Axillary bud behaviour in *Macadamia integrifolia* and its hybrids

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Abstract

Macadamia integrifolia and hybrids with *M. tetraphylla* are multiple flushing, subtropical trees which form the basis of the macadamia nut industry in Australia. However little is known about the process of inflorescence or new stem formation in these trees, including floral induction. Axillary buds form both new stems and inflorescences, so this thesis reports investigations into the effect of architectural position in the tree, and of temperature, on axillary bud release from dormancy and floral or vegetative identity.

Location of inflorescence emergence and of new stem production over one year was mapped according to their locations within tree architecture. Bud location down to node level was related to proportion of nodes branching or flowering, and to number of inflorescences or stems per node. Nodes were mapped by position along the growth unit, by growth unit position along the axis, and node position along axes. Interactions of these positions with the size of axis and the size of growth units were also examined.

Position of the node along the growth unit was the strongest factor influencing both proportion of nodes flowering and proportion of nodes branching. The proportion of nodes flowering increased with node proximity to the growth unit base. The proportion of nodes branching was highest by far on node two below the top of the growth unit.

Pruning resulted in branching on the node or two immediately below the cut, whether the cut was at the top or base of a growth unit. However pruning did not increase numbers of new stems on the axis. Pruning did not change number or location of inflorescences on the growth unit pruned, but did increase inflorescences emerging from the growth unit below it, and significantly increased the number of inflorescences emerging from the axis by around 50%.

Growth unit position along the axis also exerted a strong influence on location of inflorescences and new stems. Nodes on growth units closer to the base of an axis were more likely to flower than those on growth units towards the tip. Nodes of growth units closest to the tip were more likely to branch than those at the base.

Axis size influenced the proportion of nodes flowering in five year old trees of variety 741, in which flowering was greatest on shorter axes, but not in twelve year old variety 741 trees or thirteen year old variety 842 trees in which flowering was also studied. The effect of growth unit size on proportions of nodes flowering was different in different varieties and ages.

Axes extending were more likely to branch and less likely to flower than axes that did not extend. Branching as well as extension of an axis reduced the number of inflorescences emerging from that axis. Different applications of mineral nutrients varied the proportions of axes extending and branching in potted trees. Trees receiving moderate levels of fertiliser along with low levels of water extended more and branched less than other treatments, resulting in trees becoming more open in structure over one growth period.

In varieties A4 and A36, temperature appeared to affect flowering in two stages. Warm night temperatures (19°C) followed by cool night temperatures (11.5°C) resulted in substantial inflorescence emergence, but cool night temperatures followed by warm resulted in almost no emergence. Only-warm or only-cool night temperatures each resulted in moderate emergence. Along with the work of previous researchers, this suggests that an early stage of flowering is promoted by warm night temperatures, and a later stage by cool night temperatures. As cool temperatures are known to promote emergence of minute dormant inflorescences, the early stage may be floral induction and subsequent meristem evocation and determination.

Axillary buds were collected during months of warm night temperatures and several months following these. Microscopic examinations of buds showed morphological gradients in development along the growth unit, parallel to gradients in node likelihood of flowering. However no obvious morphological difference between the high branching node just under the growth unit tip and other nodes was detected. A small number of buds from growth units that had just finished elongation appeared to contain round, domed meristems, which are indicative of floral growth. Together with the finding that warm night temperatures promote an early stage of flowering, this prompts the suggestion that floral evocation in macadamias could occur predominantly during the summer flush growth period. One preformed node was found in most buds towards the base of growth units by the time this same flush period was ending. Two preformed nodes were found in most apical buds at this time.

The findings of clear patterns in flowering and branching, and that these change with pruning, as well as the relationship between temperature and control of flowering, may

assist orchardists in designing new orchard management programs that increase efficiency and sustainability of macadamia nut production.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

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List of abbreviations

AD	apical dominance
AIC	Akaike’s Information Criterion
° C	degrees Celsius
C	cool
C-C	cool-then-cool
cm	centimetre
CO_2	carbon dioxide
C-W	cool-then-warm
DNA	deoxyribonucleic acid
et al.	*et alia* (meaning: and others)
FT	flowering locus T gene
GLM	Generalised Linear Model
GU	growth unit
m	metre
mm	millimetres
mRNA	messenger ribonucleic acid
NPK	nitrogen phosphorus potassium
P	probability of difference being due to chance
PGR	plant growth regulators
r_s	Spearman’s correlation coefficient
SAM	shoot apical meristem
W	warm
W-C	warm-then-cool
W-W	warm-then-warm

Chapter 1. Introduction

Macadamia nuts have been part of the human diet since before written history in Australia (Australian National Botanic Gardens 2000). They are produced commercially by the trees *Macadamia integrifolia* and *M. tetraphylla* and hybrids and grafts of the two (Figure 1-1). They are the most widely commercialised Australian native food plant to date, and in 2013 ten thousand tons of nut (without shell) were produced, worth over 140 million dollars (Australian Nut Industry Council 2014).

Figure 1-1. Young *Macadamia integrifolia* trees, variety 741, in a commercial orchard.

M. integrifolia and *M. tetraphylla* are native to the subtropical coastal ranges of eastern Australia (Stephenson and Trochoulias 1994). Mature macadamias trees are over 15 m tall, and in cultivation are usually maintained in hedgerows. Hedging maintains access for machinery, and lets light onto the sides of the trees as well as onto the orchard floor. The latter keeps ground cover alive, reducing erosion and consequent medium- to long-term yield reductions (Huett *et al.* 2005). However, vegetative regrowth from axillary buds (Figure 1-2 and Figure 1-3) after hedging can create a thick layer of leaves at the new outer edge of the canopy. This can shade the interior of the canopy, which in turn can increase premature nut drop (Olesen *et al*. 2011), and is suspected of contributing to long term yield reduction. Greater understanding of the branching process and how the tree controls this may aid in balancing the need for space between trees and sustaining production levels, by reducing self-shading from hedging regrowth.

Figure 1-2. New macadamia branch emergence from axil of abscised leaf.

Figure 1-3. Axillary buds of *Macadamia integrifolia*.

In macadamia, inflorescences are also formed from axillary buds (Figure 1-4). The location of inflorescences varies with cultivar but is usually in from the edge of the canopy by a metre or two (Salter *et al.* 2005; Olesen *et al.* 2011). Hedging usually only removes outer portions of stems more distal to this. However if left unhedged, this removed portion would become that section of the canopy inside the edge by a metre or two. As hedging thus removes stem sections which hold future flowering locations, it may have long term effects on yield. Currently this is difficult to judge, as little is known about what controls the early stages of flowering. The inflorescences mature in winter with cool temperatures, but in many varieties are just visible to the unaided eye for months before this, and warm temperatures have also been shown to increase flowering, possibly promoting a separate earlier stage than the cool temperatures (Sakai *et al.* 1982; Stephenson and Gallagher 1986).

Figure 1-4. Inflorescence emerging between petiole and peduncle of older inflorescence (top of branch)

This study will investigate axillary bud behaviour in *M. integrifolia* and hybrids with *M. tetraphylla*, with a view to better understanding the processes of branching and flowering, and how orchard management techniques interact with them. It will focus on two aspects of this behaviour, release of axillary buds from dormancy, and their determination as either vegetative or floral axes. This will be undertaken through the study of variation of dormancy and determination with location in the tree, and variation with temperature, the latter being related to the cycle of tree growth activities throughout the year.

Thus the practical research of this thesis will start with three sets of investigations examining the location of branching and flowering within the structure of macadamia stems, or axes, and the relationship of axillary growth to apical growth (Chapters 3 to 5). The following group of studies will address the changes in quantity and location of inflorescences and new vegetative shoots that occur with pruning (Chapter 6). Another experiment will look at the effect of temperature on axillary bud outgrowth and identity as either floral or vegetative (Chapter 7). The final two investigations combine knowledge gained from work in temperature and in location, to search for the earliest signs of difference between floral buds (or those seemingly predisposed to become floral) and vegetative buds (Chapter 8). This is hoped to help ascertain the time of floral primordia initiation in macadamia. It is hoped the resultant collection of findings will contribute in the longer term to increased sustainability and productivity in commercial macadamia orchards, and to successful conservation of threatened wild macadamia populations.

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Chapter 2. Literature review

2.1. Macadamia structure and phenology

Macadamia integrifolia and *M. tetraphylla* form the basis of the macadamia nut industry in Australia, either as varieties of *M. integrifolia* or as hybrids of the two species. They are evergreen, subtropical trees of the family Proteaceae that reach heights of around 18 m (World Agroforestry Centre 2009). They grow mostly during synchronised bursts of stem elongation and axillary bud release, termed flushes, which occur in spring and late summer (Stephenson and Trochoulias 1994). Nodes are formed as whorls of three or four leaves, and each leaf axil contains up to five axillary buds, of which only the top two usually develop (Bennel 1984). On pruned stems, the number of axillary buds branching, i.e. forming new lateral stems, increases as temperatures rise between 16°C and 26°C (Wilkie *et al.* 2009b).

Macadamia also forms its inflorescences from axillary buds. The inflorescences are botanically described by various authors as either reduced panicles, pseudo-racemes or conflorescences (Rao 1971; Douglas and Tucker 1996; Australian Biological Rescources Study 2000). There are approximately 2,500 to 10,000 inflorescences on a mature 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). Inflorescence density is only correlated with yield up until 20/cubic metres, when yield plateaus (McFadyen *et al.* 2008). Inflorescences usually develop from buds on older wood, with highest densities on wood 3 to 9 years of age, but are also found on wood less than a year old (Nagao *et al.* 1994; Salter *et al.* 2005; Wilkie *et al.* 2009a; Olesen *et al.* 2011). The distance of flowering wood from the canopy edge usually ranges from less than a metre to more than two metres in trees over 6 m across, and is deeper in the canopy on the eastern side of the tree and on the higher branches (Salter *et al.* 2005; Olesen *et al.* 2011). All these flowering location studies show differences in fruiting wood age and canopy depth of inflorescences between varieties.

The known period of inflorescence development in macadamia is spread over many months. Immature inflorescences of many varieties become just visible as minute pale swellings behind the bud bracts in autumn or early winter (Bennel 1984; Moncur *et al.*

1985). At this stage a number of varieties have been observed to halt growth for several months (Moncur *et al.* 1985), but it is possible inflorescences of other varieties halt growth before they are large enough to part the bracts and be seen. In mid- to late-winter inflorescences elongate in response to cool temperatures (Olesen 2005), emerging from behind the bracts and growing to lengths of 15 cm or more. Anthesis occurs in late winter or early spring. However it is not known when the meristem becomes florally determined.

2.2. Axillary bud formation and dormancy

In all branching plants, axillary buds are formed as the shoot apical meristem (SAM) creates a new node – a leaf or leaves together with axillary buds on the stem just above the petiole-stem junction. From the beginning of leaf primordia formation (primordia being microscopic organs on the side of meristems), a region of tissue produced by the SAM is prevented from differentiating (Greb *et al.* 2003; Scott 2008), and this becomes enclosed in bracts to form the axillary meristem in the axillary bud.

In some species the newly formed axillary meristem is able to grow to produce lateral shoots within the flush period or season of its formation – this growth is termed sylleptic growth. In macadamia this very rarely occurs, instead the buds enter a dormant period, and lateral growth emerges in later flush seasons – termed proleptic growth (Henderson and Lawrence 1989). As per Lang’s (1987) definition, here dormancy will be defined as “a temporary suspension of macroscopic growth of predominantly meristematic structures”. Importantly, dormancy does not imply complete lack of activity - dormant axillary buds can be just as metabolically active as growing buds (Lang 1987; Stafstrom and Sussex 1988).

Axillary bud entry into and exit from dormancy is influenced by a network of factors. Dormancy may be brought on by a growth-limiting environment – termed ecodormancy - or by factors within the plant. Within the plant, dormancy can be controlled from within the dormant organ itself – endodormancy - or can be enforced by another part of the plant – paradormancy (Lang 1987). As noted by Olsen (2003), there is often overlap between these different types of dormancy – the exterior environment can act through a sensory organ to induce dormancy in a second organ. It is possible that, as well as variation in strengths of factors imposing dormancy, the sensitivities to dormancy triggers may also differ between buds of one plant (Trewavas 1982).

2.2.1. Internally regulated dormancy

The exit from dormancy of an axillary bud is termed ‘outgrowth’. It includes changes in meristem metabolism to enable growth, and initial growth to the point where the meristem is no longer sensitive to auxin-mediated suppression. At this point an independent axis[1] has been formed, and further growth is merely extension of this axis. This extension growth is a separate process to release from dormancy, and is controlled by a different set of factors termed apical control (Cline and Harrington 2007). Between the first changes to dormant meristem metabolism and reaching independence, the bud can be sent back into dormancy by plant growth regulators (PGRs) produced by other growing tissues towards the apex – including other axillary buds, new stems, and young leaves. This window of time is called ‘transition’. Buds can enter transition (i.e. begin the exit from dormancy) and be re-suppressed many times before successful outgrowth (Stafstrom and Sussex 1988; Dun *et al.* 2006), each time growing a little and thus increasing their chance of outgrowth when next released.

A major component of dormancy in axillary buds is apical dominance. Apical dominance (AD) is "the control exerted by apical portions of the shoot over outgrowth of lateral buds" (Cline 1991). Apical dominance results in relatively little branching in buds closer to the parent axis apex, and more branching further away. The strength of AD varies from species to species (Cline 1997). In macadamia the lack of sylleptic growth may be due to AD, or may be because the new buds do not form and mature fast enough to start producing a new stem within the flush period of 1 to 2 months.

Apical dominance appears to be achieved through a combination of several mechanisms. The plant growth regulators auxins, the most common of which is indole-acetic acid, have long been thought the AD signal that prevents axillary bud outgrowth (Thimann and Skoog 1933). Auxins are produced by young tissues at an axis tip and travel down its length, and not up, even from junctions with other axes. However recently it has been shown (in peas, *Pisum sativum*) that the speed of depletion of auxin and the subsequent mechanisms this depletion

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[1] An axis is an unbranched length of growth arising from one meristem. It is also often called a shoot, but this is commonly thought of as describing vegetative (as opposed to floral) structures, despite the term technically referring to either. A vegetative axis is a stem, and a floral axis is a flower or the rachis of an inflorescence. To avoid confusion when discussing canopy architecture, from here the term “axis” is used to describe the parent structure, and “new stem” is used to describe the latest generation stems arising from the axillary buds of that parent axis.

triggers are too slow to account for the initial release of axillary buds from dormancy (Mason *et al.* 2014). Instead that study found that sucrose concentration regulates the initial stage of release from dormancy, and auxin controls a second stage that can reverse the release. Increased sucrose levels, for example after removal of a growing axis apex by pruning, or when the apex finishes extension growth and new leaves begin highly efficient photosynthesis, trigger the release of many buds along the axis. As the buds grow they produce auxin, and auxin produced by upper buds on the axis indirectly sends lower buds back into dormancy, before they pass through transition.

Auxin itself does not enter the buds or itself halt axillary bud growth, but stimulates the production of a “second messenger”. Auxin reaching the larger branches or central and lower parts of the plant triggers the release of strigolactone, which appears to travel up axes from junctions with branches in which auxin is travelling down (Brewer *et al.* 2009). Strigolactone then halts growth of the bud, and the bud is by definition again dormant, re-suppressed by the apex of a mature axis.

Stafstrom and Sussex (1988) established the sequence of events that occur in the axillary meristem following decapitation of pea shoots, before growth is visible. Within minutes of decapitation, biochemical changes are detectable in the cell membranes, and increases in mRNA coding for ribosome components were detected within an hour. DNA synthesis begins and new types of proteins are expressed within three hours. Growth is microscopically detectable within six hours. By eight hours the types of protein manufactured have changed again, perhaps indicating a second stage to transition. Cell division takes twelve hours to one day. By one day after decapitation, the suite of proteins expressed is the same as mature shoots, and buds have doubled in length (the initially largest buds from 1.7 mm to 3.4 mm). Within 72 hours the largest buds have inhibited the smaller ones.

Removal of the axis apex has been used widely to study AD. It seems likely that at least some of the process of axillary bud release from dormancy when triggered by damage to the axis, is similar to release triggered by a change in biochemistry from within the plant. Dun *et al.* (2006) however pointed out that changes to auxin levels and transport with decapitation effects may be different to the changes to auxin with natural rhythms in intact plants. This may also apply to changes in sugars and other nutrient concentrations, and biochemical responses to wounding which may interact with bud outgrowth.

Plant organs other than axis apices are known to influence axillary bud dormancy. Fruit can hold axillary buds dormant in beans (Tamas *et al.* 1979), which may be through a drain on sugar levels as they develop, or via other PGRs present during fruiting. Bennel (1984), in a study of vegetative and floral growth of intact macadamia trees, noted only the top two buds in a vertical series (of three to five buds) usually develop. Dominance of buds in a series over each other has been observed in the tree *Cercis candensis* (Owens and Ewers 1997), and may be a form of AD, although as some of the buds are floral other factors may come into play. In blackcurrant (*Ribes nigrum*), subtending leaves and bud scales also inhibit outgrowth of axillary buds under conditions otherwise conducive to growth in summer (Tinklin and Schwabe 1970). Florally competent trees generally have weaker apical dominance than non-reproductive ones, and older trees generally have weaker apical dominance than younger (Cline 1991).

Many PGRs other than auxins have been suspected of contributing to axillary bud dormancy. Roles for ethylene, gibberrellic acid, and abscisic acid have been investigated but it seems unlikely they have direct effects on bud outgrowth. The ratio of auxins to cytokinins appears to be important in determining bud dormancy or activity, as cytokinins in many instances appear to promote bud outgrowth but they often cannot achieve this on their own (Cline 1991). In apples (*Malus domestica*) and peas, axillary buds in different positions on an axis have different “depths’’ of dormancy, with some positions branching often and others very rarely (Costes 2003; Gould and Cutter 1987; Renton *et al.* 2006). This could be at least partly due to gradients in cytokinin : auxin ratios.

In macadamia, the behaviour of axillary buds both with and without axis decapitation is important, as axis tips are regularly removed by pruning as well as by orchard pests. The time from pruning until axillary bud outgrowth in macadamia was positively correlated with growth rates of axis tips before pruning and age of pruned axes (Olesen *et al.* 2006), but not with carbohydrate reserves. This suggests phenological cycles of PGRs or temperature, or both, influence axillary bud outgrowth.

2.2.2. Environmentally regulated dormancy

A number of external environment factors have been related to axillary bud dormancy. Many mechanisms of axillary bud dormancy are often not described as controls in their own right but as factors of AD, although it is probably more accurate to describe them as interacting with AD.

Light is known to influence axillary bud dormancy. Reduced light levels can suppress axillary bud outgrowth (Hagen and Moe 1981; Broomhall 1987). An increase in the ratio of far-red light, the wavelengths transmitted by green leaves, has the same effect. In grasses, it has been shown that shade reduces the number of axillary buds breaking dormancy, probably mediated by phytochrome B (Kebrom *et al.* 2010). In macadamia fewer buds were released from dormancy to form branches when light was reduced to 10% incident sunlight (Broomhall 1987), and the number of buds released increased with solar radiation on pruned axes also (Wilkie *et al.* 2009b).

Nutrient availability may also affect axillary bud outgrowth (Cline and Harrington 2007). This may interact with auxin as a controller of nutrient transport. In a study of field trees, the proportion of axes flushing in macadamia canopies was dependent on leaf nutrient levels (Stephenson and Cull 1986), but there was no indication of whether the new growth units were formed as extensions of existing axes or as new stems from axillary bud outgrowth.

In macadamia, temperature affects the amount of axillary bud outgrowth from pruned stems; between 16°C and 26°C, more buds were released from dormancy at higher temperatures (Wilkie *et al.* 2009b).

2.3. Flowering

As well as exiting from dormancy, meristems that form flowers or inflorescences need to activate the gene expression patterns required to produce floral primordia, and produce them in that species' floral phyllotaxy (geometry of node production). There are a number of steps that bring about this change, collectively called 'floral transition'.

2.3.1. Floral transition

Each species of flowering plant has a set of conditions in which it will begin flowering. External conditions that are known to induce flowering include the length of the night, daylight intensity, temperature, and water availability. These can act in a plant either individually or in combination. Alternatively, induction of flowering can depend upon conditions internal to the plant such as carbohydrate availability and age (Putterill *et al.* 2004;

Taiz and Zeiger 2006; Scott 2008; Wahl *et al.* 2013). Thirdly, induction may be triggered by a combination of external and internal conditions. A network of biochemical pathways integrates the relevant environmental and/or internal flowering triggers (Kinet 1993; Shrikanth and Schmid 2011). When the condition/s a plant requires for flowering is/are met, a signal – long dubbed 'florigen' - is produced, usually in the leaves where most environmental conditions are perceived. The process of external and/or internal triggers beginning the production of florigen is termed 'floral induction' (Henderson and Lawrence 1989; Sedgley and Griffin 1989; Haghavan 1992; Taiz and Zeiger 2006; Scott 2008). Florigen travels through the vascular system, and when it is perceived by a receptive meristem floral transition begins.

Florigens have now been identified for a number of plants not closely related, including *Arabidopsis thaliana*, *Oriza sativa*, *Populus tricocarp, and Cucurbit* spp. (Putterill *et al.* 2004; Bohlenius *et al.* 2006; Corbesier *et al.* 2007; Lin *et al.* 2007), and they all appear to be small proteins of similar amino acid structure. This suggests florigen is a similar molecule in all flowering plants. The first molecule of this type to be found was from Arabidopsis, and was named FT after the gene that encodes it, *FLOWERING LOCUS T* (Amasino 2010).

When a meristem senses the presence of FT or one of its orthologs, it changes its gene expression and thus its biochemical activity to that needed to construct floral primordia. This change in the meristem is termed 'evocation'. After evocation the meristem is immediately capable of forming or 'initiating' primordia that are floral parts, a stage called 'floral initiation'. Meristems may revert to a vegetative state if insufficient FT is accumulated soon after evocation. Otherwise the meristem becomes set on its floral production patterns, and a decline in FT no longer changes the type of meristem activity. Reaching this point of no return from flowering is termed 'floral determination' (Sedgley and Griffin 1989; Taiz and Zeiger 2006; Scott 2008).

A shortcut to floral evocation and subsequent determination may occur in plants with buds that sense flowering cues for themselves, without the need for other organs or signals from them. This may occur in response to low temperatures in either apical and axillary buds (Garcia-Luis *et al.*1992; Kinet 1993; McDaniel 1994; Putterill *et al.* 2004) .

2.3.2. Flowering control in perennials

Induction of flowering in perennials has been linked to a number of environmental triggers. However many studies that have investigated the cause of floral induction of perennial plants have done so using observations of relationships between macroscopic flowering and environmental factors or physiological conditions. Using microscopic evidence of evocation – initiation of floral primordia - can provide a more accurate indication of induction time (and therefore triggers), as there can be many months or years lag between induction and macroscopic flowering (Sedgley and Griffin 1989). This said, it seems that there may also be lag between evocation and initiation of floral primordia in some species, in including olive (*Olea europaea*), in which evocation may precede visible flowering by more than six months (Rallo and Martin 1991). Control of flowering in macroscopic appearance studies is control of the sum of the processes of floral primordia initiation, entry into and exit from any dormancy, and the period of growth until flowers or inflorescences or floral buds become visible.

The length of time between floral primordia initiation and macroscopic appearance of the inflorescence in *Banksia coccinea*, in the same sub-family of Proteaceae as macadamia (Grevilliodeaceae), was observed to range from 1 to 9 months (Fuss and Sedgley 1990). For many species there is no bud dormancy between evocation and emergence, and so studies of floral triggers without detection of evocation or first floral primordia can be accurate. However in axillary flowering perennials, where flowering can occur on wood many years old, there is a large window between bud formation and macroscopic flowering in which induction and evocation may be occurring.

Banksia coccinea and *B. menziesii* floral growth becomes clear to the unaided eye in February and May respectively. They both initiate their first inflorescence primordia - involucral bracts - in late spring (November). Initiation of the common bracts of the inflorescence did not occur until mid-summer (January), and floret parts were initiated as late as mid autumn (April). Waratahs (*Telopea speciosissima*), also in the Proteaceae family, initiate microscopic floral growth in early summer (mid December), with inflorescence buds becoming visible towards the end of summer (February), but not reaching anthesis until the following winter or spring (August or later) (Dupee and Goodwin 1990). Thus there is a reasonable possibility that macadamia also would have a gap of several months or more between floral initiation and the inflorescence becoming visible to the unaided eye.

Temperature

In subtropical and tropical trees, a reduction in temperature commonly promotes flowering. Citrus, requires temperatures of between 10 and 20 ºC - low temperatures for the tropics - to promote floral initiation (Sedgley and Griffin 1989). "*Washington Navel"* cultivar oranges *(Citrus sinensis)* produce macroscopic inflorescences at day/night temperatures of 24/19 ºC but not at 30/25 ° C (Lenz 1969). Satsuma mandarins (*Citrus unshiu*) flower in warm conditions, but produce more flowers when previously exposed to a greater period of cool temperatures (Garcia-Luis *et al.* 1992). This indicates the floral induction is due to cool temperatures and the warm releases the floral growth from dormancy. Lychee (*Litchi chinensis*) and mango (*Mangifera indica*) both produce visible inflorescences when early (but not necessarily first) axillary bud growth occurs in cool temperatures (Batten and McConchie 1995). Macroscopic inflorescences formed on avocado (*Persea americana*) at temperatures of 20 ºC or under, but not at 25 ºC or over (Buttrose and Alexander 1978).

Macadamia is commonly described as being 'induced' to flower by cool temperatures in reference to macroscopic appearance of inflorescences, but the time of actual induction is not known. Stephenson and Gallager (1986) found very few inflorescences emerged from macadamias kept at night temperatures of 5, 10, 15 and 20 ºC for twelve 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, within another eight weeks. A small number if inflorescences continued to emerge from the other treatments. 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. However night temperatures in the other groups fell around 15 or 5 ºC, or rose 5 ºC, and it seems unlikely that a drop of 10°C would cause induction when a drop slightly larger and a drop slightly smaller both failed to. Day temperatures of all groups went from 25 ºC to an average of 21 ºC. Thus there was also a change in the diurnal range of temperatures, which is known to affect the rate of inflorescence development in olive (Badr and Hartmann 1971).

The effect of temperature and vegetative growth in macadamia was investigated in a field study where trees were pruned at weekly intervals from mid-February to early March (Olesen 2005) to reset flushing cycles. From mid-April to late October the regular release of groups of axillary buds, approximately a week apart, was monitored to determine the number of inflorescences produced. Trees pruned from mid- to late-February completed two vegetative flushes before producing racemes on a large proportion of pruned branches, from buds that swelled mid-July to early August. Trees pruned in early April flushed once vegetatively and then flowered well from buds that swelled in mid-July. Trees pruned around mid-March did not flower at all, undergoing three vegetative flush cycles, with vegetative buds beginning outgrowth in one of those cycles from late May to mid June and then again in early October. Trees pruned between the dates giving good flowering and no flowering had a poor flowering, with both floral and vegetative axillary growth forming in 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.

The only known study using microscopic examination to investigate the control of flowering processes in macadamia was briefly documented for an industry meeting by Sakai *et al.* (1982). They measured the time to floral primordia production of *M. integrifolia* trees at different night temperatures – 12, 15, 18 and 21°C. Trees at all temperatures produced inflorescences, suggesting that evocation occurred in the common pre-treatment (over 21°C) or in all treatments. Total inflorescence production over 40 weeks was highest in the 18°C treatment and lowest at 21°C. However microscopic floral differentiation occurred first in the cooler temperatures and slowed as temperature increased. Time to microscopic floral differentiation was reported as 26, 38, and 44 days for trees at temperatures of 12, 18, and 21°C respectively. They concluded that evocation occurred in the greatest number of buds at higher temperatures, but growth after evocation was fastest at lower temperatures. This does not fit with statistically supported evidence that visible macadamia inflorescences elongate faster at higher temperatures (Moncur *et al.* 1985). As there are many non-floral buds on a macadamia tree, there is ample opportunity to not find floral buds when they are present, and as in the very brief report of Sakai *et al.* (1982) no numbers of buds dissected were given, it is not clear if this information on speed of floral primordia appearance is definitive. However macadamia floral emergence requires cool temperatures (Olesen 2005), so perhaps Sakai *et al.'s* finding pertains to all growth up to the point of emergence, whenever the floral bud is not dormant. This suggests that, in macadamia, buds may be evoked for some time before even microscopic growth and floral primordia formation. The variety used by Sakai *et al.* was not given in this report, and it may be that different varieties, including hybrids with *M. tetraphylla,* respond slightly differently to temperature or respond at different speeds.

Only one other study has linked floral primordia initiation in Proteaceae to environmental conditions. In *Leucospermum patersonii,* a relative of macadamias among the African Proteaceae, formation of normal numbers of fully developed inflorescences in warm temperatures (27/17°C) depended on long days followed by a drop in day length (Wallerstein 1989). Long days alone resulted in good numbers of floral meristems, demonstrating they induced flowering and evocation, but without short days the meristems did not form more than a few whorls (of many) floral primordia. Substituting cool temperatures for short days resulted in further development of inflorescences, but they did not reach maturity within the duration of the experiment.

Light

In woody perennials, photoperiodic control of flowering is not common (Sedgley and Griffin 1989), and induction effects of light can be difficult to separate from effects on local photosynthate levels. However lemon *(Citrus limonum)* is one perennial evergreen in which day length is a trigger for floral emergence (Chaikiattiyos *et al.* 1994), and temperature may interact with day length to control flowering in oranges (*Citrus sinensis*) (Lenz 1969). In tropical regions, total insolation – the product of day length and light intensity – appears to control timing of both vegetative bud outgrowth and flowering in a wide range of perennial species including evergreen trees (Calle *et al.* 2010). This interaction between environmental factors has been suggested to be the trigger for floral induction in many plants that were previously regarded as flowering autonomously (due to internal triggers not environmental ones). Light intensity alone appears to play a part in control of flowering in some perennial species, including evergreen olive trees (Wilkie *et al.* 2008).

There have been no controlled studies in macadamia on involvement of photoperiod in induction, but floral induction seems to have been achieved at both short and long day lengths of 10 hours 45 minutes and 14 hours 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. Shading of individual branches for seven months did not reduce flowering on those branches (Stephenson unpublished).

Nutrients

Stephenson and Gallager (1989) found that low levels of nitrogen in macadamia trees resulted in fewer emerging inflorescences. This could be due to reduced development of inflorescences post dormancy, and not an effect on induction or determination.

Floral emergence in “Washington Navel” oranges responds to water availability (Lenz 1969). In other tropical / sub tropical species including citrus, lychee, and mango, slowing of growth, possibly water stress induced, can be a prerequisite to floral initiation (Sedgley and Griffin 1989). In macadamia it has been reported that inflorescences resumed growth (began emergence) following rain along with a rise in temperature (Moncur *et al.* 1985).

In lychee and mango, the extent of flowering may also be subject to availability of carbohydrate resources within the tree (Kotur and Murthy 2010; Li *et al.* 2000). In Hawaiian macadamias, girdling increased the number of macroscopic inflorescences produced over 27 weeks (Nagao and Sakai 1990), indicating carbohydrate levels effect macadamia flowering as well.

2.4. Conclusion

There is little information in scientific literature about the control of macadamia axillary bud behaviour, or even its variation among locations in the tree. Locations of flowering are known in terms of metres from the canopy edge, but not where flowering or branching buds are positioned among the architectural structures of the tree. Variation in behaviour with macadamia’s growth patterns around the year – the phenological cycle - is better understood, but the timing of a key event in macadamia phenology, floral induction and the subsequent floral initiation, is not known. Thus this study will examine the location of branching and flowering within architectural structures of the tree, and further investigate the effect of temperature on the flowering process, with a view to filling these gaps in our knowledge.

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Chapter 3. Vegetative axis structure and location of branching

3.1. Introduction

In orchard-grown macadamia trees (*Macadamia integrifolia* and *M. tetraphylla*), the inside of the canopy is heavily shaded, and so the lower sections receive less than three percent of ambient daylight (Huett and Smith 2008). This is due to a combination of macadamia's naturally high leaf density and high planting densities of trees that become very large early in their productive life, and it is suspected to be exacerbated by the common practise of hedging. Low light levels have been linked to increased nut drop (Olesen *et al.* 2011), so the topic is of importance to commercial production. In many plants such removal of the tip of a vegetative axis[2] results in growth of extra lateral branches from axillary buds, because it releases those buds from apical dominance. Other factors internal to the plant, such as its age and whether it is fruiting, also influence axillary bud outgrowth, as do external factors such as light levels (Cline 1991). This chapter will investigate location of macadamia axillary bud outgrowth in the architectural framework of the tree, in an effort to better understand control of branching, and subsequently shade in the canopy.

