*% outliers*
741	6	5	4 - 7	*12 - 59*	*6.9*	3	1 - 5	-	-
741	12	4	3 - 6	*11 - 83*	*8.0*	2	1 - 4	*9 - 10*	*2.2*
842	13	4	3 - 5	*9 - 41*	*6.5*	3	1 - 5	-	-

Inflorescence distribution

The vast majority of nodes did not bare inflorescences (Table 5). Of the nodes that did bear inflorescences, most commonly only one is found per node. Thus the distribution of inflorescences among nodes is not statistically normal.

Table 5. Inflorescences per node on macadamia.

Variety / age	N infs per node			% nodes bearing inflorescences	N infs on bearing nodes	
	median	IQR	outliers		median	IQR
731 / 5	0	0 – 0	1 - 6	9	2	1 – 2
731 / 12	0	0 – 0	1 - 4	5	1	1 – 1
742 / 13	0	0 - 0	1 - 5	15	1	1 - 2

Inflorescence distribution varies with cultivar / age / year grouping. Rachii from previous years' inflorescences were often still attached to nodes observed to be flowering, so it is clear that a node can flower in more than one year.

a) between nodes within a growth unit

Nodes in different positions along a growth unit, i.e. from base to collar, bear different numbers of inflorescences P<= 0.000 (Kruskal-Wallace). When data from all groups was pooled there was a positive correlation between P<= 0.000 (Pearson's) between the number of inflorescences/node and node position from the stem tip (more inflorescences were found at the base of growth units than at the collar/tip end. This was also the case within both groups of mature trees P<= 0.000 (Pearson's), but not significant in 5 yr olds .

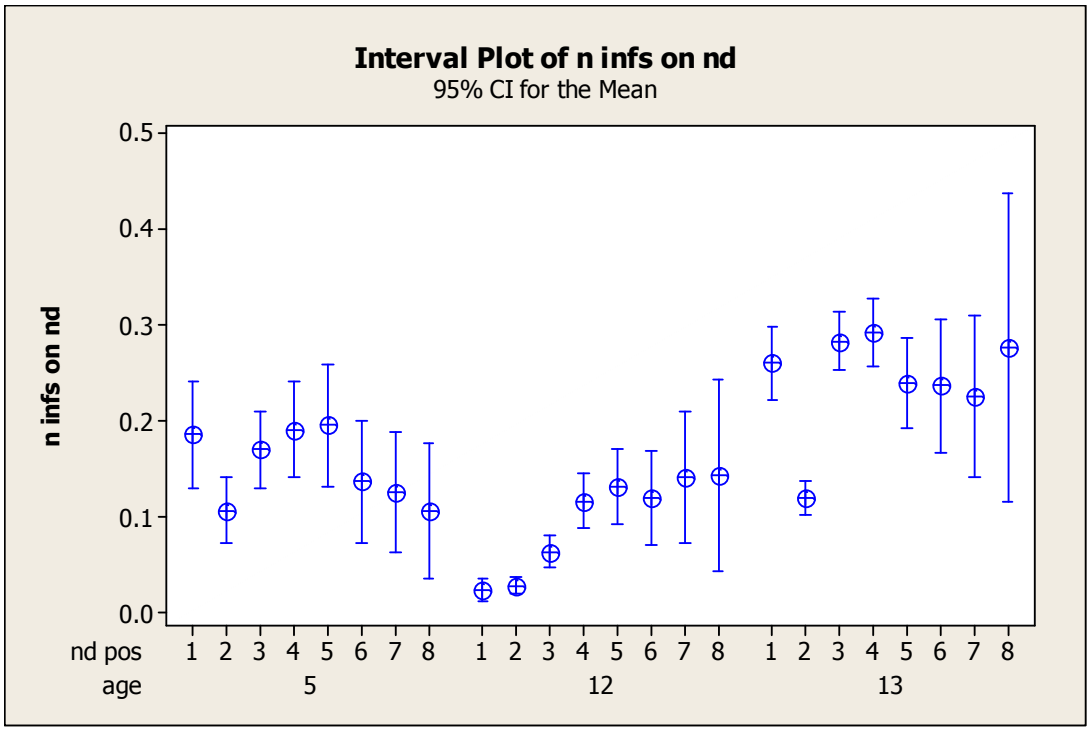


Figure 14. Effect of node position on inflorescences per node by cultivar / age groups.

Despite this correlation, the 842/13 trees had fewer inflorescences/node on node 2 than any other node, including the collar / node 1. The 741/5 trees also had a strong dip in inflorescences/node at node 2. The dip was still evident in these two groups and still not visible in 741/12 trees when stems were grouped by number of growth units (Figure 15). Overall node 2 had significantly fewer inflorescences/node than any other node P<= 0.004 (Mann-Whitney)

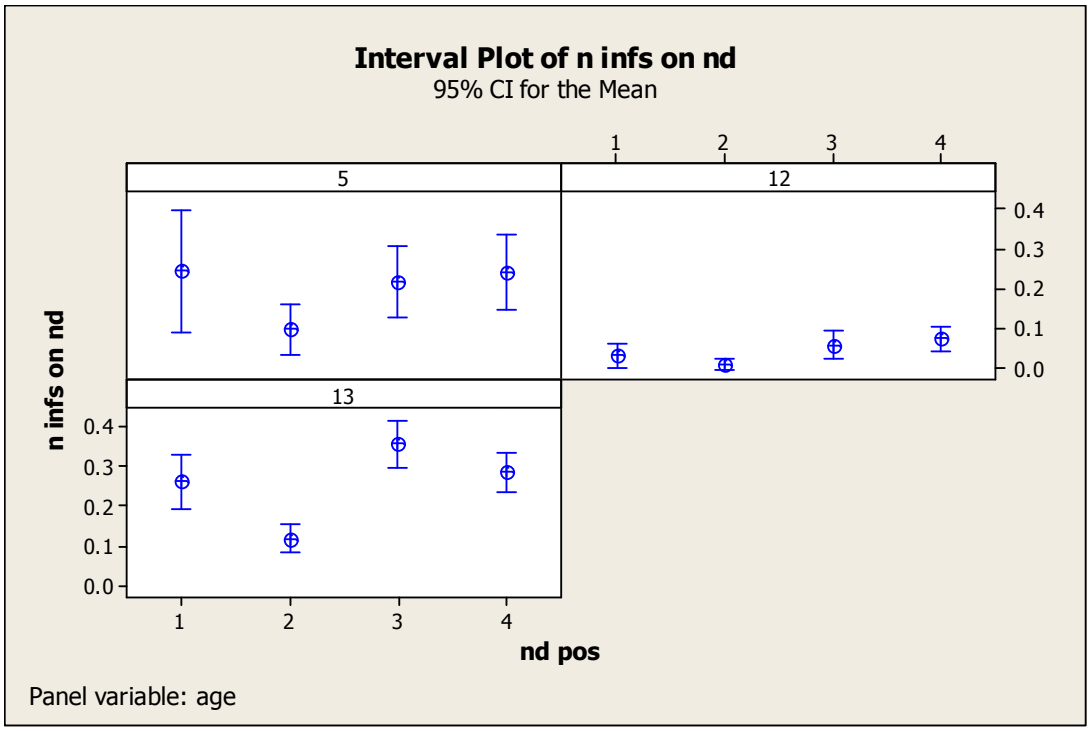


Figure 15. Effect of node position on inflorescences per node within four-growth-unit-long stems.

Differences between collar node and non-collar node were tested for because of the creation of collar nodes at a different stage in the phenological cycle and the subsequent lack of leaves at these nodes. There was no difference overall i.e. when all groups were pooled[3].

b) between growth units within a stem

Nodes on units in <u>different positions</u> from the stem tip bear different numbers of inflorescences (Kruskal-Wallace) P= 0.010. this was not due to a difference between tip nodes and non-tip nodes, despite many tips nodes being not yet hard : there was no significant difference between these two groups (Mann-Whitney test). Overall there is

[3] , and within group 842/13 no difference was detected (Mann-Whitney tests). For 731/5 and for 731/12 significant differences were found, P= 0.03 and P< 0.0000 respectively, however the magnitude of these differences are quite small. All medians were equal to zero. The mean of collars nodes of 731/5 was 0.19, slightly greater than the mean for non-collar nodes which was 0.15. However for 731/12 the collar nodes bore slightly fewer inflorescences / node than non-collar nodes, with their means being 0.02 and 0.07 respectively. Thus on the whole we are not able to conclude that there is any meaningful difference in number of inflorescences/node between collar and non-collar nodes. Nor were there any statistically significant differences between the number of inflorescences/node on collar nodes and any other individual node position (Mann-Whitney tests

a positive correlation between average numbers of inflorescences/node of a growth unit and the unit's position along the stem from its tip (Figure 16). When data from the three groups was pooled, this was significant within all four stems lengths common to all groups: in 3-unit stems P<=0.000; in 4 unit stems P= 0.012; in 5 units stems P=0.000; and 6 unit stems P=0.048 .

50% of age/cultivar/ year groups within this did not show a correlation. There was no correlation between position and inflorescences/node when all stems sizes were analysed together, nor among mature trees. In young trees there is a correlation P<= 0.000, with units at the base of a stem having fewer inflorescences/node than those at the tip.

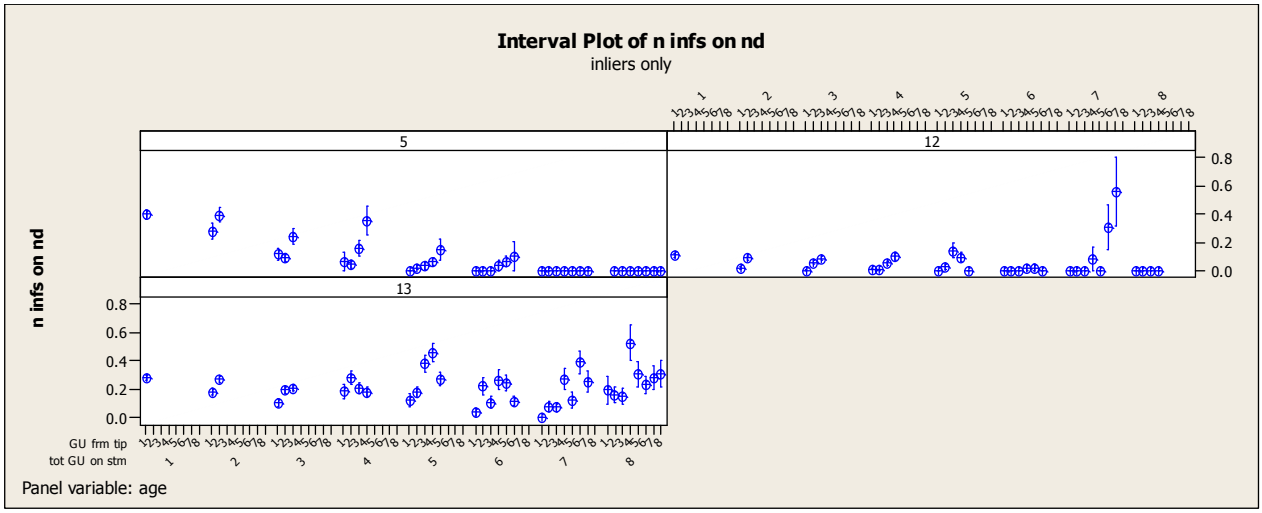


Figure 16. Effect of position of growth unit and stem length on inflorescences per node.

In 741/12, growth units with more nodes had more inflorescences/node P< 0.000. In 741/5 the correlation was opposite P< 0.000. Among the 842/13 trees there was no correlation at all (Table 4).

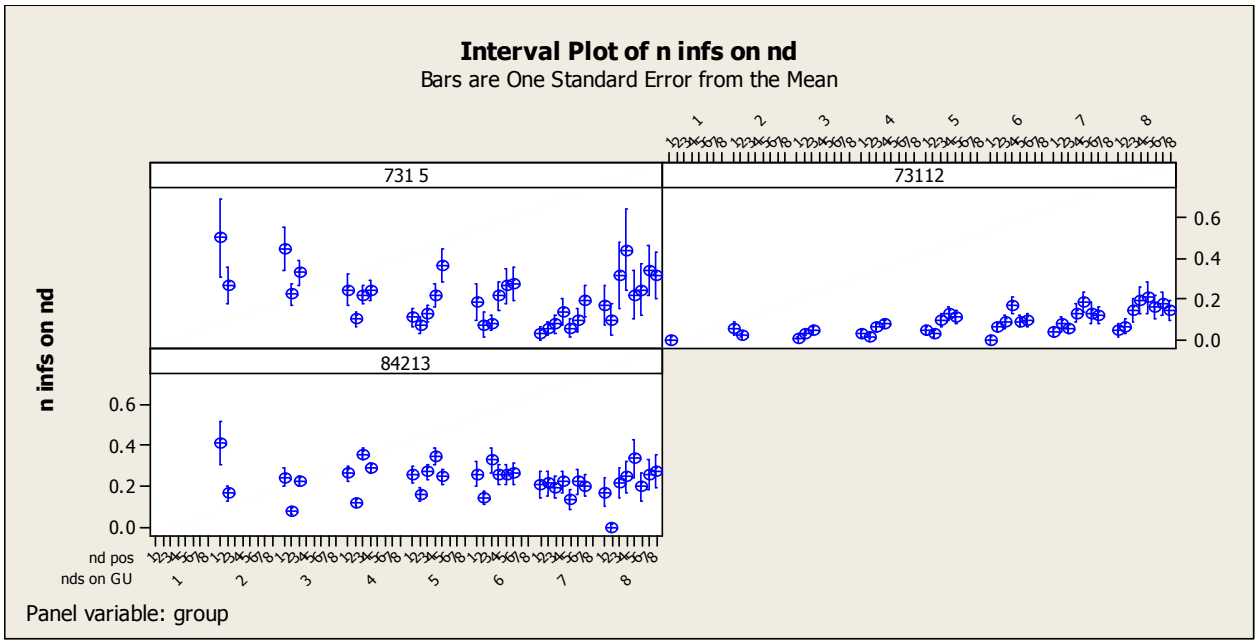


Figure 17. Interval plot of number of inflorescences per node at different node positions.

c) between stems

The number of inflorescences per node is lower on longer stems (Figure 16). The correlation is significant over all groups when pooled P<= 0.000, as well as within groups, with P= 0.022 for 842/13, and P<= 0.000 for 741/12 and 741/5.

There was also a difference between stems with different join diameter ratios. Stems with join diameter ratios over 0.5 – i.e. stems where the base was more than half the parent branch diameter at the join – did not produce any inflorescences, despite making up roughly 48% of the stem population. As a correlation the significance of this was P= 0.002. There was no correlation within flowering stems.

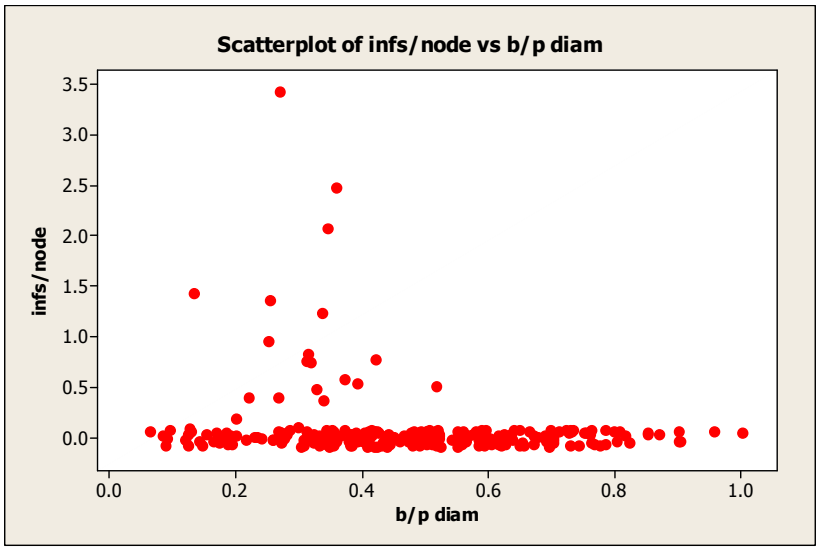


Figure 18. Scatterplot of inflorescence number per node with primary branch diameter.

d) combining factors

741/5: n infs on nd = 0.378 - 0.0106 nds on GU + 0.0101 nd pos - 0.0632 tot GU on stm

+ 0.0185 GU frm tip

Predictor	Coef	SE Coef	T	P
Constant	0.37807	0.02632	14.36	0.000
nds on GU	-0.010614	0.001805	-5.88	0.000
nd pos	0.010139	0.005016	2.02	0.043
tot GU on stm	-0.063180	0.006151	-10.27	0.000
GU frm tip	0.018467	0.008163	2.26	0.024

S = 0.564715 R-Sq = 4.8% R-Sq(adj) = 4.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	4	62.139	15.535	48.71	0.000
Residual Error	3832	1222.038	0.319		
Total	3836	1284.177			

741/12: n infs on nd = - 0.0222 + 0.0108 nds on GU + 0.0177 nd pos
- 0.0223 tot GU on stm + 0.0249 GU frm tip

Predictor	Coef	SE Coef	T	P
Constant	-0.02222	0.01306	-1.70	0.089
nds on GU	0.010818	0.002779	3.89	0.000
nd pos	0.017730	0.003094	5.73	0.000
tot GU on stm	-0.022252	0.003085	-7.21	0.000
GU frm tip	0.024925	0.004644	5.37	0.000

S = 0.310711 R-Sq = 2.7% R-Sq(adj) = 2.6%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	4	15.7597	3.9399	40.81	0.000
Residual Error	5953	574.7116	0.0965		
Total	5957	590.4713			

842/13: n infs on nd = 0.191 + 0.0163 nd pos - 0.0184 tot GU on stm + 0.0233 GU frm tip

Predictor	Coef	SE Coef	T	P
Constant	0.19079	0.01883	10.13	0.000
nd pos	0.016337	0.004561	3.58	0.000
tot GU on stm	-0.018365	0.003954	-4.64	0.000
GU frm tip	0.023296	0.005574	4.18	0.000