The strength of apical dominance (AD) and thus its effect on axillary bud outgrowth varies with species (Cline 1991), and details, including locations of buds at which most outgrowth occurs, are not known for macadamia. In many trees, apical tissues exert AD by their consumption of energy and by their production of auxins. Apical energy consumption reduces carbohydrates available to axillary buds below the apex, to less than the threshold required for buds to begin an exit from dormancy (Mason *et al.* 2014). Apical auxin production is now thought to act on buds that have already begun to exit dormancy – those in the transition between dormancy and visible growth. Auxin prevents buds from finishing this transition, halting any microscopic growth that may have begun and returning them to quiescence (Morris *et al.* 2005; Brewer *et al.* 2009; Mason *et al.* 2014). In species with

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[2] A vegetative axis or shoot is called a stem, and architecturally it is defined as an unbranched length of wood, arising from one meristem. To avoid confusion when discussing canopy architecture, here the term "axis" is used to denote the parent structure, and "new stem" is used to denote vegetative laterals arising from the axillary buds of that parent axis.

strong AD, axes with healthy apices have relatively little branching from buds closer to the apices, and more branching further away. AD becomes emphasised where lateral stems are present on a parent axis (McSteen and Leyser 2005), as the dominance of the lateral apices adds to those of their parent stems.

There are other influences on axillary bud dormancy that interact with AD. The presence of fruit, and aging or reaching floral competence can all reduce the number of buds on the tree achieving outgrowth (Cline 1991; 1997). Environmental conditions (those external to the tree) can also affect outgrowth (Cline 1991). Availability of nutrients such as water and minerals may be limiting, possibly also through competition with other tissues (Cline *et al.* 2009; Leyser 2009). Low light also reduces axillary outgrowth (Broomhall 1987; Kebrom *et al.* 2010).

Studies of branching along growth units[3] (GUs) and axes of unpruned woody perennials have found patterns in axillary bud outgrowth within the GU, regardless of position along the axis. Axillary buds at the base of peach (*Prunus persica*) GUs are predominantly vegetative, while those at the tip are less so (Kervella *et al.* 1995). In the rhythmically flushing shrub mock orange (*Choisya ternata)*, and in cherry trees (*Prunus avium*), new stems develop from upper buds of a GU, even when that GU is low on the axis (Guedes 1980; Costes *et al.* 2006). In apple (*Malus domestica*), new vegetative stems grow out mostly low on the GU (Renton *et al.* 2006). Such changes of bud fate with node position along a GU are thought, from studies of apple and peach trees, to be related to growth rates of a number of organs at the apex at the time of the new bud’s formation. Different combinations of plastochron, rates of expansion of the new bud’s subtending leaf and internode, as well as time within the growth season, have been associated with formation of floral buds, blind nodes, and vegetative buds producing sylleptic, proleptic, long and short stems (the last often being termed short shoots) (Crabbe 1984; Kervella *et al.* 1995). This suggests that some buds appear to be pre-disposed to branching from the time of their formation, long before they enter dormancy or begins outgrowth. These within-GU patterns can interact with GU size – the zones of vegetative (or floral) growth can change length or disappear with changes in GU length.

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[3] A growth unit is that segment of an axis created during one continuous growing period - Hallé F, Oldman R, Tomlinson P (1978). 'Tropical Trees and Forests.' (Springer-Verlag: New York). See Figure 3-1.

In apple, as well as the differences in branching of nodes at different position within a GU, there are also clear differences in branching between GUs. The number of branches on apple tree GUs decreases with position of the GU from the axis base . This is only partly because more distal GUs are composed of fewer nodes (observed in apple and a number of other deciduous fruit trees), and so there are fewer buds from which new stems can form in shorter and more distal GUs (Costes *et al.* 2006). The branching probability is also influenced by absolute length of GUs; in apple, GUs longer than 20 cm branch around twice as often as GUs shorter than 20 cm (Renton *et al.* 2006).

Knowledge of branching architecture in macadamia may provide insights into how the location of new stem growth is controlled by the tree. It would also enable identification of nodes most likely to branch, i.e. to form a new stem, before new stem growth become visible to the naked eye. This would permit microscopic study of the early stages of branching without the inefficiency of collecting and preparing buds from random locations, most of which would be dormant. In turn, both these types of information could assist manipulation of canopy structure to reduce orchard tree size and canopy density while maximising sustainable yield.

This chapter presents two surveys of orchard macadamias towards these ends. The first documents the architectural structure of macadamia axes as composed by nodes within GUs. The second looks at how new lateral vegetative growth is distributed along those axes, between their component structures, and between axes themselves.

3.2. Materials and methods

3.2.1. Axis structure

Two varieties of *Macadamia integrifolia* 741 and 842, growing in commercial orchards near Glasshouse Mountains Township in SE Queensland, were surveyed to ascertain aspects of their architecture. Five year old variety 741 trees were surveyed in August (when new stems and inflorescences are most visible) 2010, 12 year old variety 741 trees were surveyed in August 2011, and 13 year old variety 842 trees were surveyed in August 2012.

Four 5 year old variety 741 trees (741/5) were surveyed. For these 3.5 m tall trees, every second axis arising from the central trunk was included in the survey, and from these second-order axes every second third-order axis surveyed, and so on, so that axes representative of all of ages and positions were included. These young trees had never been hedged.

Six 12 year old variety 741 trees (hereafter abbreviated to 741/12) and six 13 year old 842 trees (842/13) were surveyed. (More mature trees were used than young trees, to reduce variation in the data.) For these 8 m tall mature trees, two main branch systems arising from the trunk on each tree were surveyed, one between 150 cm and 200 cm from the ground, growing within a 90º arc along the hedgerow, and another between 250 and 350 cm from the ground, growing within a 90º arc facing into the alley, above the skirting height. Neither variety/age group had been hedged in the year before surveying, but there were a very small number of previously hedged axes on the surveyed branch systems that were excluded from the study.

Each axis was mapped by recording the number of GUs on the axis, the number of nodes in each GU, and the position of those GUs along the axis. The bract node formed at the top of each GU, when the axis temporarily ceases growth between flushing seasons, was recorded as the most distal or top node of the GU (Figure 3-2). The node just distal to the bract node was recorded as the base node of the next GU. The axillary buds of the top node of the tip GU were always contained in the apical bud, and were unable to grow out and form new axes regardless of the GU age.

The structure of the GUs in heavily shaded parts of the canopy was sometimes obscured by moss and water-staining of bark. When nodes were obscured the axis was not used in the survey. Obscured sections were found mainly on axes seven GUs long and larger. For analyses of relationships between architectural structures (e.g. nodes per GU and GU position along axis), only axes six GUs long or less were used to ensure a large and consistent proportion of the sample branch's axes of any one size were included. Only GUs up to eight nodes long were used, as larger GUs were identified as outliers (more than 2.5 times the inter-quartile range from the median). The axes of six or fewer GUs represented 98.5, 98.9 and 96.3% of the axes mapped from the 741/5, 741/12 and 842/13 trees respectively. A total of 2341 nodes on 534 GUs on 314 axes were used in analyses of the architectural relationships in 741/5 trees, 5542 nodes on 1460 GUs on 898 axes were used in analyses of 741/12 trees, and 6341 nodes on 1618 GUs on 922 axes were used in analyses of 842/13 trees.

Differences between distributions of structures were tested for using Likelihood Ratio Chi-Square tests. Linear relationships were tested using Spearman Rank Correlation (r_S) tests. (Because non-parametric analyses were necessary, no least significant difference or standard error exists, and figures thus do not include these measures.)

3.2.2. Distribution and location of branching

Six thirteen year old trees of variety "741", around 8 m tall, were used to survey the distribution of branching within and between axes. Twenty axes, each three GUs long, were located and tagged on each tree. Axes were selected from between 1.5 m (above the skirt-pruning line) and 4 m (maximum ladder reach). Axes were selected from around 1 m into the canopy from the face of the hedgerow, where good light and little moss growth on the bark enabled clear identification of GUs. Roughly half of the axes had branched at the time of tagging, mostly producing only one lateral stem, sometimes two. All these existing lateral stems were removed at the time of tagging, so that buds of all axes were affected by the auxin production and carbohydrate consumption of the same number of apices – only the one, at its own tip. One year later the number of new stems[4] at each node was recorded, along with the position of the node along the GU and the position of the GU along the axis (Figure 3-2). Also recorded was the number of growth units of apical extension. 104 tagged axes were able to be found and mapped in this way. For axes that did not extend over the year and for axes that extended by one GU over the year, the effect of size and position of axes, GUs and nodes on probability of a node branching were examined, using binary logistic regression with a logit link function. The efficiency of multiple regression models was compared using Akaike's Information Criterion (AIC) scores (lower scores within a data set indicate a better fitting model) (Anderson 2008).

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[4] As part of this survey the number of inflorescences at each node was also recorded – see Chapter 5.

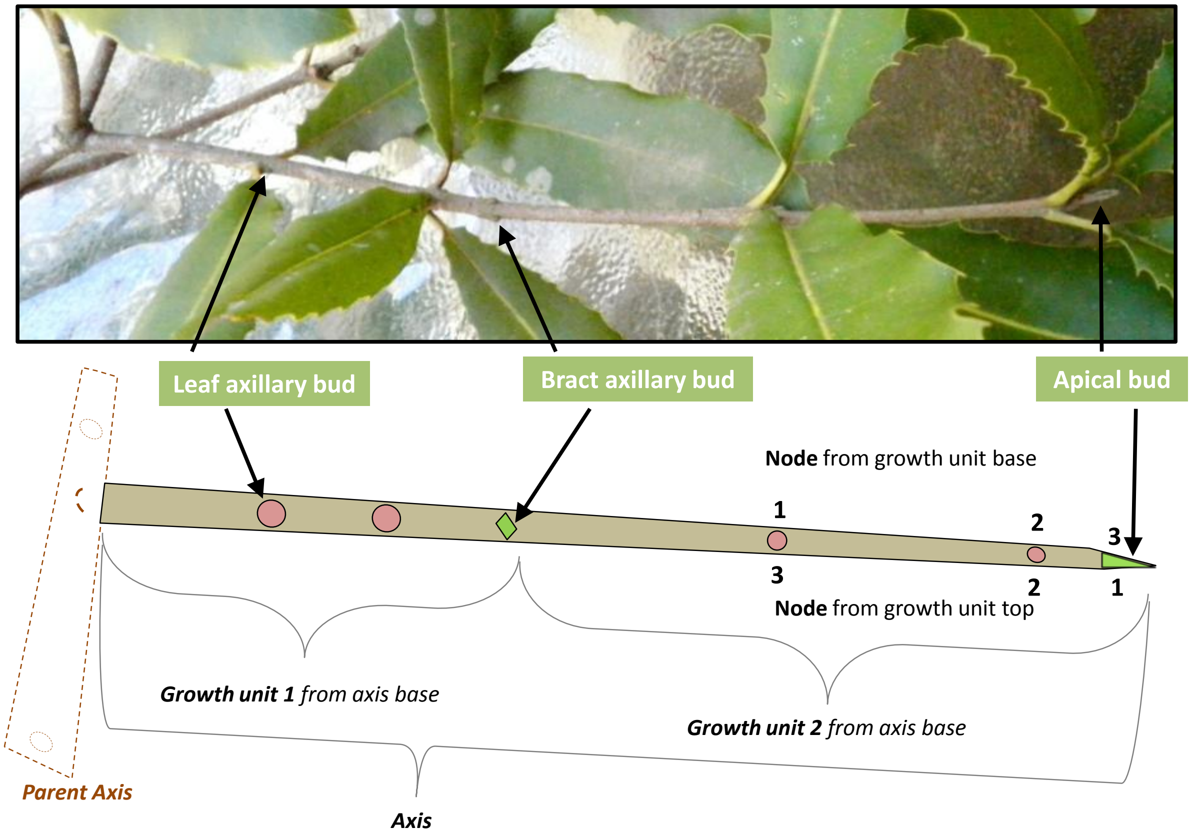

Figure 3-1. Structures of a macadamia axis, and their schematic representations.
In the schematic only one bud is shown at each node for simplicity.

Numbers of new stems emerging from a node, a GU or an axis were also analysed, both over all and for only branching nodes. Linear relationships between numbers of stems emerging and structure size or position were tested for using Spearman rank correlations (r_S). Non-linear differences between number of stems emerging per node were tested for significance using Mann-Whitney tests or Kruskal-Wallis tests (for two groups or more than two groups respectively).

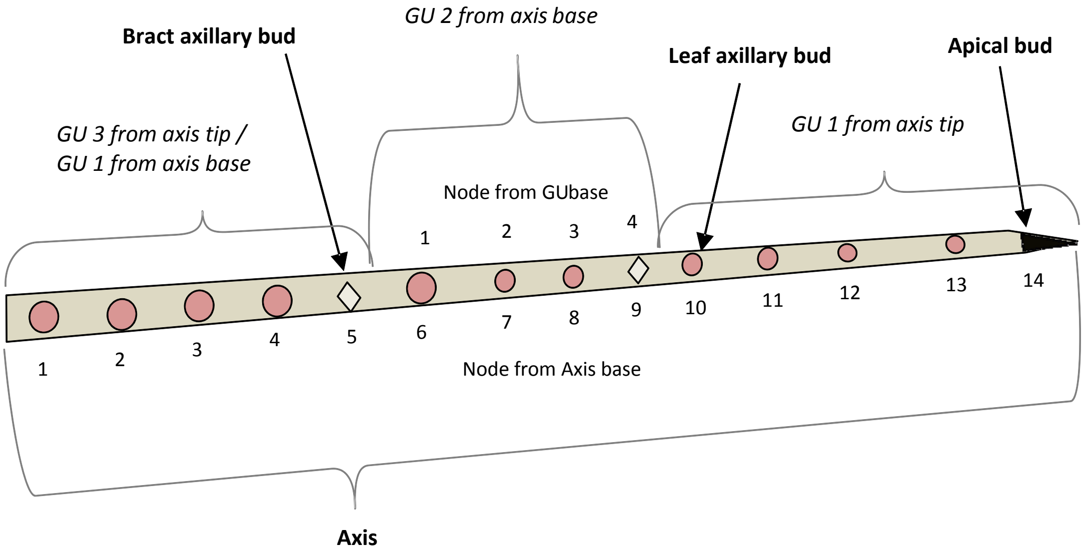

Figure 3-2. Terminology of mapping in relation to structures of macadamia axes.
GU stands for Growth Unit.

As growth units were composed of different numbers of nodes (two to eight), node position was analysed from both the top and the bottom of the GU. When analysed from the top, the most distal nodes were aligned but the bottom nodes of GUs of different lengths were not. Thus analysis from the top had the ability to detect differences and similarities related to node position from the top, but effects related to node position from the base would be lost by the distribution of bottom nodes of GUs among positions all along the range of positions two to eight, where those of shorter GUs would be aligned with middle nodes of longer GUs. Conversely, when analysed from the bottom, the most proximal nodes of different length GUs were aligned but not their top nodes, and so effects of position from the top would be disguised by top nodes of short GUs being aligned with middle nodes of longer GUs (Figure 3-3). The same approach was used when analysing node position along the axis – effect of position was analysed from both the axis base and from the axis tip. As an attempt to combine analyses from both directions, the position of a node along the axis was also calculated as a proportion of the way along the axis, i.e. second node of four (0.5) or first node of five (0.2), termed “relative node position”. This is (arbitrarily) oriented from the axis base. When analysing extended axes, position from the tip refers to position from the new i.e. the tip at the end of the survey year.

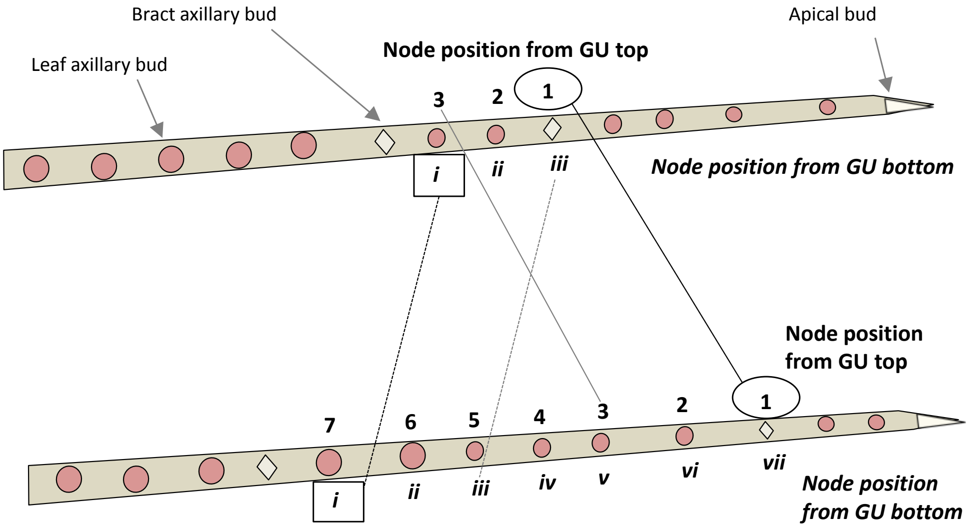

Figure 3-3. Possible alignments of node positions on GUs.
These include alignment from the GU top or most distal node, and the GU bottom or most proximal node. When analysing from the top, the most distal node of the short GU is aligned with the most distal node of the long GU, but when analysing from the base it is mis-aligned with a middle node of the long GU.

3.3. Results

3.3.1. Axis structure

Over half of the axes of each variety/age group were composed of only one GU (Figure 3-4). (These could have been formed many flush seasons or years ago and simply never extended, and so will be a variety of ages.) Around 10% of the axes had more than four GUs. There was no difference between 741/12 and 842/13 trees in the distribution of number of GUs on an axis. Young trees were not compared statistically with mature trees as the sampling procedures were not the same. 842/13 trees had GUs composed of more nodes (on average) than 741/12 trees (Figure 3-5) ($P < 0.001$). There was a very small proportion of axes composed of very large numbers of GUs and a small proportion of GUs composed of very large numbers of nodes. It seems likely that these are not all giant structures in the trees, but that bract nodes on these axes were masked by stains or growths on the bark, or that some vigorous shoots did not halt growth between seasons as most of their cohorts did.

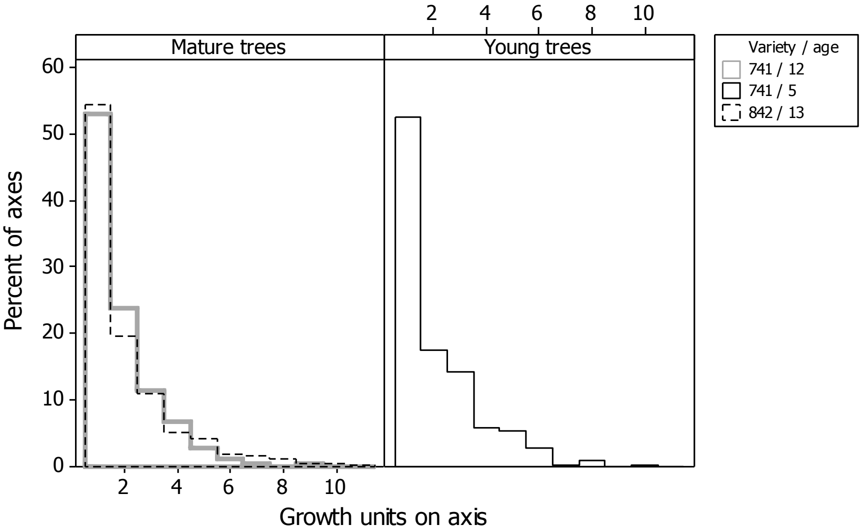

Figure 3-4. Distribution of number of growth units on all macadamia axes surveyed.
n = 922 axes for variety 841/ age 13, n = 898 axes on variety 741/age 12, n= 314 for variety 741/age 5.

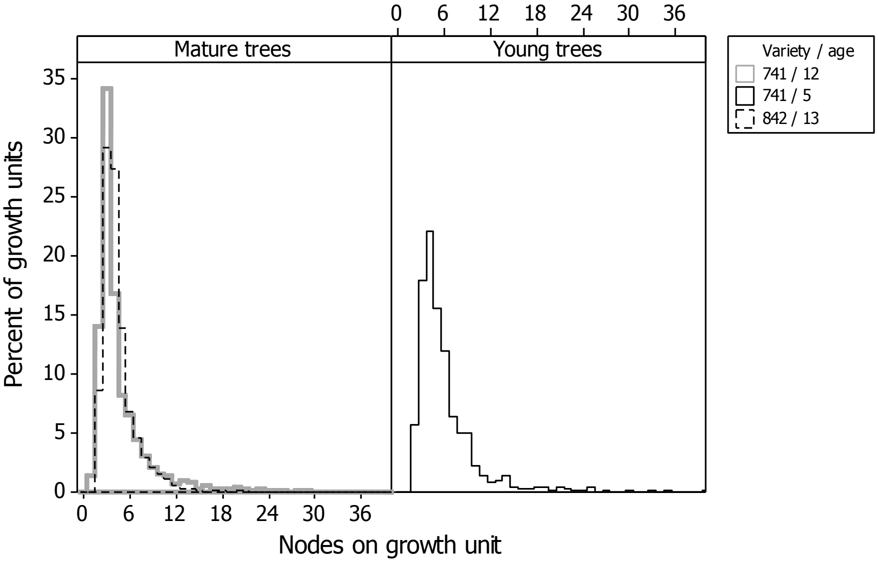

Figure 3-5. Truncated distribution of number of nodes all growth units surveyed.
Omitted are two outlying growth units of variety 741 age 12 (56 and 83 nodes) and one of variety 741 age 5 (59 nodes). N= 1618 for variety 842/age 13, n = 1460 for variety 742/age 12, n = 534 for variety 741/age 5.

There were clear relationships between the number of GUs on an axis, GU position along the axis, and the number of nodes on a GU (Figure 3-6). GUs closer to the tip were smaller than GUs closer to the base; for 741/5 ($r_S = 0.158$, $P < 0.001$), for 741/12 ($r_S = -0.178$, $P < 0.001$), and for 842/13 ($r_S = -0.177$, $P < 0.001$). These correlations were highly significant despite the pattern not being apparent in some age/variety groups for large axis sizes, probably because there were few axes in those categories.

Axes composed of more GUs had base GUs composed of more nodes in 741/5 ($r_S = 0.148$, $P = 0.009$), in 741/12 ($r_S = 0.201$, $P < 0.001$), and in 842/13 ($r_S = 0.229$, $P < 0.001$). Axes composed of more GUs also had smaller tip GUs in (741/12 $r_S = -0.94$, $P = 0.005$) and in 842/13 ($r_s = -0.107$, $P = 0.001$). The larger axes of the 741/5 trees had larger tip GUs ($r_s = 0.153$, $P = 0.007$).

Axes with more GUs had a higher average number of nodes per GU in 731/5 ($r_s = 0.222$, $P < 0.001$), in 841/12 ($r_s = 0.095$, $P < 0.001$), and in 842/13 ($r_s = 0.102$, $P < 0.001$).

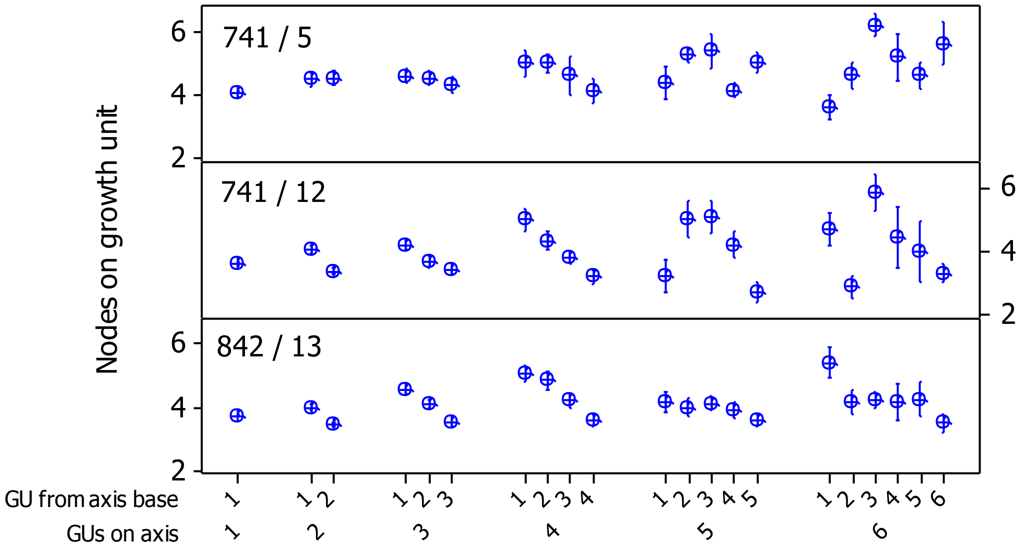

Figure 3-6. Effect of macadamia growth unit position along axis and axis size in growth units on number of nodes on the growth unit.
Note that growth units at the base of most axes have more nodes than those at the tip. Bars are one standard error from the mean. Panel labels in top left-hand corners are variety/age labels.

3.3.2. Distribution of new stems among nodes, growth units and axes

The 62 axes that did not extend during this study (out of a total of 108 axes) had a median of three nodes per GU and a median of eleven nodes per (three-unit) axis. The 36 axes that extended by one unit had a median of three nodes per GU and a median of fifteen nodes per (four-unit) axis. The proportions of nodes, units and axes branching and the average numbers of stems they produced are given in Table 3-1. The extending axes produced more new lateral stems in total ($P= 0.042$) than the non-extending axes. The number of lateral stems produced per node did not differ significantly between extending and non-extending axes, but they were spread more evenly between axes in the extending group.

Only six axes extended by two units, and only one of these branched. It branched at only one node, the second node from the top of the second unit from the axis tip (first new unit produced during this study). Three new stems were produced at this node.

Table 3-1. Percent of nodes, units and axes branching (forming at least one new stem), and the number of new stems formed, by 13 year old macadamia trees variety 741.
IQR = Inter-Quartile Range.

Axis extension	Structure	% branching	Stems on branching structures			Stems averaged over all structures		
			median	IQR	mean	median	IQR	mean
No extension	node	5.22	2	1 - 3	1.87	0	0 - 0	0.10
	unit	17.46	2	1 – 3	2.21	0	0 - 0	0.39
	axis	41.27	3	1 - 4	2.81	0	0 - 2	1.16
Extended by one GU	node	7.27	1	1 - 2	1.55	0	0 - 0	0.11
	unit	24.31	2	1 - 2	1.77	0	0 - 0	0.43
	axis	72.22	2	1 - 3	2.38	1.5	0 – 2.75	1.72

3.3.3. Location of new stems on non-extending axes

Node and growth unit position

Position of node along the axis

On those axes that did not extend, the probability of a node branching was affected by its position along an axis from the base (Figure 3-7) (P= 0.001), with nodes further from the base being more likely to branch. Relative node position along the axis (Figure 3-8) also showed this (P= 0.013). However position of a node from the axis tip did not have a significant effect on the probability of that node branching.

If a node did branch, position affected the number of new stems formed according to all three measures, nodes further from the base producing more new stems per node; position from base (r_s= 0.436, P= 0.005) (Figure 3-9), position from tip (r_s= -0.416, P= 0.008), relative position (r_s= 0.429, P= 0.006) (positive when calculated from the base end i.e. fourth node from the base of six nodes). The overall number of new stems at a node was however only weakly positively correlated with its position along an axis with position from axis base (r_s= 0.121, P= 0.001), relative node position (from base) (r_s= 0.096, P= 0.009), and position from axis tip (r_s= -0.082, P= 0.025). The weakness of this overall correlation was due to the large number of nodes at all positions not branching at all.

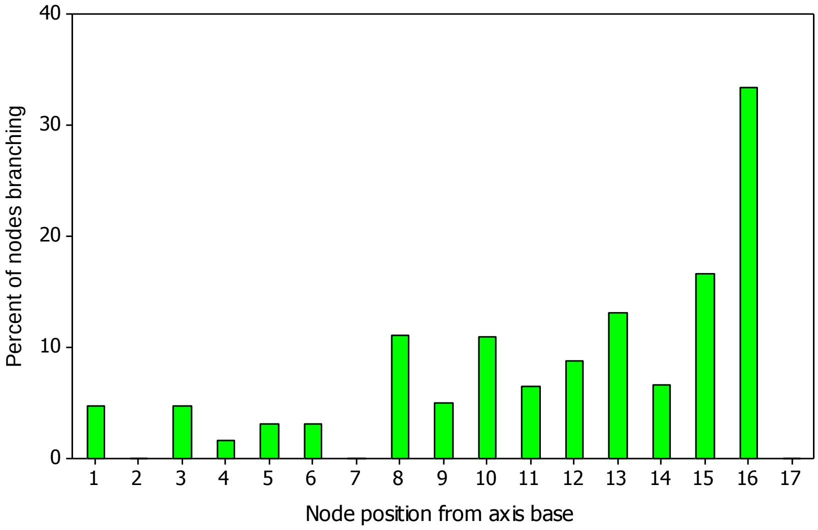

Figure 3-7. Effect of macadamia node position from axis base on probability of branching at node, in 13 year old trees of variety 741.

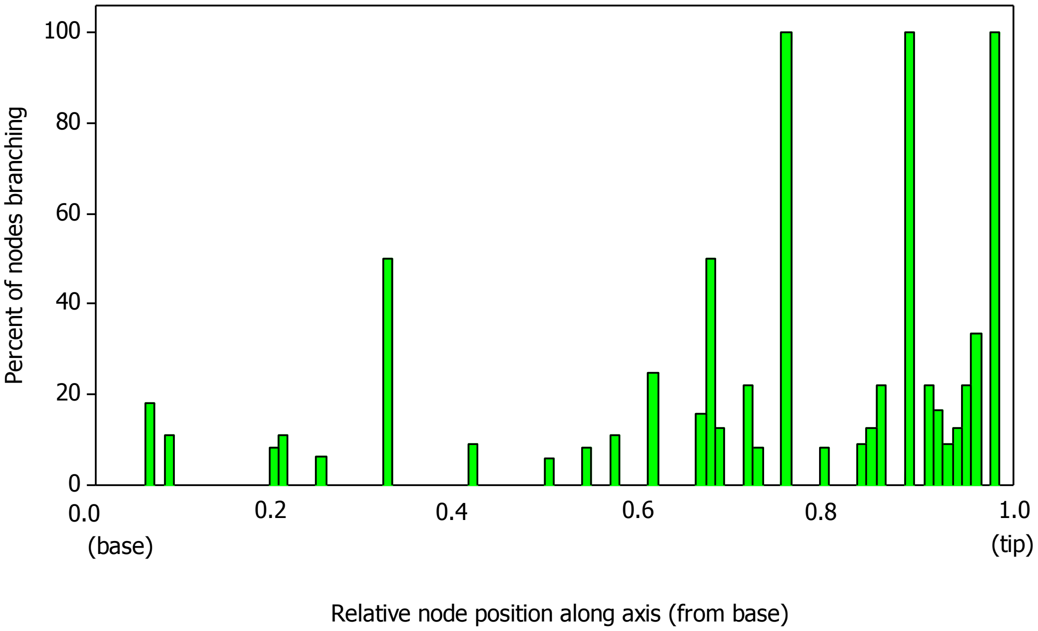

Figure 3-8. Effect of relative node position on probability of branching at a node, in 13 year old macadamia trees variety 741.

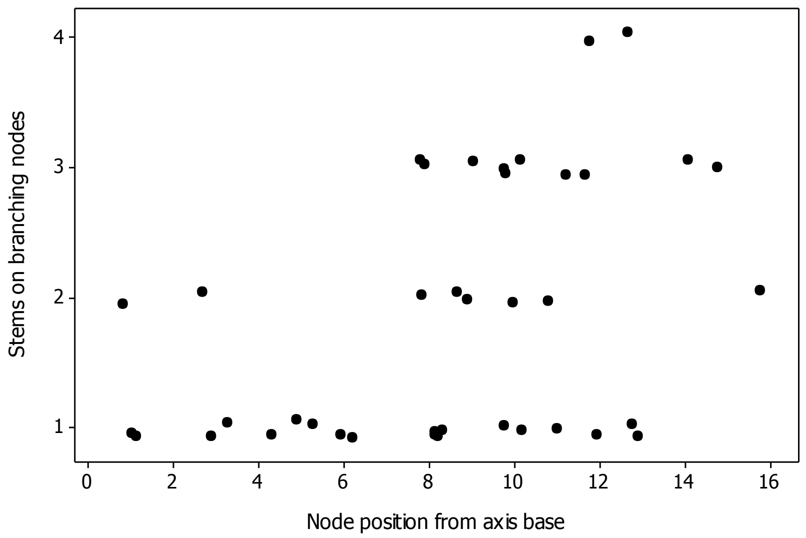

Figure 3-9. Effect of node position from base on the average number of new stems forming at a branching node, in thirteen year old macadamia trees of variety 741.
Dots represent individual values and identical values are offset to show relative frequency.