S = 0.600498 R-Sq = 0.5% R-Sq(adj) = 0.4%

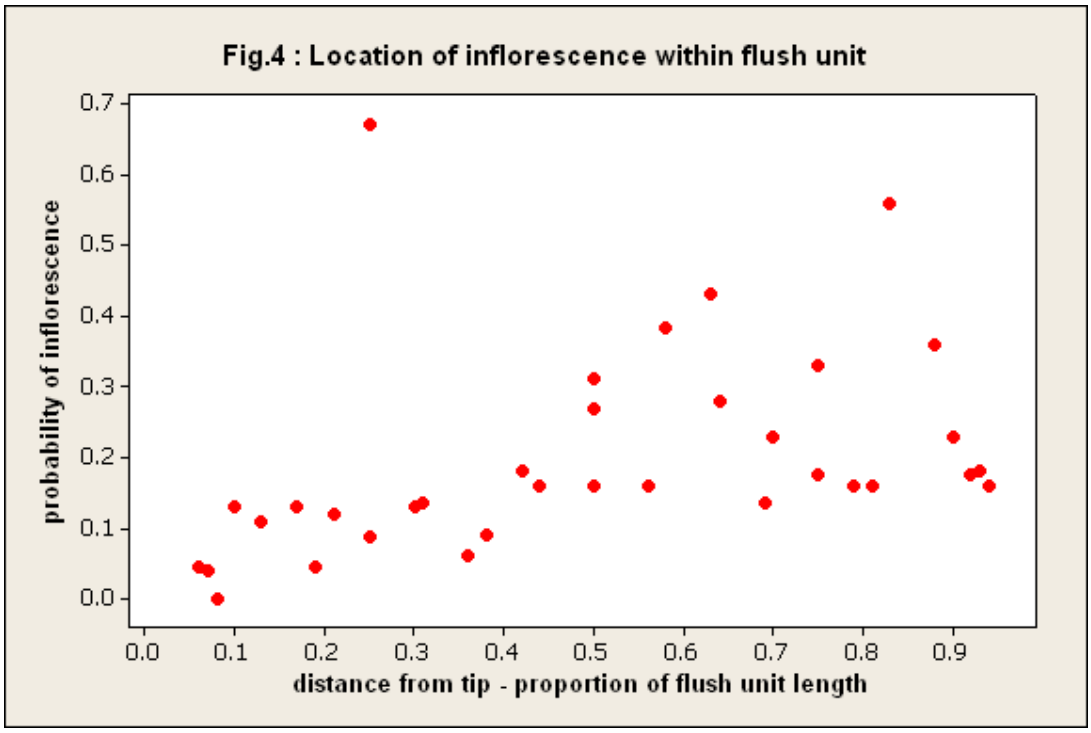
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	12.7374	4.2458	11.77	0.000
Residual Error	7510	2708.0862	0.3606		
Total	7513	2720.8237			

Chapter 4. Location of pruning and emergence of branches and inflorescences

Background:

Nodes along a growth unit increase from base to tip in probability of flowering.



Aim: To check for differences in flowering and vegetative regrowth between nodes along a growth unit after pruning

Method:

Stems 3-growth-units long were tip-pruned either at the top of the second growth unit, the bottom of the second growth unit, or not at all. 20 stems were allocated to each treatment on each of 6 trees. Trees were variety 741, 12 years old, around 8 metres high. Stems were selected from between 1.5 and 4m and tagged. Orchard rows ran E-W and stems were selected from close to the N and S faces of the rows where better light and less moss growth on the bark enabled clear identification of growth units. Pruning was carried out in mid-August when inflorescences had elongated but before anthesis.

→Count number of inf's and number of stems emerging from pruned each node, and from the remaining stem below that node.

Count at end of spring, autumn and floral flushes.

Discussion

There was considerable variety in the girth, length, node number, and number of lateral axes arising from the stems used, despite their growth-unit structure being similar. The wider stems may be more able to support new growth with translocated nutrients or local energy stores. More new axes may arise from these stems than similarly treated thin stems, but perhaps the number of stems that have any new axis arising will not be substantially affected, as the extra axes are likely to arise from the same node. Relative girth may vary due to the rate of growth, which in turn has been shown to effect lateral axis type in apple (Crabbe, 1984).

References

Abbas MF, Quinlan JD and Buckley WR (1980) Influence of early-season temperature on the growth and branching of newly-grafted apple-trees. *Journal of Horticultural Science* **55**, 437-438.

Australian National Botanic Gardens (2000) Flora of Australia - *Macadamia* (Commonwealth of Australia)

Badr and Hartmann (1971) Effect of diurnally fluctuating vs constant temperatures on flower induction and sex expression in olive (*Olea Europaea*).*Physiologia plantarum* **24**, 40-45.

Batten DJ and McConchie CA (1995) Floral Induction in growing buds of lychee (*Litchi chinensis*) and mango (*Mangifera indica*). *Australian Journal of Plant Physiology* **22**, 783-791.

Bennel MR (1984) Aspects of the biology and culture of the macadamia. Masters Thesis, University of Sydney.

Brewer PB, Dun EA, Ferguson BJ, Rameau C and Beveridge CA (2009) Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and arabidopsis. *Plant Physiology* **150**, 482-493.

Broomhall P (2009) Personal Communication: Effect of position within canopy and light levels on vegetative and floral growth of *Macadamia integrifolia*

Buttrose MS and Alexander DM (1978) Promotion of floral initiation in 'Fuerte' avocado by low temperature and short daylength. *Scientia Hortic.* **8**.

Chaikiattiyos S, Menzel CM and Rasmussen TS (1994) Floral induction in tropical fruit trees - effects of temperature and water supply. *Journal of Horticultural Science* **69**, 397-415.

Cline MG (1991) Apical dominance. *The Botanical Review* **57**, 318-358.

Cline MG (1997) Concepts and terminology of apical dominance. *American Journal of Botany* **84**, 1064-1069.

Cline MG and Harrington CA (2007) Apical dominance and apical control in multiple flushing of temperate woody species. *Canadian Journal of Forestry Research* **37**, 74-83.

Costes E, Lauri PE and Regnard JL (2006) Analyzing fruit tree architecture: implications for tree management and fruit production. *Horticultural Reviews* **32**, 1-61.

Crabbe JJ (1984) Vegetative vigour control over location and fate of flower buds, in fruit trees. *Acta Horticulturae* **149**, 55-63.

Department of Agriculture Food and Fisheries (2005) Australian Food Statistics. Commonwealth of Australia, Canberra.

Dun EA, Ferguson BJ and Beveridge CA (2006) Apical dominance and shoot branching. Divergent opionions or divergent mechanisms? *Plant Physiology* **142**, 812-819.

Fowler J, Cohen L and Jarvis P (1998) 'Practical statistics for field biology.' (John Wiley and Sons Ltd: Chichester).

Fuss AM and Sedgley M (1990) Floral initiation and development in relation to the time of flowering in *Banksia coccinea* R.Br and *B menziesii* R.Br (Proteaceae). *Australian Journal of Botany* **38**, 487-500.

Garcia-Luis A, Kanduser M, Santamarina P and Guardiola JL (1992) Low temperature influence on flowering in citrus. The separation of inductive and bud dormancy releasing effects. *Physiologia Plantarum* **86**, 648-652.

Hagen P and Moe R (1982) Effect of temperature and light on lateral branching in poinsettia (Euphorbia pulcherrima Willd.). *Acta Horticulturae*, 47-54.

Haghavan V (1992) Floral evocation and devlopment of the floral meristem. In 'Developmental biology of flowering plants' p. 145165. (Ohio State University: Columbus).

Henderson IF and Lawrence E (1989) 'Henderson's dictionary of biological terms.' (Longman Scientific: Harlow).

Huett DO (2004) Macadamia physiology review: a canopy light response study and literature review. *Aust J Agric Res* **55**, 609-624.

Huett DO and Smith G (2008a) Light distribution and flowering and macadamia canopies. *Australian Macadamia Society News* **35**, 47-49.

Huett DO and Smith G (2008b) Regrowth response to hedging. *Australian Macadamia Society News* **35**, 40-43.

Johnson LA and Briggs BG (1963) Evolution in the Proteaceae. *Australian Journal of Botany* **11**, 21-61.

Karimaei S (2012) Personal Communication: Carbohydrate sources for new vegetative growth

Kebrom TH, Brutnell TP and Finlayson SA (2010) Suppression of sorghum axillary bud outgrowth by shade, phyB and defoliation signalling pathways. *Plant, Cell and Environment* **33**, 48-58.

Kigel J, Konsens I and Ofir M (1991) Branching, flowering and pod-set patterns in snap-bean (Phaseolus vulgaris L) as affected by temperature. *Canadian Journal of Plant Science* **71**, 1233-1242.

Kotur S and Murthy S (2010) Nutrient dynamics of annual growth-flush in mango (Mangifera indica L.). *Journal of Horticultural Sciences* **5**, 75-77.

Lang GA (1987) Dormancy: a new universal terminology. *HortScience* **22**, 817-820.

Lenz F (1969) Effects of day length and temperature on the reproductive and vegetative growth of "Washington Navel" orange. In 'Proceedings of the First International Citrus Symposium' pp. 333-338.

Li Y, Davenport T and Rao R (2000) Nitrogen, flowering and production of lychee in Florida. *Acta Horticulturae* **558**, 221-224.

McFadyen L, Wilke J, Robertson D, Priddle R, Muldoon S, Sedgley M and Olesen T (2008) Orchard management for optimum production, light distribution and ground cover In 'Australian Macadamia Society Annual Conference'. Ballina. (AMS).

McSteen P and Leyser O (2005) Shoot branching. *Annual Review of Plant Biology*.

Menzel CM and Simpson DR (1988) Effect of temperature on growth and flowering of Lychee. *Journal of Horticultural Science* **63**, 349-360.

Menzel CM and Simpson DR (1995) Temperatures above 20 degrees C reduce flowering in Lychee (Litchi-Cheninsis Sonn). *Journal of Horticultural Science* **70**, 981-987.

Minitab (2007) Statistical Software Stat Guide. (Minitab Inc.: www.minitab.com).

Moncur MW, Stephenson RA and Trochoulias T (1985) Floral development of *Macadamia integrifolia* Maiden and Betche under Australian conditions. *Scientia Horticulturae* **27**.

Moss GI (1969) Influence of temperature and photoperiod on flower induction and inflorescence development in sweet orange (*Citrus sinensis* L. Osbeck). *Journal of Horticultural Science* **44**, 311-320.

Nagao M and Hirae H (1992) Macadamia: cultivation and physiology. *Critical Reviews in Plant Sciences* **10**, 441-470.

Nagao MA and Sakai WS (1990) Effects of gibberellic acid, epthephon or girdling on the production of racemes in *Macadamia integrifolia*. *Scientia Horticulturae* **43**, 47-54.

O'Hare P, Stephenson R, Quinlan K and Vock N (2004) 'Macadamia Grower's Handbook.' (Department of Primary Industries and Fisheries: Brisbane).

Olesen T (2005) The timing of flush development affects the flowering of avocado (*Persea americana*) and macadamia (*Macadamia integrifolia x tetraphylla*). *Australian Journal of Agricultural Research* **56**, 723-729.

Olesen T, Huett D and Smith G (2011) The production of flowers, fruit and leafy shoots in pruned macadamia trees. *Functional Plant Biology* **38**, 327-336.

Olesen T, Whalan K, Muldoon S, Robertson D and Mayer R (2006) On the control of bud release in macadamia (*Macadamia integrifolia*). *Aust J Agric Res* **57**, 939-945.

Oleson T, Menzel CM, Wiltshire N and McConchie CA (2002) Flowering and shoot elongation of lychee in eastern Australia *Australian Journal of Agricultural Research* **53**, 977-983.

Olsen J (2003) Molecular and physiological mechanisms of bud dormancy regulation

Acta Horticulturae **618**, 437-453.

Owens SA and Ewers F (1997) Experimentally induced changes in bud fates in *Cercis candensis* L. (Fabaceae). *International Journal of Plant Sciences* **158**, 525-536.

Robinson P (2002) Development of a phenological model of avocado tree growth in California. *Acta horticulturae*, 859.

Sakai WS, Nakata S, Nagao MA and Quedado R (1982) Further study of the effect of night temperature on flower inititiation and rate of flower growth and development in *Macadamia integrifolia*. In 'Hawaii Macadamia Producers Association 22nd Annual Meeting'. Punalu'u.

Salter B and McConchie C (2005) Differences in flower position and shoot growth between *Macadamia* cultivars. In 'Assessment protocols for selection and management in macadamia'. (Ed. Hardner C). (Horticulture Australia Ltd.: Sydney).

Schoellhorn RK, Barrett JE and Nell TA (1996) Branching of chrysanthemum cultivars varies with season, temperature, and photosynthetic photon flux. *HortScience* **31**, 74-78.

Scott P (2008) 'Physiology and behaviour of plants.' (John Wiley and Sons: Chichester).

Sedgley M and Griffin A (1989) 'Sexual reproduction of tree crops.'

Shu ZH, and Sheen, T. F. (1987) Floral initiation in axillary buds of mango *(Mangifera indica* L.) as affected by temperature. *Scientia Horticulturae* **31**.

Stafstrom JP (1995) Developmental potential of shoot buds. In 'Plant stems: Physiology and functional morphology '. (Ed. Gartner BL) pp. 257-279. (Academic Press, Inc.: San Diego).

Stafstrom JP and Sussex IM (1988) Patterns of protein synthesis in dormant and growing vegetative buds of pea. *Planta* **176**, 497-505.

Stephenson R and Cull B (1986) Flushing patterns of macadamia trees in south east Queensland. *Acta Horticulturae* **175**, 49-53.

Stephenson R and Trochoulias T (1994) Macadamia. In 'Handbook of environmental physiology of fruit crops. Volume II: sub-tropical and tropical crops'. (Eds Scaffer B, Andersen P). (CRC Press: Boca Raton).

Stephenson RA and Gallagher EC (1983) A preliminary evaluation of the timing of nitrogen application to macadamias. In 'Proceedings of the first Australian macadamia research workshop'. Marcoola. (Eds Stephenson RA, Gallagher EC).

Stephenson RA and Gallagher EC (1986) Effects of night temperature on floral initiation and raceme development in macadamia. *Scientia Horticulturae* **30**, 213-218.

Stephenson RA and Gallagher EC (1989) Timing of nitrogen application to macadamias 3.

Stephenson RA, Ko HL and Gallagher EC (1989) Plant-water relations of stressed, non-bearing macadamia trees. *Scientia Horticulturae* **39**, 41-53.

Storey (1985) Macadamia. In 'CRC Handbook of flowering'.

Taiz L and Zeiger E (2006) 'Plant Physiology.' (Sinauer Associates: Sunderland).

Tamas IA, Ozbun JL, Wallace DH, Powell LE and Engels CJ (1979) Effect of fruits on dormancy and abcisic acid concentration in the axillary buds of *Phaseolus vulgaris* L. *Plant Physiology* **64**, 615-619.

Taylor BK (1969) The role of nutrition in fruit set and fruit growth. . *Journal of the Australian Institute of Agricultural Science* **35**, 168-174.

Trochoulias T and Lahav E (1983) The effect of temperature on growth and dry matter production of macadamia. *Scientia Horticulturae* **19**, 167-176.

Wallerstein I (1989) Sequential photoperiodic requirement for flower initiation and development of leucospermum-patersonii (proteaceae). *Israel Journal of Botany* **38**, 25-34.

Warner R and Gitlin H (1971) Effect of hedge pruning on macadamia nut yields. *Hawaii Farm Science* **20**, 8-9.

Wilkie J (2009) Interactions between the vegetative growth, flowering and yield of macadamia (Macadamia integrifolia, M. integrifolia x M. tetraphylla), in a canopy management context. SUBMITTED / ACCEPTED PhD Thesis, University of New England.

Wilkie JD, Sedgley M and Olesen T (2008) Regulation of floral initiation in horticultural trees. *Journal of Experimental Botany* **59**, 3215-3228.

Wilkie JD, Sedgley M and Olesen T (2009) A model of vegetative flush development and its potential use managing macadamia (Macadamia integrifolia) tree canopies. *Crop and Pasture Science* **60**, 420-426.

Zamski E, Oshri S and Zieslin N (1985) Comparative morphology and anatomy of axillary buds along a rose shoot. *Botanical Gazette* **148**, 208-212.

Technology Transfer

Information from both PhD projects was provided to the AMS through presentations to the Annual Industry Conference and at a workshop held at the Maroochy Research Station on 13th February 2013. The main purpose of these studies was to provide information leading to the development of better functional-structural models for macadamia, a longer term project.

Recommendations for Future Research

1. Further development of the functional structural model being developed by Dr Neil White so that a variety of different tree management (pruning) strategies can be assessed for macadamia orchards and the most promising selected for more intensive research.

2. Develop the functional structural modelling capability as a platform for identifying and prioritising physiological research needs Convenient and consistent remote measures of crop load on sentinel trees should be investigated to help refine models.

3. Further develop the functional structural modelling capability to fill the critical gaps in knowledge on floral initiation, floral development and fruit development.

Publications from this Project

Apart from the two PhD theses that will be published from this research, the following scientific papers have been written or are in preparation for publication:

Carbohydrate sources in vegetative growth of macadamia – A novel indirect method to determine CHO source contributions

Sadegh Karimaei, Jim Hanan, Susan Schmidt and Russ Stephenson

Background and Aims Vegetative growth in macadamia as a recurrent flushing tree depends on the supply of carbohydrate (CHO) from reserves and current photosynthate. Understanding the contribution level of these sources in vegetative growth of macadamia tree will advance our knowledge in canopy management. There are controversial theories about the level of reserved CHO and current photosynthate contributions in vegetative growth of macadamia tree in literatures. Destructive analysis of CHO level in tissues and organs of fruit trees during vegetative growth period is complicated and challenging, either directly through a variety of analytical lab methods (i.e. non-structural carbohydrate analysis) or simply measuring dry weight as an indirect method. This study tries to demonstrate a novel indirect method to evaluate CHO source contribution by measuring growth length and girth of a growing flush on girdled and non-girdled parent shoots during vegetative growth period in macadamia tree.

Methods Two experiments were conducted at times when the major flushes occur in macadamia (March and September). Based on these experiments a novel indirect method to calculate the contributions of the carbohydrate sources (reserves and current photosynthate) to vegetative growth was adopted. Data were collected by measuring new flush length and girth, internode length and girth and number of internodes over their growth period and implementation of equations to calculate the sources contributions. Girdled and non-girdled parent shoots were decapitated and a single new flush was allowed to grow with or without growing leaves. In single new flush with growing leaves, leaves were matured during 1/3 of the total period of experiment, and in defoliated flushes newly growing leaves were removed in early stage of growth. Equations are established to find the level of CHO source (reserve and photosynthate) contributions to vegetative growth of new flush. Girdled parent shoot with defoliated new flush considered as having no contribution (SDg) and the same flush on non-girdled parent shoot (SDng) considered as the reserve contribution (pool). New flush with growing leaves on girdled parent shoot (SLg) considered as current photosynthate and on non-girdled parent shoot (SLng) considered as the

contribution of both reserve and current photosynthate on vegetative growth. Two pools and two current photosynthates were calculated from equations.

Key results

Conclusions

Key words

Introduction

Macadamia (*Macadamia integrifolia* Maiden and Betche, *M. tetraphylla* Johnson, and hybrids, Proteaceae) is the only native Australian tree commercially produced for its edible nut. Although crowded high density macadamia orchards have little impact on immediate yield potential of trees, crowding does affect management practices and long term reductions in yield (Huett et al., 2005). Using regular pruning and annual hedging to resolve these issues stimulates undesirable vegetative re-growth and increases fruit abscission. Here we aim to advance the development of long-term solutions for efficient canopy management by understanding of macadamia tree canopy development.

The energy for vegetative growth and canopy development is provided by carbohydrate resource that is distributed through the carbohydrate allocation process (Figure 1). Knowledge about the balance between carbon allocation and tree architectural development as a result of vegetative growth will support development of techniques for efficient canopy management.

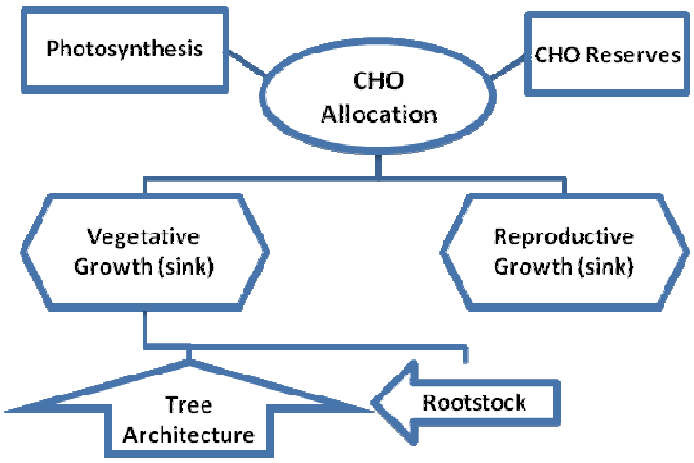


Figure.1. Schematic relationship between carbohydrate allocation and vegetative growth in macadamia tree

Annual carbohydrate cycle in macadamia

Carbohydrates are quantitatively the most important components of woody plants. They comprise almost 75 percent of their dry weight and are the primary organic source of energy (Costes et al., 1999). Woody plants use and accumulate different amounts of carbohydrates annually according to species and genotype.

In macadamia, carbohydrate accumulation occurs in autumn and winter and depletion in spring (Stephenson et al., 1989b). Vegetative flushing occurs during nearly all periods of the year, however, two major flushes happen in late summer (March) and early spring (September) (Stephenson et al., 1986b, Stephenson et al., 1986a, Nagao et al., 1994, Stephenson R., 1994). During the spring, photosynthesis cannot provide sufficient carbohydrate to meet the demand of both vegetative flushing and flowering, therefore the plant uses its reserves. Carbohydrate reserve was highly depleted during oil accumulation into nut, while in trees with aborted raceme (absence of reproductive sink) depletion of CHO was lower (Figure 2) (Stephenson et al., 1989a).

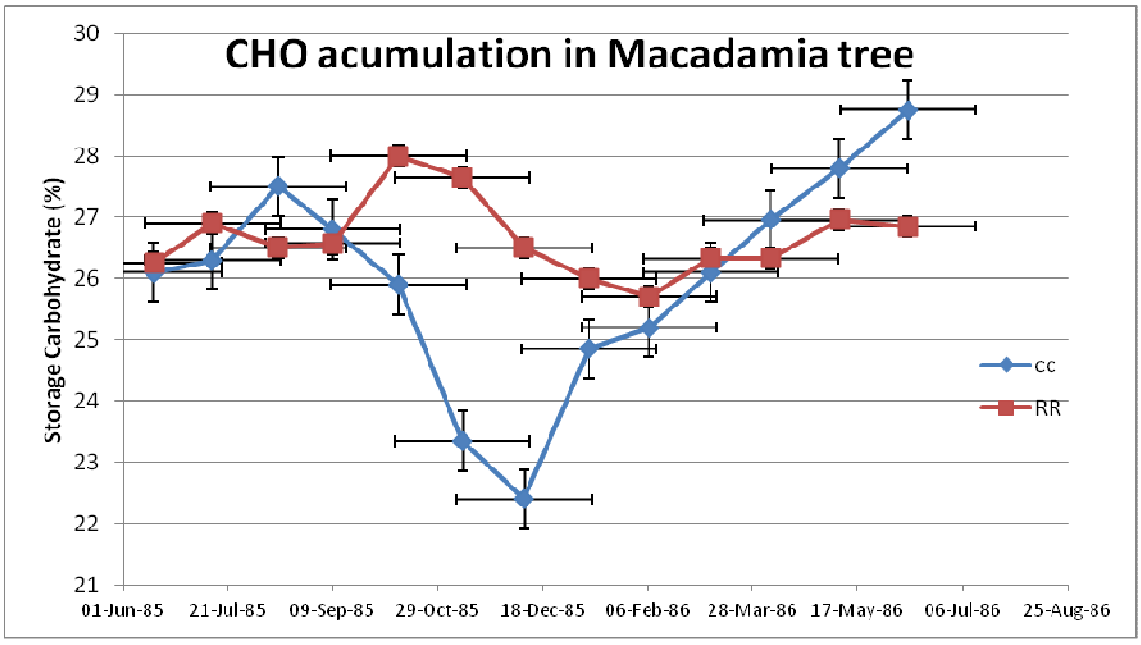


Figure.2. Seasonal pattern of storage carbohydrates in trunk wood tissues of macadamia trees subjected to growth manipulation treatments (RR= removed raceme), from Stephenson et al. (1989a) with permission.

Photosynthesis in macadamia

Macadamia generally has a lower photosynthesis rate (7.5-14.5 µmol $CO_2/m^2.s$) (Lloyd, 1991) than temperate fruit and nut trees (7-20.6 µmol $CO_2/m^2.s$) (Flore and Lakso, 1989).

In most temperate zone crops, assimilates loading from leaf to phloem is through apoplast, while in some tropical woody plants it seems to be symplastic, which is a less efficient loading mechanism of assimilates.

This might be due to the different pathways of loading sucrose from leaves to phloem. While in temperate zone trees carbon is loaded to phloem through the apoplast, tropical trees use less efficient symplastic loading mechanisms through the plasmodesmata (Voitsekhovskaja et al., 2006). On the other hand, macadamia's long-lived leaves have a low light compensation point with a degree of shade tolerance (Demmig-Adams et al., 1997), which can explain its maximal canopy photosynthesis in lower light intensity (in 30–40% of total light) (Flore and Lakso, 1989). However, seasonal differences in the environment (i.e. temperature and light) should be considered, as they will affect the amount of assimilates that are produced and modify the proportion of new growth occurring between winter and spring. More knowledge is needed to explain the relationship between current photosynthate and vegetative growth in macadamia.

Carbohydrate reserves or current photosynthate?

There are contradicting arguments about the effect of carbohydrate (CHO) reserves versus current photosynthate on the vegetative growth of subtropical evergreen fruit trees. Previous studies claim that the level of CHO reserves is responsible for flush development in evergreen trees including macadamia (Cormack and Bate, 1976), avocado (Liu et al., 1999) and lychee (Menzel et al., 1995). In contrast, Olesen et al. (2008) considered current photosynthates to be the main source of carbohydrate for new growth in macadamia and it was proposed that carbohydrate reserves play a secondary but important buffering role during periods of high carbon demand. Stephenson et al. (1989a) asserted that major vegetative flushing activity in macadamia did not coincide with significant depletion of reserves (Figure 2), although the secondary decline in stored CHO in July may be due to maturing of vegetative growth that commenced the previous May.

Reproductive sink versus vegetative sink

Compared to vegetative sinks, reproductive sinks have a priority in attracting carbohydrates from limited sources (Soderstrom et al., 1988). Although the results from the Sakai (1981) study in kiwi fruit showed that the vegetative sink had a higher priority, different sink types have different priorities when they are supplied with different levels of carbohydrate (Saleem and Buxton, 1976, Stephenson et al., 1986c, Lacointe and Minchin, 2008). There are no published studies describing the relationship between sources and sinks in macadamia and we cannot explain sink priority without a detailed experiment having both vegetative and reproductive sinks simultaneously.

We take a new approach to evaluate the contribution of two major carbohydrate sources (reserves and current photosynthate), which affect vegetative growth in macadamia tree.

In order to investigate the relationship between the current photosynthate and carbohydrate reserves and their impact on vegetative growth and canopy development in mature macadamia trees, an experiment was designed to study those relationships and the effect of each carbohydrate source. A vegetative sink was created by allowing vegetative regrowth to develop after pruning, and current photosynthate source was adjusted by retaining different number of mature leaves on each parent shoot

(Stephenson et al., 1986c). Phloem girdling of shoots was implemented to produce different CHO reserve level treatments. In this study carbohydrate is considered as the only source of energy responsible for vegetative growth in macadamia (Costes et al., 1999) and comes from two sources: current photosynthesis by the leaves, and CHO reserves (Figure 1). Several growth parameters of the new single flush were measured over time. This study investigates and quantifies how the current photosynthate and/or carbohydrate reserves can affect the vegetative growth of macadamia tree and the level of their contributions. We hypothesised that in limited/girdled parent shoots, current photosynthate from leaves is the only CHO source responsible for vegetative growth, while in non-limited/non-girdled parent shoots, CHO sources are current photosynthate from leaves on parent shoot plus CHO reserves.

Material and methods

Two experiments were conducted in mid March and mid September 2011, when macadamia has its two major flushing (O'Hare et al., 2004, Wilkie et al., 2009).

Parent shoot treatments

For the first experiment (mid-March 2011), twenty trees and 40 trees for second experiment (mid-September 2011) were selected from a macadamia orchard in Beerwah at Glasshouse Mountain, Queensland, Australia (26.856447S, 152.919633E). Trees were planted in February 2009 from two year old nursery plants variety "816" grafted on "H2" rootstocks. Treatments initiated March 16 for the first experiment and September 14 for the second experiment, the times when macadamia trees start to accumulate CHO in their tissues. Trees were selected based on the availability of branches similar in girth and length. To prevent competition between reproductive growth and vegetative growth, all developing fruits (either in March or September) or flowers (mid-September) were removed.

Three pairs of branches, between 21cm and 48cm length and 3.9 and 5.9 mm girth diameter and without lateral branches were selected on each tree. Branches were selected from outer canopy positions at a similar height from the ground (160 to 200 cm) without considering branch orders. Then they were decapitated to stimulate new growth immediately below the cut surface. These branches, which were holding the new growth are named hereafter "parent shoot". The length of parent shoots was between 17.5cm and 29cm from base after decapitation. Two, four and six leaves were left on each parent shoot. Leaves were considered as the source for current photosynthate. One of each pair of parent shoots was girdled and the other left intact without girdling (Figure 3). Girth diameter of first or second internode of parent shoots was ~ 5cm from the base, measured by a calliper.

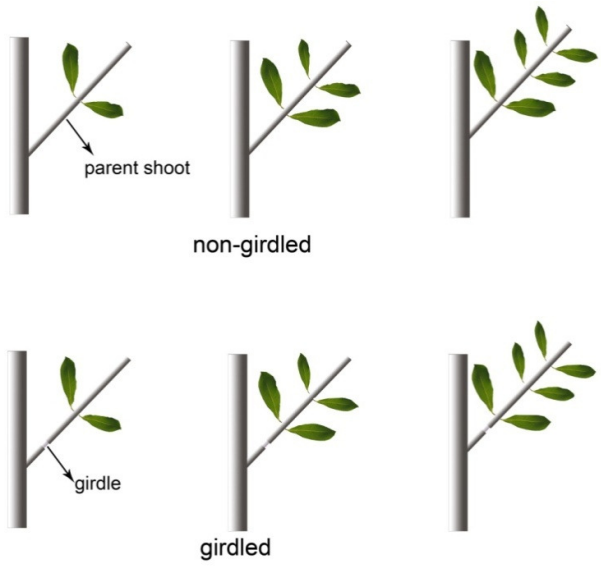


Figure 3. Background treatments on each tree; 3 pairs of branches (parent shoots) were selected in a tree with 2, 4 or 6 leaves retained on each and with or without girdling.

New growth type treatments

After selecting the parent shoots and global treatments were applied, treatments of new growth type grown from bud/buds under the cut surface on parent shoots were conducted on randomly selected trees. Ten trees for March experiment and 20 trees for September experiment were selected randomly for each treatment in a completely randomized design (CRD) (in total twenty trees for March and 40 trees for September experiments). Every individual tree was treated to manipulate the new growth (flush) with one of the below treatments of new growth:

4. A single new shoot with growing leaves (SL); all new growing shoots from axillary buds under the cut surface were pinched off except one.
5. A single new shoot without leaves (SD); all new growing shoots were pinched off except one and the growing leaves on this shoot were removed during the growth of this shoot.

In single new shoot treatments (SL and SD) any subsequently growing shoots other than the selected one were removed by early pinching. Growth parameters of the new shoot/shoots was measured 20 days after treatments 15 times for the first experiment (mid-March) in one week intervals from date one (5/4/2011) to date eleven (8/6/2011) and three weeks between date eleven and date twelve (29/6/2011) and one week intervals between date twelve and date fifteen (21/7/2011) of growth, over a 5 month timeframe. For the second experiment (mid-September), growth parameters were measured 20 days after treatments for 11 times……

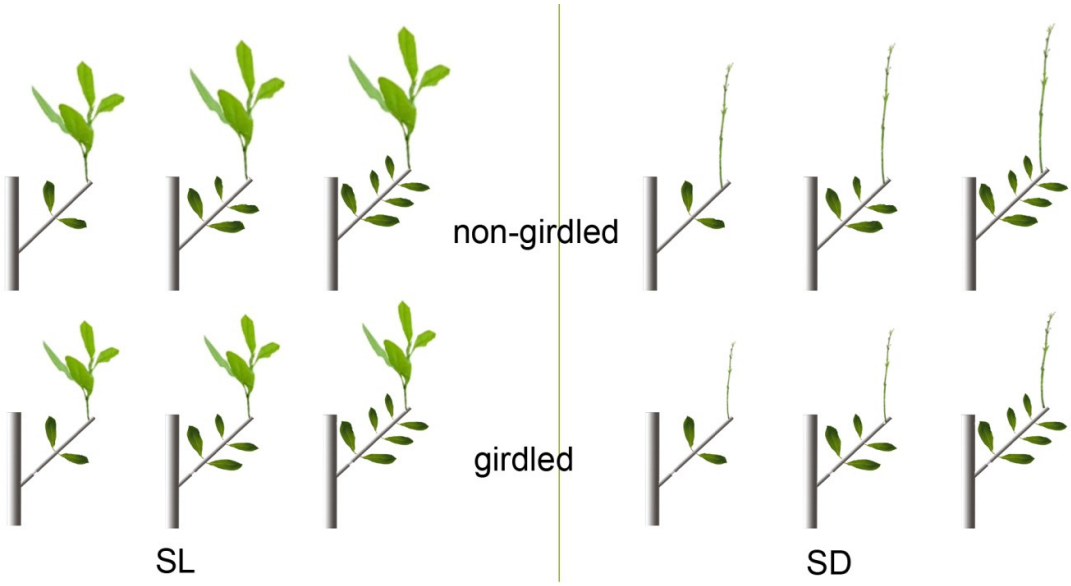


Figure 4. Schematic of the new growth type (NGT) treatments; SL: a single new shoot with growing leaves, SD: a single new shoot without growing leaves.

Girdling treatments

Girdling prevents the translocation of CHO from leaves to the other parts of tree, therefore current photosynthate from leaves and CHO of the parent shoot above the girdled point can be considered as the main sources of energy for vegetative growth on girdled parent shoots and the carbohydrate reserves plus current photosynthate for those that were not girdled. Girdling was applied by removing a 5 mm wide strip of bark from the middle of the first internode approximately 5cm from the branch base.

Measurements

Measurements were started 20 days after treatments in both experiments. Internodes' lengths and girths of new shoot without growing leaves (SD) and with growing leaves (SL) were measured on non-girdled and girdled parent shoots (PSs) every one or two weeks until 127 days after treatments were applied for March experiment and 119 days for September experiment.

Growth measurements over time included the measurement of length and girth of internodes on new growing shoot and the total number of internodes. After the last measurement of length and girth of growing flush, branches were harvested in the early morning and put into boxes filled with ice and transferred to the lab and their fresh weights were measured the same day. Parent shoot length and girth was measured at the beginning and their fresh and dry weight at the end of the experiment. Before drying parent shoots, leaves on them (PSL) were removed and their area was

measured by scanning. New growths and parent shoots were dried in an oven for two weeks at 60°C and then weighed to determine their dry weight.

New growth volume (FV) was calculated from new flush length and girth as a cylinder ($\pi r^2 h$). New growth wood density was calculated from new growth fresh or dry weight and their volume (m/v).

Statistical analysis

For statistical analysis, linear mixed effects models were used in R software of "nlme package" (Pereira et al., 2011). Randomly selected trees for "new growth type" (NGT) is the random factor. Fixed factors are the independent variables (treatments) which applied, were "girdling" (girdle), "parent shoot girth" (PSG), and "parent shoot dry weight" (PSdw). Dependent variables (responses) which, were used in this study are "flush length (FL)" and "new growth volume" (FV).

lmmfit package was used to evaluate the goodness-of-fit measures of models (r-squared) (Gent and Seginer, 2012).