Position of node along the growth unit

Node position along a GU also affected probability of a node branching (Figure 3-10). There were several positions, including the collar, where the node never branched, and among those GUs that branched at least once, the position of a node from the GU top had a highly significant effect on whether the node branched or not ($P = 0.001$). Nodes at position 2 from the top had the highest probability of branching, for all sizes of GU, although this was not significantly different to that of nodes at position 6 from the tip. There was no significant relationship between node position from GU base and probability of a node branching, nor between relative node position along a GU and probability of branching.

Among branching nodes, there was no relationship detected (linear or otherwise) between node position along GU and number of new stems growing from the node. Node position from the tip of a GU did affect the number of stems emerging overall ($P < 0.001$). The highest number of new stems emerged from the second node from the GU tip (Figure 3-11), and this was the case for GUs of all sizes except for 5-node GUs where it was second highest and statistically similar to the highest, which was position 3. There was no significant difference in number of stems at a node when analysed by node position from GU base.

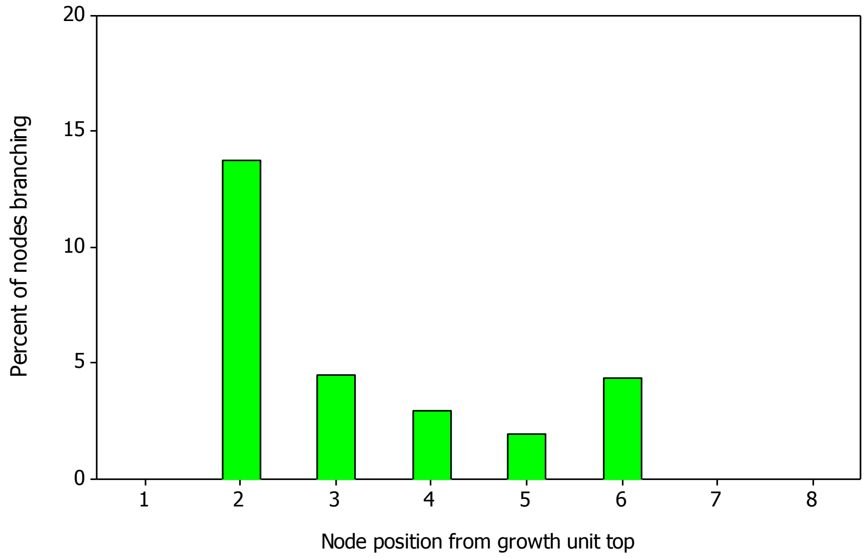

Figure 3-10. Effect of node position from growth unit top on probability of branching at node, in thirteen year old macadamia trees of variety 741.

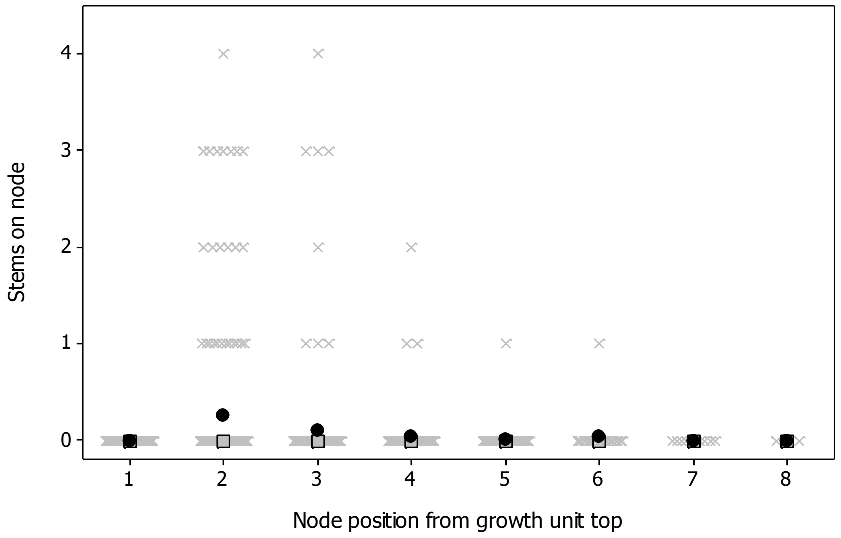

Figure 3-11. Number of stems emerging at different node positions from growth unit tops, in thirteen year old macadamia trees of variety 741.
Squares represent medians, circles represent means, crosses are individual values.

Position of growth unit along the axis

The probability of a node branching depended upon which GU it was part of (Figure 3-12) (*P*= 0.009). Among branching nodes, those on GUs closest to the tip produced more new stems (r_s= -0.399, *P*= 0.012) (Figure 3-13). In both cases the linear relationship was stronger than non-linear relationship. The overall number of stems per node also had a negative linear relationship, although a weak one, with GU position from the tip (r_s= -0.101, P= 0.006).

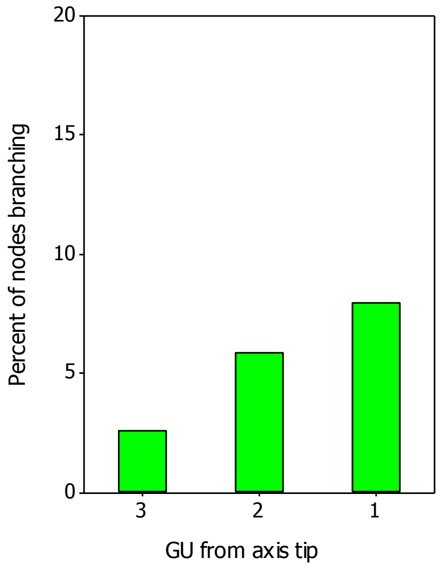

Figure 3-12. Effect of GU (Growth Unit) position along axis on probability of branching from a node, in thirteen year old macadamia trees of variety 741.

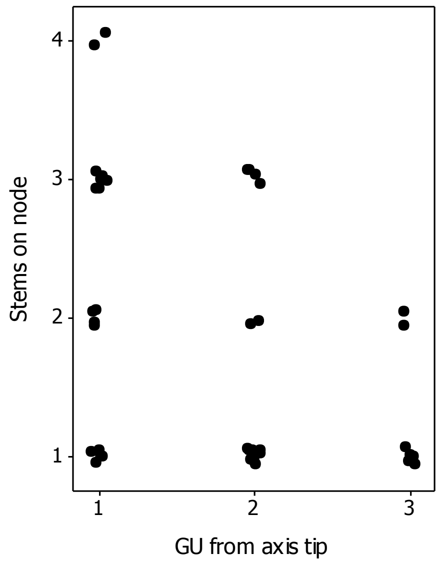

Figure 3-13. Effect of GU (Growth Unit) position on number of new stems emerging from branching nodes, in thirteen year old macadamia trees of variety 741.
Dots represent individual values, and identical values are offset to show relative frequency.

Growth unit and axis size

Size of growth unit in nodes

The number of nodes on a GU did not affect the probability of those nodes branching, the number of stems forming if they did branch, or the overall numbers of stems on a node. When nodes on a GU was incorporated into multiple regression analyses (see below) the effect on probability of branching was still not significant .

Size of axis in nodes

The probability of a node branching increased with the number of nodes on its axis (P= 0.024) (Figure 3-14). There was no relationship between axis size and number of stems per node among just branching nodes, but among all nodes combined there was a weak correlation: each node on larger axes produced more stems on average than smaller axes (P= 0.029 r_s= 0.080).

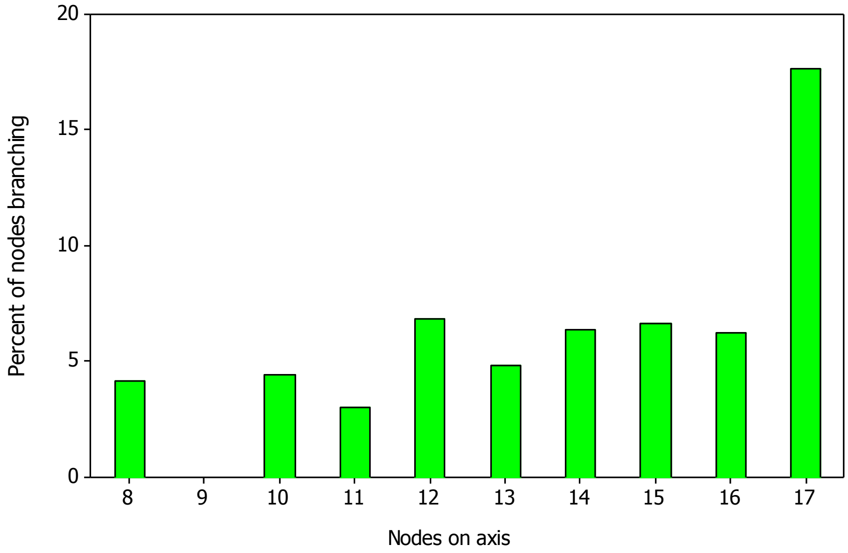

Figure 3-14. Effect of axis size (in nodes) on probability of node branching, in thirteen year old macadamia trees of variety 741.

Multiple regression using position and size variables

Multiple regression analyses were conducted to find the combination of variables that best described branching probability. Node position along a GU plus GU position along an axis (Figure 3-15) together produced a better explanation (lower AIC score) of probability of a node branching than they did individually. Among branching nodes, there was no difference in number of stems emerging between nodes at different node-plus-GU positions. There were significant differences between node-plus-GU positions in the overall number of new stems emerging (Figure 3-16) (*P*< 0.001).

Using size as well as position, all combinations of the variables studied individually were also analysed with multiple regression. The probability of a node branching was modelled best by node position within a GU, GU position along an axis, and axis size in nodes – in that order of importance. Other variables measured, found to have significant relationships with node probability of branching individually, did not add to this combination's efficiency of determining flowering proability (results section above), indicating that they were measuring the same source of variation in a different way, or represented an interaction between these variables.

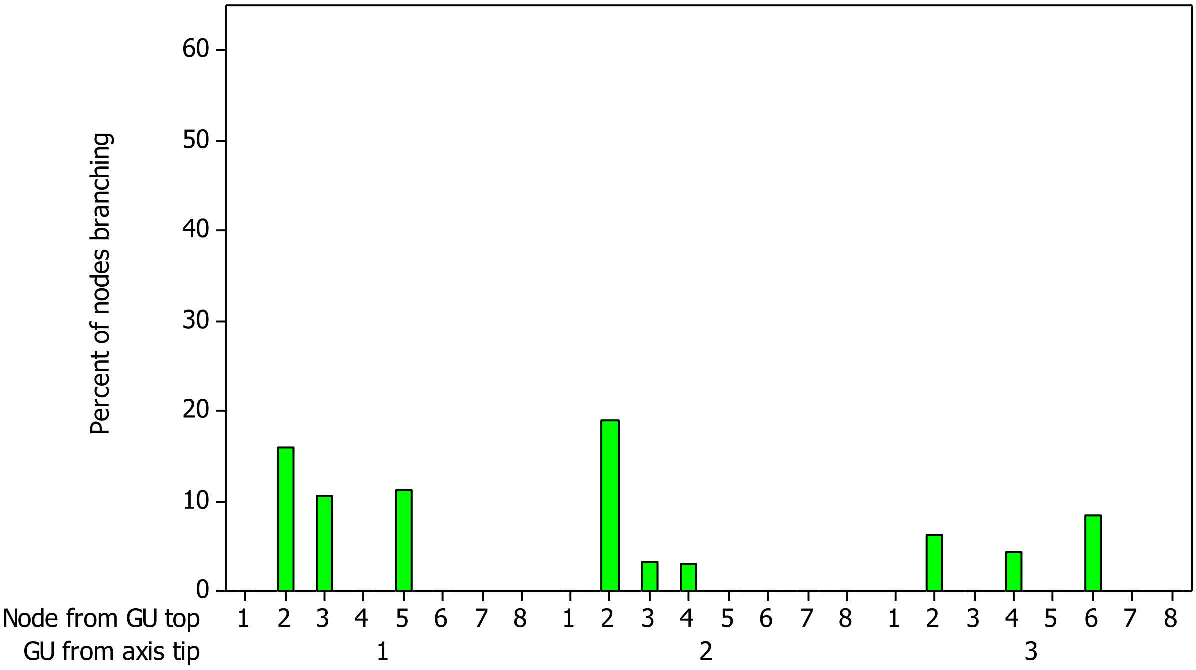

Figure 3-15. Percent of nodes branching at different node position along a growth unit (GU) together with position of that GU along an axis, for GUs of all lengths combined, in thirteen year old macadamia trees of variety 741.

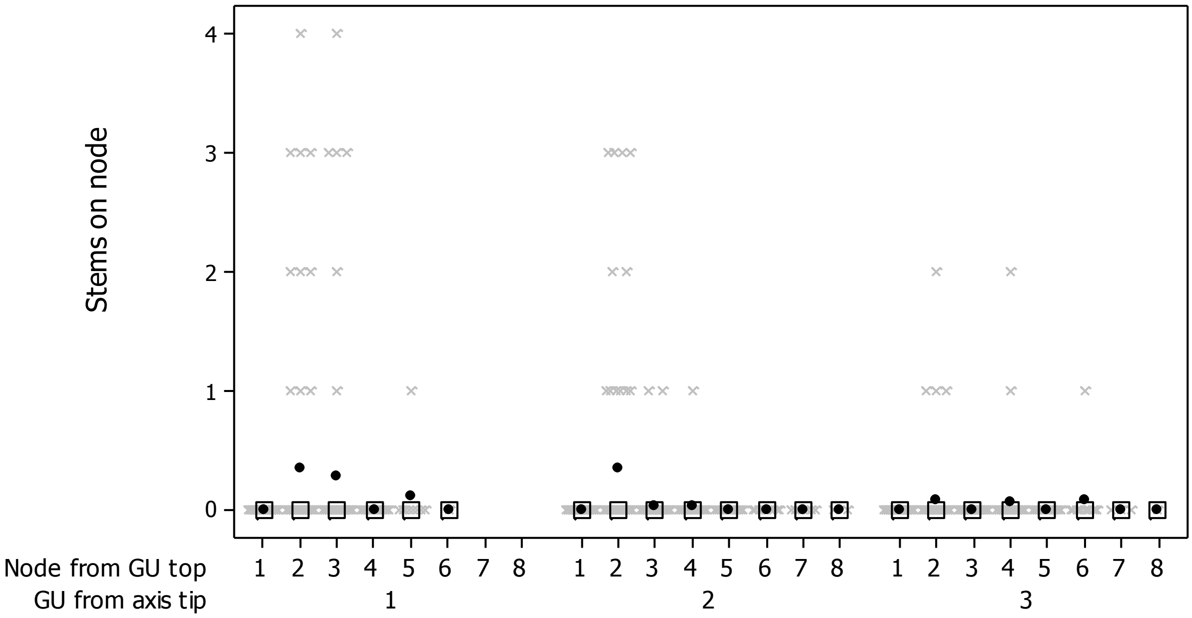

Figure 3-16. Effect of position of node along a growth unit (GU) together with position of GU along axis on number of new stems emerging from a node, for GUs of all lengths combined, in thirteen year old macadamia trees of variety 741.
Squares represent medians, circles represent means, crosses are individual values.

Predictions of proportions of nodes branching were made using the multiple regression model. Taking into account axis size, the most likely place to find a new stem on non-extending axes was on node two from the top of the GU at the axis tip. For the average three-unit axis of 11 nodes, 16% of nodes at this position formed at least one new stem. This is four times the average for all nodes on three unit axes, which is 4%. On a longer three-unit axis of 15 nodes, the proportion of nodes at this same position predicted to branch increases to 34%, and for an axis of 18 nodes it becomes 52%. Figure 3-17 shows the predicted likelihood of node branching with position along a non-extending axis for 15-node axes, as calculated by the multiple regression model.

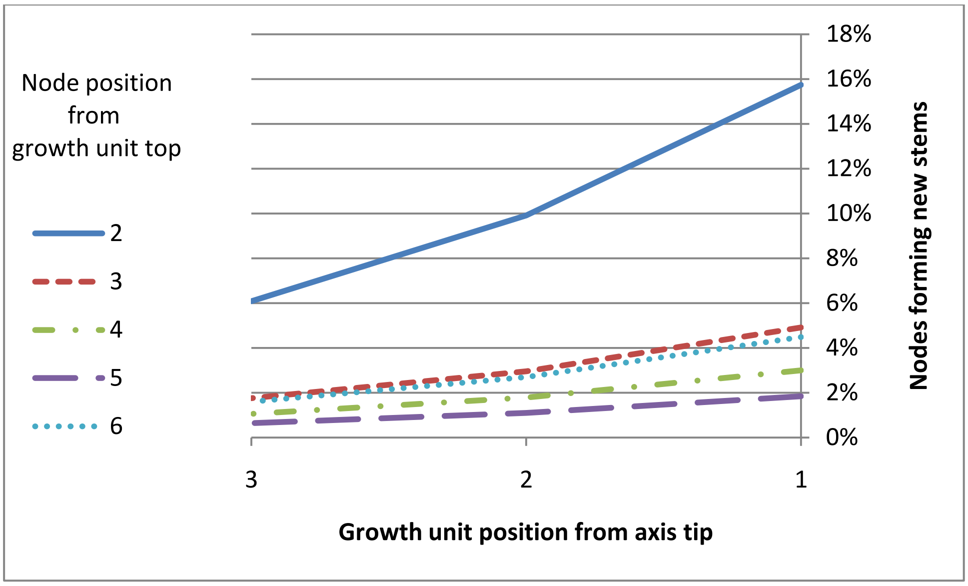

Figure 3-17. Predicted effect of node position from growth unit (GU) top, and of GU from axis tip, on the percent of nodes forming new stems, for axes 15 nodes long, in thirteen year old macadamia trees of variety 741.
Nodes at position 1,7 and 8 did not branch.

3.3.4. Location of new stems on extending axes

Node and growth unit position

Position of node along the axis

On those axes that extended by one unit, no relationship was detected in individual regression analyses between the probability of a node branching and its position along the axis. This was the case whether using relative position (Figure 3-18) or absolute position from base or tip. However a significant effect of relative node position along the axis was detected when this variable was part of a multiple regression analysed with node position along the growth unit and growth unit position along the axis (section below); nodes further from the axis base were less likely to branch.

Among branching nodes there was a correlation between number of new stems on a node and relative node position: there were more new stems on each branching node when nodes were further from the axis base ($P= 0.003$ $r_s= 0.462$). Over all nodes however there was no significant relationship detected between position of a node along the axis and the average number of stems formed.

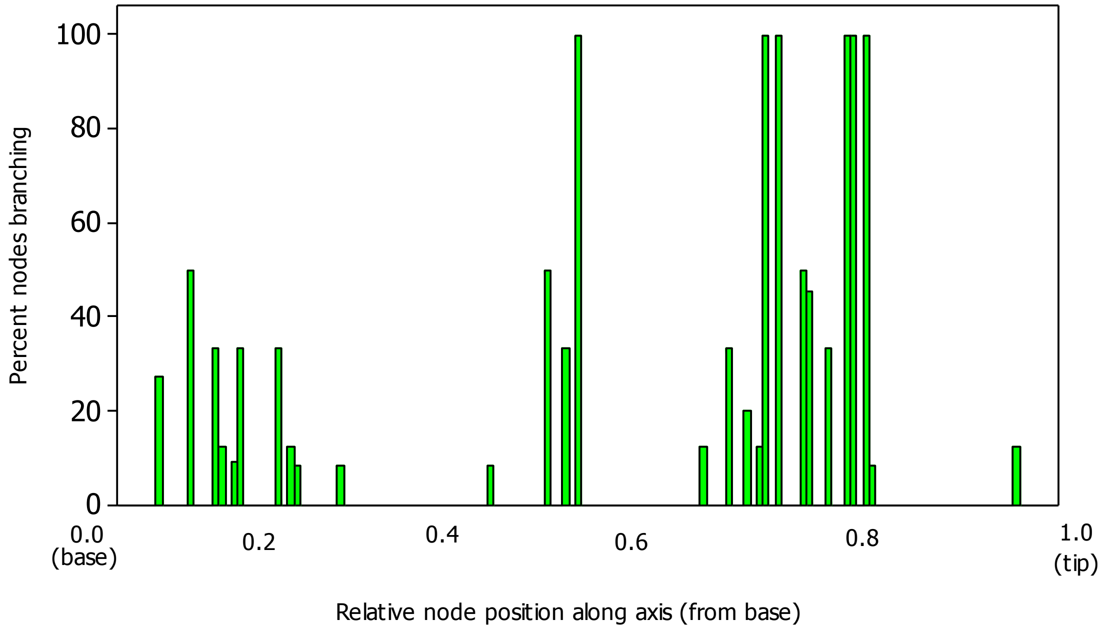

Figure 3-18. Relationship between relative node position and probability of branching among axes extending by one unit, in thirteen year old macadamia trees of variety 741.

Position of growth unit along the axis

Position of GU along the axis did affect the probability of a node branching ($P < 0.001$), with the probability of GU2 from the tip significantly higher than that of all other GUs (Figure 3-19). The probabilities of nodes on GU3 and GU4 branching were statistically similar, and that of GU1 was lower than any other GU. GU position also affected the number of new stems forming on those branching nodes ($P = 0.008$), in a pattern similar to that of branching probability, although there was a stronger trend toward more stems emerging from units closer to the tip (Figure 3-20) than the units at the base. Over all nodes however there was no difference between unit positions in the numbers of new stems emerging from a node.

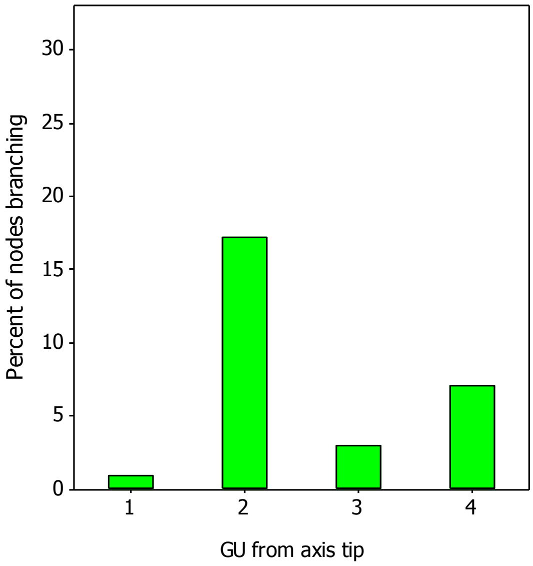

Figure 3-19. Relationship within extending axes between probability of a node branching and the position of its GU (Growth Unit) from the axis tip, in thirteen year old macadamia trees of variety 741.

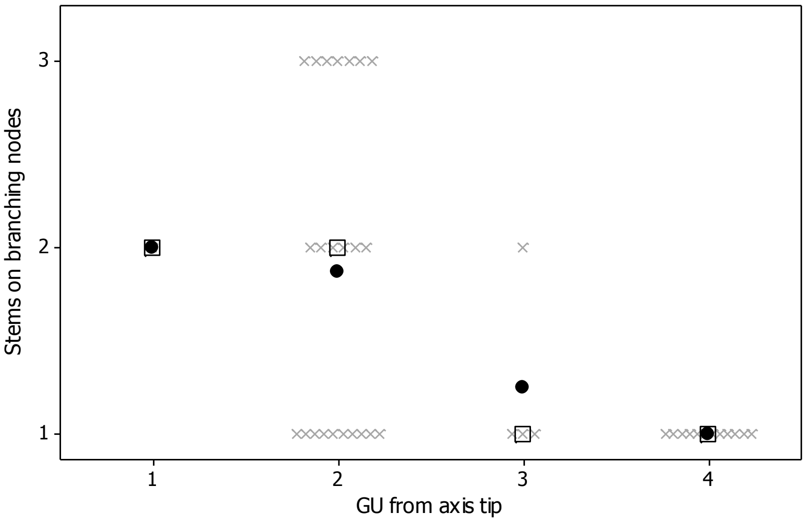

Figure 3-20. Relationship within extended axes, between GU (Growth Unit) position from the axis tip and number of stems formed on branching nodes, in thirteen year old macadamia trees of variety 741.
Squares are medians, circles are means, crosses are individual values.

Position of node along the growth unit

Position of a node along its growth unit also affected the probability of a node branching ($P < 0.001$). Nodes 2 positions from the top of a unit had a significantly higher probability of branching than nodes at any other position except 4 from the top (Figure 3-21). Positions 1, 3 and 5 had similar probabilities of branching, and were significantly lower than position 4 (as well as position 2). Among branching nodes, there was no difference between node positions on the GU in numbers of stems emerging from each node, but over all nodes the position on the GU did effect the average number of stems emerging from different positions (*P*< 0.001) (Figure 3-22).

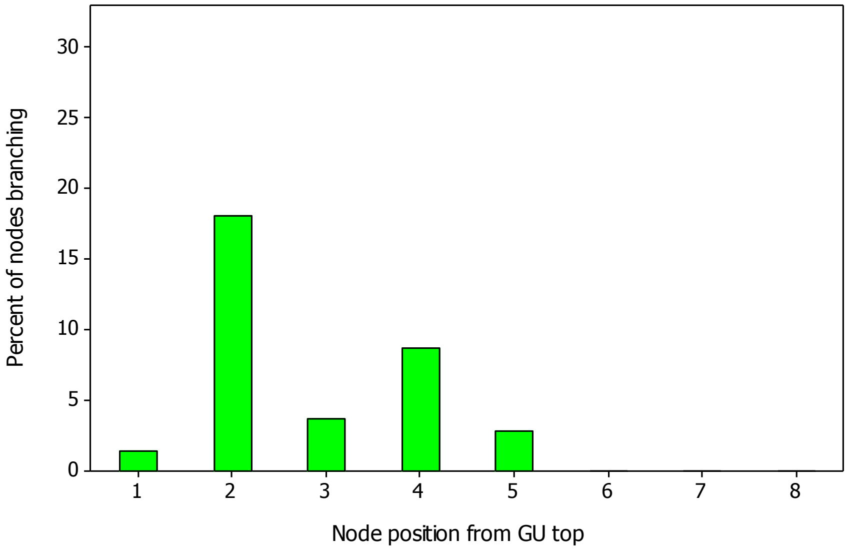

Figure 3-21. Relationship among axes extending by one unit, between probability of node branching and node position from top of its GU (Growth Unit), in thirteen year old macadamia trees of variety 741.

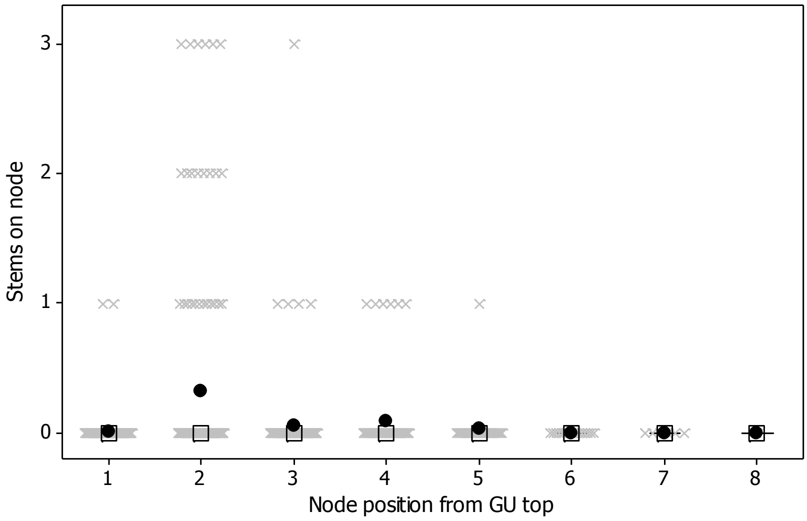

Figure 3-22. Effect within extended axes of node position from the top of a GU (growth unit) on number of stems emerging from the node, in thirteen year old macadamia trees of variety 741.
Squares are medians, circles are means, crosses are individual values.

Growth unit and axis size

No relationship was detected between the number of nodes on an axis or the number of nodes on a GU and the probability of a node branching (data not presented). There was no correlation between either axis size or GU size (in nodes) and number of stems emerging from those nodes that did branch, or from all nodes in general.

Multiple regression using position and size variables

Multiple regression analyses were conducted to find the combination of variables that best described branching probability. Node position along a GU plus GU position along an axis together produced a better explanation (lower AIC score) of probability of a node branching than they did individually. Node two of GU2 – the tip-most of the 'old' units (those present at the beginning of the survey) had by far the highest probability of branching ($P = 0.007$) – 56% - and number of stems per node ($P < 0.001$) – 1.06 - of any node-within-GU-along-axis (Figure 3-23 and Figure 3-24 respectively).

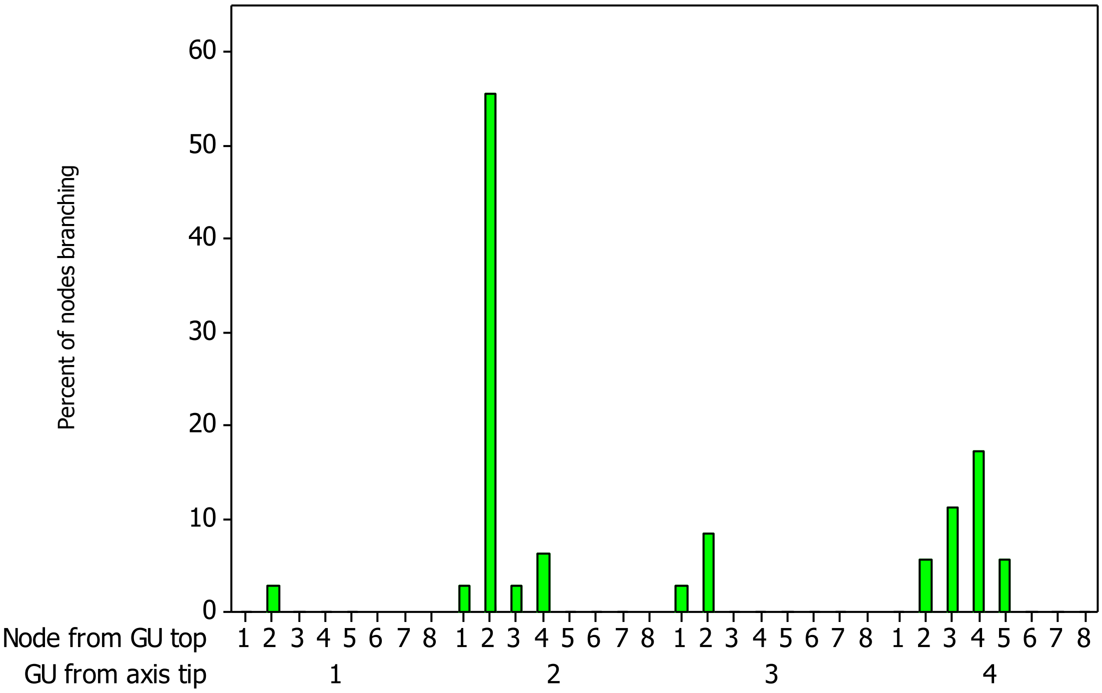

Figure 3-23. Effect among extending axes of node position and GU (Growth Unit) position on probability of the node branching, for GUs of all lengths combined, in thirteen year old macadamia trees of variety 741.