Linear mixed models were used for both experiments based on the responses, treatments and random effect (randomly selected trees for treatments).

For March experiment we used "fit <- lme(FL/FV~(NGT + girdle + PSdw + PSG)^4, random=~1|Tree)" models with R-squared of 0.28 and 0.45 respectively.

For September experiment we used "fit <- lme(FL/FV ~(NGT + girdle + PSdw + PSG)^3 random=~1|Tree)" models with R-squared of 0.69 and 0.70 respectively.

Results

Number of leaves on parent shoot (PS) didn't have a significant effect in our statistical models for both experiments, which seems to be due to the variation of areas of individual leaves. Therefore we used leaf area instead of number of leaves on parent shoot as an independent variable. However, we didn't use leaf area on PS to calculate the contributions of CHO sources in this study. Instead, we compared the growth of new flush with or without growing leaves (new growth type; NGT).

Experiment 1 - March

For statistical analysis we used the flush length (FL) and volume (FV) of new single flushes grown on parent shoots in date 25 May of growth measurement period, when they didn't show significant growth afterward. FL was affected by NGT significantly ($P<0.007$) while FV was not affected significantly. Girdling affected FL and FV significantly, $P<0.002$ and $P<0.004$ respectively. The interaction between girdle and NGT was only significant for FL ($P<0.02$).

Parent shoot dry weight (PSdw) had no significant effect on FL and FV but the interaction between girdle and PSdw significantly affected them ($P<0.005$ and $P<0.003$ respectively). Interaction between NGT and PSdw didn't show any significant effect on FL and FV. Parent shoot girth (PSG) had significant effect on FL ($P<0.03$) and FV ($P<0.04$) and its interaction with NGT was only significant for FL. Interaction between NGT, girdle and PSdw, interactions between NGT, girdle and

PSG and interaction between NGT, PSdw and PSG had significant effects only on FL ($P<0.04$, $P<0.01$ and $P<0.02$ respectively). However, interaction between girdle, PSdw and PSG significantly affected both FL and FV ($P<0.003$ and $P<0.002$ respectively).
Finally, interaction between all independent variables (NGT, girdle, PSdw and PSG) affected significantly FL ($P<0.02$) but without significant effect on FV.

P-values of different treatments' effects on FL and FV in March and September experiments resulted from statistical models

	March		September	
Treatments	(FL)	(FV)	(FL)	(FV)
NGT*	0.0063**	0.5028ns	0.0258*	0.0963ns
girdle	0.0016**	0.0032**	<.0001***	0.0002***
PSdw	0.2107ns	0.4676ns	0.1909ns	0.6404ns
PSG	0.0256*	0.0392*	0.0192**	0.1136ns
NGT:girdle	0.0109**	0.2561ns	0.0001***	0.0290*
NGT:PSdw	0.0562ns	0.7250ns	0.3645ns	0.8133ns
NGT:PSG	0.0010**	0.3393ns	0.0183**	0.0816ns
girdle:PSdw	0.0046*	0.0023**	0.0561ns	0.3250ns
girdle:PSG	0.0014**	0.0037**	0.0003***	0.0002***
PSdw:PSG	0.1007ns	0.2335ns	0.1821ns	0.6984ns
NGT:girdle:PSdw	0.0303*	0.4797ns	0.0477*	0.8122ns
NGT:girdle:PSG	0.0074**	0.1951ns	0.0007***	0.1266ns
NGT:PSdw:PSG	0.0176**	0.9464ns	0.2029ns	0.5831ns
girdle:PSdw:PSG	0.0026**	0.0017**	0.9961ns	0.1504ns
NGT:girdle:PSdw:PSG	0.0139**	0.3509ns		

*NGT: new growth type, girdle: girdling, PSdw: parent shoot dry weight, PSG: parent shoot girth

The growth patterns of single defoliated flush (SD) and single flush with growing leaves (SL) grown on parent shoots with or without girdling were almost similar except for SD non-girdled. However, their growth rate was significantly different for instance in day 70. SD non-girdled showed a slightly increase in growth 70 days after when treatments were applied.

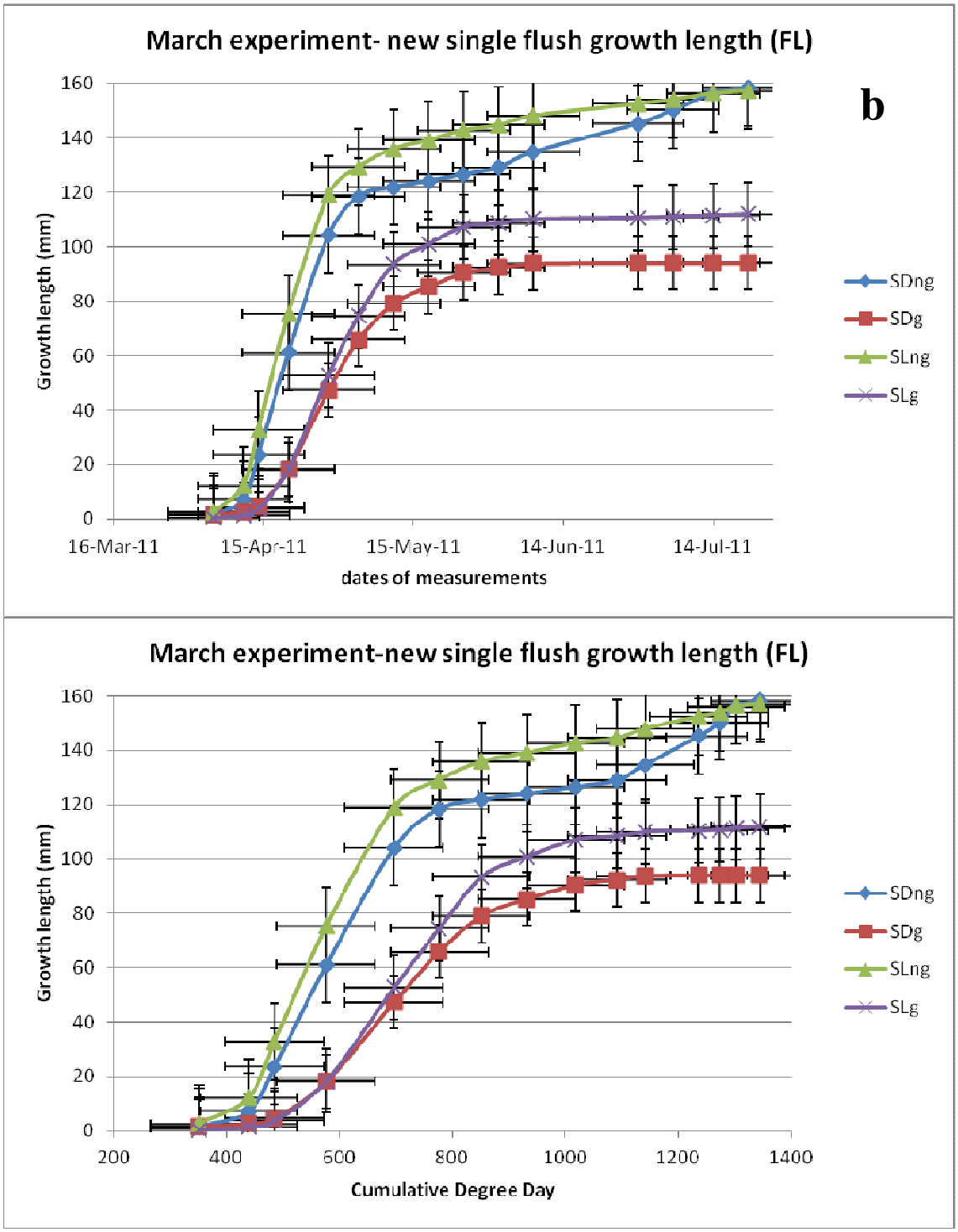


Figure 5. Growth pattern of new single flush length (FL) of different shoot types on girdled and non-girdled PSs for dates of measurement (a) and for cumulative degree day (b)

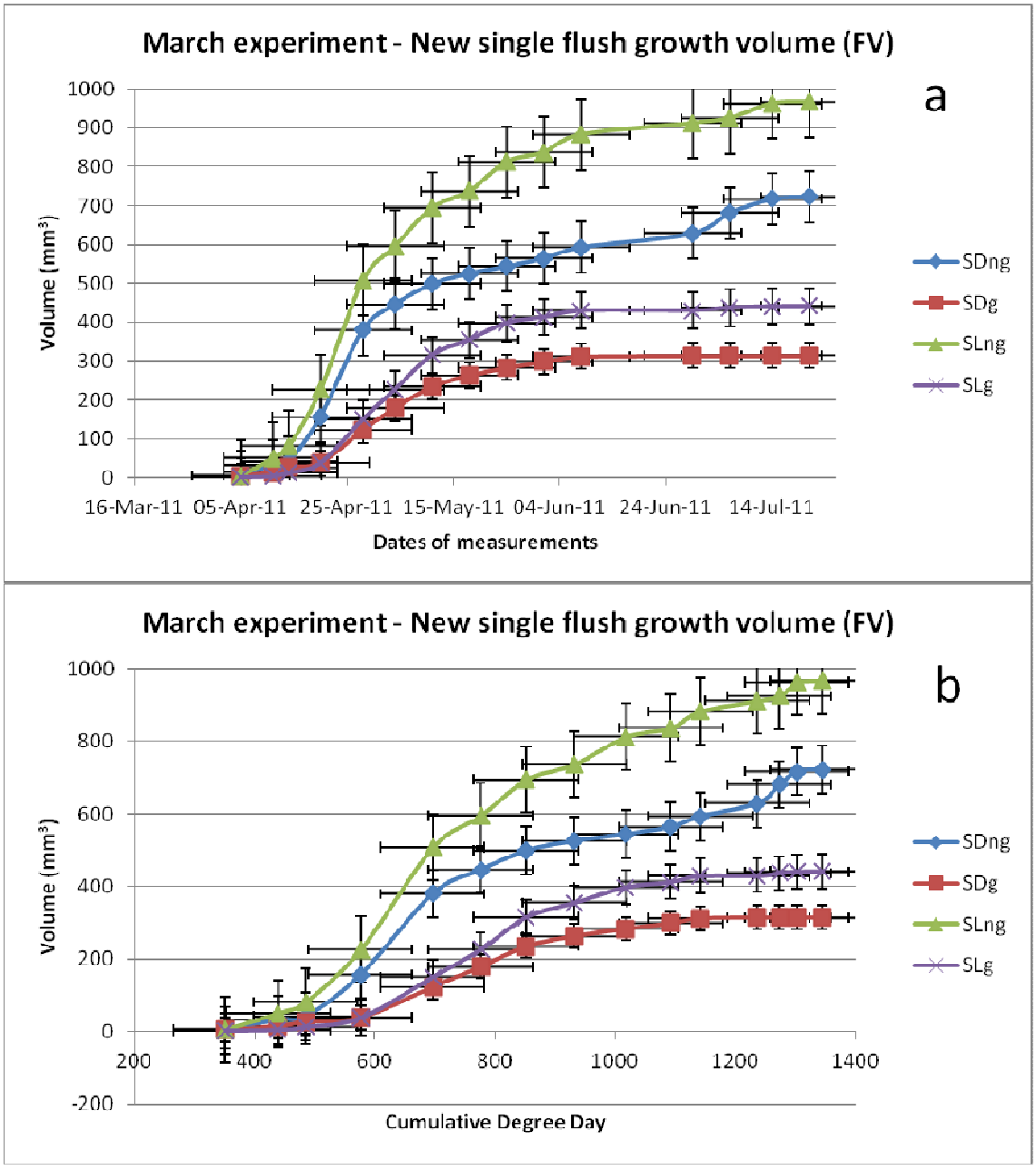


Figure 6. Growth pattern of a new single flush volume (FV) of different shoot types on girdled and non-girdled PSs for dates of measurement (a) and for cumulative degree day (b)

Experiment 2 – September

For statistical analysis, we used growth data in date 28 September. FL and FV of new single flush grown on PS were also considered for this experiment in our models. NGT had only significant effect in FL ($P<0.03$). Girdling had strong effect either on FL ($P<0.0001$) or FV ($P<0.0003$). PSdw affected neither FL nor FV. PSG only affected FL significantly ($P<0.02$). The interaction between NGT and girdle significantly affected FL ($P<0.0002$) and FV ($P<0.03$). Only FL was affected

significantly by the interaction between NGT and PSG ($P<0.02$). Interaction between girdle and PSG strongly affected either FL ($P<0.0004$) or FV ($P<0.0003$). Three way interactions between NGT, girdle and PSdw and between NGT, girdle and PSG significantly affected only FL respectively $P<0.05$ and $P<0.0008$.

Unlike March experiment, the growth patterns of single new flushes of defoliated (SD) and with growing leaves (SL) on non-girdled PSs are similar. This similarity is even more on girdled PSs as is shown in Figure 7. This level of similarity might be due to the double number of samples in September experiment, which means more accuracy with higher R-squared values in comparison to March experiment. R-squared of 0.69 and 0.66 for September experiment models vs. 0.28 and 0.45 for March experiment models.

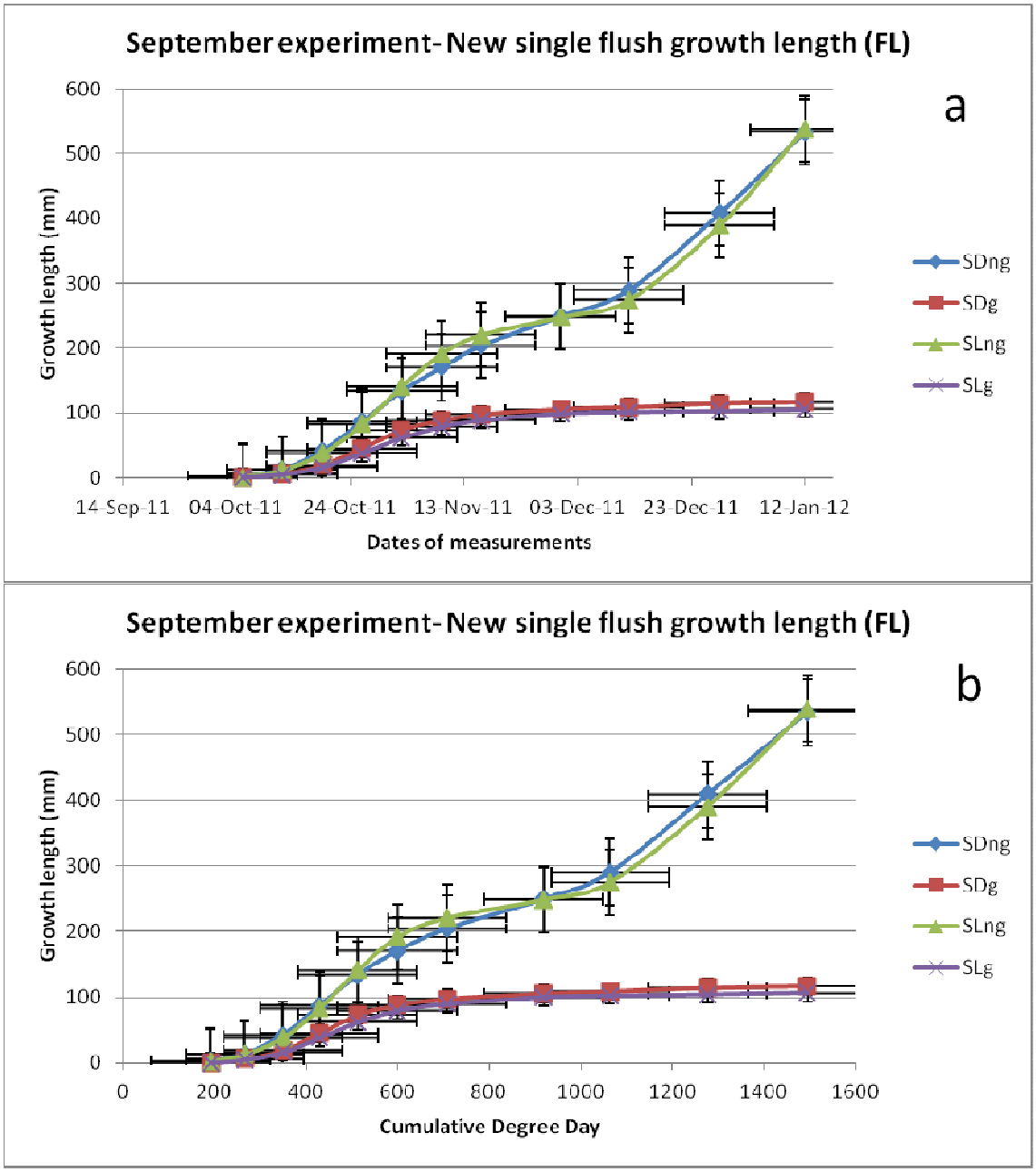
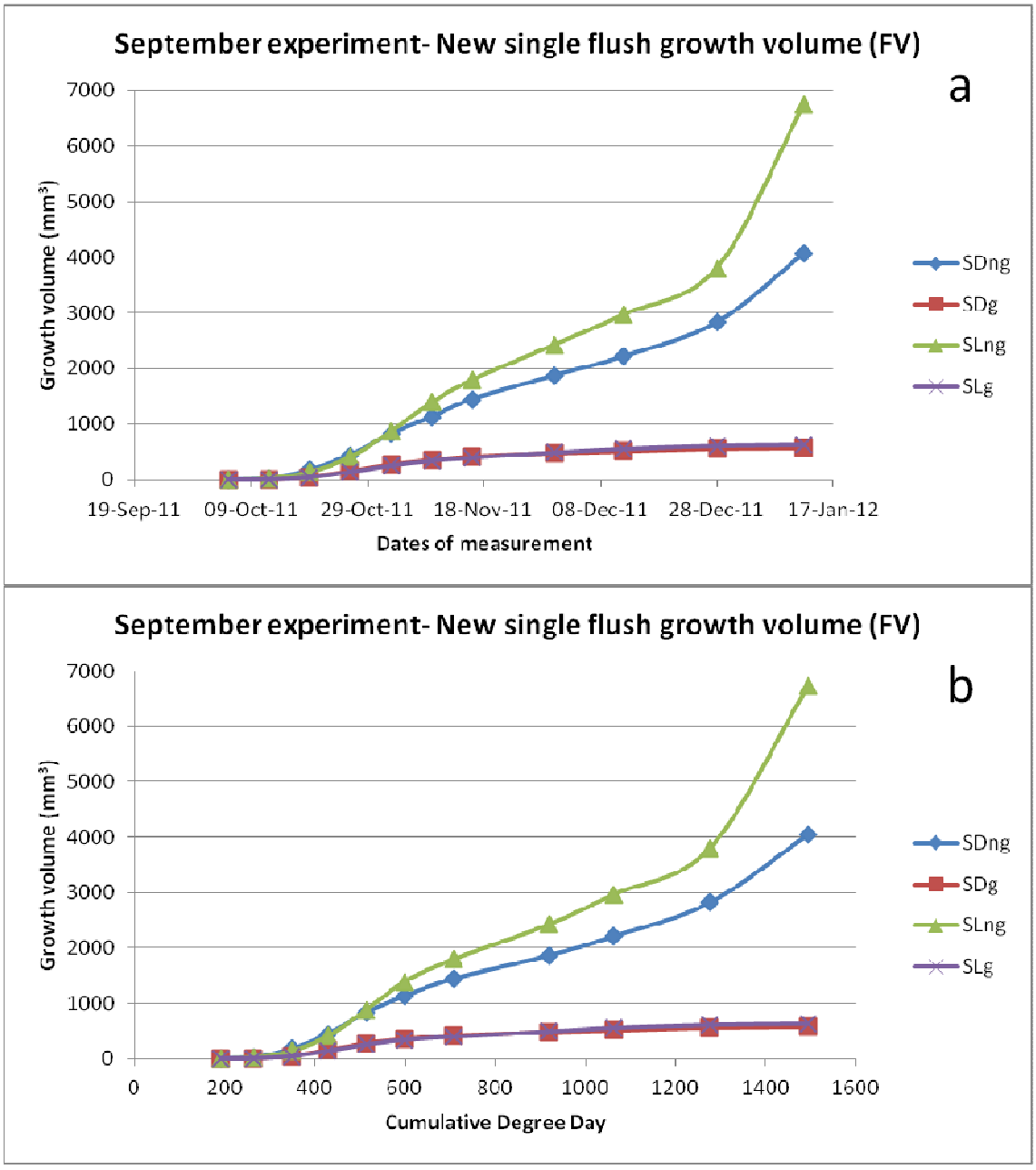