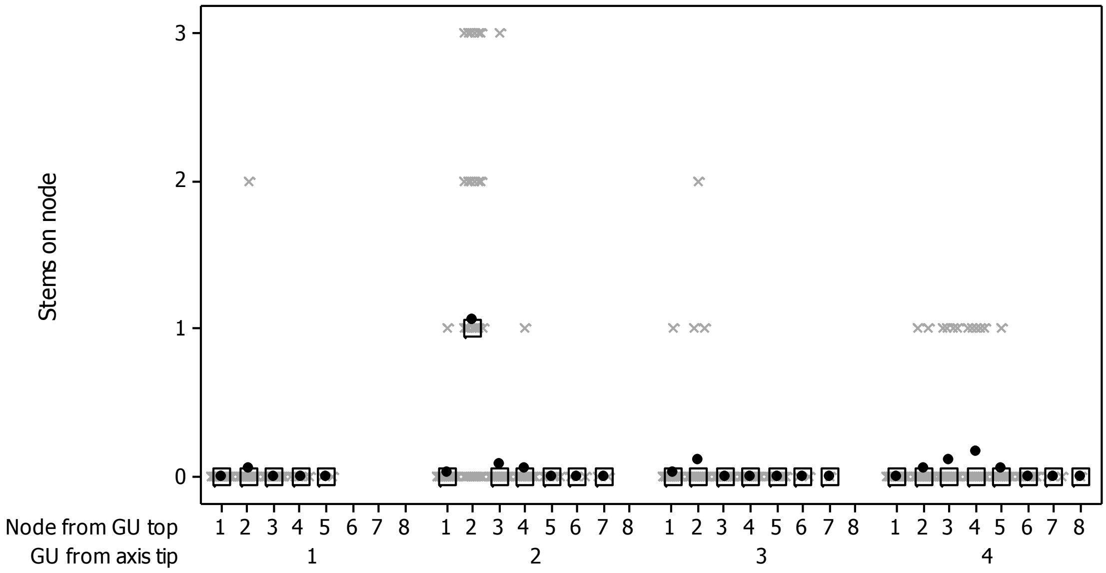

Figure 3-24. Effect among extending axes of node position and GU (Growth Unit) position on number of stems emerging from a node, for GUs of all lengths combined, in thirteen year old macadamia trees of variety 741.
Squares are medians, circles are means, crosses are individual values.

Using size as well as position, all combinations of the variables studied individually were also analysed with multiple regression. The best fitting model of branching probabilityon extending axes included node position within the GU, GU position along the axis and relative node position along the axis. In the multiple regression analysis, node probability of branching increased with node position along the axis from the axis tip. However it may be that relative node position is reflecting what is already told by node position and GU position, and so calculations of node probabilities of branching for different node positions along an axis have not been presented .

Predictions of proportions of nodes branching were made using the multiple regression model. On the extending axes studied here, the node most likely to branch was the node two below the GU top, of the second GU from the axis tip. Forty-two percent of these nodes branched, compared to the average of six percent, offering a seven-fold increase in the likelihood of finding a branching node and therefore branching bud.

3.4. Discussion

In the macadamia axes surveyed here, larger axes had larger base GUs. This may be because GU size and probability of an axis extending are both limited by similar resources, and a good first season's growth on an axis lays down good vascular connects and sinks to enable greater growth in subsequent seasons. The pattern was similar for all three groups of trees, and was similar to the pattern found in many deciduous fruit trees including apple (Costes *et al.* 2006). The sampling method used to survey the axis composition of young trees probably included a slightly greater proportion of longer axes (axes composed of more growth units) than were present on the whole tree, because a greater proportion of lower order axes were surveyed than are present over the whole tree. Mature trees data collection resulted in the opposite imbalance – fewer larger axes were surveyed because of moss and water staining on their older bark. Thus the absolute values of architectural relationships involving axis length calculated here may vary slightly from the whole tree values. However the patterns and trends of the axes examined – such as increase in axis size with base GU size - are accurate within the size groups examined, and so are likely to be able to be extrapolated to the whole range of axis sizes found in a tree.

The location of branching was similar along the old wood of extending axes and non-extending axes. The biggest difference is that the new wood (the new GU of the extending axes) did not usually branch. The numbers of new stems were greater on extending axes, but their distribution along the old wood of these axes was quite similar to that along non-extending axes.

The non-extending axes had a linear relationship between position of GU along the axis and probability of branching, as apple does (Costes *et al.* 2003), but in these macadamia axes the trend ran in the opposite direction to apple, i.e. they had more branching on GUs closer to the axis tip. As there were only three GUs on these axes it is hard to be sure that the relationship is truly linear, but it does match up with the greater probability of branching at nodes further from the axis base. If it is a linear relationship, the dominant influence driving it may be light levels, which will be higher closer to the edge of the canopy (where axis tips are in comparison to their bases). Increased light is known to weaken apical dominance (Cline 1991), and a higher proportion of direct sunlight to reflected light increases lateral outgrowth in some species (Kasperbauer 1987). In macadamias new vegetative shoot production was positively correlated with light levels, although it is unclear whether this was just axillary shoots of also included axis extension (Olesen *et al.* 2011). In this situation apical dominance may be negligible due to the lack of growth at the apex, as it is new tissues that consume sugar and produce auxin.

In the extending axes, the highest branching probability in the second GU is probably also (as for non-extending axes) due to higher light increasing branching towards the axis tip, combined with the inability of buds on the new (most distal) GU to grow out. This may have been either due to immaturity or AD. Extension may have taken place mostly in the summer flush, and so the new GUs had no opportunity to branch, perhaps simply because they do not grow quickly enough. In the spring flush, branching would have been in a similar pattern to the non-extending axes. Possibly newly formed axillary buds also need more than the few months between the spring and summer flush to mature. Alternatively, the growing apex may be suppressing axillary buds on the new growth unit in classical apical dominance, but not appearing to exert much influence further away. When multiple factors were analysed simultaneously, nodes closer to the base did branch more often in extending axes. This was not part of the multiple regression model for non-extending axes, and was the only difference between the models of the two axis types. The inclusion of observations of branching at time intervals of one growth flush (instead of one year) in future research would be useful in further understanding such interactions between locations of new stems.

Node position along a GU had the strongest effect of any one factor on branching in extending axes and was part of the best fitting model for both extending and non-extending axes. In both types of axis, probability of branching and number of stems per node were highest at node two from the top of GUs. The non-linear relationship between branching and node position within a GU is similar to the findings of zones of branching in apple, apricot, cherry and peach (Kervella *et al.* 1995; Costes and Guedon 1996; Costes *et al.* 2006; Renton *et al.* 2006). If this is a branching 'zone' in macadamia, it is very short – usually only one and occasionally two nodes - compared to other species – three to twenty nodes in apple (Renton *et al.* 2006). A shorter branching zone may simply be formed because each macadamia growth unit is much shorter than those of the deciduous species in which this zonation has been studied previously – around four nodes, as opposed to 10 to 70 in 'Fuji' apple (Renton *et al.* 2006). Shorter branching zones were found on shorter axes of the Fuji apples. Fulford (1965) showed that the type of bud being produced changes quite abruptly, so a zone of one node seems possible. Regardless of the mechanism behind the fact, the influence of nodes position along a growth unit on probability of a node branching seems likely to be due to the growth dynamics it that part of the season when a node is created, even though new stems may now grow out until many years later.

Buds on node two from the GU top of GUs in all positions along axes may have been equally predisposed to greater branching than the rest of their units, from the time of their formation. In peach (Kervella *et al.* 1995) the different combinations of vigours of the various parts of the metamer at the time of its formation are associated with different types of lateral growth. Vegetative buds had a higher rate of internode elongation than floral buds or blind buds, with dormant vegetative buds having a longer plastochron[5] – i.e. slower node formation – than nodes producing sylleptic[6] shoots (new lateral stems produced in the same growth period). It may be that buds formed under these conditions do not enter as deep a dormancy as others, and / or have a reduced sensitivity to florigen. However the nodes at position two on different GUs and on different types of axes in these macadamias may vary in branching probability due to a combination of GU age, GU distance from the axis tip, axis vigour or light exposure. Branching on the older GUs may be less because the nodes there have branched or flowered in previous seasons, leaving fewer buds to form new stems or inflorescences – collectively termed lateral shoots. However, because there are six to eight buds that are clearly capable of growth at each node (Bennel 1984), the forming of one or two lateral shoots previously may not reduce branching probability at the node substantially. At the beginning of the survey, lateral stems were removed by pruning, to remove the effects of old lateral stem apices and their sugar and auxin flows, and thus give all buds on the axis a more even chance of escaping apical dominance. However, those axes and nodes that had laterals to remove may have been more likely to have thicker vessels connecting them to their parent axis, in order to support the growth of more stems and fuelled by those stems.

A number of node positions on the longer GUs (towards their base) never branched. This raises the possibility that they are not capable of branching. Alternatively, buds in these locations may have begun forming new stems but aborted before maturity – this has been observed in apples, in which stem abortion between growing seasons is known to

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[5] A plastochron is the period of time between formation of nodes by the meristem.

[6] Sylleptic shoots are those that grow in the same season as the buds from which they grow are formed.

contribute to acrotony[7] patterns (Lauri 2007). Microscopic examination would reveal if this is also the case in macadamia (see Chapter 8 of this thesis). Pruning immediately above these nodes could test their capacity to branch, and as most GUs on a tree are less than five or six nodes long, a comparison of pruning above node 2 to pruning above node 6 could yield information on regrowth constraints (see Chapter 6 of this thesis for further examination of this area).

On non-extending axes, nodes on larger axes branched more often and had a higher number of stems per node overall than nodes on smaller axes. This may also be due to the effect of light – longer axes are more likely to have their tip in more distal regions of the canopy with higher light levels, or because axes with more nodes have more leaves providing more fuel to support more branching.

The number of new stems emerging a node once it was branching varied only with node or GU position, not with GU or axis size. The effect was always one of more stem emerging from branching nodes closer to the GU or axis tip, in both extending and non-extending axes.

Among both extending and non-extending axes, the number of nodes on a unit did not affect node branching probability or stems per node. This is another difference to the patterns found in apple, where the number of nodes in a branching zone increases with GU length (Renton *et al.* 2006). It may be that the shorter GUs of macadamia have less capacity for detectable change in their branching 'zone'.

Among extending axes, in the most favourable node position for branching – the node two below the top of GUs two below the axis tip - 42% of nodes, or around 14% of buds, formed new stems in the year studied. This knowledge of branching distribution increases the chance of collecting a microscopically branching node, and therefore bud, seven-fold. This will help make viable the study of microscopic development of a bud as it forms a new stem, as it reduces substantially the number of buds needing to be dissected to study one newly growing vegetative bud. As there are around two new stems produced by each branching node, and usually 3 axils at each node, 2/3 of the axils at a branching node will be forming new stems each year (in this type of axis). Branching usually occurs at the highest bud in the axil – as with buds along a stem, lower buds in an axil can be suppressed

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[7] Acrotony is the tendency of stems to grow longer towards the top of a parent axis.

by upper ones (Owens and Ewers 1997). Thus each bud at this top position of an axil has just under a 10% chance of producing a new stem in a year. The absolute of these proportions may vary from year to year, with environmental conditions, but it is expected the relationships and relative amounts would remain similar.

The greater overall quantity of branching in extending axes than non extending axes suggests that extending axes have a greater supply of carbohydrates than non-extending axes, and that there is excess supply which is channelled into branching after extension has used what it needs. The consistent factors determining new stem location in macadamia trees are the position of a growth unit, or its maturity, and the position of nodes along that growth unit. The distance from the axis apex appears to exert a significant influence on the outgrowth of macadamia buds only when it is extending, which is partly the absence of branching on the tip unit and partly a subtle gradient below that. But in these axes as well as the non-extending ones, the tip half of the axis always produced the most branches.

If the extending axes displaying some apical dominance branch more than non-extending axes which do not display AD, AD may not be reducing the amount of branching. This depends on interactions with carbohydrate supply and other bud dormancy controls. But if AD is not reducing numbers of stems, or if it is only doing so to a small extent, tip removal may not release many more buds from dormancy. This is a question that will be investigated in Chapter 6.

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Chapter 4. Relationships between the axis apex and axillary buds

4.1. Introduction

In many species of plants, outgrowth of axillary buds from dormancy has long been known to be controlled by activity of their axis apex. Axillary bud outgrowth is often suppressed by young growing leaves and internodes at the axis tip, a relationship termed apical dominance (Thimann and Skoog 1933; Cline 1997; Cline and Harrington 2007). There appear to be many interacting factors contributing to apical dominance (AD), and competition for nutrients, both organic and inorganic, emerges as the common theme (Cline *et al.* 2009; Leyser 2009). This probably involves coordination of growth in different parts of the plant through signalling with plant growth regulators as well as direct competition through consumption of sugars, water and minerals. The extent to which apical extension is preferred over axillary outgrowth varies with species (Barthelemy and Caraglio 2007) and the conditions faced by the plant or axis (Cline and Harrington 2007; Cline *et al.* 2009).

There is no record of work directly measuring the extent of apical dominance in macadamia trees, but while working on other canopy research (Chapter 5) the author noticed that axes missing apical buds appeared to flower more than those with intact apices. Thus the work of the first part of this chapter set out to investigate possible relationships between the axis apices and axillary flowering.

As with apical dominance of vegetative axillary buds, a number of mechanisms have been proposed in the literature that may result in vigorous axis apices suppressing axillary flowering. The role of auxins in axillary flowering has been long discussed, but without firm conclusions being reached. One suggestion is that auxins act as a signal to inhibit evocation (Teltschrova *et al.* 1976). However Jacobs (1985) concluded the most likely role for auxin in evocation was that of a background suppressor that is over-ridden by conditions that promote evocation/determination. He noted that the research is difficult to interpret, as auxin can be toxic at high levels and will change in concentration as it moves from its site of application. Auxin may also act indirectly on evocation: in the axillary flowering species of *Pharbitus nil* (Japanese Morning Glory), more flowers are produced when the apex is removed a day before exposing the plants to their floral stimulus of long nights (Ono *et al.* 1993). This suggests that buds need to be released from dormancy before they can respond to floral stimulus, and so low auxin levels – and high sugar levels - would be a requirement for good flowering.

Evocation in the transitioning or newly independent axillary bud may also be controlled by local sugar levels, which decrease with axis apex growth. In *Arabidopsis thaliana,* trehalose-6-phosphate – a molecule proposed to represent carbohydrate availability in plants – is essential for the production of FT and therefore floral determination of buds under the normally inductive long day conditions (Wahl *et al.* 2013).

After floral determination and initial growth of the inflorescence, floral macadamia axillary buds re-enter dormancy. Emergence of inflorescences, or growth of florets, or both, occurs on exit from this second dormancy, and the axis apex could also influence the release from this. Here again, both sugar and auxin could be some of the signals through which the bud detects when to resume growth. Macadamia inflorescences resume growth and emerge in cool temperatures (Sakai *et al.* 1982; Stephenson and Gallagher 1986), but only at times well removed from vegetative flushing (Olesen 2005). Inflorescences also are removed in space from recent vegetative growth: with greater elongation of the parent axis inflorescences were found further back from the axis tip (Salter *et al.* 2005). It seems that a likely mechanism could be the same as that for vegetative bud outgrowth from dormancy – competition for sugars between the apex and the axil (Mason *et al.* 2014).

The second part of this chapter continues to examine the relationships between the shoot apex and the axillary buds, looking at their interaction with mineral nutrients and water. Competition between apices and axillary buds for nutrients, including water, are suspected of contributing to axillary bud dormancy (Cline 1991), with the growing apex depriving axillary buds of materials necessary for growth. In the herbaceous perennial *Epilobitum ciliatum,* increased application of 1:1:1 nitrogen : phosphorus : potassium fertiliser (NPK) for four months from germination, increased branching in both intact and tip-pruned seedlings (Irwin and Aarssen 1996). In the herb *Verbascum thapsus*, 1:1:1 NPK and additional water were applied to mature plants (3g in 1 L every week), for 15 weeks to decapitated plants and 6 months to intact plants (Lortie and Aarssen 1997). The treatment increased branching in decapitated plants, but not in intact plants. In ten-node tall *Triplochiton scleroxylon* tropical hardwood cuttings, 0.4g or 4 g of dissolved 23:19.5:16

NPK fertiliser was applied for 11 weeks. Trees were decapitated and leaves removed from the lower halves. No effect on release of axillary buds from dormancy was found (Leakey and Longman 1986). Thus the effect of mineral nutrition on AD may depend on species, on damage to the plant, and possibly on the ratio of nutrients applied. In orchard macadamias, the proportion of surface axes flushing in a canopy increases with improved mineral nutrient status of the tree (Stephenson and Cull 1986), but it is not known whether the new growth units form as extensions of existing axes, or as new axes from axillary buds leaving dormancy.

The case for an effect of water availability on apical dominance seems weaker than that of nutrient availability. McIntyre and Damson (1988) postulated that low hydrostatic pressure in an axis, resulting from the high consumption of water by growing apex tissues, may leave axillary buds unable to access enough water for outgrowth, and thus competition for water may maintain AD. In bean seedlings (*Phaseolus vulgarus*) they found removal of the entire shoot 5 mm above the cotyledons resulted in cotyledon axillary bud outgrowth, along with a higher-than-normal water potential in the axis. The authors interpreted the increase in water potential as at least part of the cause of bud outgrowth. No control for other factors such as auxin levels was used however, so it is not clear whether other mechanisms played a more important role in triggering the outgrowth of these axillary buds. Lortie and Aarssen’s (1997) work with *V. thapsus* looked also for an effect of supplementary watering on axillary bud outgrowth after apex removal, but found none.

A higher amount of apical extension relative to new lateral stem formation would produce more open trees, desirable for their increased light distribution throughout the canopy and also to the orchard floor. Thus the ability to manipulate this aspect of architecture through nutrient and water application could be of broad interest. However if this ability came at the cost of reduced flowering the effort may not be beneficial overall. This chapter examines both the effect of apex damage and growth on flowering, using orchard surveys, and the effect of nutrient supply on branching and extension via a potted plant experiment.

4.2. Materials and methods

4.2.1. Damage to apical buds and axillary flowering

In August 2010, apical bud damage and extent of flowering of macadamia trees were surveyed in a commercial orchard near Woodford in south-east Queensland. Four 5 year old macadamia trees of variety 741 were used, none of which were on orchard row ends. Every second axis growing from the leader was included in the survey, and then every second axis growing from them and so on. In this way 216 axes from all canopy positions were included. For each axis, the number of inflorescences was counted, and the apical bud condition was classified as either intact or damaged. Damaged apices included those which had been bitten into by insects, were necrotic from fungal rot, or missing completely (e.g. nipped off by birds). Effect of tip condition on proportion of axes flowering was analysed using a Likelihood Ratio Chi-Square test, and effect on density of inflorescences was analysed using Mann-Whitney tests.

4.2.2. Axis extension and axillary growth

Six 13 year old trees of variety 741 were used to survey the distribution of flowering and branching within and between axes. Twenty axes, each 3 growth units (GUs) long, were located and tagged on each tree. Trees were around eight metres high and axes were selected from between 1.5 m (above the skirt-pruning line) and 4 m (maximum ladder reach). Axes were selected from between 50 cm and 100 cm into the canopy from the face of the hedgerow, a zone where good light and little moss growth on the bark enabled clear identification of GUs. Roughly half of these axes had previously branched, mostly producing only one lateral stem. All these existing lateral stems were removed at the time of tagging, so that buds of all axes were affected by the auxin production and carbohydrate consumption of the same number of apices – only the one, at its own tip. One year later, 104 tagged axes were able to be found and the number of inflorescences and new stems[8] at each node and number of growth units of apical extension was recorded. Also recorded

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[8] As part of this survey, the influences of GU and axis size and node and GU position on new stems distribution was analysed – see Chapter 3.

was the position of the node along the GU and position of the GU along the axis (see Figure 3-1). Differences between extending and non-extending axes in proportions producing lateral shoots were tested using Likelihood Ratio Chi-Square tests. Differences between extending and non-extending axes in numbers of stems or inflorescences emerging were tested for significance using Mann-Whitney tests.

4.2.3. Effects of water and mineral nutrients on extension and branching

Twenty-four 3 year old macadamia trees of variety A38 were grown in 30 cm diameter pots, in the open, near Nambour in south east Queensland for 2 years. The pot media was a sandy loam (appendix 1) and 20 g of slow release Australian native fertiliser (65:5:30 NPK with trace minerals, see appendix 1) was added every four months. Plants were drip irrigated with 600 ml water once per day, and no saucers were used so that pots drained freely.

In July 2012, when the plants had not been fertilised for four months, the plants were randomly allocated to one of four groups – fertilised (F), high water (W), both fertiliser and high water (F+W), or a ‘control’ (C) group. The C trees were supplied with the minimum input judged necessary for tree condition – the continuation of 600 mL of water each per day, but no saucers or fresh application of fertiliser. Trees in the F group were each given 20 g of the same fertiliser as above. Trees in the W groups had their irrigation allocation increased to 1200 ml once per day, and saucers placed under the pots to provide a reservoir of water that could be wicked up into the media over the following 24 hr. Rainfall for the six weeks of the experiment averaged 0.8 mm per day.

After six weeks each tree was examined for new vegetative growth. New stems emerging from axils and apical extensions to existing axes were counted. Only the 238 axes with undamaged apices were included in the analyses. Differences between treatments in proportions of stems extending or branching were analysed using Chi-Square tests. Differences between treatments in numbers of new stems or extending apices per axis, and ratios of stems-to-apices were analysed with Mann-Whitney tests (for two groups) or Kruskal-Wallis tests (for three groups or more).

4.3. Results

4.3.1. Damage to apical buds and axillary flowering

Of the axes surveyed, 14% had damaged apices (30) and 86% had intact apices (186). Axes with damaged apices were three times more likely to flower ($P = 0.021$) (Figure 4-1), and had a higher mean number of inflorescences per node than those with intact tips ($P = 0.007$) (Figure 4-2). Among flowering axes alone there was no significant difference between the inflorescences per node of axes with damaged and intact tips (data not shown).

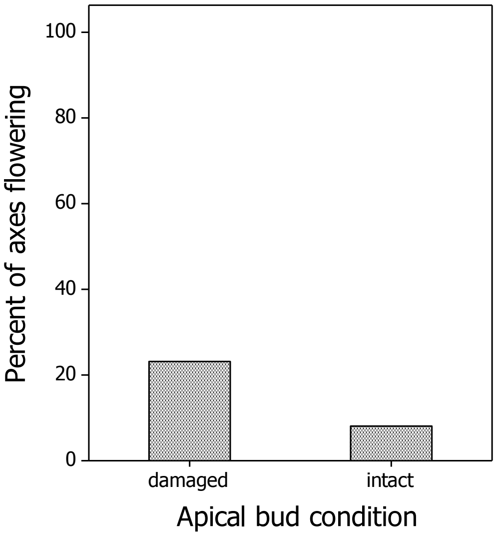

Figure 4-1. Effect of damage to apical bud on percent of axes flowering, in five year old macadamia trees of variety 741.

4.3.2. Axis extension and axillary growth

Sixty-two axes (59.6%) did not extend during the course of the year, 36 axes (34.6%) extended by 1 unit, and 6 axes (5.8%) extended by 2 units. Because of the low number of axes extending by two GUs, these axes were excluded from further analyses.

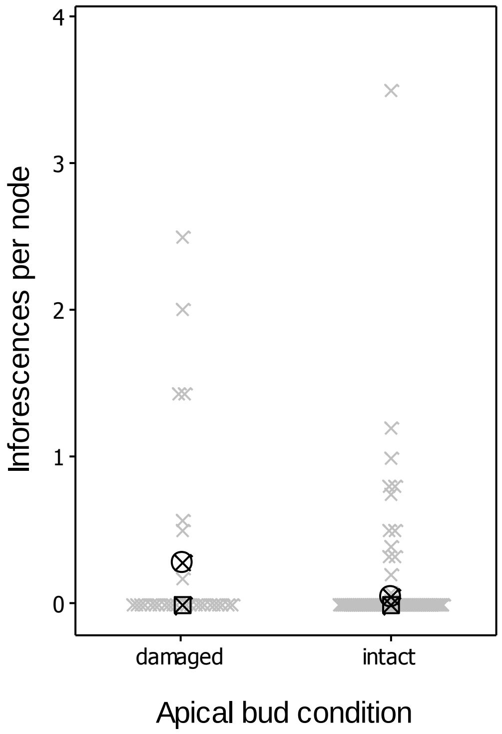

Figure 4-2. Effect of damage to axis apical bud on number of inflorescences emerging per node, in five year old macadamia trees of variety 741.
Circles are means, squares are medians, and crosses are individual values.

Axes extending by one growth unit were more likely to branch than those not extending ($P < 0.001$) - 74% compared to 41% - and were less likely to flower than those not extending ($P < 0.001$) - 15% compared to 54%. Axes extending by one growth unit over the year produced around a third more lateral stems per node than non-extending axes ($P = 0.0423$) and less than one tenth of the inflorescences of non-extending axes ($P < 0.001$) (Figure 4-3). Overall 54% of axes branched and 39% of axes flowered.

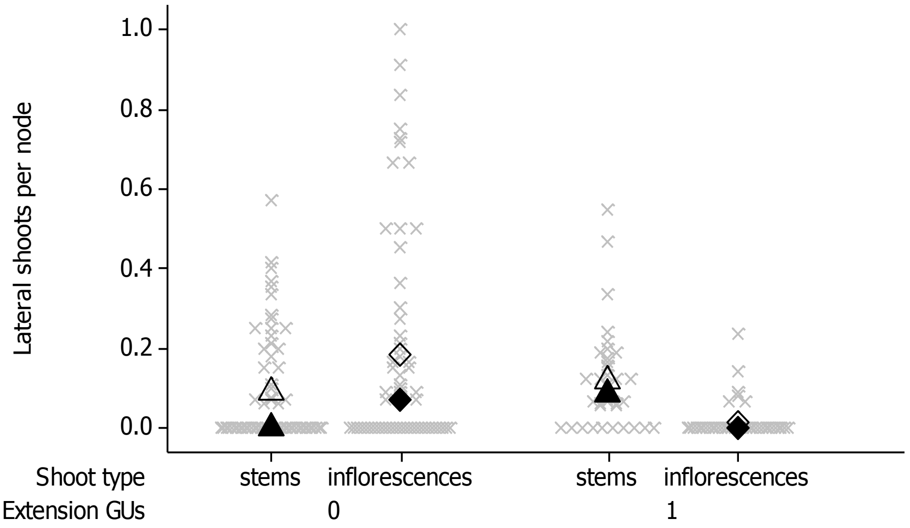

Figure 4-3. Effect of axis extension on lateral growth over one year, in thirteen year old macadamia trees of variety 741.
Filled shapes are medians, outlined shapes are means, crosses are individual values. Triangles represent new stem emergence and diamonds represent inflorescence emergence.

The drop in number of inflorescences per node with axis extension appeared to be spread all along the axis (Figure 4-4), although statistical analysis of this pattern was not capable of discerning differences because of low numbers of extending axes[9].

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[9] A larger survey addressing this is detailed in Chapter 5.

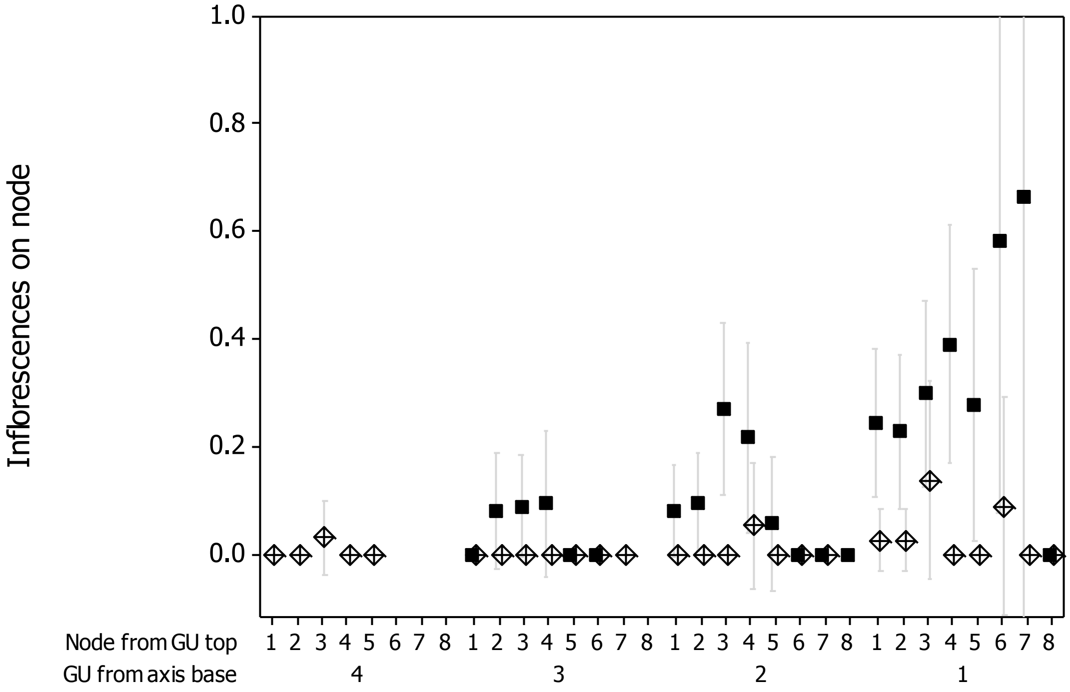

Figure 4-4. Effect of axis extension on distribution of inflorescences along that axis, in thirteen year old macadamia trees of variety 741.
Squares represent figures for non-extending axes, diamonds represent extending axes, and bars represent 95% confidence limits of the mean.

Extension and branching both reduced the number of inflorescences per node on an axis (Figure 4-5), ($P = 0.0238$ and $P < 0.001$ respectively). However there was no significant difference in inflorescences per node between axes extending-but-not-branching and axes branching-but-not-extending.

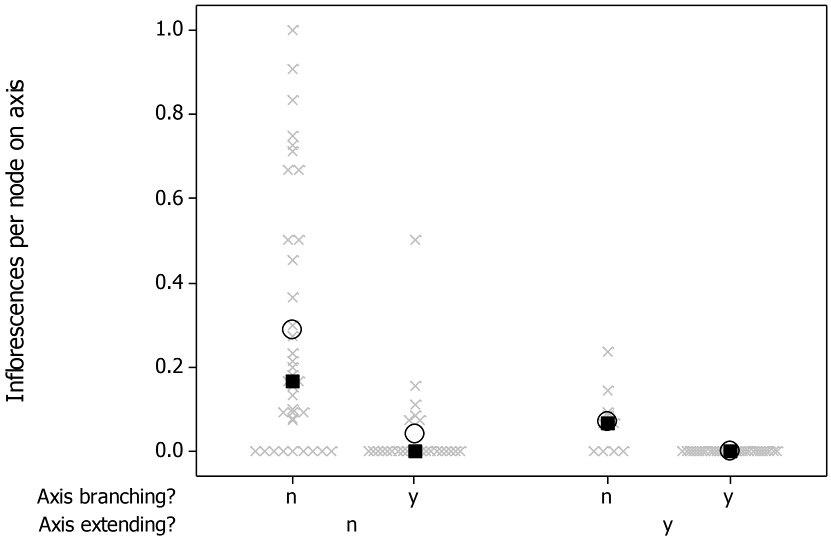

Figure 4-5. Effect of axis extension and branching on inflorescences per node on axis, in thirteen year old macadamia trees of variety 741.
Circles are means, squares are medians, and crosses are individual values.

4.3.3. Effects of water and mineral nutrients on extension and branching

The proportion of axes branching was greater among trees of the F (minimum water, fertilised) group than C (minimum water, no fertiliser), and greater again in F+W (fertilised plus extra water) ($P< 0.001$) (Table 4-1). There was also a difference between treatments in proportion of axes growing in general i.e. when axes were categorised into those neither branching nor extending, compared to those either branching or extending or both (Figure 4-6) ($P< 0.001$). In trees of the C groups and the W (extra water) group most axes neither extended or branched, but in those of the F and F+W groups more than half of the axes grew, either through extension or through formation of new stems. More axes of F+W grew than did F axes.

When axes were categorised into either extending or not extending, the differences between treatments were again significant ($P= 0.001$). The proportion of axes extending was similar in C and W (Table 4-1). The F treatment and the F+W treatment had greater proportions of axes extending than the C group. W had a lower proportion of axes extending than either F or F+W, while F and F+W were similar to each other. Many more axes extended without branching (between ten- and twenty-fold) than axes branching without extending, in all groups. The highest proportion of axes that branched over all, as well as branched without extending, was in F+W.

Table 4-1. Percent of axes branching, extending, or not growing, in 3 year old potted macadamia trees variety A38.

Treatment	Percent of axes					
	Branching	Extending	Neither (no growth)	Both branching and extending	Only extending	Only branching
Control	1.6 a	20.4 a	79.6 c	1.9 ab	18.5 ab	0.0 *
Extra Water	3.2 a	12.9 a	85.5 c	1.6 a	11.3 a	1.6 b
Fertiliser	12.9 b	54.3 b	42.9 b	10.0 b	44.3 c	2.9 b
Fertiliser + extra water	48.1 c	61.5 b	21.2 a	30.8 c	30.8 bc	17.3 c

* Significance of difference could not be computed due to the branching percent of 0. Different letters in cells within a column indicates a statistically significant difference (percent of axes flowering/branching tested using Chi-Square $P< 0.05$; numbers of new shoots tested with Mann-Whitney, $P< 0.05$).

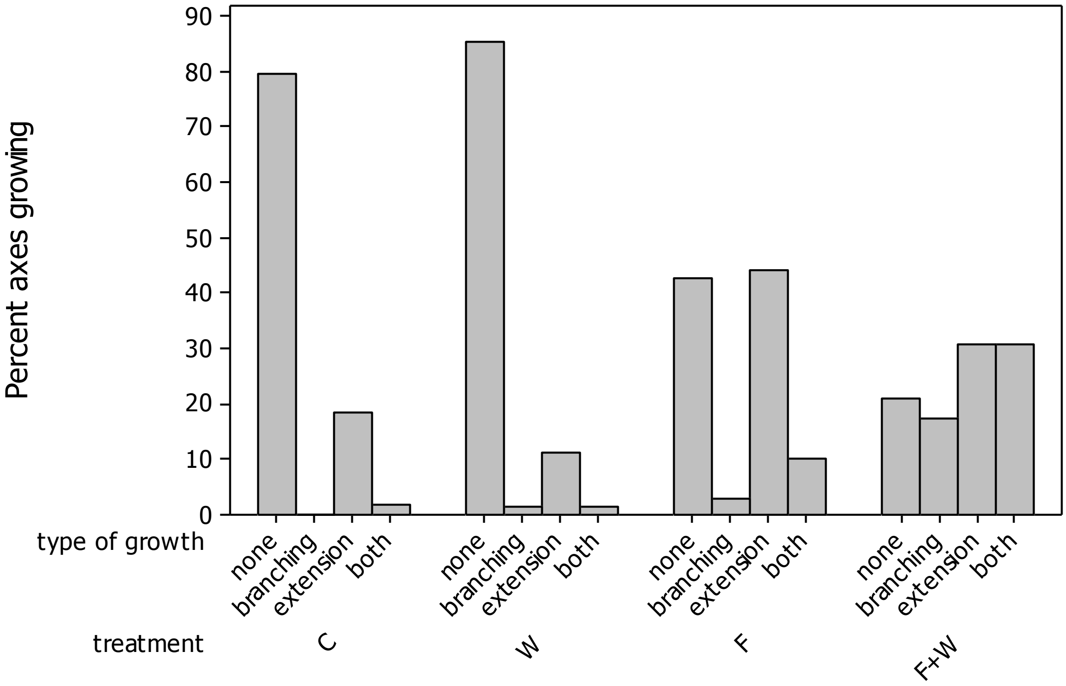

Figure 4-6. Effect of input treatments on percent of axes branching and extending, in five year old potted macadamia trees of variety A38.
C = control, W = extra water, F = fertilised, F+W = fertilised plus extra water.

The total number of buds growing out from dormancy varied with treatment ($P < 0.001$), in a similar pattern to the proportion of axes growing – F was greater than C, and F+W was greater again. The number of axillary buds growing out to form new lateral stems (Figure 4-7) also varied with treatment ($P = 0.003$), in a similar pattern to the proportion of axes branching. The number of lateral stems emerging from an axis increased with fertiliser, and increased again with a combination of fertiliser and water, but water alone did not increase the number of new stems. Of those axes that did branch, there was no significant difference between treatments in the number of new stems per axis.

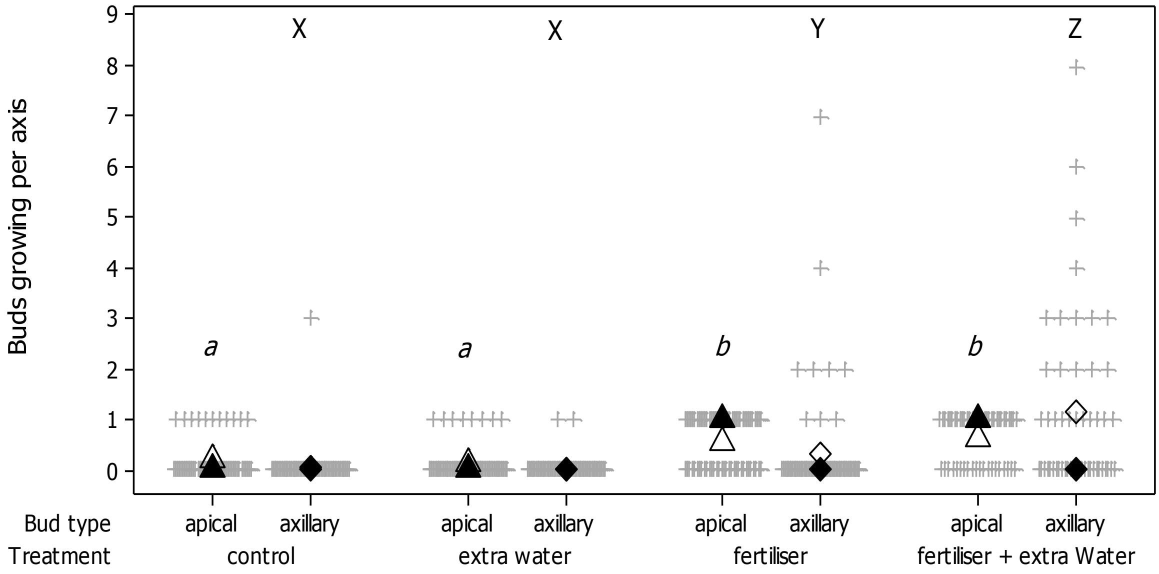

Figure 4-7. Effect of input treatment on number of apical buds and axillary buds exiting dormancy to form new growth units, in five year old potted macadamia trees of variety A38.
Filled shapes are medians, outlined shapes are means and crosses are individual values. Triangles represent apical extension growth and diamonds represent axillary growth forming new stems. Treatments labelled with different lower-case letters had significantly different numbers of apical buds growing. Treatments labelled with different upper-case letters had significantly different numbers of axillary buds growing.

4.4. Discussion

Among the axes on the 5 year old orchard trees, axes with apices that were intact were less likely to flower and produced fewer inflorescences per node than those with damaged apices. Among the axes on the 13 year old trees, extending axes – i.e. those currently vigorous as well as intact – were less likely to flower and produced fewer inflorescences than axes that did not extend. Thus there appears to be a negative relationship between apex vigour and amount of flowering on an axis, in both young and mature trees. The reductions in flowering with extension were similar to the reductions in flowering with branching, indicating that a more open tree need not be a less floral one. Reductions in flowering with increased branching would be via competition and not auxin. Thus reductions in flowering with axis extension, which must also be due to competition to some extent, need not involve auxin to have the same magnitude of flowering decline as competition with branches. The difference between these two groups of trees may have been partly due to age, as younger trees generally produced more inflorescences per node on axes with average (orchard) amounts of damage and extension (see Chapter 5).

Parent axes extending over the year branched more frequently and produced more lateral stems than non-extending axes. This appears to contradict the apical dominance theory that vigorous apical tissues suppress lateral growth below them (Cline 1991; Mason *et al.* 2014), but as macadamias undergo two or more flushes of growth over a year, the extension and lateral growth may have occurred at different times of the year. Growth, either as extension or branching, during the first flush period of the survey year could have increased the number of new, highly productive leaves and thus provided more energy for any subsequent flush growth. Alternatively, high vigour that enabled one type of vegetative growth could also have enabled the other.

If this reduction in flowering is via evocation it would fit with the theory that evocation may be suppressed by high auxin levels (Teltschrova *et al.* 1976; Jacobs 1985), and with the findings that high sugar concentrations have been shown to contribute to the production of FT (Gisbon 2005; Wahl *et al.* 2013). As this reduction (Figure 4-4) is distributed among all the original growth units, if it is via evocation it would indicate that determination could occur in buds on hardened wood.

The reduction of flowering with axis extension may also be effected via a reduction in emergence of dormant inflorescences. They may not be sensitive to auxin, either because they are not vegetative, or because they have already exited dormancy once, and so they may effectively be very small but independent axes. Emergence of the inflorescence may be more similar to axis extension than vegetative bud exit from dormancy, despite the inflorescence being hidden by buds bracts in a similar manner to vegetative buds which have not grown since the season of their formation. If carbohydrates were the means by which apical extension suppresses emergence, low carbohydrate conditions created during this extension have to persevere some time – vegetative flushing is removed from emergence by many months. This infers that the carbohydrates depleted by extension would have to be stored ones. A limiting effect on emergence of stored carbohydrates, more of which are found in larger axes of the canopy and the roots than the tips of these small axes (Newell *et al.* 2002), fits the distribution of flowering, which is greater at the bottom of an axis (Chapter 5). Further investigations separating location of inflorescences on stems with branching from that on stems without branching, and growth in the first flush period from those in the second flush period, would be a useful next step in addressing these issues.

The potted tree experiment provided evidence of relationships between outgrowth of macadamia buds and supply of minerals, and possibly supply of water. The addition of fertiliser resulted in branching, and also increased extension of existing axes. Increasing the amount of water appeared to increase the proportion of axes that branched but did not extend, but as proportion doing this in the control group was zero, it may be concluded that these plants were suffering water stress, or that the experiment duration was too short to clearly discern between these groups. There is also the possibility that different amounts of water applied did not result in different amounts of water available to the trees, and if this were the case differences in branching and extending would be due to differences in nutrient availability only.

The extra water did not change the number of new stems formed per axis or the number of axes extending, while the addition of fertiliser increased both. The addition of fertiliser-plus-extra-water together increased the number of new stems per axis more than the addition of only fertiliser. One interpretation of these findings is that well nourished trees could make use of more water to form new growth than could poorly nourished ones, perhaps via increased photosynthesis. An alternative interpretation is that the trees may not have actually used the extra water supplied, but the extra water made available more of the added nutrients - more water may have transported more of the fertiliser (via osmosis) from granules to root surfaces, making the nutrients more available to the trees. If the extra water in the fertiliser-plus-extra-water was not actually used by the tree, this experiment effectively tested two levels of fertiliser (high and low), and the amount of branching continued to increase with the extra supply of mineral nutrients. But whether testing one or two levels of fertiliser addition, the increase in branching with increased supply of mineral nutrients is the same positive relationship as that found in *Epilobitum ciliatum,* the only other study examining these variables in intact perennials (Irwin and Aarssen 1996).

While these results point towards effects of nutrients on branching rates, studies of soil and plant nutrient status are necessary to be more confident of a cause and effect relationship. Future research determining the role of water in the mineral nutrient relationship with branching would be helpful to the macadamia industry, as would investigation into which minerals or combinations of minerals are having this effect.

The differences in amount of branching between treatments were not due just to differences in overall growth. The higher proportion of axes branching in fertiliser-plus-extra-water group than the fertiliser-only group was not accompanied by a higher proportion of axes extending (Table 4-1). Numbers of new stems relative to numbers of extending parent axes were also were greatest in this treatment – the only one where new stem numbers were greater than extension numbers (Figure 4-7). This suggests that apical dominance in macadamia varies with nutrient status of the tree. The increase in NPK fertiliser application appeared to reduce apical dominance, increasing the branching of these trees as a proportion of overall vegetative growth. If this occurs in commercial trees it would increase the density of macadamia canopies and thus self-shading. This may lead to decreased fruit retention (Olesen *et al.* 2011). Future work should address whether these apparent effects on canopy architecture are sustained over the productive life of a tree. Further studies including analyses of soil and plant nutrient contents, as well as soil water availability, would also be required to confirm that both water and nutrients are affecting branching. It is also necessary to investigate the transferability of these results obtained in potted trees to orchard trees - rooting conditions, including being potted, influence plant behaviour (Poorter *et al.* 2012), and so a difference in the effect on architecture of these nutrient applications between potted-trees and field-trees is possible.

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Chapter 5. Location of flowering on axes

5.1. Introduction

The flowering process in macadamia is known to be spread over many months, with the first visible signs of inflorescences appearing in autumn, and elongation and anthesis occurring over winter and early spring. However neither the time of induction – when the tree senses environmental or internal cues to begin the flowering process – nor the time of evocation – when the meristem cell genetic and biochemical activity changes in preparation for floral organ formation – are known. In trees where this has been studied evocation can occur many months or years before visible floral growth (Sedgley and Griffin 1989; Okuda *et al.* 2004).

Knowing the seasonal timing of these processes would help orchardists most efficiently fertilise, irrigate, and otherwise manage their trees to optimise flowering and maximise sustainable nut production. As evocation is confirmed by morphological changes to the meristem (Sedgley and Griffin 1989), monitoring of microscopic morphological features of buds through the seasons until meristems are seen to become floral would identify more closely the time of year of evocation. However given the enormous numbers of axillary buds on a macadamia tree, microscopic studies into the timing of the very first stages of bud differentiation could only be made feasible if locations fairly likely to flower could be identified.

Such knowledge of the distribution of inflorescences in macadamias may help improve understanding about their flowering controls. Distribution of inflorescences between and within structural components of a tree could provide indications of which aspects of the tree's physiology influence the production of inflorescences. There have been quite a few studies of macadamia inflorescence distribution along the axes. In unpruned, eight year old trees of variety '849', Olesen *et al.*(2011) found that inflorescence production in the upper part of the tree increased towards the inside of the canopy (from less than one metre from the edge to over two metres from the edge in trees over six metres across), and was greater on wood older than 3 years. Inflorescence production was relatively constant across the canopy in the lower part of tree. In five year old trees of a wide selection of varieties, including '842', 'A4' and 'A38', Salter *et al.* (2005) found that flowering usually occurred

40 cm to 150 cm back from the tip of the axis, typically on wood more than 2 years old. This also would fit with a pattern of more flowering at the base of the axes than the tips. Similarly, Wilkie *et al.* (2009) found in one year old axes of variety 849, that inflorescences formed more often on the first growth units of an axis than on the second growth units (see Chapter 3 for explanation of architectural structures such as growth units). In varieties ‘344’, ‘660’, and ‘558’, Nagao *et al.*(1994) documented an increase in amount of flowering as growth units aged up to 3 years old, and then a decline as the growth units aged further.

Wilkie *et al.* (2009) also studied the distribution of inflorescences between one year old macadamia axes of different sizes. They found flowering was more frequent on shorter axes (less than 20 cm) in varieties ‘A4’, ‘675’ and ‘A38’, or intermediate length axes (around 20 cm) in variety ‘660’. McFadyen *et al.* (2008) also found that longer macadamia axes were less likely to bear inflorescences than shorter ones. This is opposite to the findings for pecan trees (*Carya illinoensis*), in which longer axes produced more inflorescences (Malestrom and McMeans 1982).

In other perennials, flowering location has been studied at the level of bud location within a growth unit. In mock orange (*Choisya ternata*) inflorescences always develop from the axils of the first nodes formed in a growth unit - the most proximal or base nodes - whereas the axils of the last nodes formed produce new stems (Guedes 1980). Apricot (*Prunus americana*) produces more inflorescences in the axils of the centre nodes of its floral growth units than at either end (Costes and Guedon 1996). Peach (*Prunus persica*) produces floral axillary buds mostly in the middle of the growth unit, with a smaller number near the distal end (Kervella *et al.* 1995; Fournier *et al.* 1998). In “Fuji” apple (*Malus domestica*), zones of floral shoots (short with apical flowers,) occur at different locations along axes - depending on the length of the axis, its branching order and the year (Renton *et al.* 2006). These floral shoot zones occurred less frequently in medium-length growth units than in long growth units. The length of the floral shoot zone was also flexible, and tended to increase with the total growth unit length, but only for growth units with less than 25 nodes. In apple, these patterns in bud ‘fate’ have been linked to rates of growth of different tissues at the axis tip when the nodes are formed by the apical meristem (Fulford 1965; Kervella *et al.* 1995). However no links were found between mature size of metamer organs and bud fate (Lauri and Terrouanne 1998). In mango (*Mangifera indica*), growth units themselves differ with those at the base of an axis less likely to flower than other GUs on the axis (Normand *et al.* 2009).

To provide more detail on location of flowering in macadamia, this study investigated patterns of inflorescence occurrence within and between the architectural structures of axes, growth units and nodes. Surveys were conducted in both young and mature macadamias, examining axes of all ages, to include a wide range of flowering wood found in commercial orchards. The study used axes that had not been pruned to understand the behaviour of axillary buds before direct manipulation.

5.2. Materials and Methods

Two varieties of macadamia, ‘741’ and ‘842’, growing in commercial orchards near Glasshouse Mountains Township in south-east Queensland, were surveyed to determine the distribution of their inflorescences within and between the architectural components of their canopies. Five year old 741 trees were surveyed in August 2010, 12 year old 741 trees were surveyed in August 2011, and 13 year old 842 trees were surveyed in August 2012. None of the trees had been hedged in the year preceding survey. These were the same trees that were used for the study on vegetative architecture detailed in Chapter 3 of this thesis.

Four five year old 741 trees, hereafter abbreviated to 741/5, of similar size were used for the survey of young trees. The trees were growing in two rows separated by a row of another variety, and not on the end of either row. Every second axis growing from the leader was included in the survey, and then every second axis growing from them and so on. In this way axes from all canopy positions in the 5 year-old-trees were included.

Six trees each of 12 year old 741 trees, hereafter abbreviated to 741/12, and 13 year old 842 trees, hereafter abbreviated to 842/13, were used for the survey of mature trees. For each mature variety the survey used six adjacent trees, none of which was on the end of the row. As the mature trees were over 8 metres tall a different sampling method was used to that of the young trees. Two main branch systems growing from the leader (trunk) on each tree were surveyed. One branch was low in the canopy, beginning around150 cm from the ground, growing within a 90º arc around the eastern side of the tree (along the row). The other branch was from the middle of the canopy, between 250 and 350 cm from the ground, growing within a 90º arc around the north side of the tree (into the alley). Every axis on the branch system was surveyed, except for a very small number of axes with small cuts from hedging two or more years beforehand.

For each node of an axis, the number of inflorescences was recorded, along with the node's position along the growth unit and the position of the growth unit along the axis (Figure 5-1). The bract node formed at the top of each growth unit, when the axis temporarily ceases growth between flushing seasons, was recorded as the most distal node (from the main trunk) of the growth unit. Thus the axillary buds of the top node of the tip unit were always contained in the apical bud, and were unable to grow out and form new axes regardless of the growth unit age.

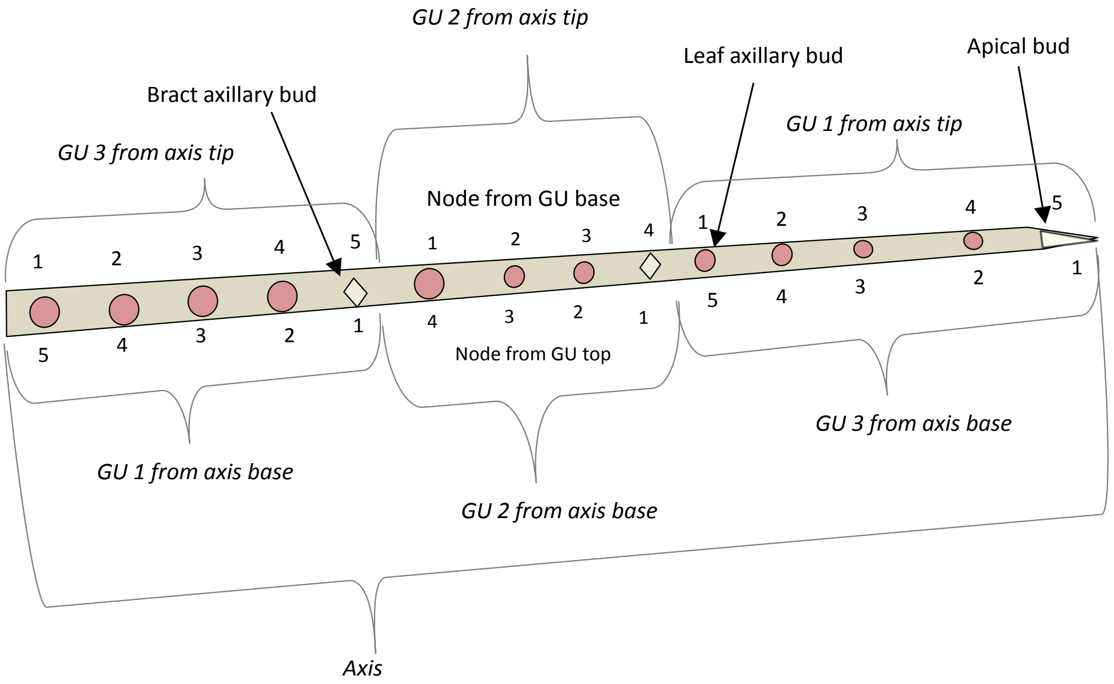

Figure 5-1. Macadamia axis structures and mapping terminology.
Bract buds and apical buds form the distal end of each growth unit (GU). (Only one bud is shown at each node for clarity.)

For the 741/5 trees, the diameter of each axis at its base, where it joined its parent axis, was measured, as was the diameter of its parent axis at this join. The diameter ratio was calculated by dividing axis-base diameter by the parent axis diameter.

The structure of the growth units in heavily shaded parts of the canopy was sometimes obscured by moss and water-staining of bark. The accuracy of data collected from mossy / stained sections, which were found mainly on axes seven growth units long and larger, could not be known. As this study was interested in patterns and trends of flowering within the tree architecture, only whole axes six units long or smaller were used in the analyses of patterns. The axes of six or fewer growth units represented 98.5, 98.9 and 96.3% of the axes surveyed from the 741/5, 741/12 and 842/13 trees respectively. Growth units of more than eight nodes were also omitted from the analysis, as this was the most conservative calculation of outliers in the distribution of nodes/growth unit. Growth units of up to eight nodes made up 84.4%, 88.4% and 93.5% of the growth units surveyed on 741/5 trees, 741/12 trees, and 842/13 trees respectively. Thus a total of 2341 node on 534 growth units on 314 axes were used in analyses of 741/5 trees, 5542 nodes on 1460 growth units on 898 axes were used in analyses of 741/12 trees, and 6341 nodes on 1618 growth units on 922 axes were used in analyses of 842/13 trees.

Correlations were analysed using Spearman's rank correlation co-efficient (r_s), to accommodate derived, skewed and non-continuous data (Sokal and Rohlf 1981). Differences between medians of two categories were analysed using Mann-Whitney tests, and of more than two categories using Kruskal-Wallis tests. The proportion of architectural structures – nodes or units or axes - flowering was analysed by logistic regression using a logit link function. Effect of growth unit position along the axis was analysed from both the distal end of the structure and the proximal end, as depicted for growth unit position in Figure 5-2. Because of varying numbers of GUs on axes, when aligning axes at the tip, the base GUs of short axes are mixed in with middle GUs of long axes, and effects that are most pronounced at the base are diluted and masked. The opposite happens when axes are aligned from the base. Effect of node position was similarly analysed from both ends for the same reason.

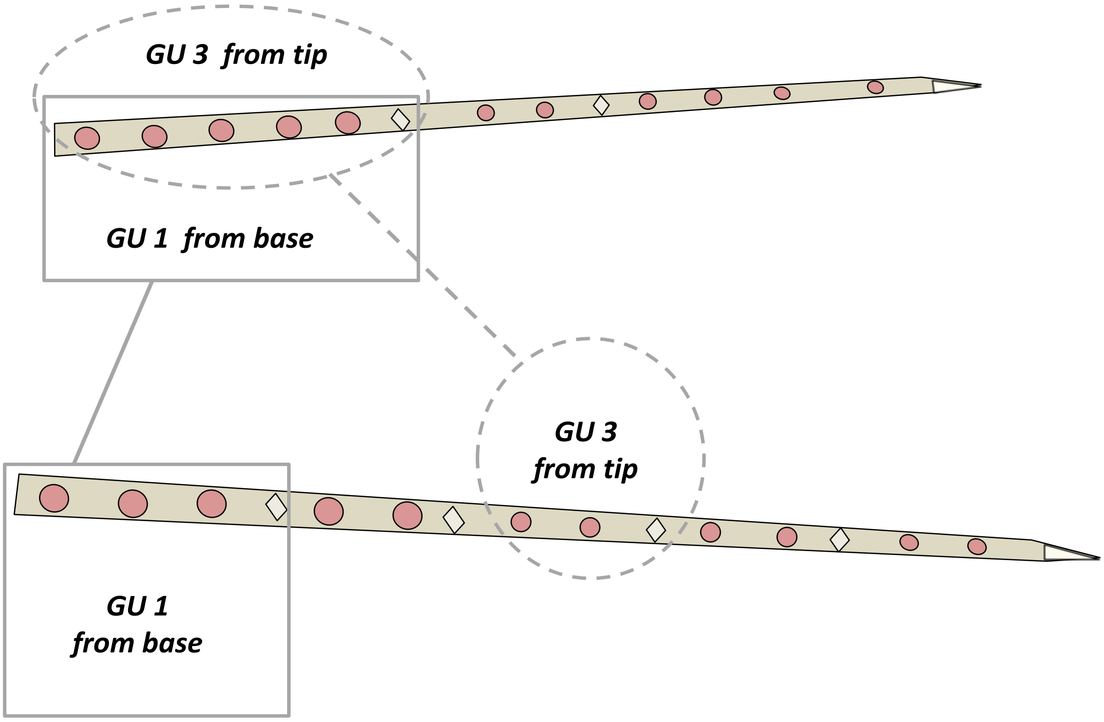

Figure 5-2. Growth unit position as counted from the tip compared to as counted from the base.
GU stands for growth unit. Diamond shapes represent bract axil buds, the triangle represents the apical bud, and circles represent leaf axil buds (only one bud is shown at each node).

5.3. Results

5.3.1. Distribution of inflorescences among nodes, growth units and axes

The number of inflorescences at a node is a function of the likelihood the node flowering and the number of inflorescences produced if it does flower. Inflorescences emerged from one to ten percent of nodes (Table 5-1). On the nodes that did flower, a median of only one or two inflorescences was produced. Between a tenth and a third of growth units flowered, depending on variety and age. Among those that did flower, one or two inflorescences emerged per growth unit (Table 5-2). Less than half of axes flowered. Among those that did flower, there was a median of one to three inflorescences per axis (Table 5-3).

Table 5-1. Inflorescence count statistics for macadamia nodes

Variety / age	% nodes flowering	Inflorescences on flowering nodes			Overall inflorescences per node		
		median	IQR*	mean	median	IQR*	mean
741 / 5	9	2	1 – 2	1.83	0	0 – 0	0.16
741 / 12	1	1	1 – 2	1.32	0	0 – 0	0.01
842 / 13	10	1	1 - 2	1.54	0	0 - 0	0.15

*IQR = Inter-quartile range

Table 5-2. Inflorescence count statistics for macadamia growth units

Variety / age	% growth units flowering	Inflorescences on flowering growth units			Inflorescences on all growth units		
		median	IQR*	mean	median	IQR*	mean
741 / 5	30	2	1 - 4	3.32	0	0 - 1	1.01
741 / 12	11	1	1 – 2.5	2.01	0	0 - 0	0.22
842 / 13	23	2	1 - 3	2.43	0	0 - 1	0.79

*IQR = Inter-quartile range

Table 5-3. Inflorescence count statistics for macadamia axes

Variety / age	% axes flowering	Inflorescences on flowering axes			Overall inflorescences per axis		
		median	IQR*	mean	median	IQR*	mean
741 / 5	42	3	2 - 5	4.05	0	0 - 2	1.71
741 / 12	16	1	1 - 3	2.23	0	0 - 0	0.36
842 / 13	31	3	1 - 4	3.31	0	0 - 2	1.39

*IQR = Inter-quartile range

5.3.2. Effect of position on location of flowering

Effect of position of node along the axis

In the 741/5 trees the position of nodes from the base of the parent axis did affect the proportion of nodes flowering (Figure 5-3) ($P <= 0.001$). There was no significant relationship between position of node from the axis base and proportion of nodes flowering in either variety of mature tree, although there did appear to be a negative trend in the 741/12 trees.

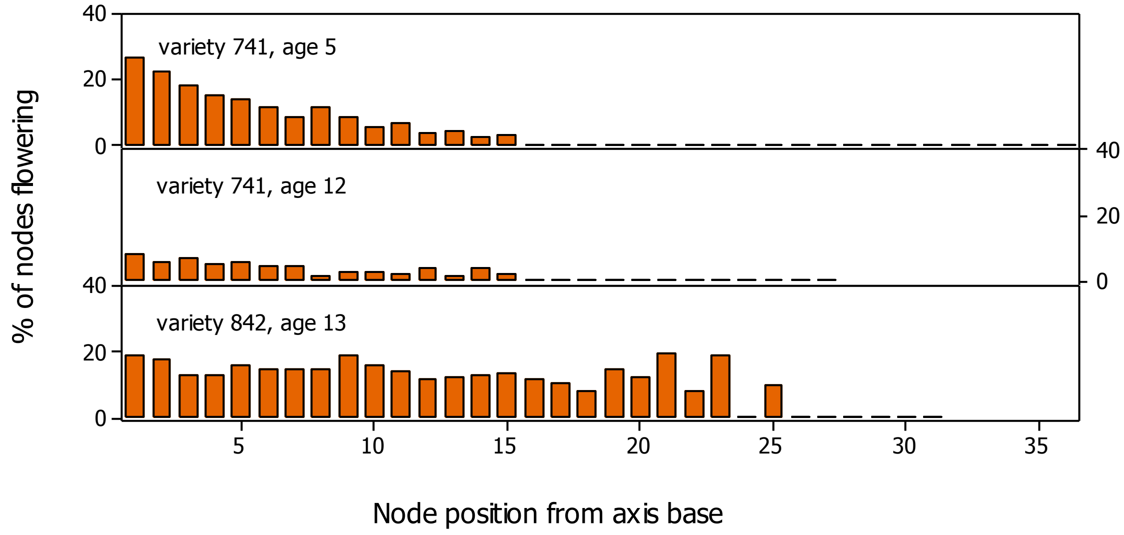

Figure 5-3. Relationships between macadamia node position from base of axis and percent of nodes flowering at that position.

Among flowering nodes of 741/5 there was a weak positive correlation between node position from axis base and probability of flowering ($r_s = 0.160$ and $P = 0.006$) (data not shown). Among those mature axes that did flower, there was no significant relationship between position of node from axis base and inflorescence density.

Relative node position along axis (from the tip) affected probability of a node flowering in both age groups of 741 trees (Figure 5-4). The closer to the base and further from the tip a node was located, the higher the probability of flowering – ($P < 0.001$) for both age groups of 741.

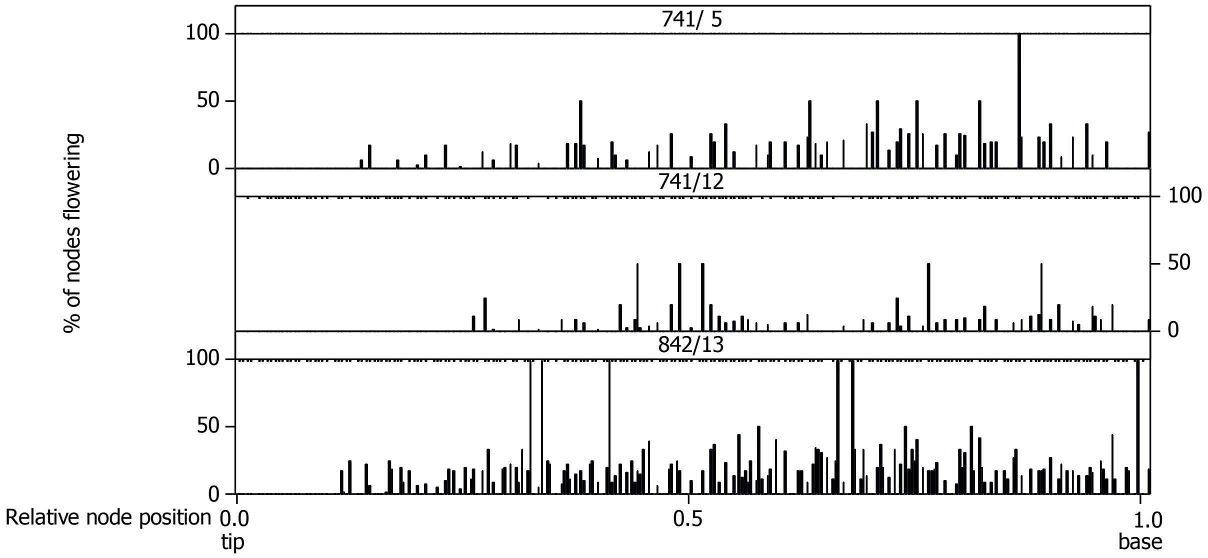

Figure 5-4. Relationships in macadamia trees between relative node position and probability of nodes flowering. Labels at the top of each panel show variety / age.