Figure 7. Growth pattern of new single flush volume (FV) of different shoot types on girdled and non-girdled PSs for dates of measurement (a) and for cumulative degree day (b)



Discussion

The contribution of a CHO source in vegetative growth is actually equal to the amount of CHO level is spent by a specific source or depleted by the growing flush for a certain amount of growth.

Flush length (FL) and new growth volume (FV) were the variables in our study that their responses to girdling and independent variable of new growth type (NGT) were

considered to evaluate the level of CHO contribution of two CHO sources (reserved CHO and current photosynthate) responsible for vegetative growth in macadamia trees. Growth length and girth of single flushes grown on girdled or non-girdled PS with or without growing leaves was measured during the growing period. Also, their fresh and dry weight was measured once only after harvest.

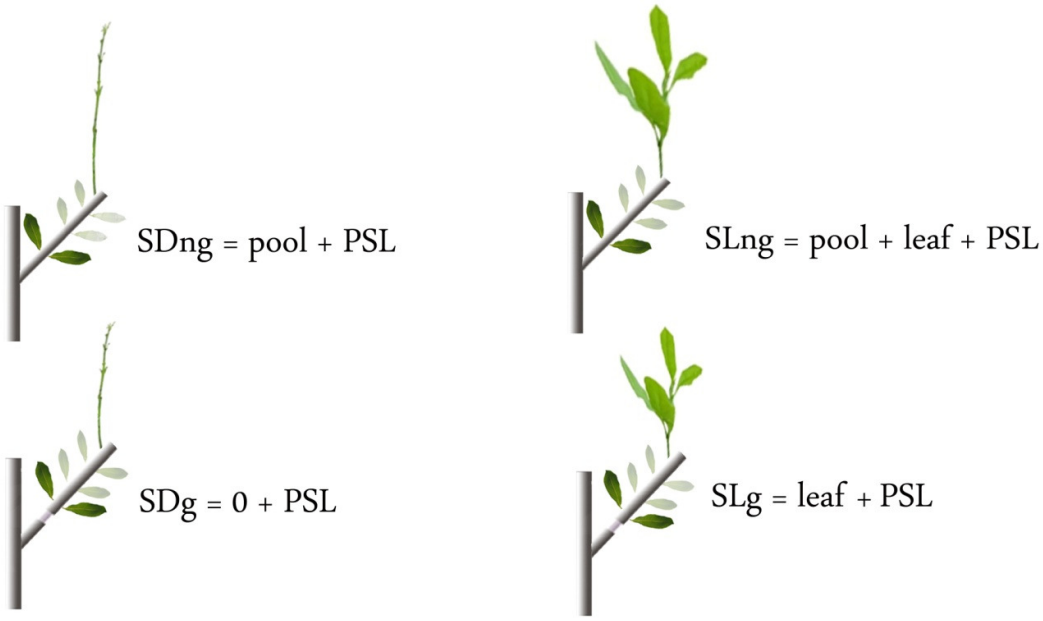
Equations are developed from the relationships between girdled and non-girdled SD and SL growth types for different growth traits. Contributions of CHO sources in FL and FV are determined during the growth period based on these equations as well as FL and FV values at specific dates alongside with fresh and dry weight of new growth at the end of growth period. Graphs of their contributions in growth are produced during the growth period. Proportions of CHO sources contributions were also determined for specific dates of growth and fresh and dry weight of growth at the end of growth period.

We considered two growth types with girdling treatments as below (Figure 8):

- *SDng*: single shoot without growing leaves (defoliated) and non-girdled
- *SDg*: single shoot without growing leaves (defoliated) and girdled
- *SLng*: single shoot with growing leaves and non-girdled
- *SLg*: single shoot with growing leaves and girdled

Then we considered different sources of energy (probably non-structural carbohydrate and/or current photosynthate) for growth of these new shoot types on PSs.

Following equations are made for each single shoot with or without girdling and with or without having growing leaves on new growth (new flush):



We did not consider the number of leaves (2, 4 or 6) on PSs in our assumptions, as PSL exists in all equations. Therefore, flush length (FL) and other growth traits of new single flush on PSs with different treatments are summed up for PSLs and PSL effect can be omitted in our sources' CHO contribution determination.

We define pool as an energy source (CHO reserve) from whole tree.

Through the following equations the sources of energy derived from reserves (pool) or current photosynthate (leaf), responsible for the growth of the new shoot were calculate.

5. **Pool 1** = SDng – SDg = pool – 0
6. **Leaf 1** = SLng – SDng = (pool + leaf) – pool
7. **Leaf 2** = SLg – SDg = leaf – 0
8. **Pool 2** = SLng – SLg = (pool + leaf) – leaf

We used the above equations to calculate two sources' contribution including reserve (Pool) and current photosynthate (leaf). Each CHO source contribution was calculated for two instances (Pool 1, Pool 2, Leaf 1 and Leaf 2). Values of flush length (FL) of new single flush over time (Figures) were used to produce vegetative growth contribution of each source.

We also used FL, FV, NGfw and NGdw values at the end of experiment to calculate the percentage of sources' contributions for these growth attributes in both experiments in March and September 2011 (Table 3).

Experiment 1. March

The above equations were used to calculate the contribution of each source in vegetative growth of macadamia in two instances for each; Pool1 and Pool2 for reserved CHO and Leaf1 and Leaf2 for current photosynthate. Four curves related to these sources are shown in Figure 8.

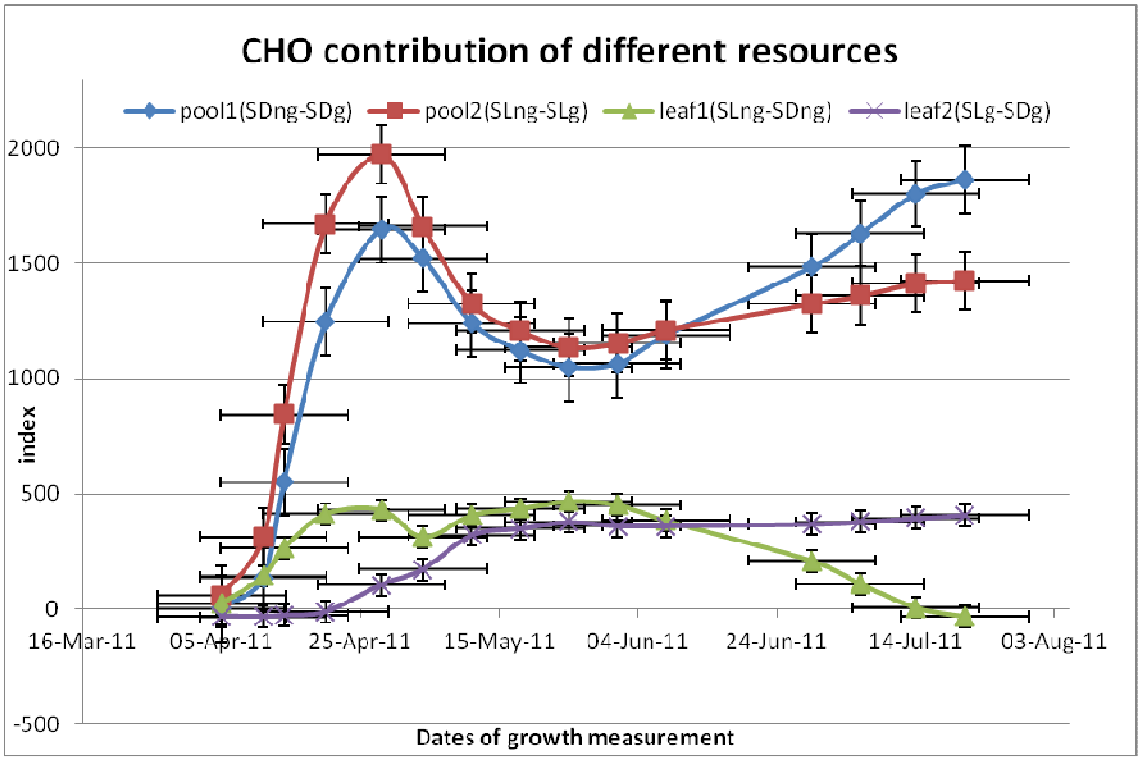


Figure 8. Contribution of different CHO sources (pool or leaf) in growth length of new shoot over the time

Different pool contributions (pool 1 and pool 2) and different leaf contributions (leaf 1 and leaf 2) which were used to calculate the growth length of new shoot showed similar patterns (Figure 8). These similar curves for each CHO source contribution increase the confidence of our assumptions. Therefore, an average of two instances used for each CHO source contribution is made to produce the final graph (Figure 9).

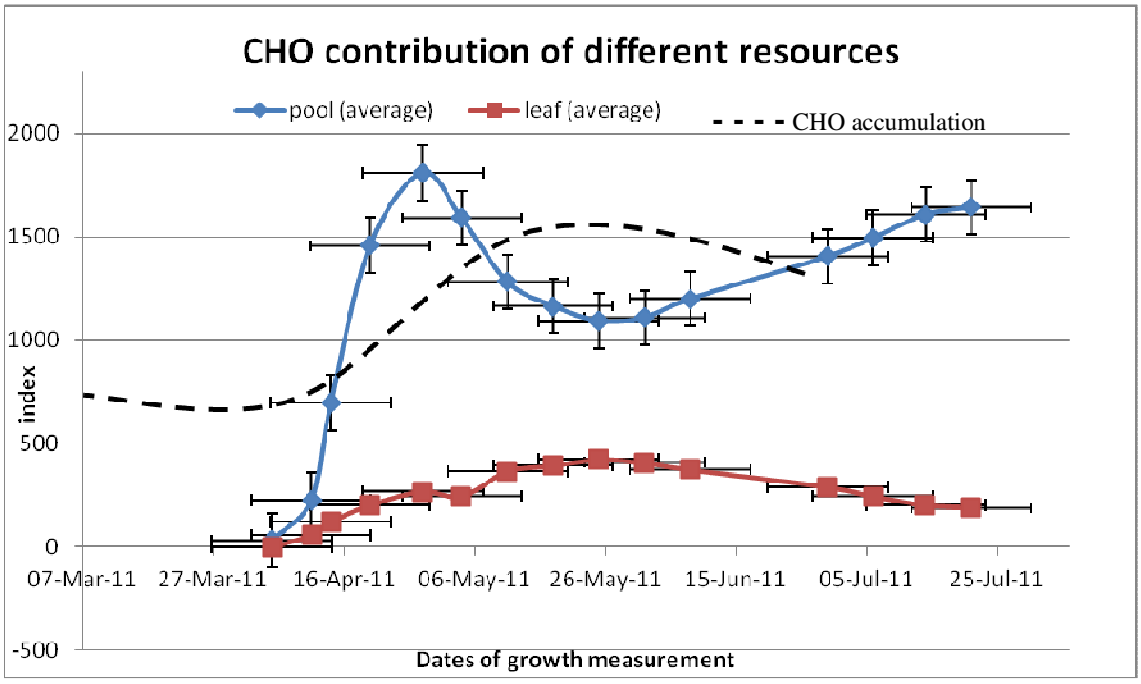
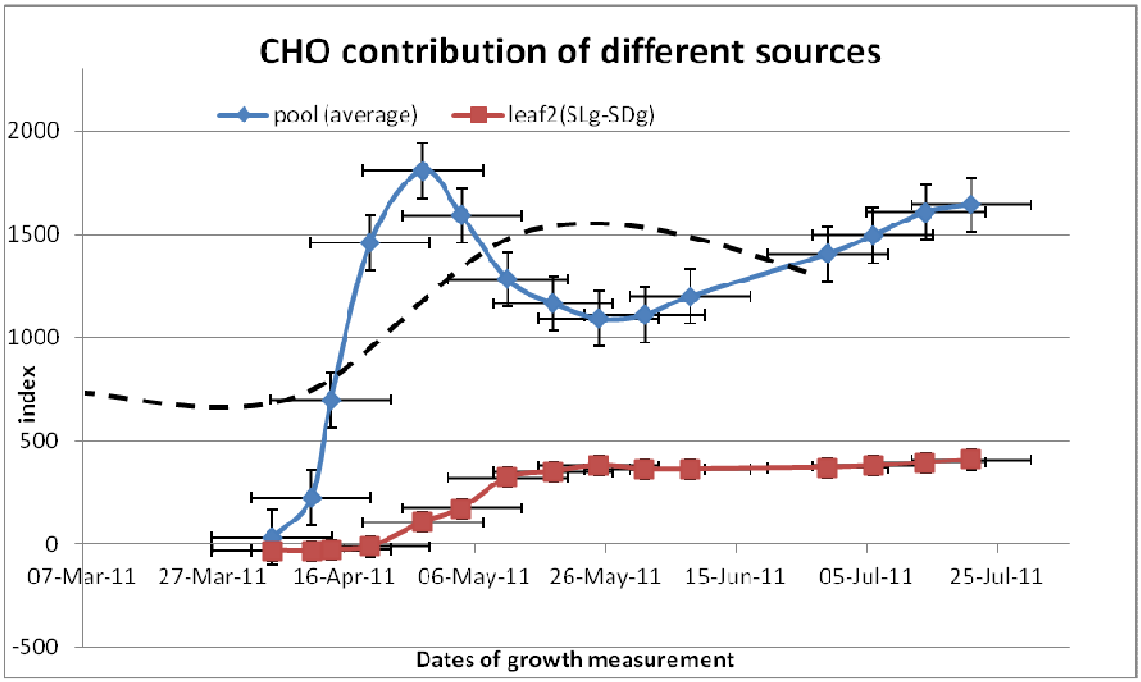


Figure 9. Reserved CHO (pool) and current photosynthate (leaf) contributions in vegetative growth of macadamia (growth length of a single flush; FL). Dashed curve shows carbohydrate accumulation in macadamia tree (trunk wood tissues) in absence of reproductive sink, this curve is extracted from the graph produced by Stephenson et al. (1989a) for the same period of time of current study.

Pool 1 and pool 2 show more similarity than the contributions from leaf 1 and leaf 2. Source contribution from leaf 1 shows a consistent pattern rather than leaf 2. This is due to the equations we used for their calculations. Leaf 1 is calculated from the difference between girdled branches while leaf 2 is calculated from the difference between non-girdled branches. Non-girdled branches are affected by pool while girdled branches are not. Girdled branches are completely separated from pool in girdling point. On the other hand we cannot consider the leaf contribution in shoot growth from the beginning of experiment (day zero) until 4 May, 49 days after treatments were applied. At this time leaves on SL flushes were fully expanded and mature enough to be productive (Figure 9). Therefore, for our concluded graph of CHO contributions of two sources responsible for vegetative growth of macadamia in March experiment, we have considered the average of pool 1 and pool 2 for CHO reserve, and only leaf 2 for current photosynthate (Figure 10).



Dashed curve in figures 9 and 10 is released from graph 2 for the non-structural carbohydrate storage in macadamia tree between March and July, when our study was occurred. It shows consistency with our graph, which shows depletion of source of energy (Figure 11).

In the first 23 days of vegetative growth in macadamia the main source of energy (carbohydrate) is from reserves (pool) (94.5%), then it is dropped down when leaves (current photosynthate) activity is increased until day 50 (74.2%). From day 50 until the end of growth period, reserves contribution to the vegetative growth in macadamia is increased (89.71% at the end of experiment). Leaves or current photosynthate showed a different and constant effect on the growth of the new shoot when they were matured and fully productive after day 36.