In the 741/5 trees, the number of inflorescences per node - inflorescence density - decreases with node position from the axis base (Figure 5-5) (r_s = -0.234 and $P < 0.001$). Mature trees also displayed a highly significant negative correlation between node position from the axis base and number of inflorescences ($P < 0.001$ in both varieties), but in these groups it was weaker; for 741/12 trees r_s = -0.086 and for 842/13 trees r_s= -0.052.

The effect of node position on probability of a node flowering was also analysed from axis tip, and in these analyses no difference in either proportion of nodes flowering or inflorescence density between node positions was detected in any of the variety/age groups.

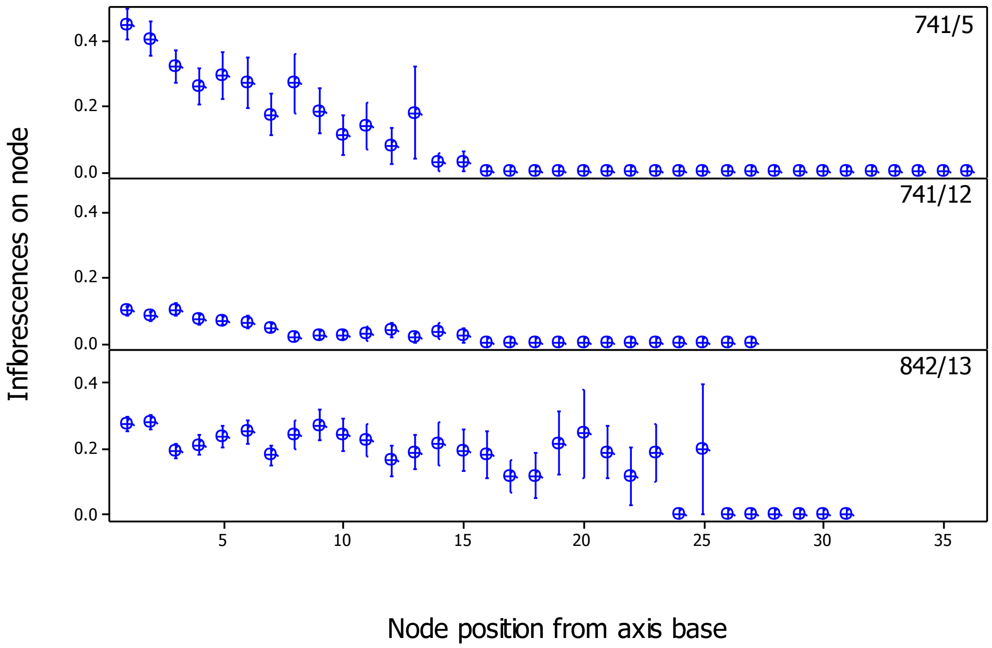

Figure 5-5. Relationships in macadamia trees between node position from base of axis on average number of inflorescences on that node. Labels in the top right of each panel show variety / age.
Bars are one standard error from the mean.

Effect of position of node along the growth unit

The effect of node position along its growth unit on the proportion of nodes flowering was significant in all ages and varieties, when analysed from the top of the growth unit (Figure 5-6) as well as from the base of the growth unit. Analysis from the top gave more information on flowering location for the mature trees than for young trees, but there were no significant differences. In the 741/5 trees the relationship between node position and flowering probability was linear. In the 842/13 trees the relationship was curved due to the dominant effect of long axes on the node positions furthest from the GU top, and those long axes tended to flower less than the mid-length ones (see section 5.3.3 below). In longer units of the 741/12 trees the relationship was not linear, due to interactions with GU size (see section 5.3.3 below, and multiple regression results in section 5.3.4). Over all three groups of trees, among axes up to four units long – 88% of axes – the probability of flowering was related linearly to position from the unit tip.

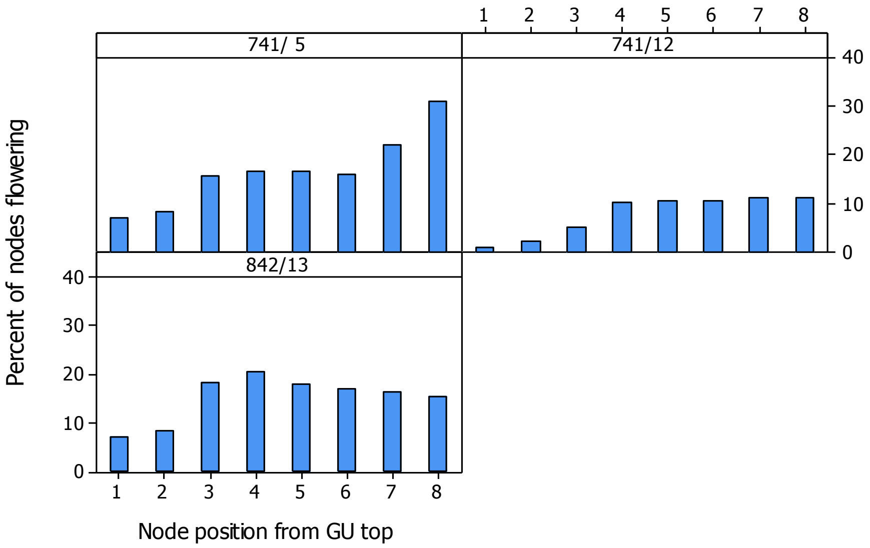

Figure 5-6. Relationships in macadamia between position of node along a growth unit from its top and probability of nodes on that growth unit flowering. Labels at the top of each panel show variety / age.

Among only those nodes which did flower, no relationship between the number of inflorescences and node position was detected. However, over all nodes (flowering and non-flowering), weak correlations between the number of inflorescences at a node and the node position from the growth unit top were present in all the groups (for 741/5 trees $r_S = 0.065$, $P = 0.004$, for 741/12 trees $r_S = 0.141$, $P < 0.001$, for 842/13 trees $r_S = 0.072$ $P < 0.001$). This relationship appeared linear only in the 741 trees (Figure 5-7). Position within a unit may interact with the effect of unit size in mature trees – longer growth units have a greater inflorescence density, and nodes can be further from the unit top on longer growth units. In 741/5 trees and 842/13 trees, node two averaged the lowest number of inflorescences on a growth unit. This weakened an otherwise consistent correlation in the 741/5 trees. Among the 842/13 trees inflorescence densities appeared similar at all positions other than node 2.

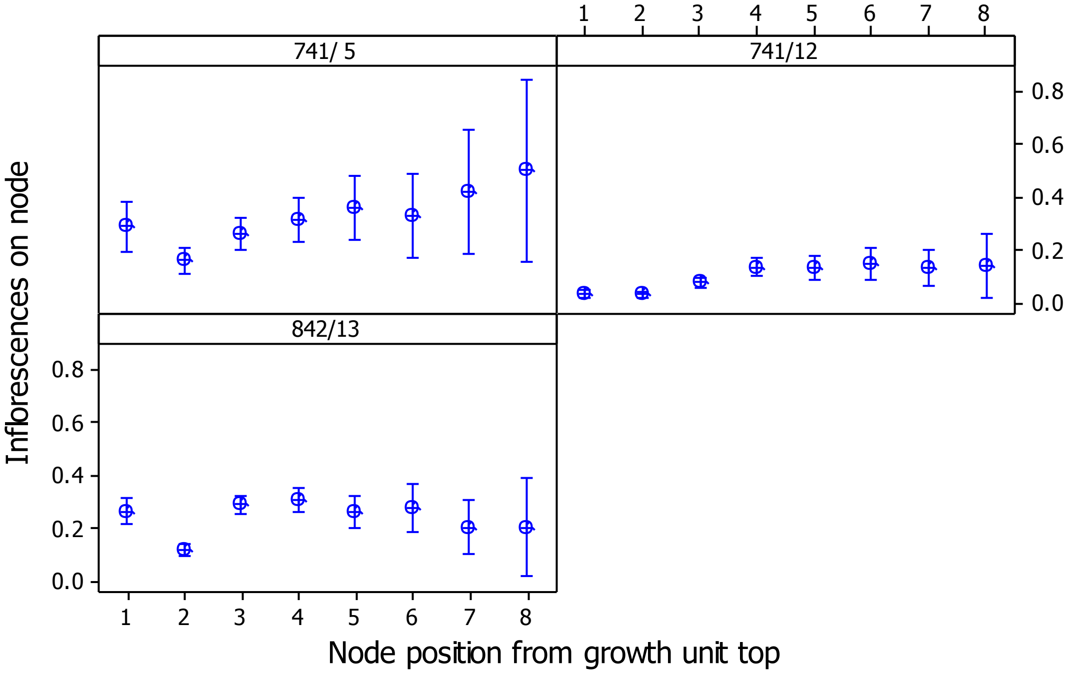

Figure 5-7. Relationships in macadamia between the position of a node along its growth unit and the number of inflorescences on that node. Labels at the top of each panel show variety / age.
Bars are one standard error from the mean.

Effect of position of growth unit along the axis

The position of the GU on which a node was found affected the probability of that node flowering, in all groups. Nodes on GUs further from the axis base were less likely to flower for both varieties and for mature as well as young trees ($P <= 0.001$) (Figure 5-8). GU position interacted with axis size, as nodes on longer axes were less likely to flower (see below), and so when graphed by GU position alone the slope of GU position's relationship with flowering probability is exaggerated.

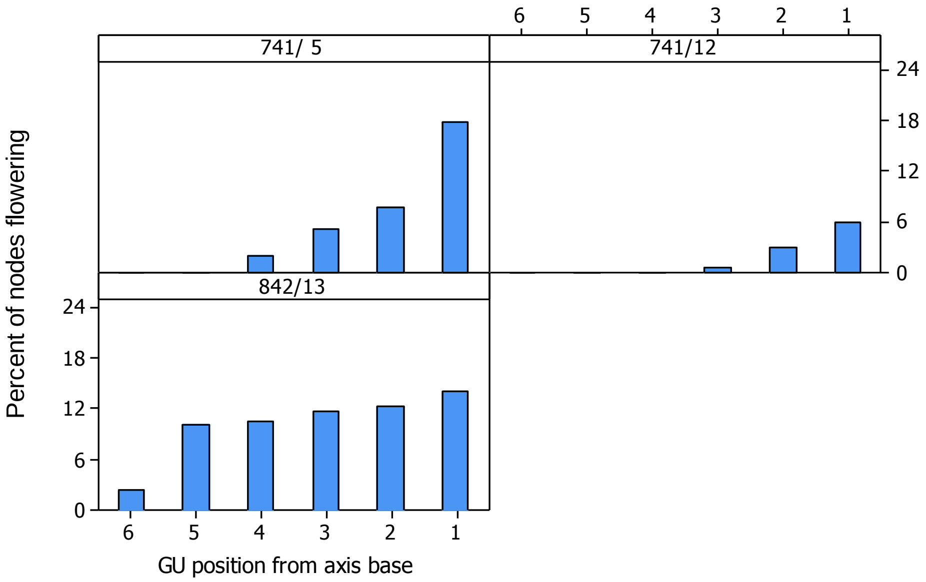

Figure 5-8. Relationships in macadamia between growth unit (GU) position from the axis base and probability of nodes on that GU flowering. Labels at the top of each panel show variety / age.

Among flowering nodes there was no clear relationship between the number of inflorescences on a node and GU position along an axis in any of the groups (data not shown).

In 741/5 trees there was a moderate negative correlation between the number of inflorescences on a node and the position of the node's GU along the axis from the base ($P < 0.001$ $r_s = -0.204$) (Figure 5-9). In the older trees there were also negative correlations but (without co-analysis with other factors) these were weaker (for 741/12 $P < 0.001$, $r_s = -0.108$; for 842/13 $P = 0.001$ and $r_s = -0.045$).

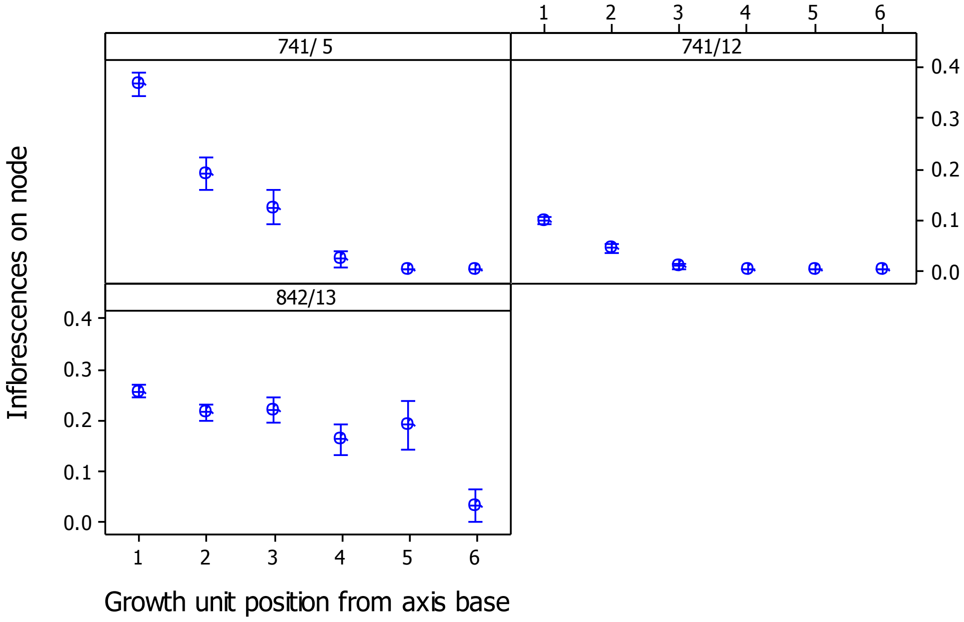

Figure 5-9. Relationships in macadamia between growth unit position from axis base and number of inflorescences emerging from a node. Labels at the top of each panel show variety / age.
Bars show one standard error from the mean.

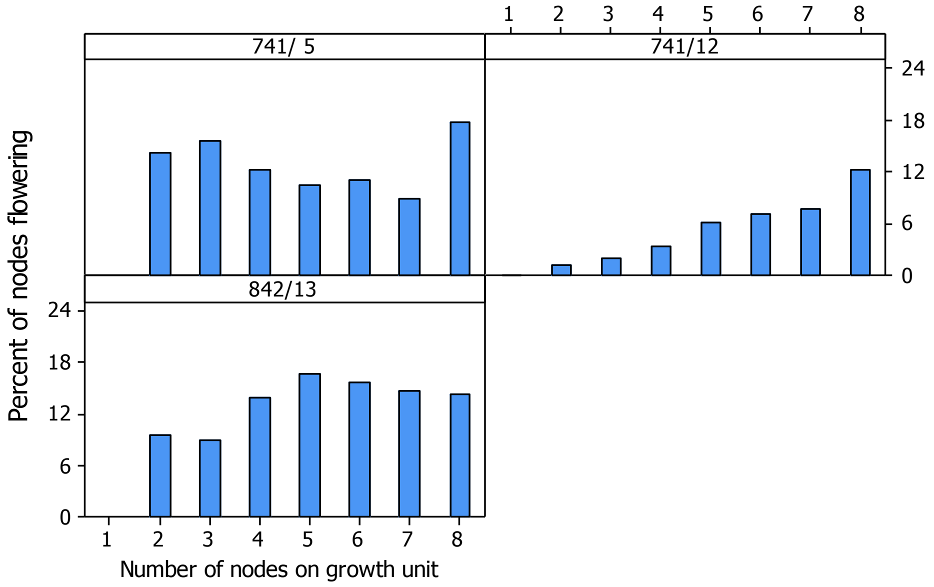

Figure 5-10. Relationships in macadamia between growth unit size (in nodes) and probability of nodes on that growth unit flowering. Labels at the top of each panel show variety / age.

5.3.3. Effect of growth unit and axis sizes on location of flowering

Effect of size of growth unit in nodes

The probability of a node flowering was also affected by the size of the GU (measured in nodes) on which it was found (Figure 5-10). In 741/12 trees, nodes on larger units were more likely to flower ($P< 0.001$). In the 741/5 trees and the 842/13 trees the relationship was not linear but GU size still effected the probability of nodes flowering (for 842/13 $P= 0.001$ and for 741/5 $P= 0.037$).

GU size interacted with the effect of node position along the GU on flowering probability in 741/12 trees (Figure 5-11) – see also multiple regression results in section 5.3.4. The effect of node position from GU top is linear for the majority of units (four or fewer nodes long), but in the largest units middle node positions have the highest likelihood of flowering.

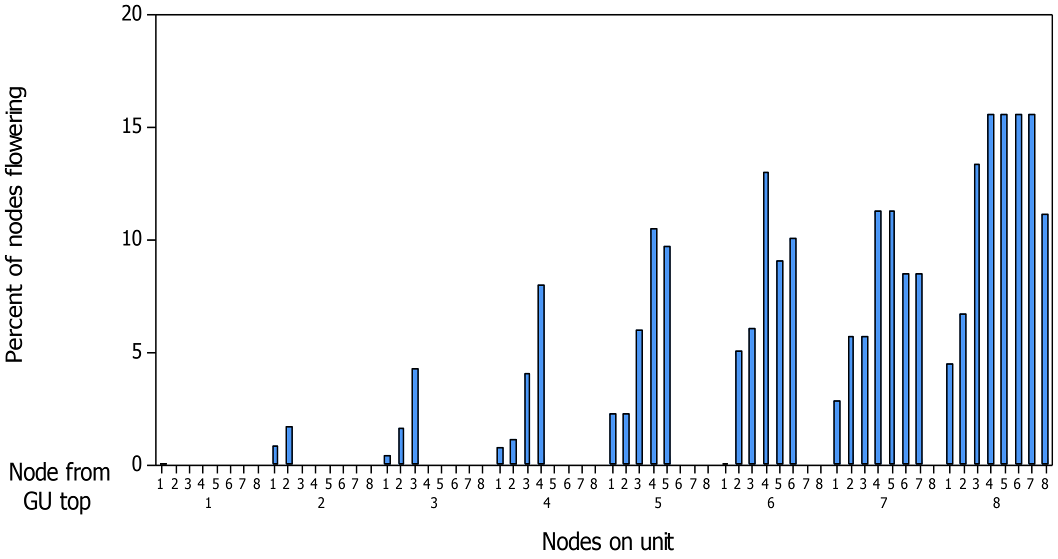

Figure 5-11. Interaction in twelve year old macadamia variety 741 trees, between growth unit (GU) size (in nodes) and position of node on the GU, in effect on probability of nodes on that growth unit flowering.

Among flowering nodes there was a weak positive correlation between the number of inflorescences on a node and the size of its GU (in nodes) in the 741/5 trees (P=0.002 r_S= 0.179) (data not shown). There was no clear relationship between these variables in the mature groups of trees.

Among the 741/12 trees there was a weak correlation between the overall number of inflorescences on a node and the size of that node's GU, measured in nodes ($P < 0.001$ and $r_s = 0.126$). Among the other groups there was no clear relationship between inflorescences on a node and nodes on a unit (Figure 5-12).

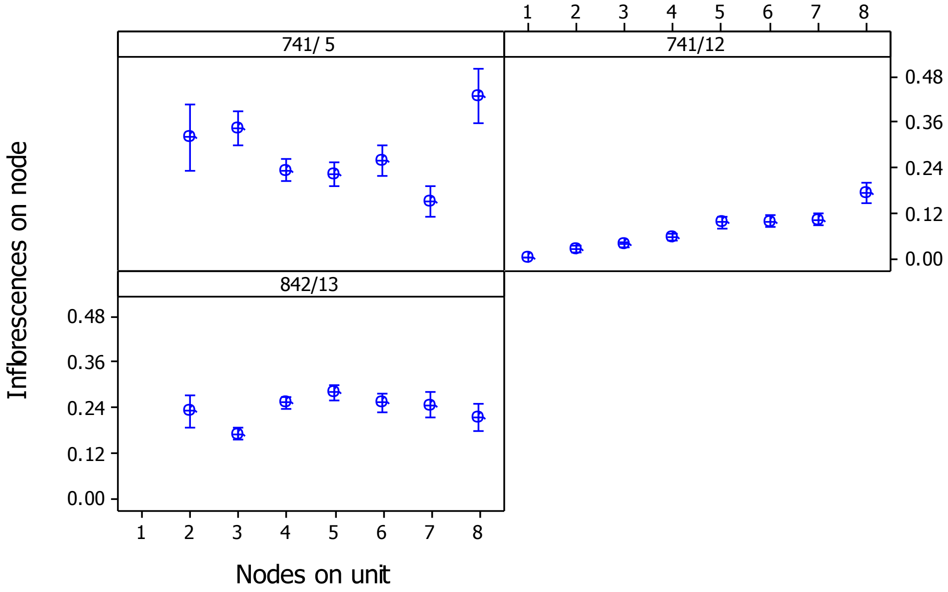

Figure 5-12. Relationships in macadamia between growth unit size (in nodes) and number of inflorescences on a node. Labels at the top of each panel show variety / age.
Bars are one standard error from the mean.

Effect of size of axis in nodes

When axis length was measured in number of nodes, it had no statistically significant effect on the probability of a node flowering in any group (data not shown). When looking from the level of whole axes, larger axes appeared to flower more often than smaller ones in the lower range of nodes per axis (Figure 5-13), although no significant difference was detected over the whole range of axis sizes. However among the smaller 90% of axes, which were those up to and including 13 nodes long, 741/12 and 842/13 both had significant positive relationships between axis size in nodes and proportion of axes flowering ($P < 0.001$).

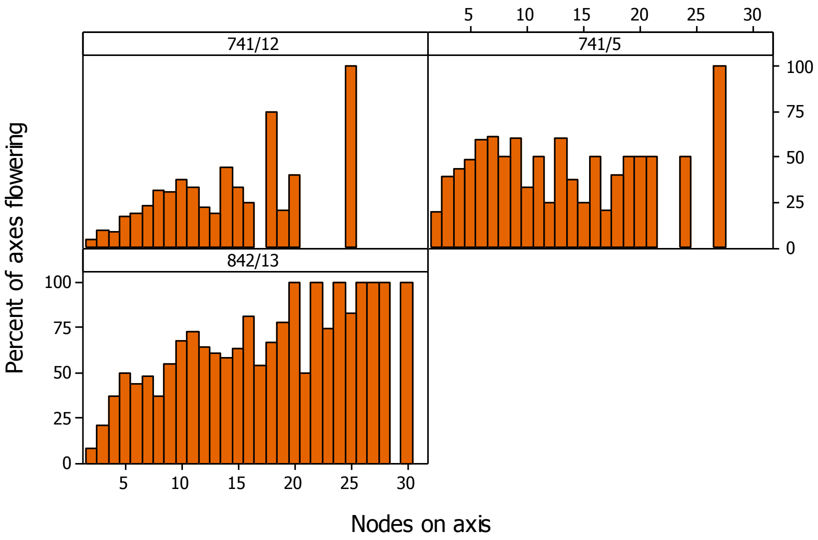

Figure 5-13. Relationships in macadamia between size of axes in nodes and percent of axes flowering. Labels at the top of each panel show variety / age.

Among nodes which flowered, in 741/5 trees there was a weak positive correlation between number of inflorescences on a node and the length in nodes of an axis it was found on. However, among axes that flowered, larger axes had lower densities of inflorescences in all groups of trees (Figure 5-14) (for 741/5 r_s= -0.295 P= 0.001, for 741/12 r_s= -0.555 P< 0.001, for 842/13 r_s= -0.444 P< 0.001).

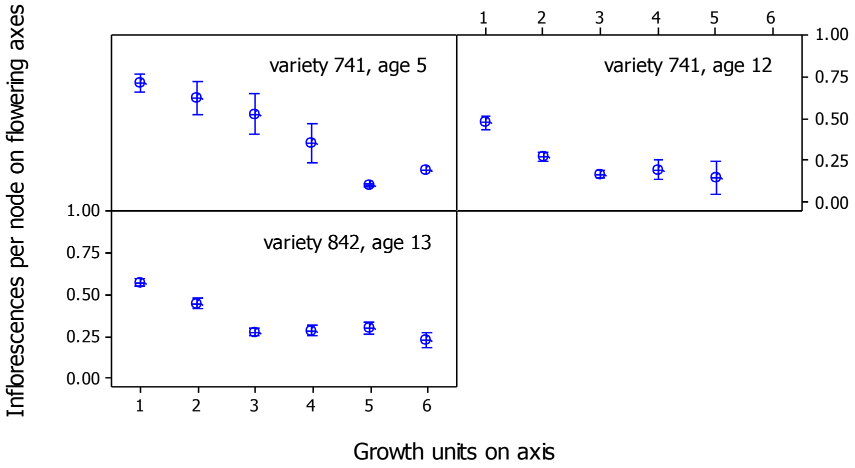

Figure 5-14. Relationships in flowering axes of macadamia, between axis size in growth units and inflorescence density on axis.
Labels at the top of each panel show variety / age. Bars are one standard error from the mean.

Despite this, the length of an axis length in nodes was negatively correlated with overall number of inflorescences on a node among 741/5 trees ($P < 0.001$ and $r_s = -0.230$) (Figure 5-15). There was no clear relationship between these variables in the other groups however. At the scale of whole axes, inflorescence densities were not correlated with the number of nodes on an axis (data not shown).

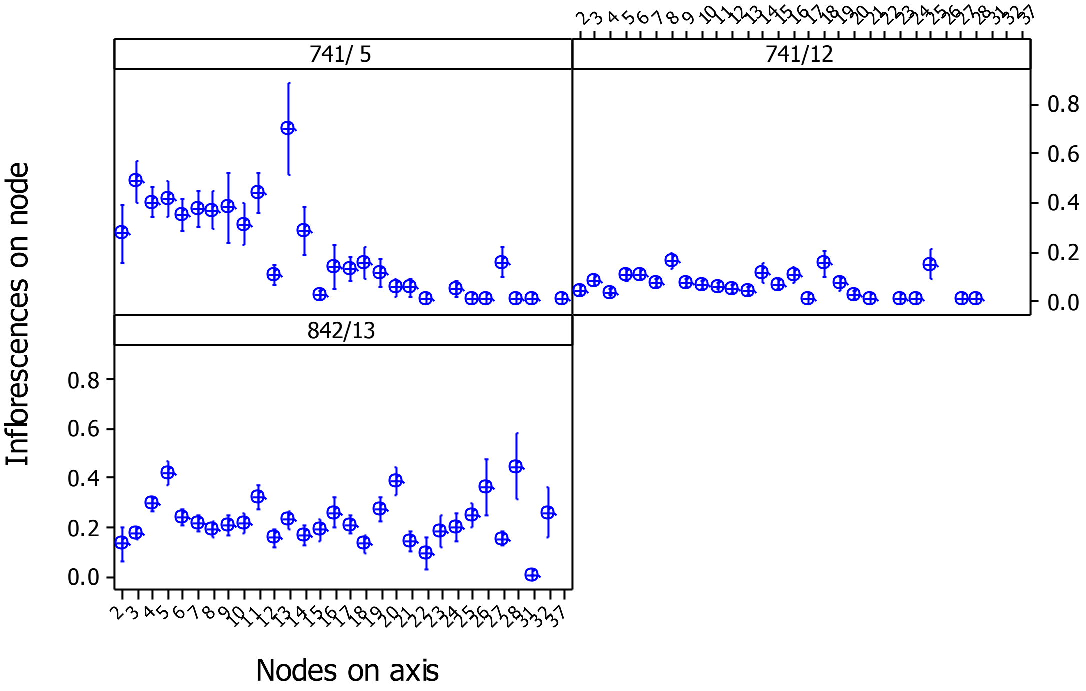

Figure 5-15. Relationships in macadamia between axis size (in nodes) and number of inflorescences on a node.
Labels at the top of each panel show variety / age. Bars are one standard error from the mean.

Effect of size of axis in growth units

In 741 trees of both ages, nodes on axes composed of fewer GUs were more likely to flower than nodes on longer axes (Figure 5-16) ($P <= 0.001$ for both ages). In variety 841 there was no linear relationship although there were differences between some sizes ($P = 0.001$).

When examining whole axes, those with more nodes or growth units have more potential flowering sites. In the 741 trees, because nodes on longer axes were less likely to flower, this meant that the probability of an axis as a whole flowering did not change substantially or significantly with size in nodes or growth units. In 842 trees the more constant proportion of nodes flowering over axes of different sizes meant that longer axes, with more nodes, flowered more often than shorter axes (*P*< 0.001) (Figure 5-17).

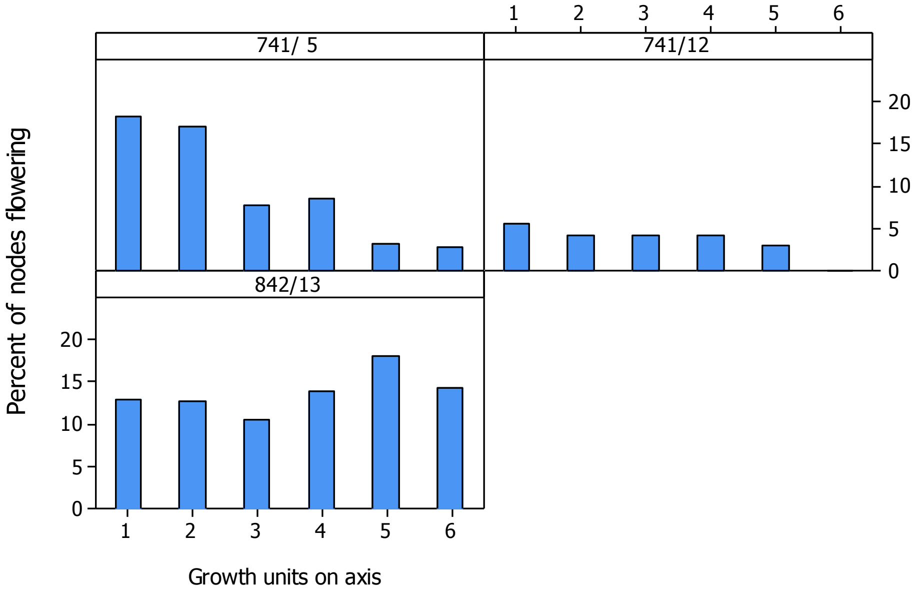

Figure 5-16. Relationships in macadamia between axis size (in growth units) and probability of nodes on that axis flowering.
Labels at the top of each panel show variety / age.

Figure 5-17. Relationships in macadamia between number of growth units on axis and percent of axes flowering.
Labels at the top of each panel show variety / age.

There was no clear relationship between the number of inflorescences emerging from flowering nodes and the size of the axis (in GUs) on which the node was found, in any of the groups (data not shown).

Over all nodes (flowering and non-flowering), among 741/5 trees there was a weak negative correlation between the size of an axis on which a node was found, as measured in GUs, and the number of inflorescences emerging from that node ($P < 0.001$ and $r_s = -0.221$) (Figure 5-18). There was also a significant negative correlation among 741/12 trees but this was even weaker ($P < 0.001$ and $r_s = -0.072$). There was no significant correlation among 842 trees.

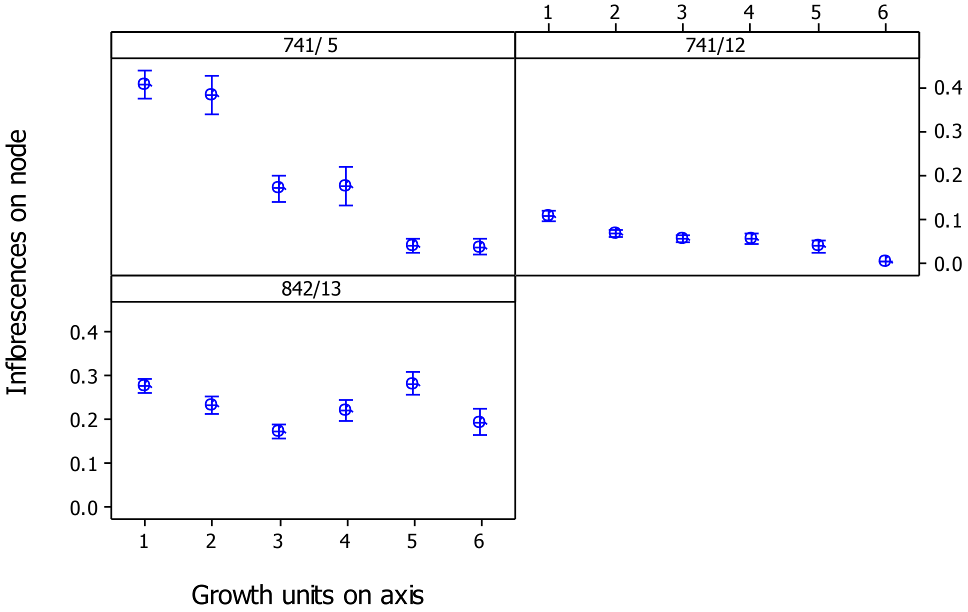

Figure 5-18. Relationships in macadamia between axis size (in growth units) and number of inflorescences on nodes of that axis.
Labels at the top of each panel show variety / age. Bars are one standard error from the mean.

The total number of inflorescences was greater on axes with more growth units of 842/13 trees ($P < 0.001$, $r_s = 0.341$). A highly significant correlation was also found in the mature 741 trees ($P < 0.001$, $r_s = 0.121$), however this trend was not upheld by the axes with the highest number of growth units, which did not flower at all (Figure 5-19). Longer axes did not have more (or fewer) inflorescences than shorter ones among the 741/5 trees.

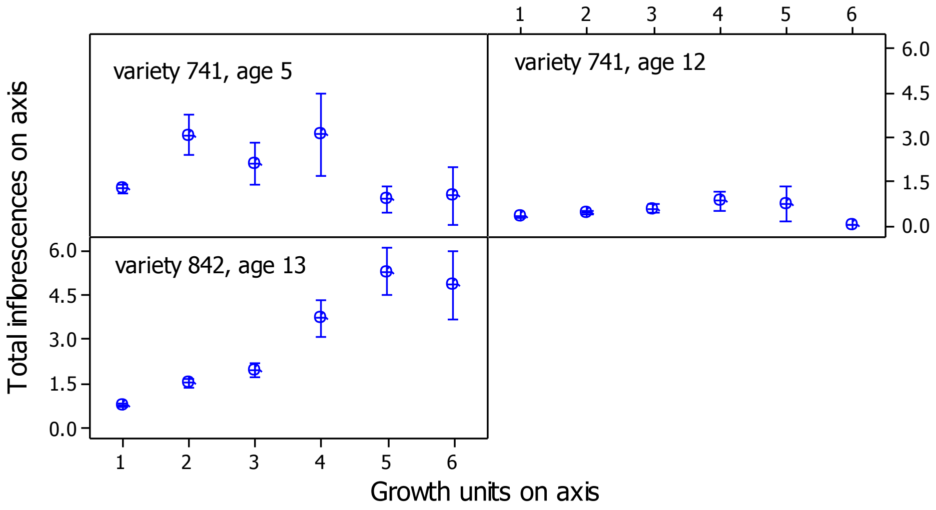

Figure 5-19. Relationships in macadamia between axis size and number of inflorescences on axis. Labels at the top of each panel show variety / age. Bars are one standard error from the mean.

5.3.4. Multiple regression using position and size variables

The probability of a node branching was also analysed by multiple logistic binary regression with a logit link funtion. Node position along a GU plus GU position along an axis together produced a better explanation (lower AIC score) of probability of a node branching than they did individually. Figure 5-20 shows the effect of position in flowering probability, presented for axes composed of one, two, and three GUs, when those GUs were composed of up to five nodes. 741/12 axes three GUs long can be compared with branching location probabilities from Chapter 3, keeping in mind the GUs presented here are made up of fewer nodes. As position along GU or GU position along axis did not affect the number of inflorescences emerging at a flowering node, the final number of inflorescences emerging from any node followed a similar pattern to that of probability of flowering. As around half of the axes on a tree are only one GU long, the distribution on those axes strongly influences the overall pattern.

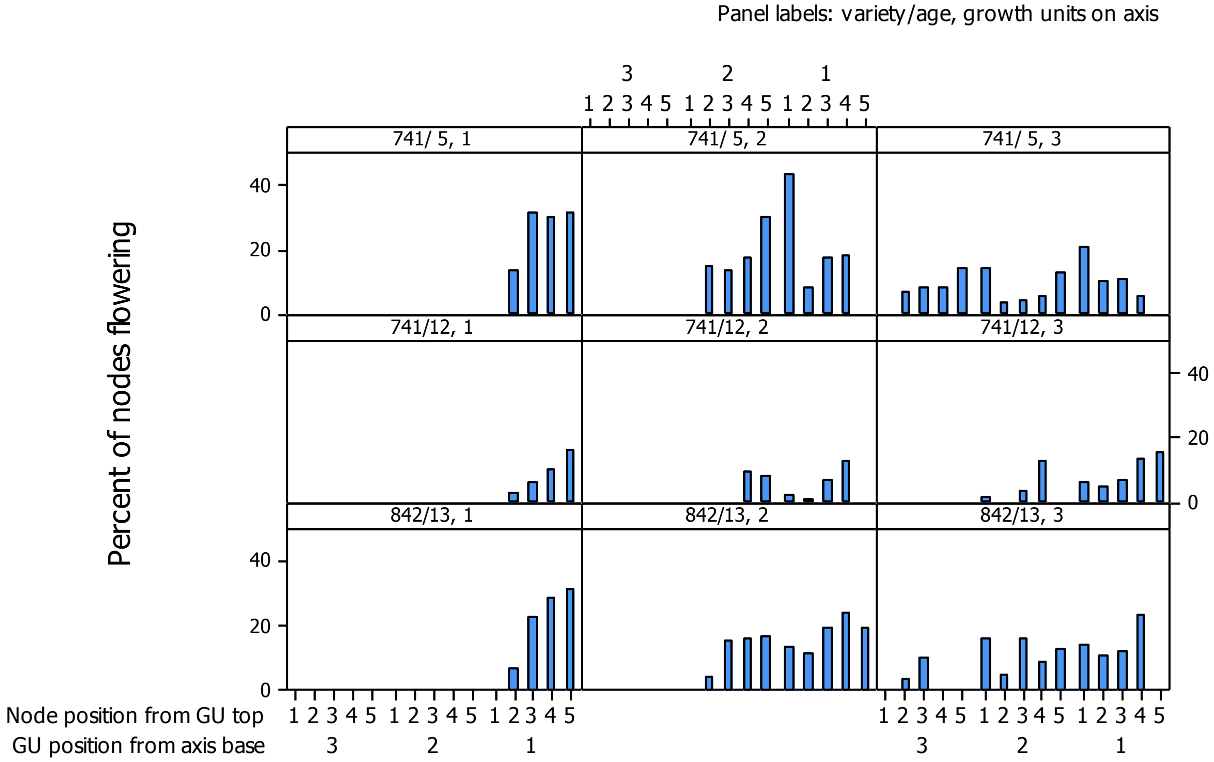

Figure 5-20. Percent of macadamia nodes flowering at different node positions along a GU (growth unit) and GU position along an axis, in GUs up to five nodes long on axes up to three GUs long.
The top row shows data from variety 741/ age 5 trees, the middle row shows data from variety 741/ age 12 trees and the bottom row shows data from variety 842/ age 13 trees. The left column shows axes one GU long, the middle column shows axes 2 GUs long and the right column shows axes 3 GUs long.

Using size as well as position, all combinations of the variables studied individually were co-analysed with multiple regression. The probability of a node flowering was best modelled in mature trees by node position from the GU tip with GU position from the axis base and GU size in nodes. In the young trees the best model included these same three variables plus axis size in GUs. Other variables measured and found to have significant relationships with node probability of flowering individually (results section above), did not add to these combinations’ efficiency of determining flowering proability. This indicates that they were measuring the same source of variation in a different way, or represented an interaction between these variables.

Mature 741 tree nodes were most likely to flower on a node in the middle of longer growth units, where those growth units are found at the base of the axis. However on the majority of 741 GUs, which were shorter, the likelihood of flowering increased with proximity to the GU base. Mature 842 tree nodes had similar flowering probabilities except that the effect of unit size was less clear. For young 741 tree nodes, flowering probability was highest at nodes furthest from the unit top, except for on the longest GUs studied – eight nodes long – in which it was greater on the node one up from the base. Like mature trees, young tree nodes also had an overall greater likelihood of flowering on GUs at the base of axes, but the young tree nodes were alone in being more likely to flower on axes with only one or two GUs than on larger axes. In the young trees smaller GUs – of two or three nodes - were more likely to flower than longer ones.

Predictions of proportions of nodes flowering were made using the multiple regression model. An example of two dimensions of this model for 741/12 nodes is represented graphically in Figure 5-21. On 741/12 trees, nodes four positions from the tip of eight-node GUs at the base of an axis, have the highest likelihood of flowering in this age / variety group: 22%. At node four of five-node GUs in the same position this figure is 13%. These figures are both large increases from the average node - from all eight-node GUs and six-GU axes - flowering rate of 1%. They respectively offer increases of over twenty-fold and ten-fold in the likelihood of finding a flowering node. On 842/13 trees, nodes four from the tip of eight-node GUs at the base of an axis, have a 20% likelihood of flowering. 842/13 nodes at position four from the unit tip of a four-node GU, in the same position along the axis, have the highest likelihood of flowering in this age/variety group: 21%. The average proportion of nodes flowering in the 842/13 axes used for this location survey was 10%, so the proportions flowering at these nodes are double that of the average. On 741/5 trees, nodes at the base of three-node GUs, where those GUs are at the base of two-GU axes, have the highest likelihood of flowering in this age/variety group: 29%. Compared to the average node's likelihood of 9%, this is an increase of over three-fold.

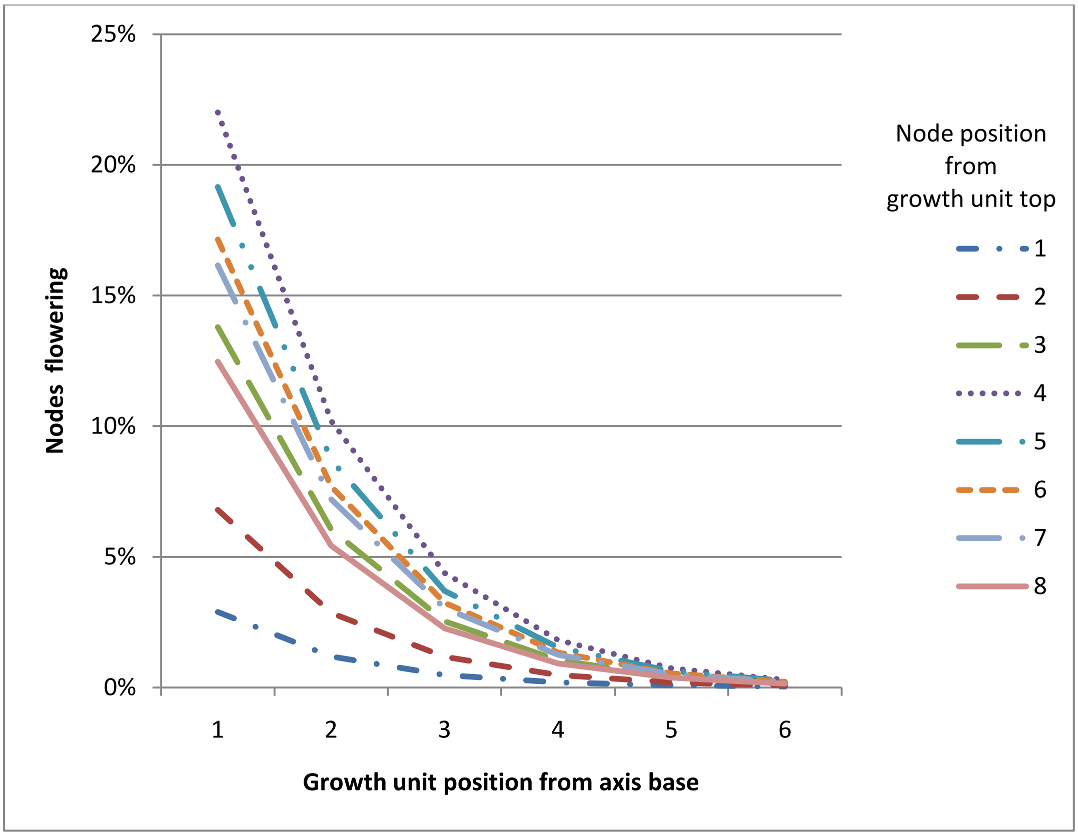

Figure 5-21. Effect on percent of macadamia (variety 741, age 12) nodes flowering, of node position from Growth Unit (GU) tip and GU position from axis base, among GUs eight nodes long.

5.3.5. Effect of axis relative diameters on flowering

Axes producing inflorescences had smaller diameter ratios – a median of 0.40 - than vegetative axes did – a median of 0.50 ; (*P*= 0.0003). Axes with a diameter ratio of between 0.1 and 0.3 were more likely to flower than branch, while those with larger ratios were more likely to branch than flower (Figure 5-22).

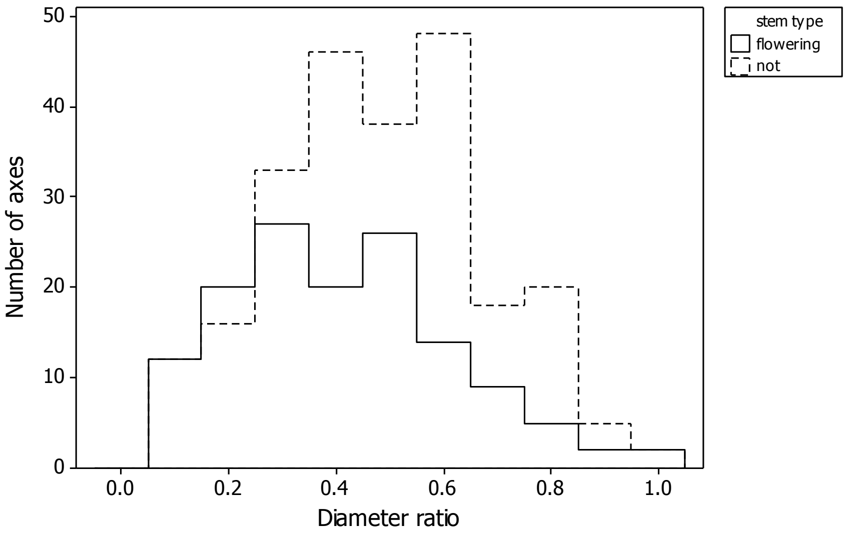

Figure 5-22. Distribution of diameter ratios of flowering and non-flowering axes, of five year old macadamia trees variety 741.

5.4. Discussion

Proportion of nodes flowering and flowering density

The overall number of inflorescences on a node (inflorescence density) is due to a combination of i) the proportion of nodes flowering, and ii) the number of inflorescences forming on those that did flower. The proportion of nodes flowering was dominant in explaining the effects of structure size as well as position, because production of more than one inflorescence on a node was rare. Significant trends in inflorescence density on flowering nodes were only found on the young trees, and none were strong correlations. Density increased with size of GU and size of axis (measured in nodes), although neither of these variables were related to flowering probability in these young trees. This could be due to the high vigour involved in forming these largest structures on the youngest trees. The young trees also had higher density on flowering nodes that were further from the axis base, although there was an opposite trend in probability of flowering. In all these relationships the effect of probability was the dominant, being reflected in the patterns of numbers of inflorescences per node over all nodes (flowering and not).

The Spearman rank correlation coefficients reveal that density of overall flowering in both varieties of mature trees responds most strongly to the size of growth units. This was also one of the variables influencing probability of a node flowering, as determined by the multiple regression analysis. In younger trees, overall flowering density is most influenced by position of nodes along the axis or position of growth units along the axis, again detected as a component also of flowering probability for this group.

Size

Only among the young trees was the probability of a node flowering substantially and significantly influenced by the size of its axis, as measured by number of growth units; nodes on longer axes were less likely to flower. This may be due to the base units of the larger axes being older than those of shorter axes, and perhaps having been formed before the trees were florally competent. However the probability of an axis flowering – i.e. that at least one node on an axis flowered – was not influenced by axis size in the young trees. This indicates that axis size did not change the number of axes with flowering nodes, but did change the number of flowering nodes on the axes that did flower. In the 842/13 trees, axes composed of more growth units were more likely to flower, and had higher densities of inflorescences. This was simply due to the longer axes having more potential flowering sites, as unlike the young trees, in this group the probability of a node flowering did not decline with axis length. The relationship among the 741/12 trees between axis size and flowering was somewhere in-between these other two groups – no clear trend in flowering with axis size, probably peaking in the middle size range.

The variation between varieties in relationships of axis length to flowering probability, is similar to the variation found between varieties by Wilkie *et al.* (2009). The 2009 study found the highest proportion of axes flowering in mid-length axes in cultivar “660”, but on shortest axes in cultivars “695’, ‘A4’ and ‘A38’, although they found no variety in which axis flowering probability increased with axis length. The difference may lie in the types of axes studied. Here axes up to six GUs were used of up to 13 years old, and Wilkie *et al.* used axes of up to two GUs all one year old. The longer axes among these one year olds were 2 growth units long, and thus had extended within the last year. Extension of axes in the year previous to counting inflorescences was found to reduce the amount of flowering (Chapter 4). *Salter et al.*(2005) also found inflorescences more proximal following more parent axis elongation. Extension among axes surveyed here had been spread over much of the life of the 13 year old trees, and so few of the axes would have extended in the previous year, long or short.

A mature tree's growth unit is more likely to flower when larger. However on growth units which flower, the inflorescences per node declines with growth unit size. This may be due to units with greater numbers of nodes having a greater number of potential flowering sites. This could increase the chance of a flowering node being on a growth unit, but also spread available fuel for flowering between more buds. Overall the average number of inflorescences per node increases with the size of growth units. The increased proportion of units flowering when larger (in mature trees) concurs with Renton *et al.*'s (2006) findings in apple. Growth unit size may be related to flowering via temperature. Growing seasons with a longer duration of warm weather may result in more nodes being added to a growth unit. (Unfortunately the year and season of formation of each growth unit in this study is not known, so examination of this hypothesis is not currently possible. This would be a useful topic for further research.) At the same time more of the meristems in the nodes may undergo floral evocation, as there is evidence that evocation occurs at a higher rate during warm nights (Sakai *et al.* 1982; Stephenson and Gallagher 1986; Chapter 7 of this thesis). This may be because a prolonged warmer growth season may result in higher carbohydrate production. Carbohydrate availability is now known to be a factor contributing to floral induction in *Arabidopsis thaliana* (Wahl *et al.* 2013), which shares many flowering triggers with distantly related plants such as rice (Putterill *et al.* 2004), and so could be expected to share them with other dicots such as macadamia. Increased carbohydrate production could also increase flowering via increased fuel available for inflorescence emergence during autumn or winter.

When analysing GU size as part of multiple regression, the young tree nodes were found to flower more often when part of a shorter GU of two or three nodes than when part of longer GUs. This is the opposite of the relationship between GU size and flowering in mature trees. It may be indicating that vegetative growth competes more strongly with floral growth in younger trees.

The lack of an increase in density of inflorescences with growth unit size in the young trees may be due to a greater general vigour than the older trees, partly because of that age and partly because of less shading. This may result in emergence being at maximum levels even on small growth units, with no room for increase. Flowering density in mature trees increased with both size of axes (i.e. number of growth units) and size of growth units to some extent. The presence of more inflorescences on axes with small relative diameters may however be due to limiting competition between floral growth and later stages of vegetative growth e.g. secondary thickening. If an axis has many floral buds along its length from formation, the use of resources in the outgrowth, elongation and maturation a high number of inflorescences and nuts in its first year of life may reduce resources available locally for vegetative growth. Alternatively, small relative diameter may be the cause and not effect of a high density of inflorescences. Smaller relative diameters may result from lateral buds growing out long after their creation when their parent axis has already thickened considerably, and subsequently larger volumes of resources are flowing through the larger vessels close to the new axis. If carbohydrates promote floral induction and evocation in macadamia, as has been found in *A. thaliana* (Wahl *et al.* 2013), such relatively large supply vessel could result in more inflorescences forming (at least microscopically) on such axes.

Position along axis

Growth unit position along the axis from its base affected both inflorescence density and proportion of nodes of flowering. Nodes flowered more often on the growth units closest to the axis base, and overall there were more inflorescences per node on base units. This pattern fits with the findings of Wilkie *et al.* (2009) that in variety “849”, inflorescences were more likely to form on the initial flushes than on the second (more distal) flush, as well as (Salter *et al.* 2005) who found inflorescences more proximal following more parent axis elongation. The fact that the correlation between growth unit position and flowering was stronger when analysed from the tip for the 842/13 trees, and stronger when analysed from the base for the others, may indicate that a combination of mechanisms is at work, with at least one of them acting from the base and one from the tip in each group. The strongest flowering at the base units in this study could also be due to the buds needing to pass through a number of stages to form mature inflorescences, each of which may require cues not present every year.

However the stronger flowering at base units may also be due to proximity to stored carbohydrate sources towards the centre of the tree (Newell *et al.* 2002). This would account for the effect of node position having an effect separate to GU position in 741/5 trees. Node position along the whole axis was also correlated moderately with the number of inflorescences on the node in 741/5 trees. This decline in inflorescence density with position from base was accompanied by a decline in probability of flowering. In both varieties of mature trees the correlation was weaker, and no difference in flowering probability between nodes along the axis was detected. The stronger relationship in 741/5 trees fits with the more linear relationships between flowering and growth unit position along the majority of axes (those up to 4 growth units long).

The stronger effect on the probability of a node flowering of GU position along the axis than node position along the axis could be due to either of two reasons. i) The node position is interrupted by regions of branching where node two of GUs occurs for the most common axis structures ii) the aging of nodes occurs in batches by GU.

Olesen *et al.* (2011) found little variation in inflorescence density with distance from edge of the lower portion of macadamia canopies. The study here found significant variation in inflorescence location along axes in the lower portion of the canopy, more similar to Salter *et al.*'s (2005) findings. This is likely to be because position from the axis tip is not the same as distance in from the canopy edge, as many axes do not grow straight to the canopy edge from their point of origin, but at an angle.

Position along growth unit

Position of a node along the growth unit also affected flowering. In all groups, both proportion of nodes and density of flowering were lowest at the growth unit top, the final nodes to elongate before the apex ceases growth at the end of the flush period. Greater emergence of inflorescences may be due to a higher concentration of carbohydrates closer to the axis base, due to axis extension using carbohydrates at the top of the axis and the re-supply coming from more central structures of the tree (Newell *et al.* 2002). When this gradient exists along the axis it will also exist along any individual GU.

It is also possible that the pattern of inflorescence density along growth units may also be due to differences in location of emergence. Increased sugar availability closer to the axis base may increase emergence directly by providing energy for growth. Alternatively, the dip in flowering may be due to a change in meristem activity as the growing season closes, changing the nature of the buds formed. In apple and apricot, patterns of floral axillary growth within growth units have been related to the speed of growth of the parent axis at the time of axillary bud formation (Costes and Guedon 1996; Crabbe 1984; Fulford 1966).

Variety and age, and rootstock

Surveying the three groups in three different years introduces the possibility of differences in flowering due to environmental conditions, preventing comparisons of absolute values of flowering measures between the variety/age groups. However trends and patterns in flowering within tree architecture have been shown to remain consistent between years when flowering does occur in apple (Renton *et al.* 2006). The effect of the individual tree (within variety/age groups) on the probability of node or growth unit flowering was always highly significant. The variation between the trees is most probably due to the root stock, which is from seedling plants.

Of the different measures of size and position studied, only node position along a growth unit and growth unit position along an axis affected all three groups of trees in similar ways. Axis size affected flowering differently in the different varieties – axes were more likely to flower when longer in the 842/13 trees, while there is no clear relationship in the 741/12 trees. The probability of a node flowering in 741 trees of different ages differed in response to position of the node along the axis. Among the 741/5 trees the probability of flowering was greater on nodes towards the base, but position along the axis did not affect the proportion of nodes flowering in the mature 741 trees. However the overall density of flowering decreased with node position from the base in both ages, and on the older trees the probability of a node flowering was higher on base GUs, so perhaps the difference in probability with node position was just a matter of degree, and thus statistical detectability. The multiple regression picked up another difference between the mature and young trees in probability of flowering at a node – probability of flowering in a young node was dependent on axis size. Nodes on smaller axes were more likely to flower in this group, whereas when all other factors were accounted for this variable did not play an important part in the older trees' flowering.

Implications

The identification of major factors in the location of inflorescences suggests mechanisms behind flowering control, although there is little previous research in the area to aid in interpretation of these results. Node position along a growth unit may be dependent on time of the season in which the node was formed, and thus temperature, speed of growth, or both. Number of nodes on the growth unit would be associated with the conditions during the season of node formation, and the most floral nodes being on the largest growth units suggests that a good season for vegetative growth – optimum temperatures, nutrient supply and light – is in some way related to good flowering on that unit. Both these factors suggest that events or conditions during GU formation may be directly related to flowering, even many years after formation, when the GU is distant from the tip. This would imply that at the time of their formation buds are pre-disposed to either floral or vegetative growth for the life of the tree.

The effect of growth unit position along the axis on flowering seems most likely to be associated with concentration gradients, of either floral promoters or suppressors. These could be either internal such as nutrients or hormones, or external such as light, the latter perhaps then creating internal sugar gradients. These gradients could influence flowering either at bud formation or long after, or both. The number of growth units on the axis affected probability of a node flowering only in the young trees. This may be related to the way resources are shared between and among axes, but also may be related to the average age of the growth units on the axis. This latter possibility is the one that offers a clear explanation as to why this variable is relevant only in the younger trees – the older wood was formed before the trees reached floral competency. Again this would point to buds becoming pre-disposed to flowering at the time of GU formation.

These results enable the locating of nodes far more likely to flower than an average node, before inflorescences become visible. The average overall proportion of nodes branching is 9, 1 and 10% in 741/5, 741/12 and 842/13 trees respectively. The nodes in locations found to be those most likely to branch within these groups, flower at rates of 29%, 22% and 21% respectively. This gives increases in the likelihood of sampling from a flowering node of three times, twenty times and two times respectively. Practical considerations may favour sampling from nodes of slightly lower flowering probability. Sampling from four node GUs at the base of an 842/13axis gives a relatively good chance of finding inflorescences – 21% - without being overly time consuming. This is an increase of over twenty fold compared to the average node on the lowest inflorescence producing group 741/12, and of three-fold compared to the average of all groups of 7%. Such increases in rates of finding microscopic inflorescences among dissected nodes could save copious amounts of time in studies looking for the onset of flowering. Thus investigations which were not viable in the past could now be manageable.

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Chapter 6. Effect of pruning location on axillary growth

6.1. Introduction

Hedging is used in macadamia production to maintain alleys for machinery access and for light penetration to sustain ground cover plants. Over a six year study, yield of hedged trees declined by 4% relative to unhedged trees in densely planted orchards (McFadyen *et al.* 2004; McFadyen *et al.* 2005). it is thought that many lateral branches are created at the edge of the canopy by hedging, decreasing internal light levels, and so this yield decline may be because fruit set is lower in more shaded parts of canopy (Olesen *et al.* 2011). Alternatively, hedging may reduce yield because of removal of leaves and subsequent reduction in photosynthate production.

Damage to the axis apex increases flowering in macadamia (Chapter 4), so it is likely that pruning would have a similar effect. However previous studies have suggested that apical dominance is weak on mature wood of macadamia (Chapter 3), and so there may not be a big branching response to pruning. The response may depend on location of pruning however. Some nodes and GUs of a macadamia axis produce more lateral stems or inflorescences than others (Chapters 3 and 5). In the GUs of twelve year old trees of variety 741, more inflorescences emerged from nodes closer to the unit base. Another pattern interacts with this; more inflorescences emerge from GUs closer to the base of the axis. Branching occurs most often at the node below the top of the GU, and if the axis is not extending more new stems are formed towards the tip of the axis. As pruning often releases from dormancy the buds found immediately below the cut, pruning above macadamia nodes or GUs of greater flowering or branching may increase the amount of inflorescences or lateral stems emerging respectively.

The style of pruning and the location of the cut in the tree are known to affect post-pruning regrowth in a number of species (Mika 1986). In Eastern Redbud trees (*Cercis canadensis*), deeper summer pruning cuts reduced the number of mature inflorescences per node in the following spring, and changed the location of new stem emergence to nodes lower on the axis (Owens and Ewers 1997). Although no statistical analysis was given, Chandler reported that apple (*Malus domestica*) stems pruned near the tip release more lateral shoots than those pruned near the base (Chandler 1960). In macadamias, pruning immediately above existing laterals restricted regrowth to elongation of those lateral shoots, whereas cuts at random locations made by hedging resulted in four to six extra shoots being formed (Huett and Smith 2008).

The experiments reported here were conducted to determine if pruning at different locations along an axis affects flowering or branching on the axis, and if so whether in number or distribution, and in similar ways for the two types of axillary growth or differently. Examining such changes in axillary bud behaviour with pruning will hopefully give information about the physiological control of flowering and branching in these trees. This is turn may provide the background for orchard management techniques in the medium- to long-term future.

6.2. Materials and methods

6.2.1. Effect of node of pruning

To investigate the effect of pruning at different nodes along a GU, stems which were three GUs long were pruned either at the top or the bottom of the middle GU, or not pruned at all. Variety 741 trees, 12 years old and around 8 metres high, were selected from the middle of the row in a commercial orchard near Glasshouse Mountains Township in south-east Queensland. Orchard rows ran east-west, and stems were selected from close to the north and south faces of the rows where less moss/lichen growth and less water staining on the bark enabled clear identification of nodes. Stems were selected from between 1.5 m and 4 m above the ground. Twenty stems on each of six trees were allocated randomly to each node-of-pruning treatment and controls, and tagged. For “top-node” pruning the cut was made immediately below the bract node, and for “base-node” pruning the cut was made immediately above the node at the proximal end of the GU (Figure 6-1). Pruning was carried out in mid-August, when inflorescences were elongating but before anthesis has begun. Existing lateral stems were removed at the time of pruning, to enable clear identification of new stems formed after pruning. After one year, the number of new inflorescences and number of new stems that had grown from each parent axis were counted, and their GU and node of origin recorded.

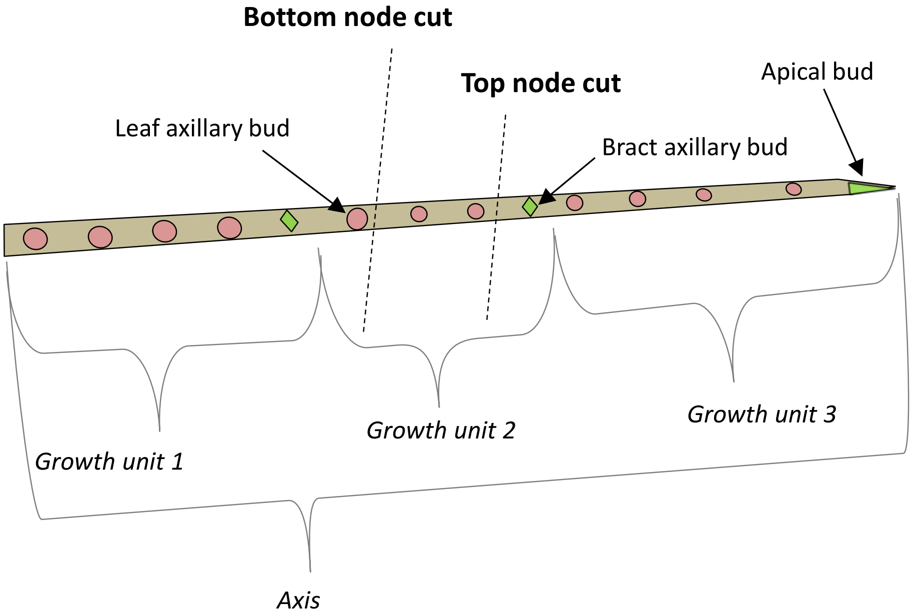

Figure 6-1. Location of pruning cuts for node of pruning experiment.

6.2.2. Effect of growth unit of pruning

A second experiment investigated the effect of pruning at different GUs along a stem. Here GU position along the axis is the position as counted from the base. Stems which were four GUs long were selected (on different trees to the node-of-pruning experiment), and pruning cuts were made in the middle of one of the four GUs. The axis base GU was designated GU1 and the axis tip GU was designated GU4. GUs were a median of four nodes long. They were pruned in their middle, giving axes with medians of two, six, ten and fourteen nodes after pruning at GU 1, 2, 3 and 4 respectively. If there were an odd number of nodes on the GU the cut was made above the middle node. Trees and stems were selected from the same location in the same manner as described above for the “Node of pruning experiment”. Only the number of stems used per treatment varied - nine stems were allocated randomly to each GU-of-pruning treatment on each of six trees.

6.2.3. Analyses

For both node-of-pruning and GU of pruning experiments, only control stems which did not extend over the year (Figure 6-2) were used as controls in the analysis of pruning effects. Shoot numbers (on GUs or axes) and densities of shoots (on GUs) were analysed using generalised linear models (GLM). Step one of the these analyses assessed the proportion of stems producing new lateral shoots, using a GLM with binomial distribution and logit link, and step two assessed the number of shoots on those stems that did shoot, using a GLM with a Gamma distribution and log link. Densities of shoots on axes were analysed with Mann-Whitney tests.