Table 3. Contribution of each CHO source for different growth traits

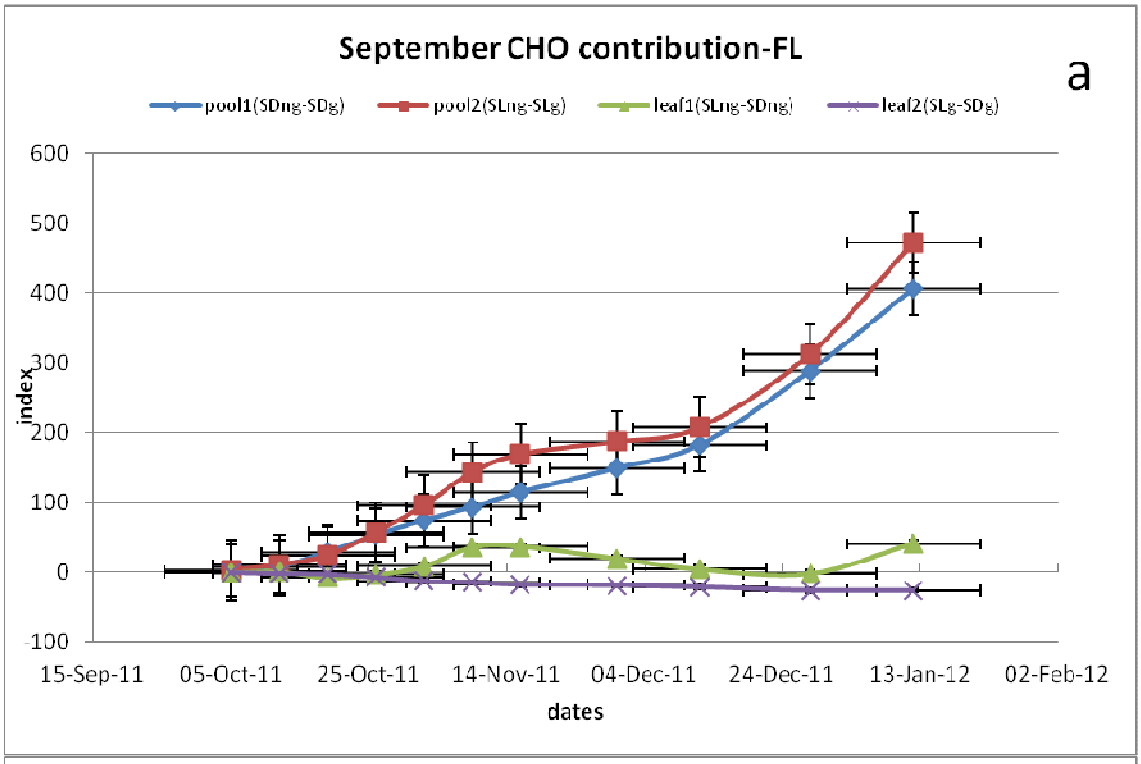
	pool%	leaf%	pool1%	leaf2%
NGfw	73.07567	26.92433	85.93195	14.06805
NGdw	56.34074	43.65926	62.2534	37.7466
FL - 18May2011	74.75946	25.24054	76.18725	23.81275
FL -1Jun2011	73.15303	26.84697	74.61431	25.38569
FL - 8Jun2011	76.25556	23.74444	76.64516	23.35484
FL-21Jul11	89.7107	10.2893	82.03435	17.96565
FV-21Jul11	74.32897	25.67103	80.5437	19.4563
FL-sum	82.53504	17.46496	83.35241	16.64759

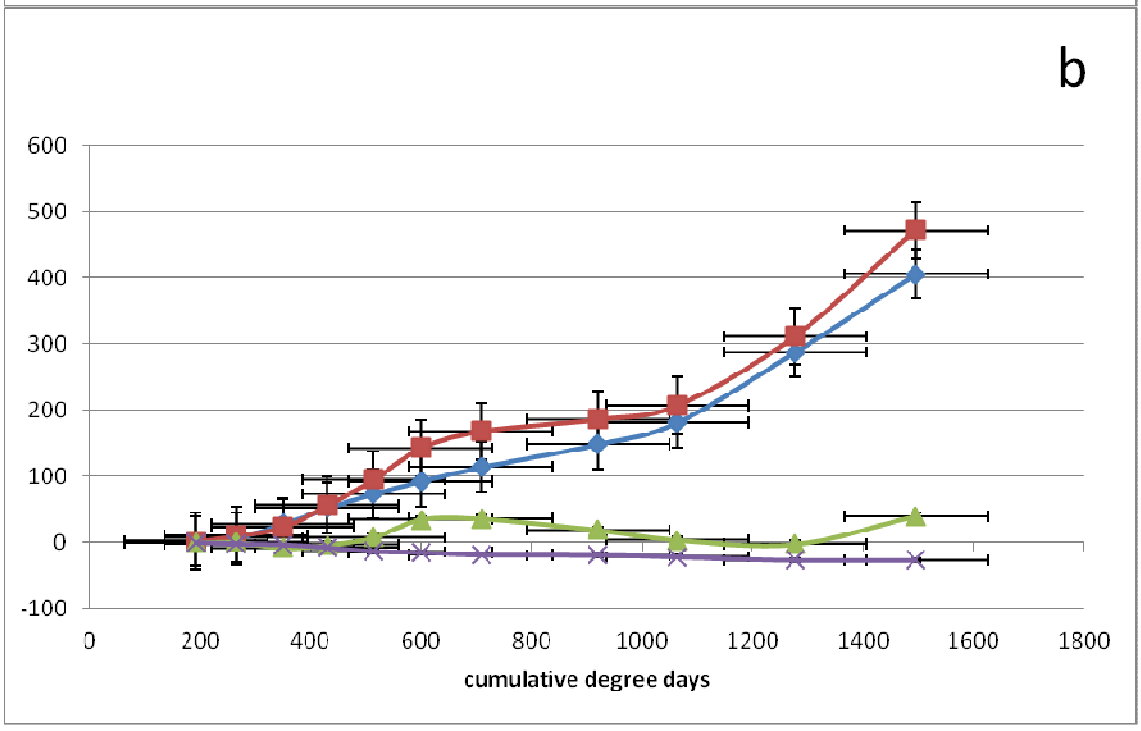
FV-sum	71.60399	28.39601	79.14322	20.85678
Total average	74.64035	25.35965	77.85619	22.14381

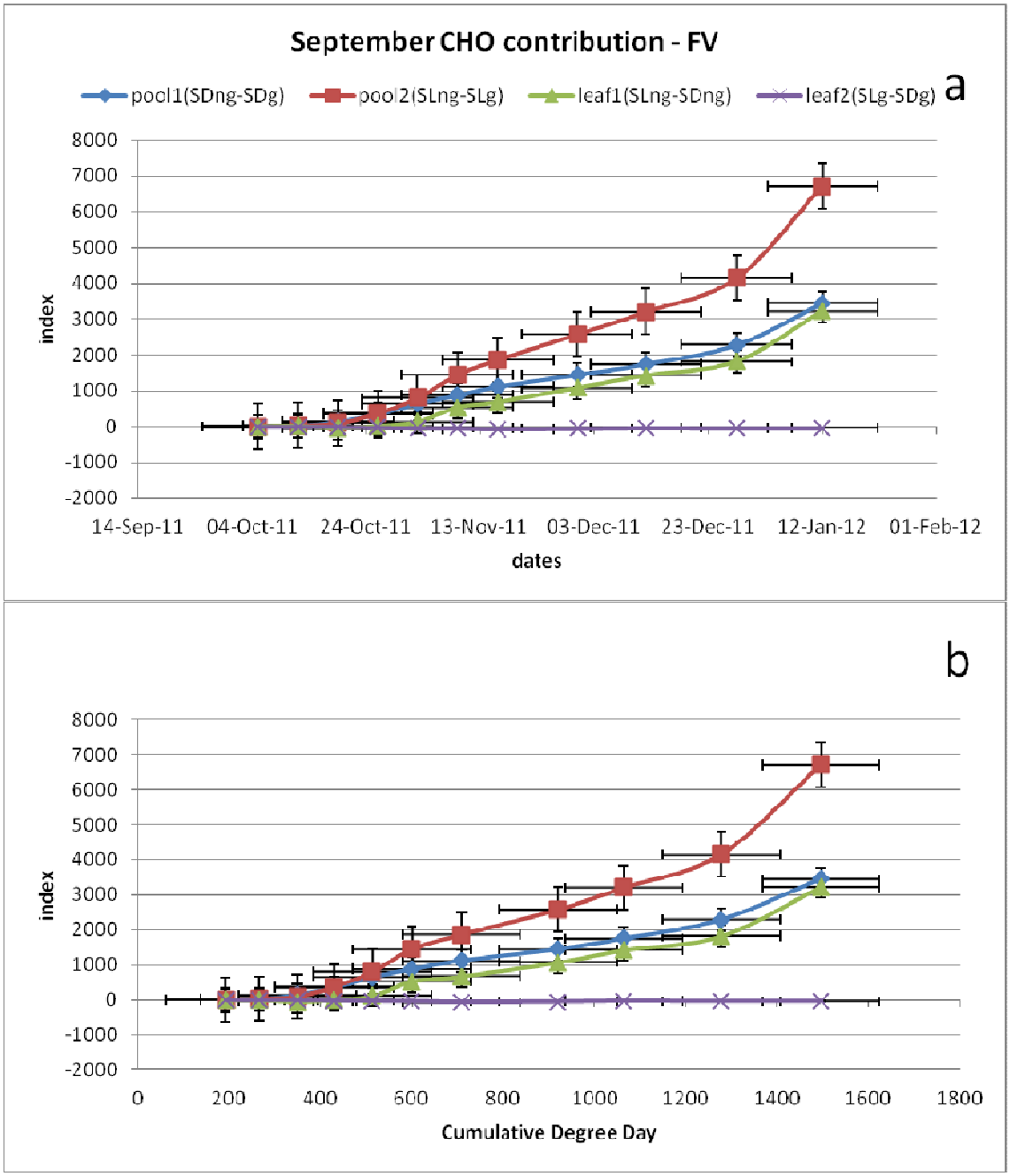
We can generalise the level of contributions of two sources of energy in macadamia for vegetative growth. According to the figure 8, both pool 1 and pool 2, and leaf 1 and leaf 2 crossovers in day 64, so we can consider this point for their contributions (76.26% for reserve and 23.74% for current photosynthate). On the other hand "new growth fresh weight" as we discussed before, is the variable with the highest R-square that we can rely for our assumption of the sources' contributions in vegetative growth of macadamia (73.08% for reserves and 26.92% for current photosynthate) (table 3). So we can say generally that between 73% and 76% of the energy needed for vegetative growth is from reserved CHO and 24% to 27% from current photosynthate. However there is a constant effect from current photosynthate on the vegetative growth of macadamia when the leaves are matured, whereas the effect of reserved CHO fluctuates during the season.

Experiment 2. September

We used the same definitions, equations and calculations for CHO sources contribution like the first experiment in March.


September CHO contribution-FL
a
pool1(SDng-SDg)
pool2(SLng-SLg)
leaf1(SLng-SDng)
leaf2(SLg-SDg)
600
500
400
300
200
100
0
-100
index
15-Sep-11
05-Oct-11
25-Oct-11
14-Nov-11
04-Dec-11
24-Dec-11
13-Jan-12
02-Feb-12
dates


b
600
500
400
300
200
100
0
-100
0
200
400
600
800
1000
1200
1400
1600
1800
cumulative degree days



Conclusion

As it can be seen in growth pattern graphs of September experiment, growth curves of single flushes on girdled PSs are almost overlapped.

The lower photosynthesis rate in macadamia than temperate fruit and nut trees (Flore and Lakso, 1989) is due to a slow and less efficient symplastic loading of photosynthate mechanism through the plasmodesmata can describe the lower contribution of current photosynthate source in vegetative growth of macadamia. On the other hand, macadamia's long-lived leaves have a low light compensation point

with a degree of shade tolerance (Demmig-Adams et al., 1997), which can explain its maximal canopy photosynthesis in lower light intensity (in 30–40% of total light) (Flore and Lakso, 1989).

References:

Cormack DB, Bate GC. 1976. Seasonal fluctuation of total Nonstructural carbohydrate levels within the macadamia-integrifolia cultivar Kakea and its relation to shoot extension. *Rhodesian Journal of Agricultural Research* 14: 39-45.

Costes E, Sinoquet H, Godin C, Kelner JJ. 1999. 3D digitizing based on tree topology: Application to study the variability of apple quality within the canopy. *Proceedings of the Fifth International Symposium on Computer Modelling in Fruit Research and Orchard Management*: 271-280

Demmig-Adams B, Adams WW, III, Grace SC. 1997. Physiology of light tolerance in plants. *Horticultural Reviews* 18: 215-246.

Flore JA, Lakso AN. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. *Horticultural Reviews* 11: 111-157.

Gent MPN, Seginer I. 2012. A carbohydrate supply and demand model of vegetative growth: response to temperature and light. *Plant Cell and Environment* 35: 1274-1286.

Huett DO, McFadyen LM, Olesen T. 2005. Physiological constraints to macadamia (Macadamia integrifolia) production. *Proceedings of the International Symposium on Harnessing the Potential of Horticulture in the Asian-Pacific Region*: 243-246

Lacointe A, Minchin PEH. 2008. Modelling phloem and xylem transport within a complex architecture. *Functional Plant Biology* 35: 772-780.

Liu X, Robinson PW, Madore MA, Witney GW, Arpaia ML. 1999. 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology. *Journal of the American Society for Horticultural Science* 124: 671-675.

Lloyd J. 1991. Modeling Stomatal Responses to Environment in Macadamia-Integrifolia. *Australian Journal of Plant Physiology* 18: 649-660.

Menzel CM, Rasmussen TS, Simpson DR. 1995. Carbohydrate Reserves in Lychee Trees (Litchi-Chinensis Sonn). *Journal of Horticultural Science* 70: 245-255.

Nagao MA, Ho-a EB, Yoshimoto JM, Yoshimura ER, Notley E, Fuchigami LH. 1994. Relationship between vegetative flushing and flowering of Macadamia integrifolia in Hawaii. *Scientia Horticulturae* 60: 9-16.

O'Hare PJ, Queensland. Dept. of Primary I, Fisheries, New South Wales Dept. of Primary I, Australian Macadamia S. 2004. *Macadamia grower's handbook / Paul O'Hare ... [et al.],* Nambour, Qld. :, Dept. of Primary Industries and Fisheries in collaboration with New South Wales Dept. of Primary Industries and the Australian Macadamia Society.

Olesen T, Robertson D, Muldoon S, Meyer R. 2008. The role of carbohydrate reserves in evergreen tree development, with particular reference to macadamia. *Scientia Horticulturae* 117: 73-77.

Pereira CS, de Siqueira DL, Salomao LCC, Cecon PR, dos Santos D. 2011. Fruit Production and Carbohydrate Contents in the Leaves of Acid Lime Tree 'Tahiti' Girdled and Treated with Gibberellic Acid. *Revista Brasileira De Fruticultura* 33: 706-712.

Sakai S. 1981. Preservability and Carbohydrate Reserve of Tea Vegetative Organs in a Long-Term Storage. *Jarq-Japan Agricultural Research Quarterly* 15: 39-45.

Saleem MB, Buxton DR. 1976. Carbohydrate Status of Narrow Row Cotton as Related to Vegetative and Fruit Development. *Crop Science* 16: 523-526.

Soderstrom B, Finlay RD, Read DJ. 1988. The Structure and Function of the Vegetative Mycelium of Ectomycorrhizal Plants .4. Qualitative-Analysis of Carbohydrate Contents of Mycelium Interconnecting Host Plants. *New Phytologist* 109: 163-166.

Stephenson R. A, Trochoulias T. 1994. Macadamia. In: Schaffer B, Andersen PC eds. *Handbook of environmental physiology of fruit crops.* Boca Raton, CRC Press.

Stephenson RA, Cull BW, Mayer DG, Price G, Stock J. 1986a. Seasonal Patterns of Macadamia Leaf Nutrient Levels in South East Queensland. *Scientia Horticulturae* 30: 63-71.

Stephenson RA, Cull BW, Stock J. 1986b. Vegetative Flushing Patterns of Macadamia Trees in South East Queensland. *Scientia Horticulturae* 30: 53-62.

Stephenson RA, Cull BW, Stock J. 1986c. Vegetative flushing patterns of macadamia trees in south east Queensland. *Scientia Horticulturae* 30: 53-62.

Stephenson RA, Gallagher EC, Rasmussen TS. 1989a. Effects of Growth Manipulation on Carbohydrate Reserves of Macadamia Trees. *Scientia Horticulturae* 40: 227-235.

Stephenson RA, Rasmussen TS, Gallagher EC. 1989b. Timing of nitrogen application to macadamias 2. Storage carbohydrates. *Australian Journal of Experimental Agriculture* 29: 575 - 579.

Voitsekhovskaja OV, Koroleva OA, Batashev DR, Knop C, Tomos AD, Gamalei YV, Heldt HW, Lohaus G. 2006. Phloem loading in two Scrophulariaceae species. What can drive symplastic flow via plasmodesmata? *Plant Physiology* 140: 383-395.

Wilkie JD, Sedgley M, Olesen T. 2009. A model of vegetative flush development and its potential use managing macadamia (Macadamia integrifolia) tree canopies. *Crop & Pasture Science* 60: 420-426.

Effects of order of exposure to temperature on flowering and branching in macadamia

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Abstract

Effects of the sequence of exposure to warm and cool temperature regimes on macadamia axillary bud behaviour were investigated, to increase understanding of this tree's flowering and branching processes. Three-year-old trees were exposed to sequences of day / night regimes of 25 / 19 ºC ("warm") and 19 / 11.5 ºC ("cool") in controlled environment chambers, beginning after the spring flowering. Each exposure period was two months long, and four sequences of two periods each were investigated: cool-then-cool, cool-then-warm, warm-then-cool, and warm-then-warm. Tip pruning was used to increase number of shoots. After each exposure period the numbers of inflorescences and vegetative stems emerging were counted, and the proportion of trees branching and flowering was calculated. Over the whole four months, there was no difference between warm-then-warm and cool-then-cool treatments in the numbers of inflorescences emerging, but more emerged from the warm-then-cool sequence than the cool-then-warm sequence. These results suggest that two stages may exist in inflorescence development, the first favoured by warm temperatures and the second favoured by cool, supporting the hypothesis of Sakai *et al*. (1982) that macadamia floral evocation is maximised by warmer night temperatures and emergence by cooler ones. Trees in the warm-then-cool sequence also produced more new stems than those in the cool-then-warm sequence, although there was no accompanying difference in proportion of trees branching. More buds becoming inflorescences in a treatment group did not mean fewer buds becoming stems, which suggests that different buds become inflorescences to those that become stems.

Introduction

Commercial orchards of macadamia nut trees (*Macadamia integrifolia* Maiden & Betche and *M. tetraphylla,* and hybrids of the two) are planted densely in order to increase profits early in the life of the orchard. These densities necessitate pruning later in the life of the orchard (hedging, topping and skirting, carried out mechanically) to enable machinery access. As a result of the pruning, many axillary buds are released from apical dominance, growing out to form a thick outer layer of regrowth, shading the tree's interior and eventually reducing flowering and yield (Olesen *et al.* 2011). If bud outgrowth from dormancy could be better understood, self shading or inhibition of flowering may be mitigated, and the macadamia industry and consumers would benefit from more efficient management of orchards for optimum sustainable nut production.

In their natural geographical distribution - the sub-tropical coastal hinterland of eastern Australia - macadamia trees grow mostly during synchronised bursts of tip elongation and axillary bud release, termed flushes. Similar to lychee (*Litchi chenensis)* and avocado (*Persea americana*) (other sub-tropical perennials), macadamia flushes occur at regular periods, several times a year (Stephenson and Trochoulias 1994; Oleson *et al.* 2002; Olesen 2005). The trees are evergreen and there is no phase of whole-plant dormancy enforced by conditions prohibitive to growth, such as freezing or dehydrating.

Both vegetative and floral shoots arise directly from axillary buds. The term shoot is here used to describe the growth arising from just one axillary bud, and not from the secondary axillary buds found along its length. Vegetative shoots are termed stems and floral shoots are the backbone of an inflorescence. The number of new shoots (either vegetative or floral) forming in macadamia during any one flush has been shown to be affected by temperature. At mean temperatures of 16 ºC to 26 ºC, the number of stems emerging per day increased with temperature in orchard macadamias (2009). This is similar to poinsettia (*Euphorbia pulcherrima*) and beans (*Phaseolus vulgaris*), which respond to increased temperature with increased branching (Hagen and Moe 1982; Kigel *et al.* 1991). In other species the relationships are less clear - in chrysanthemums (*Dendranthema grandiflorum*) and apple trees (*Malus domestica*) no consistent trend relating branching to temperature was found (Abbas *et al.* 1980; Schoellhorn *et al.* 1996).

It is not clear when macadamia buds are first evoked, i.e. set on the biochemical path to forming floral growth. However immature inflorescences become visible as the bud bracts open in autumn or winter (Bennel 1984; Moncur *et al.* 1985). In mid- to late winter they emerge from behind these bracts and elongate to 15 cm or more, before anthesis in late winter or early spring. Cool temperatures will result in emergence of inflorescences from macadamia buds if they coincide with the phase of the phenological cycle when flushing is beginning (Olesen 2005).

Other sub-tropical species are known to use temperature as a cue for the beginning of flowering. In lychee, mango (*Mangifera indica*) and avocado, cooler temperatures of the annual range in which they are grown are needed for inflorescences to be produced (Buttrose and Alexander 1978; Shu 1987; Menzel and Simpson 1988; 1995). In citrus, "Washington Navel" variety oranges produce more inflorescences at lower temperatures, within the range 15/8 ºC to 30/25 ºC days/nights, but day length seems also to influence flowering time (Lenz 1969; Moss 1969; Garcia-Luis *et al.* 1992). In lemon, temperature seems to have only a limited effect on flowering and water stress seems the main trigger for floral growth (Chaikiattiyos *et al.* 1994).

Glasshouse studies of the relationship between temperature and flowering in macadamia suggest that temperature affects inflorescence formation in more than one way. Sakai *et al.* (1982) investigated the effect of temperature on the number of inflorescences and the speed of their production. They treated macadamia trees with night temperatures of 12, 15, 18 or 21 ºC, while exposing all to ambient temperatures of around 28 ºC during the day. No statistical analysis of their data was published, but they noted a number of interesting trends. They reported that inflorescences emerged sooner at lower night temperatures, but that the total number of inflorescences emerging in the 31-week-long experiment appeared to increase with night temperature

up to 18 ºC. Very few inflorescences were produced at 21 ºC, and microscopic observations found no floral differentiation of buds in trees at night temperatures of over 21 ºC. The authors put forth two linked hypotheses; i) warmer night temperatures, up to 18 - 21 ºC, favoured evocation, while ii) cooler night temperatures favoured emergence.

Stephenson and Gallagher (1986) found that macadamia trees exposed to night temperatures of 5, 10, 15 or 20 ºC did not differ in numbers of inflorescences emerging over 10 weeks. However, at the end of the experiment the trees were transferred outside to night temperatures of around 11 ºC, and subsequently trees in the group that had been at 20 ºC produced around 5 times the numbers of inflorescences than those of other treatments. The difference was obvious within seven weeks after transfer. These findings fit well with Sakai *et al.*'s (1982) theory (above).

This study set out to make a direct comparison of the effects on macadamia flowering of warm temperatures, cool temperatures and different sequences of the two, following up on Sakai *et al.'s* 1982 theory that warm temperatures favour evocation and cool temperatures favour emergence. This comparison was made by exposing trees to warm or cool temperatures or both, over two exposure periods each two months long. As emergence must be preceded by evocation, if these two processes do have different optimum temperatures, then the sequence in which trees experience warm and cool temperatures should affect the number of inflorescences produced – the hypothesis is that a different order of exposure to the temperature regimes will result in different numbers of inflorescences emerging. Our study aimed to investigate whether temperature regime sequences affect the number of axillary buds forming new stems. As axillary buds that form inflorescences are no longer available to form stems, it may be that conditions resulting in trees forming a greater number of inflorescences would also result in fewer new stems. Clarification of these relationships between temperature and macadamia growth and flowering would assist commercial growers and managers of threatened wild populations to balance vegetative and floral growth for most efficient nut or seed production, as well informing where and how to adapt to climate change.

Materials and Methods

Fifty-six three-year-old *Macadamia integrifolia x tetraphylla* trees, grown from cuttings for three years in an outdoor nursery in south-east Queensland, were transplanted into pots, 33 cm diameter and 35 cm depth, of sandy-loam pot media in spring 2009. Half of the trees were cultivar A38 and half were cultivar A4. The trees were about 150 cm tall. At the end of the flowering season in early spring, they were moved into a controlled environment facility with day / night temperatures of 26 / 23 ºC to prevent floral evocation (Sakai *et al.* 1982). Three walls of each controlled environment chamber and their roofs were made of translucent plastic, and together with overhead shade-cloth these resulted in the trees receiving light at 60% of outdoor levels. Inflorescences and young fruit were removed, to prevent their inhibition of any new growth. The trees were kept at this temperature for two months and the few new inflorescences emerging during this time were removed.

"Night" temperatures in the glasshouse were set for eight hours during dark, "day" temperatures were set for eight hours during light, and transitions from day to night temperatures and back again were gradual over the intervening four hours. They received one litre of water – sufficient to saturate the media - up to three times per week, when the media surface and saucer were both dry. Each tree received 20 g of low-phosphate slow-release fertiliser every three months.

After the two-month pre-treatment, trees were exposed to one of four treatments, each a sequence of temperature regimes; cool-then-warm (C-W), warm-then-warm (W-W), cool-then-cool (C-C), and warm-then-cool (W-C). This was achieved by exposing half of the trees to warm temperatures and half to cool temperatures for two months, then half of each of these groups stayed under their initial temperature regime and the other half were exposed to the other temperature regime for two months. Four months was chosen as the total experiment duration as this was sufficient for effects of temperature on flowering to be seen in previous experiments (Sakai *et al.* 1982; Stephenson and Gallagher 1986).

At the beginning of the first two-month exposure period an equal number of trees of each cultivar was randomly allocated to either the cool (C) temperature regime - 19.0 / 11.5 ºC - or warm (W) regime - 25.0 / 19.0 ºC. Initial sizes were approximated by summing the length in centimetres of the most central (leader) axis and the secondary axis arising from this. Differences were examined using a Mann-Whitney test. A4 trees had greater initial stem lengths than A38 trees, with means of 645 and 421 cm respectively. This difference in size was highly statistically significant ($P < 0.001$). Each regime was maintained in its own chamber of the building. Trees and temperature settings were swapped between chambers every two weeks to ensure even exposure of treatments to any undetected differences between the apparently identical chambers. At these times, positions of trees within each chamber were re-randomised.

Trees were pruned at week five of the first exposure period, to encourage growth of more new shoots (either stems or inflorescences) to increase statistical clarity (Batten and McConchie 1995). The leader and every second first-order branch was pruned, one growth unit back from the tip, where a growth unit is the portion of a stem created by one continuous period of tissue production by the apical meristem, usually during a spring or summer flush. Typically it consists of about four nodes. This pruning removed any green stem and new leaves towards the end of the parent shoot as well as the apical bud, all of which contribute auxin to maintain apical dominance, which prevents the outgrowth of axillary buds (Warner and Gitlin 1971; Cline 1991).

At the end of the first two-month period, the number of new shoots emerging from each tree was counted. Every inflorescence on each tree was counted, while only stems on the main leader and the first-order branches were counted (around 1/3 of the tree). Small shoots counted at the end of this first period were marked to ensure they were not also counted at the end of the second period. Any tree from which one or more inflorescences emerged was classified as "flowering". Any tree from which one or more new stems emerged was classified as "branching".

The trees were then exposed to the second two-month period of temperature treatment. The leader and every first-order branch were pruned, again at five weeks into the treatment period, one growth unit back from the tip.

Glasshouse chambers' temperatures were each monitored with a pair of "Tiny Tag" brand electronic temperature recorders, each accurate to 0.5 ºC and programmed to record every 15 min. Eight instances of departure from set temperatures reduced the temperature differences between treatments by 2 - 5 ºC, for periods of up to eight hours. None of these departures occurred on consecutive nights and most occurred more than a week apart.

At the end of the second exposure period a large cohort of swollen buds, not yet identifiable (by eye) as either vegetative or floral, was noticed. No such cohort of swollen buds was detected at the end of the first exposure period. The trees were kept outside for six weeks, where temperatures averaged 24.6 / 14.7 ºC, until shoots could be identified as stems or inflorescences, at which time the new shoots were counted and classified as in the first count detailed above. This second count included only shoots that had emerged since the end of the first period.

The effect of temperature regime sequence on both the proportion of trees flowering and the number of inflorescences emerging was analysed. Also, the effect of temperature regime sequence on both the proportion of trees branching (forming new stems) and number of new stems emerging was analysed. Normality of datasets was investigated using Anderson-Darling tests, with non-normal data being transformed (see regression below) or analysed with non-parametric methods.

Differences in the proportion of trees producing new shoots under different treatments were analysed using Pearson chi-squared tests. Differences in new shoot numbers between cultivars were analysed with Mann-Whitney tests. Differences in second period new shoot numbers between either first or second period temperatures were analysed using Mann-Whitney tests. Differences in total new shoot numbers between temperature sequence treatments were examined using ordinal logistic regression, with initial tree size included as a predictor. For inflorescences, regression analyses used a complementary log-log link function, while for stems a probit link function was used. Correlations between stem numbers and inflorescence numbers were investigated using Pearson's coefficient. Effect of temperature on new shoot numbers within cultivars or treatments were examined using Mann-Whitney tests (for comparisons between two groups) or Kruskal-Wallis tests (for comparisons between more than two groups). $P < 0.05$ was used as the indicator of a significant relationship in all analyses. (Fowler *et al.* 1998; Minitab 2007)

Results

Inflorescences

First period: During the first two-month period, more trees flowered in the warm (W) treatment than in the cool (C) treatment (Figure 7, Table 1). There was also a greater number of inflorescences per tree emerging from trees under the W regime. There was no difference between cultivars in the number of inflorescences, and no correlation between tree size and number of inflorescences (data not shown).

Second period: The proportion of trees that flowered in this period (Figure 7) depended on the previous period's temperature, as well as the current period's. More trees flowered in this second period after being exposed to warm temperatures in the

first period (W-C together with W-W) than after being exposed to cool (C-W with C-C). Second period temperatures had the opposite effect, with more trees flowering under cool temperatures in this period (W-C with C-C) than those under warm temperatures (W-W with C-W).

The number of inflorescences emerging during the second period (Figure 9) was different between every pair of temperature sequences except W-W and C-C. More emerged from trees exposed to the warm regime during period one (W-C and W-W) than trees exposed to the cool (C-C and C-W). More emerged from trees exposed to the cool temperatures in the second period (C-C and W-C) than trees exposed to the warm (C-W and W-W).

There was no correlation between plant size and inflorescence number, either overall or within temperature regimes (data not shown). There was no difference between cultivars in numbers of inflorescences emerging during the second period (Figure 9).

Combination of periods: The proportion of trees flowering in total i.e. over the combination of both periods (Figure 7), did differ between temperature sequences. The number of inflorescences emerging in total (Figure 10) was also different for every pair of regimes except C-C and W-W. There was a positive correlation (P=0.009) between tree size and number of inflorescences emerging in the W-W treatment (data not shown), but not in any other treatment. There was no difference between cultivars in the number of inflorescences emerging (Figure 4).

Relationships between first and second period inflorescence emergence: A correlation between the number of inflorescences emerging from a tree in the first period and that emerging in the second period was not detected, over all treatments or in any one treatment.

Stems

First period: After the first period, there was no difference between treatments in the proportion of trees branching (Figure 8, Table 1). There was also no difference in the number of new stems emerging per tree. There was no correlation between the number of stems emerging and the initial size of the trees, either overall or within treatments. Cultivar made no significant difference to the numbers of new stems produced.

Second period: The proportion of trees branching in the second period (Figure 8) was not dependent on temperatures experienced in the previous period, nor was it dependent on temperatures experienced in the second period. The number of new stems (Figure 11) was also similar between trees with different first period temperature regimes, as well as between trees with different second period regimes.

There was a difference between numbers of stems emerging in this period from trees of different cultivars (P= 0.040), with a median of 10 emerging from A38 and of 6 emerging from A4.

Combination of periods: When flowering data for both periods was combined there was little difference between temperature sequences in the proportion of trees branching (Figure 8).

There was a positive correlation between tree size and number of new stems emerging in group W-C (P= 0.021), and a negative correlation between size and number of stems emerging in C-W (P= 0.005) (Figure 13). Temperature sequence and size interacted to effect the number of new stems over the combination of periods (*P*= 0.004) (Figure 12, Figure 13). Fewer stems emerged from C-W than from W-C . The W-W stem emergence was no different to that of C-C.

There was no significant difference between the numbers of stems emerging from trees of different cultivars (Figure 12).

Relationships between first and second period stem emergence: The number of stems emerging in the first period from a tree under any temperature sequence treatment was not correlated with the number in the second period, over all treatments or in any one treatment.

Relationship between vegetative and floral growth

There was no correlation between the number of emerging stems and numbers of emerging inflorescences within either period or overall. Neither was there any correlation within any of the treatment groups.

There was no overall correlation between numbers of stems emerging in the first period and the number of inflorescences emerging in the second period, or vice versa. Only trees in the C-C group had a correlation (P= 0.032) between new stem numbers in the first period and inflorescence numbers in the second (data not shown).

Discussion

Flowering

The order of exposure to warm and cool temperature regimes affected flowering, as over the combination of treatment periods a greater number of inflorescences emerged from trees in the W-C sequence than C-W, and a greater proportion of trees flowered in W-C than C-W. This is despite the W-C treatment and the C-W treatment receiving the same total degree days, over the same duration of exposure to the warm regime and the cool regime. Some trees in the W-C treatment did not flower at all. This is possibly because they were still immature and flowering-incompetent, as some of the trees in other treatments may also have been. As a result, the variation as a proportion of the mean for the CW treatment is about twice that of the other treatments.

The number of inflorescences emerging during the second period was greater when the trees had been exposed to warm temperatures in the first period. Also, cooler temperatures in the second period resulted in more inflorescences emerging during this time. These two observations added together logically support the overall difference between W-C and C-W.

The difference between C-W and W-C fits with the hypothesis that an early stage of inflorescence formation is favoured by warm temperatures and a later stage by cool temperatures. As suggested by Sakai *et al.* (1982), the early stage could be evocation and the later stage growth and subsequent emergence from behind the bracts.

Dormancy between evocation or initial floral development and emergence have been well documented in Eastern Redbud trees (*Cercis canadensis)* and roses (*Rosa spp*.) (Zamski *et al.* 1985; Owens and Ewers 1997).

If Sakai's theory is correct, when cool temperatures preceded warm in this experiment, there were few buds evoked in the first period and thus few able to emerge as inflorescences during the second period. Then warm temperatures in the second period would result in a small proportion of these few evoked buds growing to become visible. This would explain the low numbers of inflorescences seen in the C-W treatment. It would also explain the high numbers seen in the W-C treatment; warm temperatures favouring evocation would start many buds along the first, microscopic and hidden stages of floral development, and subsequent cool temperatures would result in a large proportion of those many evoked buds emerging.

An alternative mechanism that could explain these results is that trees build more reserves during warm temperatures than cool, and cool temperatures promote evocation (with emergence following without a temperature trigger). This evocation could occur in more buds of W-C trees than of C-C trees, due to the former having more energy to fuel any sort of growth that occurs. However this scenario would probably have resulted in a linear relationship between initial temperature and number of inflorescences in the experiment of Stephenson and Gallagher (1986), where as only the warmest treatment produced a sizeable number and the rest produced close to none. To determine which mechanism is behind the behaviour of macadamia axillary buds, further work investigating biochemical or microscopic morphological changes to the bud accompanying changes in temperature regime would be useful.

Trees in the W-W treatment did not produce more inflorescences overall than those in the C-C treatment. In contrast, after only two months of exposure to either a warm or a cool temperature regime, more inflorescences had emerged from the warm regime. In the second period, more inflorescences emerged from the cool regime, thus cancelling out the first period results and resulting in no difference overall. This may be due to weak evocation of the cool regime being more limiting over two months than weak emergence in the warm regime, with the evocation catching up over the second two months.

Branching

The number of stems emerging over the combination of the two periods was also dependent on the sequence of temperature regimes: more stems emerged from W-C trees than C-W. However as the magnitude of the difference is small, it may not have a substantial biological impact. The lack of difference in proportion of trees branching over the combination of periods supports further the cautious use of these results as preliminary. If a substantial difference in temperature relationships between emergence and number of trees flowering is accepted, it could be due to temperature acting in tandem with other factors to control stem outgrowth. Release of axillary buds from dormancy may have occurred in most of the trees studied here simply through tip-pruning, removing the tissues imposing apical dominance (Cline 1991). The effect of temperature may not have been strong enough to modify the response of whole trees to pruning, only the response of some axillary buds within responding trees. Such types of interaction between a number of dormancy controls are regarded

as common (McSteen and Leyser 2005). In macadamia, the first flush may be cued by temperature (Stephenson and Cull 1986), but latter flushes are triggered internally and are related to the growth rate of the previous flush (Olesen *et al.* 2006). Water supply is thought to affect the number of buds emerging from macadamia, but has not been observed to control the timing of release from dormancy (Stephenson and Trochoulias 1994). Low light levels reduced the number of flushing stems in macadamia (Broomhall 2009; Olesen *et al.* 2011). In lychee and mango, nutrient supply is known to affect release of axillary buds from dormancy (Li *et al.* 2000; Kotur and Murthy 2010). In avocado increased starch concentrations were found immediately before spring flushes and may be part of the axillary bud release mechanism (Robinson 2002). However these factors have been found to play minor or negligible roles in triggering flushing of macadamia (Stephenson and Cull 1986; Olesen *et al.* 2006).

The finding that there was no difference in overall stem emergence or proportion of trees branching between trees exposed to the W-W temperature regime sequence and trees exposed to the C-C sequence (or between W and C) is surprising, given Wilkie *et al.*'s (2009) results that temperature does effect stem emergence. It is possible that the response of vegetative growth to temperature varied between the two studies due to differences between tree maturity, time of year, cultivar, pruning history, and / or orchard versus pot conditions. In particular the second tip-pruning of these plants may have removed many of the nodes most likely to produce new stems – those of the previous growth flush. The subsequent reduced proportion of stems available to count may have reduced the magnitude of difference between these treatments to the point where it was not statistically detectable. In Wilkie *et al.*'s work each stem was only pruned once, and so the stems emerging from the first flush were included in their count. Repetition of this work with a less severe pruning regime would be useful in resolving this issue.

Relationship between vegetative and floral fates

No consistent relationship was found between growth in the first period - either floral or vegetative - and growth in the second period. Logically it could be expected that trees with higher vigour than others or larger trees (with more energy stores and buds, and greater photosynthetic capacity) would produce more growth in both first and second periods, either vegetative or floral. If the trees were using up stored energy to grow, as observed by Karimaei (2012), it could be expected there would be an inverse relationship between first and second period growth, as there would then be less fuel for second period growth. One possible explanation is that trees used stores for first period growth, but second period growth was not less as the new leaves of the first period were fuelling the trees in the second period.

No consistent relationship was detected between the number of inflorescences and the number of stems emerging in either period or the combination of periods. More buds becoming inflorescences did not mean fewer buds becoming stems in the same growth period. This can be read as an indication that different buds become inflorescences to those that become stems.

Methodology

At the end of the second period of this experiment, trees were moved out of the chambers to an outside site (to await differentiation of swollen buds) where the ambient temperatures were in between those of the treatment temperatures. This meant that those trees moved out of the glasshouse from the warm regime experienced a decrease in temperature (of 0.4 / 3.3 ºC), while those moved from the cool regime experienced an increase in temperature (of 5.4 / 3.2 ºC). The effect of this difference in direction of temperature change on bud development, despite all trees being held at the same absolute temperatures, needs to be considered. If this difference in change direction did affect the numbers of emerging shoots, it would reduce or negate the differences between the groups moved from warm treatments and the groups moved from cool treatments. As there were still highly significant differences between these treatments, it seems that either the effect of the different direction of temperature change at this point was negligible, or that the treatment effect was even larger before storage. Comparing time to bud differentiation in this study and the studies of Stephenson and Gallagher (1986) and Sakai *et al.* (1982), it is clear that the time taken for shoot emergence following a temperature trigger can vary, no doubt with factors such as plant health, light levels, and water availability.

Implications

The relationships between temperature and the extent of flowering in *Macadamia integrifolia x tetraphylla* orchard trees, and wild plants of their ancestor species, will clearly have implications as global climate changes. The detail of such effects cannot be reliably predicted without further study. One area of investigation would need to be the relationship between cool temperatures and the speed and extent of emergence. Warming may decrease or delay emergence, more so at the warmer end of the geographical range of macadamia.

If a two-step flowering process is confirmed, more knowledge of the timing of evocation, especially whether it is a continuous phenomenon or something that occurs in a narrow window, will also be important to forecasts of change. In cooler regions of the range increased temperatures may increase evocation. In warmer regions, increasing temperatures may decrease evocation, where the upper temperature limit of evocation (23 ºC) is approached or exceeded. Alternatively, evocation may occur at different times of the year. The sum of such changes to evocation and emergence is flowering intensity. This may increase or not change in cooler regions, but may decrease in warmer regions. This may be compensated for by longer durations of flowering. Where emergence times are altered, differences in response between varieties may mean those varieties previously matching in flowering time may now not overlap, interrupting cross-pollination in some multi-variety orchards.

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Table 6. Effect of the first period of exposure to either cool or warm temperatures on the number of new shoots and proportion of trees shooting in that period.

Temperature regime	Trees flowering (proportion)	Trees branching (proportion)	Inflorescences emerging per tree (median, *percentile*)	Stems emerging per tree 25^{th}-75^{th}
C	0.04	0.78	0 *0 – 0*	7 *1 – 10*
W	0.28	0.88	0 *0 – 1*	8 *3.75 – 18*
P of difference between C and W	0.018	0.010	0.019	0.093

Table 7. P values of effects of first and second period exposure to either warm or cool temperature on second period growth.

Period of exposure to cool or warm	*P* values of effect of temperature on second period growth			
	Trees flowering	Trees branching	Inflorescences emerging per tree	Stems emerging per tree
First	0.007	0.700	0.027	1.000
Second	0.032	0.186	0.044	0.085

Table 8. *P* values of differences between temperature sequences in proportion of trees branching or flowering and number of inflorescences or stems per tree, over the combination of both periods

Treatments compared	Trees flowering	Trees branching	Inflorescences emerging per tree	Stems emerging per tree

C-C and W-W	0.561	1.000	0.800	0.280
C-W and W-C	0.000	[Insufficient variation to test]	0.000	0.001

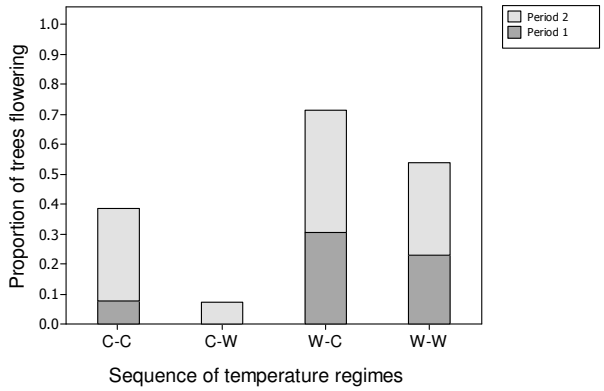

Figure 19. Cumulative effect of temperature regime sequence on proportion of trees flowering over both exposure periods.

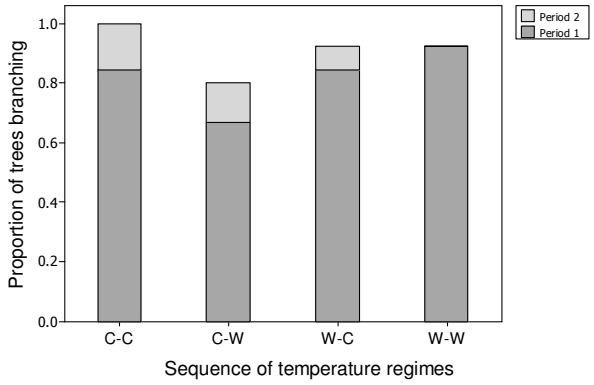

Figure 20. Cumulative effect of temperature regime sequence on proportion of trees branching over both exposure periods.

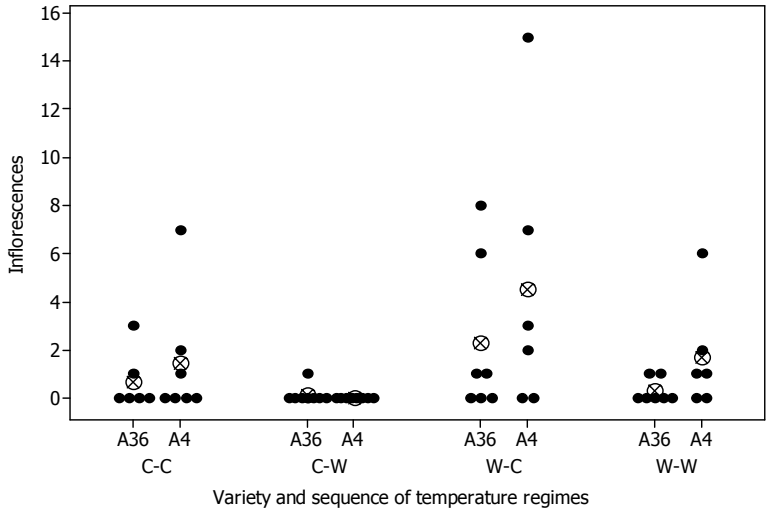

Figure 21. Effect of temperature regime sequence and variety on the number of inflorescences emerging during the second exposure period. *Dots are individual values (trees), crossed circles are means.*

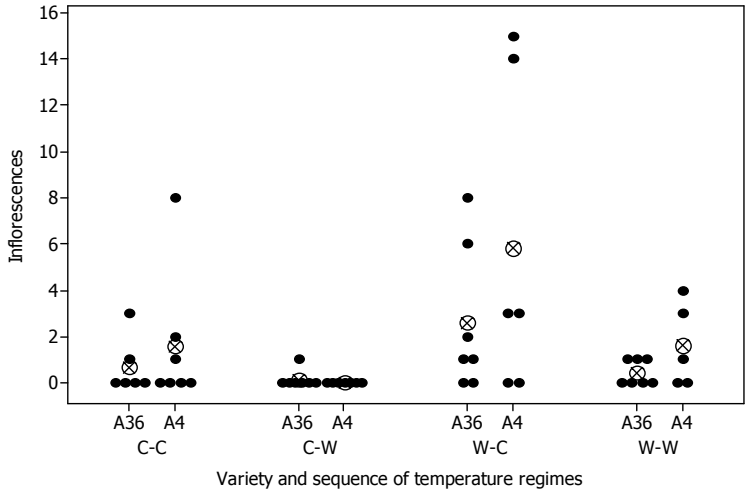

Figure 22. Effect of temperature regime sequence and variety on number of inflorescences emerging in total over both exposure periods. *Dots are individual values (trees), crossed circles are means.*

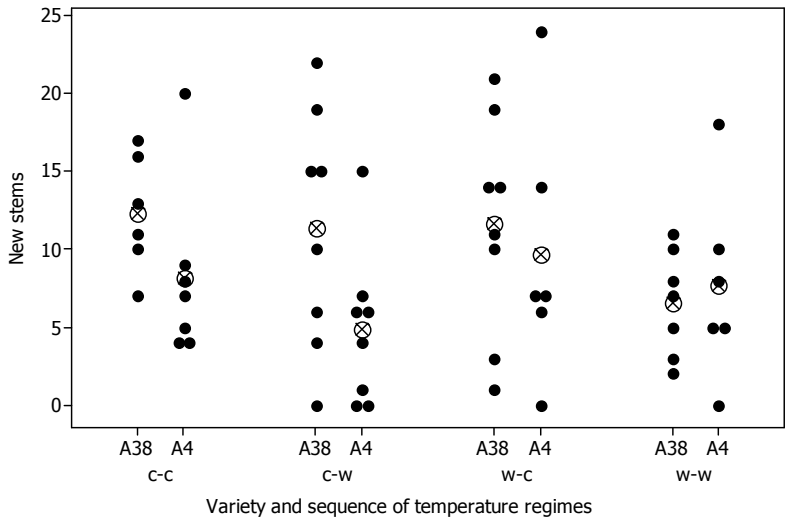

Figure 23. Effect of temperature regime sequence and variety on number of new stems emerging during the second period. *Dots are individual values (trees), crossed circles are means.*

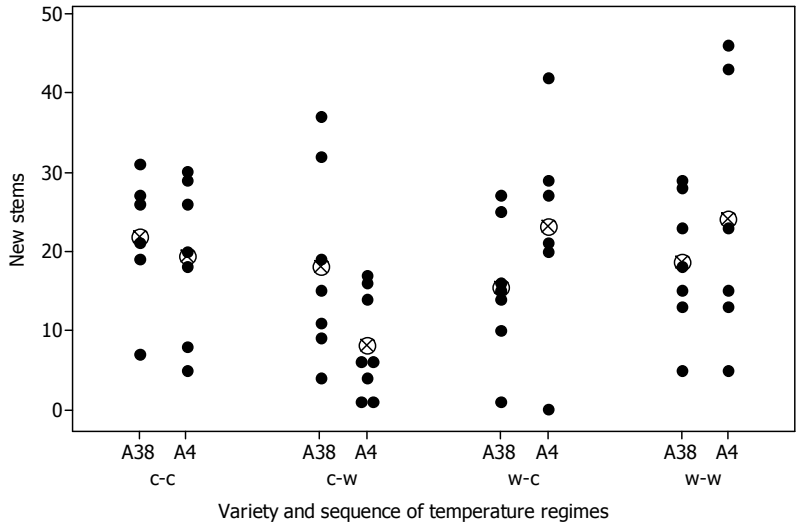

Figure 24. Effect of temperature regime sequence and variety on number of stems emerging in total over both periods. *Dots are individual values (trees), crossed circles are means.*

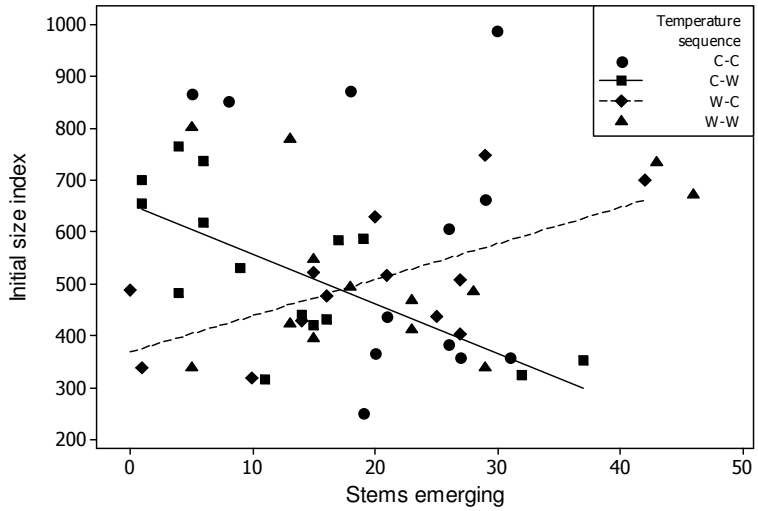

Figure 25. Effect of temperature and size on total stems emerging over both periods. *Initial sizes were approximated by summing the length in centimetres of the most central (leader) axis and the secondary axes arising from this.*

Appendix 1. PhD Work Summaries

PhD Project No. 1. Implications of tree architecture and carbohydrate allocation in Macadamia tree development – Sadegh Karimaei

Activity / chapter	Stage	Remaining
Introduction (investigation industry needs and definition of project)	Written-up.	Proof read.
Literature review (investigation of existing knowledge of relevant works)	Final edit.	Add any relevant new knowledge and proof read.
Digitizing assessment – architectural studies	Data analysing and writing the result	Writing results and conclusion
Micrografting	Data analysing	Writing results and conclusion
Orchard experiments	Finalised data analysis	Publish two possible papers
Carbohydrate sources in vegetative growth of macadamia – A novel indirect method to determine CHO source contributions	First draft is almost ready to submit to Annals of Botany in two months.	Some parts should be reviewed.
Modelling – orchard experiments	A prototype model is developed from orchard experiments in L-systems	Develop a model based on orchard experiments results
Modelling – glasshouse experiments	Data analysing	Models from digitized plants
Modelling – final model		Finalise macadamia tree model
Conclusions	Initial understanding of interactions between results drafted.	Integrate remaining results including modelling outcomes.

Compilation of thesis document including abstract, referencing, appendices	References largely catalogued and formatted. Documents for thesis publication constructed/connected.	Finalise cataloguing and formatting after 'Discussion' sections completed.
University presentations	First and second year results / research presented to interview panel and discussed.	Pre-thesis-submission presentation and interview.

PhD Project No. 2. Axillary bud behaviour in Macadamia - Janine Conway

Activity / chapter	**Stage**	**Remaining**
Introduction (investigation industry needs and definition of project)	Written-up.	Proof read.
Literature review (investigation of existing knowledge of relevant botany)	Final edit.	Add any relevant new knowledge and proof read.
Temperature sequences	Paper submitted. Results presented at Industry conference.	Possible changes (if suggested by reviewers).
Location of Flowering/Branching	Results analysed. Chapter written up to discussion. Preliminary results presented at industry conference.	Write 'Discussion'.
Pruning at key locations	Preliminary analysis of results completed. Chapter written-up to 'Results'.	Confirm and develop statistics with biometrician. Write 'Results', and 'Discussion'.
Modelling	Basic skills attained. Skeleton model tree created. Modelling potential of data collected discussed.	Add research results to skeleton model.
Conclusions	Initial understanding of interactions between results drafted.	Integrate remaining results including modelling outcomes.
Compilation of thesis document including abstract, referencing, appendices	References largely catalogued and formatted. Documents for thesis publication constructed/connected.	Finalise cataloguing and formatting after 'Discussion' sections completed.
University presentations	First and second year results / research presented to interview panel and discussed.	Pre-thesis-submission presentation and interview .