For the node-of-pruning experiment, amounts and densities of shoots on all pruned axes (base-node plus top-node treatments) were compared to those of unpruned axes using Mann-Whitney tests. Differences in shoot production between nodes immediately under a pruning site and nodes at the same position on un-pruned axes were analysed with Mann-Whitney tests. Differences in shoot numbers and densities (shoots per node) between node positions were analysed using positions as counted from the distal ("top") end of GUs and then separately analysed again using positions as counted from the base, as the former gave clearer results for nodes at the distal end of the GU and the latter gave clearer results for nodes at the proximal end. This is because of varying numbers of nodes on GUs. When aligning GUs at the tip, the base nodes of short GUs are mixed in with middle nodes of long GUs, and effects that are most pronounced at the base are diluted and masked. The opposite happens when GUs are aligned from the base.

6.3. Results

6.3.1. Effect of node of pruning

Distribution of all new shoots along all three GUs of the unpruned controls are presented (Figure 6-2), to enable comparison of flowering and branching on the same axes. More new stems emerge than inflorescences at just one node, node two of the middle growth units. More inflorescences emerge than new stems on every node of the base growth unit. At the remaining node positions, around half of the nodes on an axis, the number of inflorescences and new stems emerging are similar.

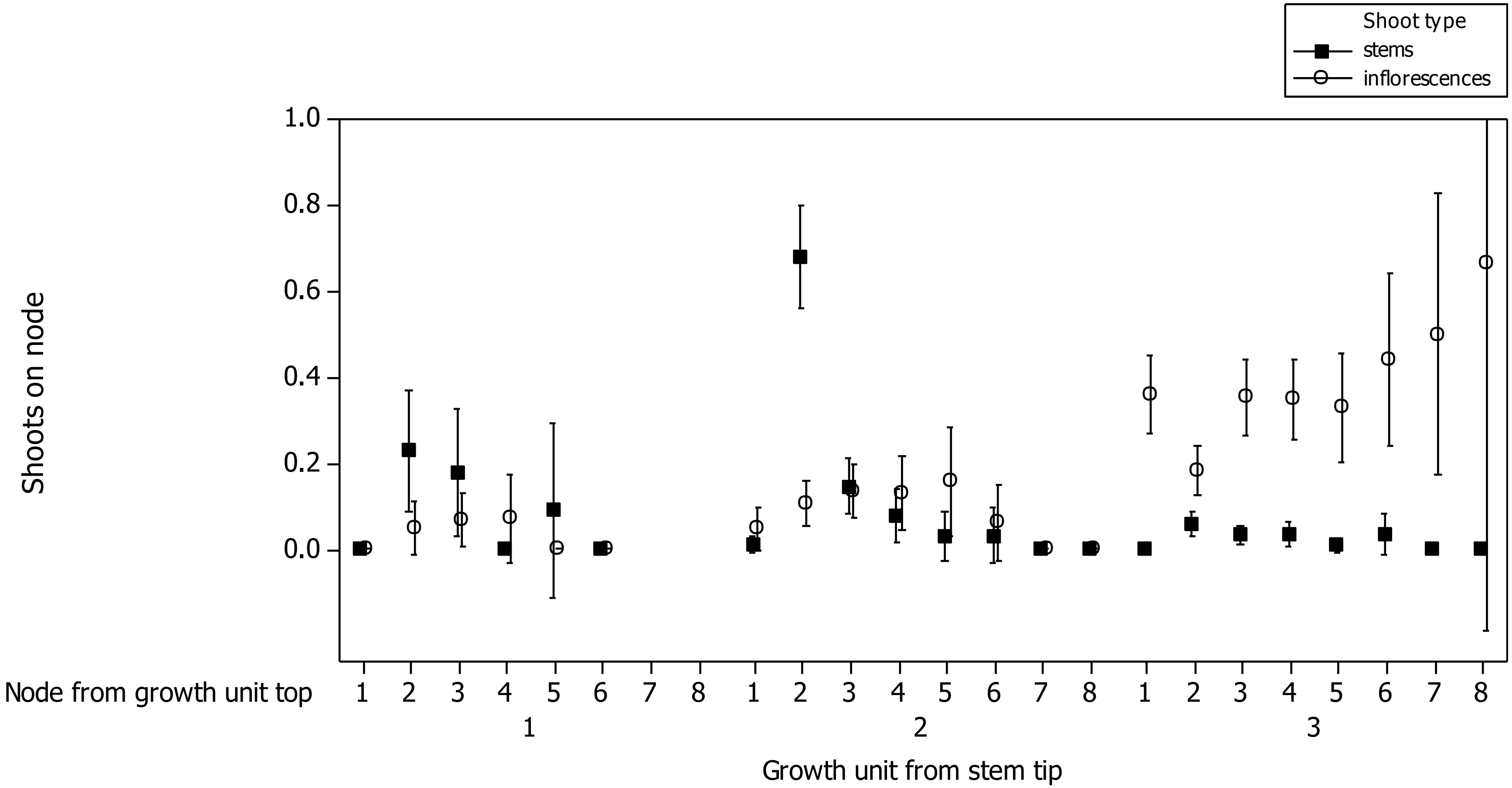

Figure 6-2. Distribution of lateral stems and inflorescences formed in one year on axes without extension growth. GU = growth unit, in thirteen year old macadamia trees of variety 741.

Quantity of flowering and branching

Pruning did increase the proportion of axes producing inflorescences (Figure 6-3) (*P*= 0.010 for top-node pruning and *P*= 0.021 for bottom-node pruning). However it did not significantly change the proportion of axes branching.

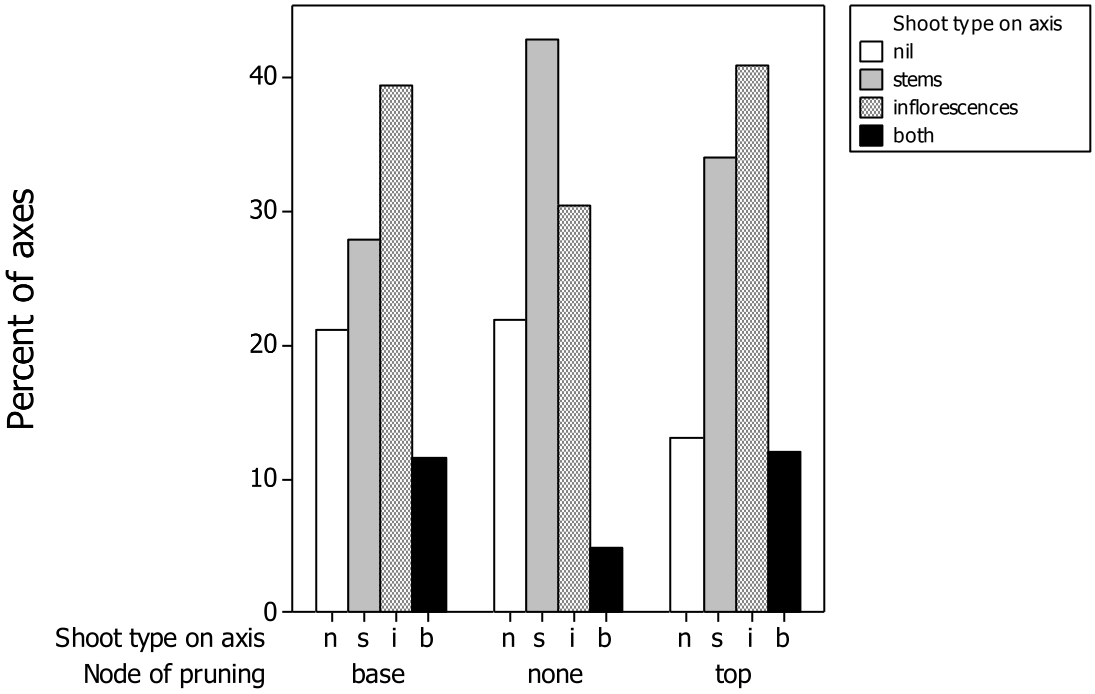

Figure 6-3. Effect of node of pruning, on percent of axes producing axillary shoots broken down by shoot type, in thirteen year old macadamia trees of variety 741.

The number of inflorescences emerging (Figure 6-4) from the pruned axes was more than 50% greater than that of unpruned axes (*P*= 0.038 for top-node pruning and *P*= 0.012 for base-node pruning). There was no significant difference in the number of new stems emerging between pruned and un-pruned axes, regardless of location of pruning, or if both pruning locations were combined.

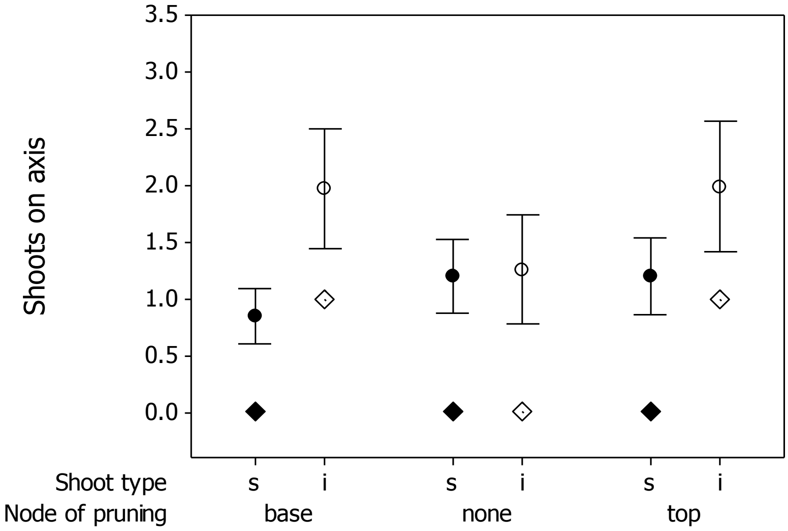

Figure 6-4. Effect of node of pruning, on number of axillary shoots emerging from axis broken down by shoot type, in thirteen year old macadamia trees of variety 741.
Shoot type “i” = inflorescence and “s”= new stems. Bars show the confidence interval of the mean. Circles are means and diamonds are medians.

Location of flowering and branching

Branching

Pruning in the middle unit did not change the number of stems emerging on the unit, but as the units were shortened, it did change the number of stems emerging per node. As there was only one remaining node after bottom pruning, the stems per node were far higher on the that base-most node of the GU after bottom-node pruning ($P < 0.001$). Stems per node on this middle GU were around 50% higher after top-node pruning ($P = 0.048$).

Top-node pruning resulted in no significant difference in the amount of branching at the node below the cut (Figure 6-5), node two from the top of the middle GU ($P > 0.05$). However at the next node down, node three from the top, top node pruning produced around four times as many stems as were found on the control ($P = 0.0003$). When analysed from the bottom of the unit, node 4 also appeared to produce a greater number of stems with top node pruning, but given the average length of a unit is four nodes, this may have been due to node s two from the top mixing with lower nodes. At all node positions that usually branched, there appeared to be more branching with top-node pruning than without pruning, on the GU which was pruned.

Base-node pruning left only one node on the middle GU. As GUs were anywhere from two to eight nodes long, the base-node was at a mixture of positions counting from the GU top, and examining nodes by position from the base of the GU (Figure 6-6) aligned all the base nodes for comparison. Base node pruning increased the number of stems emerging from the node below the cut around twenty-fold P <0.001. Top-node pruning did not significantly affect the number of stems emerging from the base-node of the middle GU. On the base GUs, neither top-pruning or base pruning produced a difference in new stem emergence from any one node . However on base GUs six, seven or eight nodes long, control GUs produced no new stems (detail not shown) while some pruned six- and seven node-long GUs did.

Pruning in the middle unit, either at the top of the bottom, made no difference to the number of stems emerging from the unit below the cut.

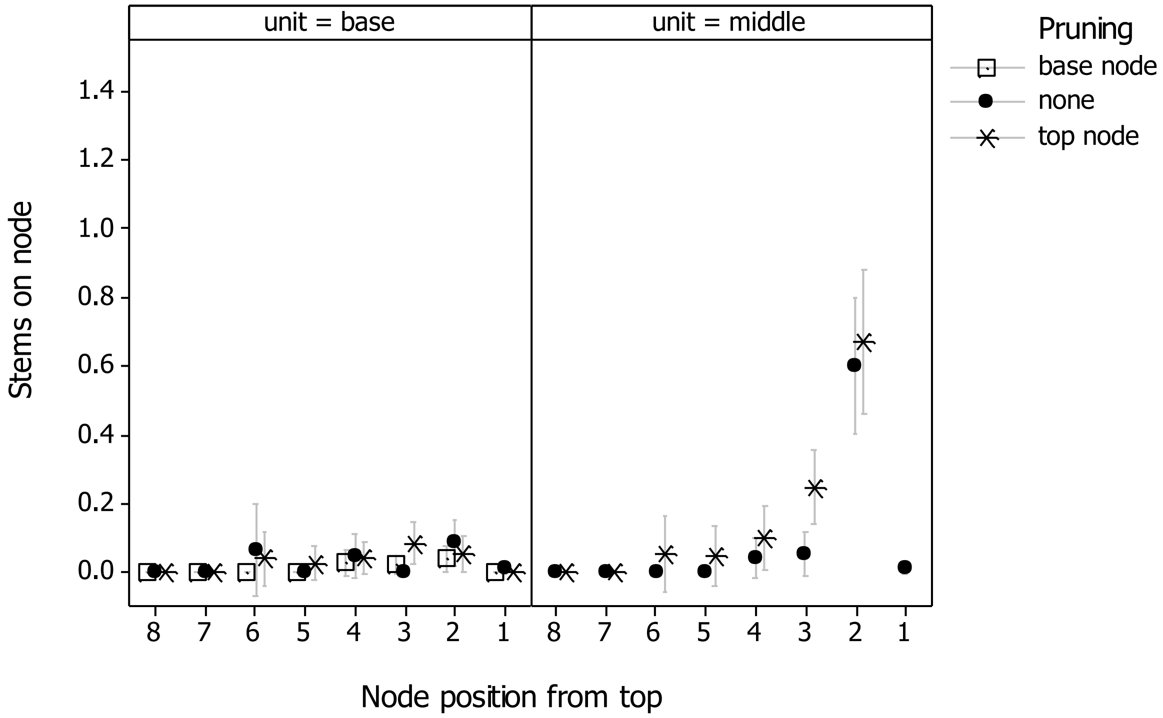

Figure 6-5. Effect of node of pruning, on number of stems emerging from nodes, with position counted from the GU top, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean.

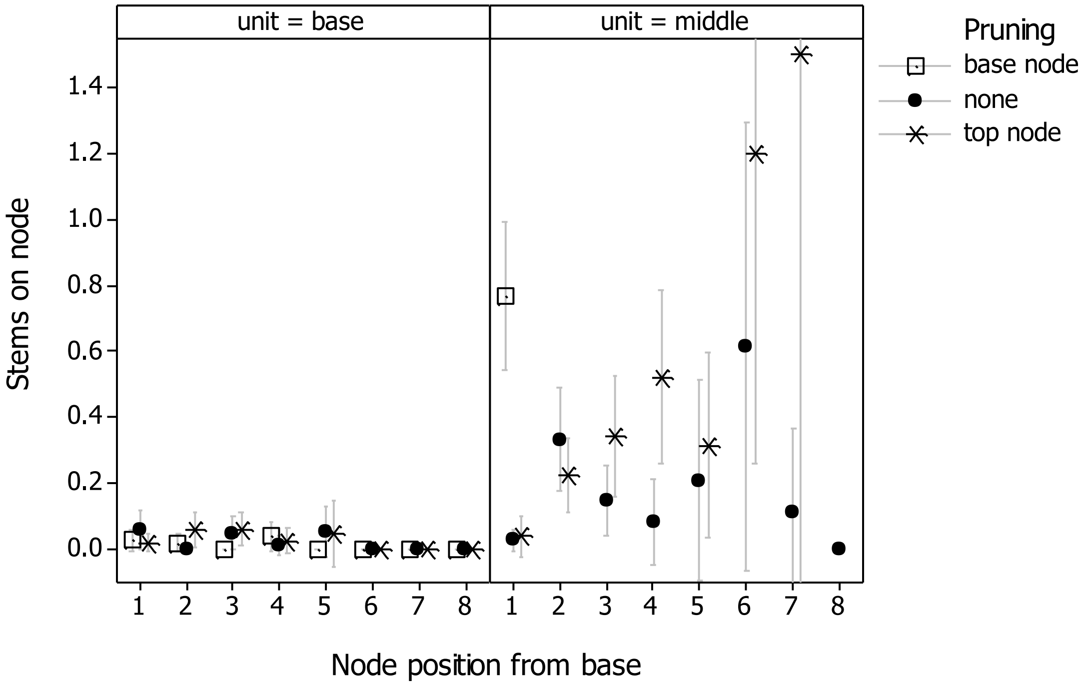

Figure 6-6. Effect of node of pruning, on number of stems emerging from nodes, with position counted from the GU base, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean.

Flowering

Pruning in the middle unit, at either the top-node or bottom-node, did not make any significant difference to the number of inflorescences on the pruned unit. Nor did it make any difference to the number of inflorescences per node. The number of inflorescences was not affected by pruning even at the nodes immediately below the cuts (Figure 6-7 and Figure 6-8).

There was no flowering at all on middle GUs of unpruned axes that were six, seven or eight nodes long (detail not shown). However top-pruned axes flowered on six-node long GUs, mostly at the base of the GU. Top-pruned middle GUs produced more inflorescences than the bottom-pruned middle GUs ($P = 0.0286$), probably only because more nodes were left on the unit.

Both top-node and bottom-node pruning in the middle unit increased the number of inflorescences emerging from the base unit ($P = 0.0003$ for the base-prune and $P = 0.0015$ for the top-prune). The number of inflorescences per node was also increased by both GU-top pruning ($P = 0.009$) and GU-bottom pruning ($P < 0.001$). This was reflected in most node positions on the base GU producing more inflorescences. Axes up to five nodes long comprised 86% of the sample, and among these axes only node two, where stems have been observed to commonly emerge (Chapter 3), did not show a substantial difference in flowering between the control and pruned axes.

When aligned from the base-node of the GU, the effect of the cut at this end of the unit can be more clearly compared between GUs of different sizes (Figure 6-8). It can then be seen that pruning also increases flowering at the base-nodes of the base GU. The base-node of the base GU produced more inflorescences when the stem was pruned at the base of the middle GU than when not pruned ($P = 0.0312$). There was no significant difference in base node inflorescence numbers between top-node pruning and no pruning. There was also no difference in these between the two pruning methods.

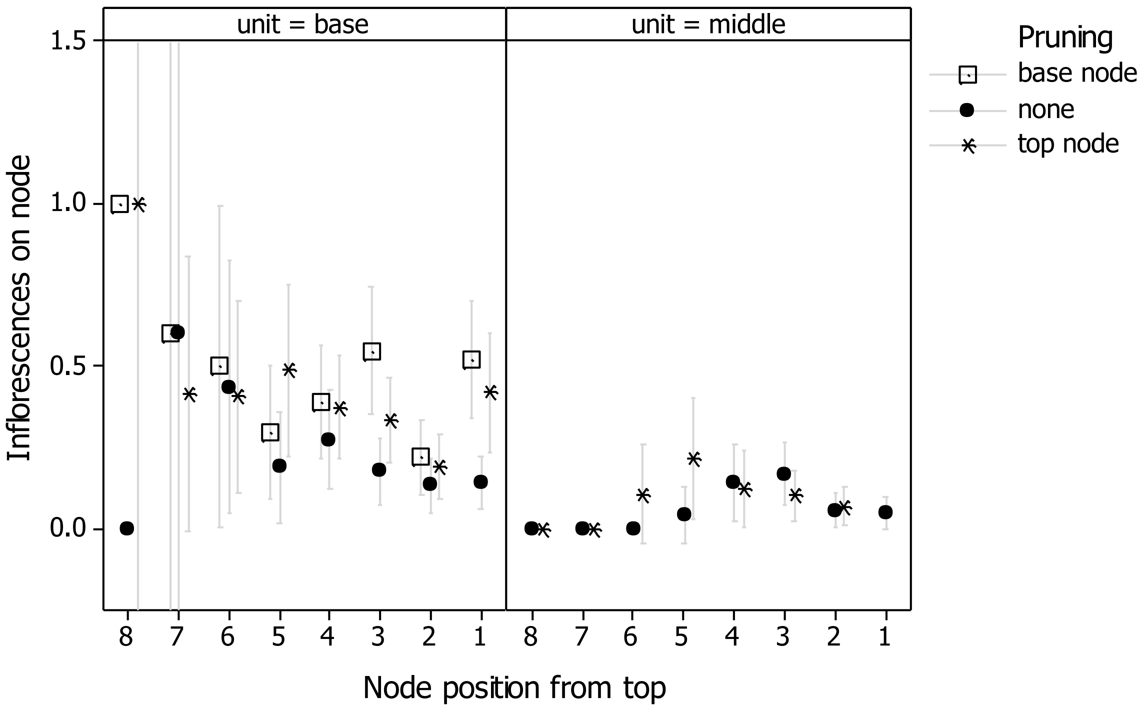

Figure 6-7. Effect of node of pruning, on number of inflorescences emerging from nodes, with position counted from the GU top, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean.

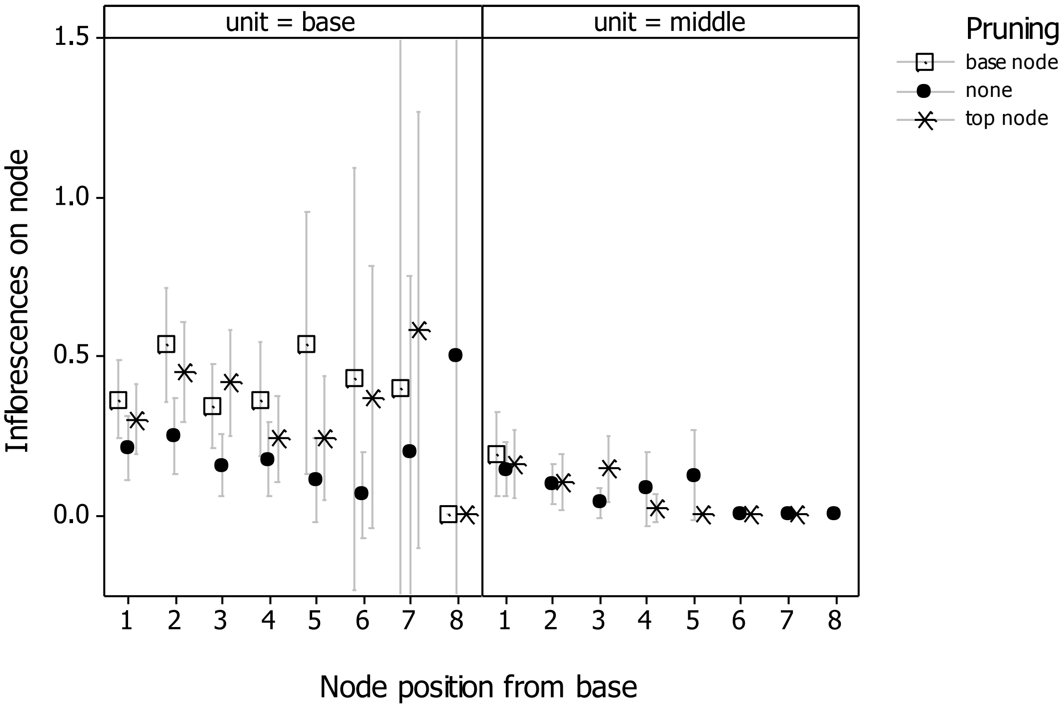

Figure 6-8 Effect of node of pruning, on number of inflorescences emerging from nodes, with position counted from the GU base, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean.

6.3.2. Effect of growth unit of pruning

Quantity of flowering and branching

The proportion of axes producing new stems changed with GU of pruning ($P < 0.001$), with more stems branching when pruned closer to the tip i.e. in GU3 and GU4. The proportion producing inflorescences did not change significantly with GU of pruning (Figure 6-9). The proportion of axes producing lateral growth, either vegetative or floral, was dependent on GU of pruning ($P = 0.011$), again increasing as pruning got closer to the tip.

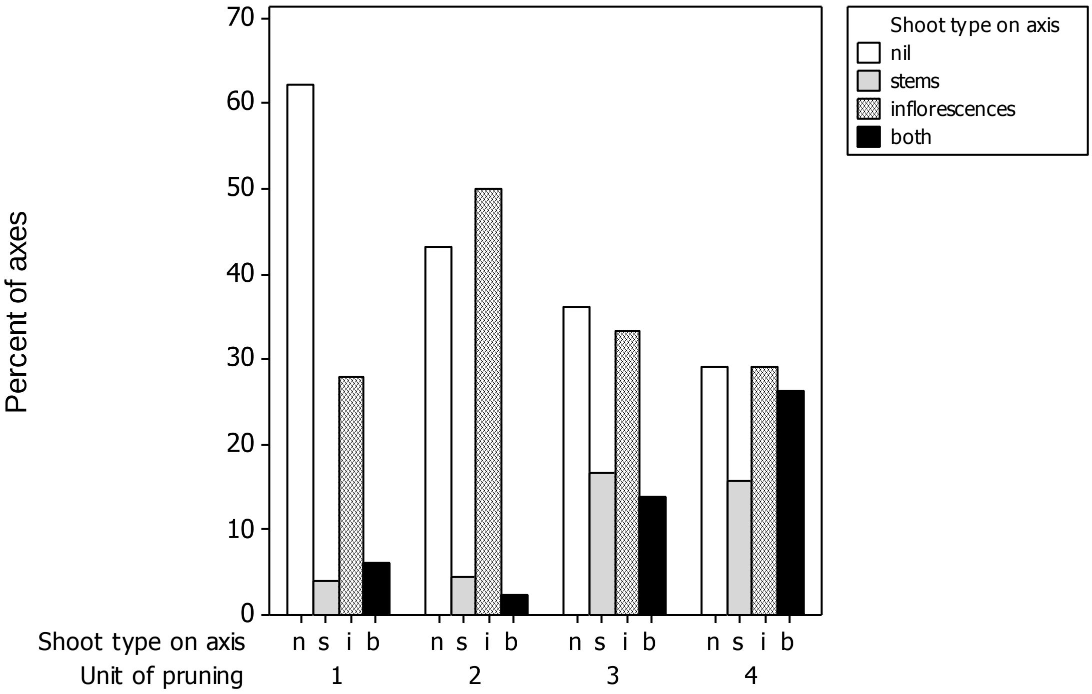

Figure 6-9. Effect of GU of pruning, on percent of axes producing axillary shoots and shoot type, in thirteen year old macadamia trees of variety 741.

The total number of new stems growing out from a parent axis (Figure 6-10) was greater when pruning took place in a GU closer to the tip. Pruning in the two GUs closest to the base gave similar numbers of new stems to each other, as did pruning in the two GUs closest to the tip, but all the other pair-wise combinations of pruning locations were significantly different ($P < 0.05$). However the stems per node (Figure 6-11) did not vary significantly with GU of pruning.

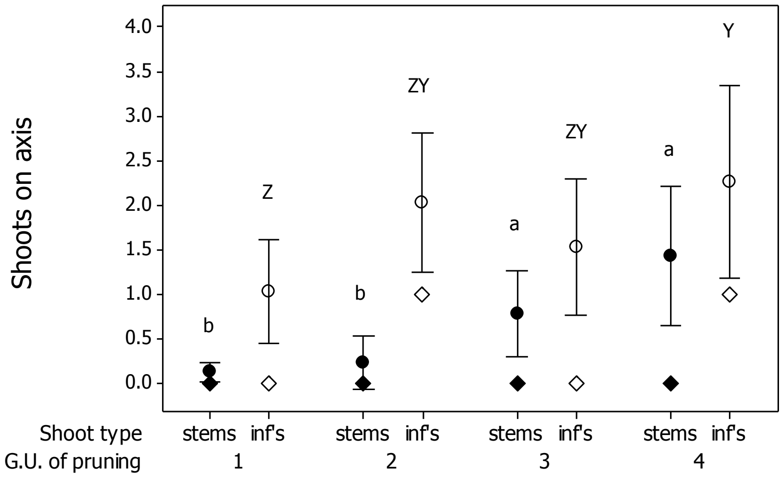

Figure 6-10. Effect of GU of pruning, on number of shoots emerging from an axis and shoot type, in thirteen year old macadamia trees of variety 741.

"inf's" = inflorescences. Bars show the confidence interval of the mean. Circles are means and diamonds are medians. Treatments labelled with different lower-case letters had significantly different numbers of stems emerging. Treatments labelled with different upper-case letters had significantly different numbers of inflorescences emerging.

The total number of inflorescences emerging from an axis (Figure 6-10) was greater when the axis was pruned at GU4 than when the axis was pruned at GU1. However there were more inflorescences per node (Figure 6-11) when axes were pruned at GU2 than when pruned at GUs closer to the tip.

That total number of shoots emerging from an axis was greatest with pruning at GU1, but there was no significant difference between treatments in shoots per node.

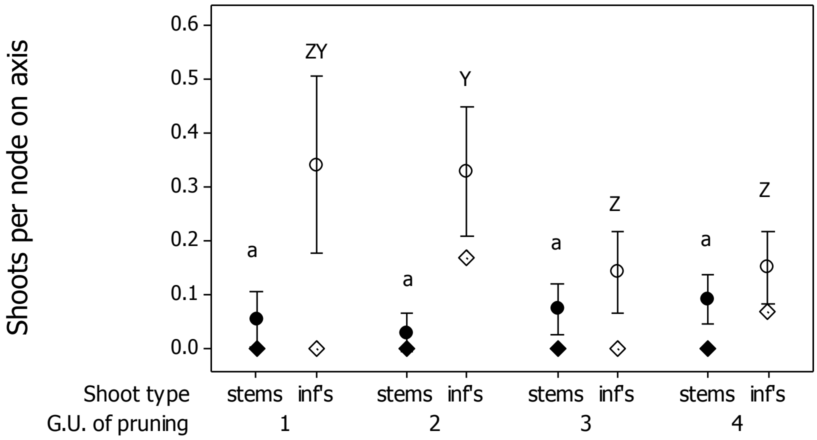

Figure 6-11. Effect of GU of pruning, on density of axillary shoots emerging on an axis and shoot type, in thirteen year old macadamia trees of variety 741.

Bars show the confidence interval of the mean. Circles are means and diamonds are medians. Treatments labelled with different lower-case letters had significantly different numbers of stem per node. Treatments labelled with different upper-case letters had significantly different numbers of inflorescences per node.

Location of flowering and branching

The number of stems growing (Figure 6-12) from GU1 varied significantly with GU of pruning, but numbers of stems growing from other GUs did not. Pruning at GU1or GU2 resulted in fewer new stems growing from GU1 than pruning at GU3 or GU4. The number of stems growing from other GUs did not change with pruning.

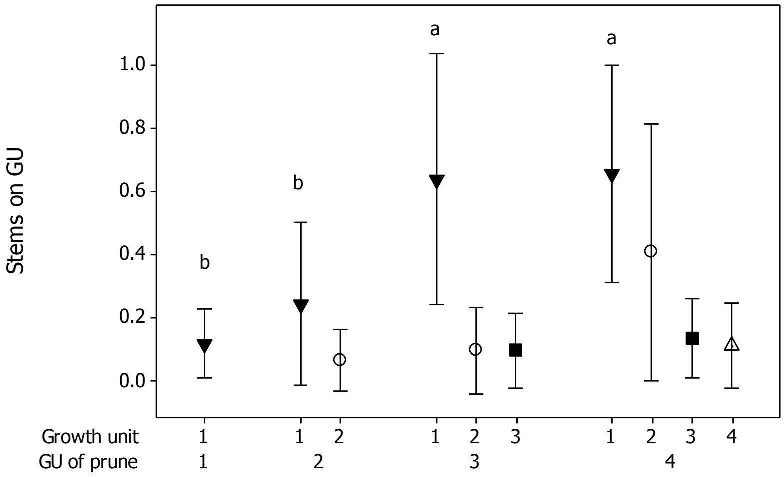

Figure 6-12. Effect of Growth Unit (GU) of pruning, on number of stems emerging from each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different letters had significantly different numbers of stems on the GU.

Pruning at GU1 and GU2 also resulted in fewer stems growing per node (Figure 6-13) from GU1 than pruning at GU3 or GU4. Among those axes pruned at GU4, more new stems emerged on GUs closer to the base (r_s= -0.260, P= 0.001). The stems per node on other GUs did not change with pruning.

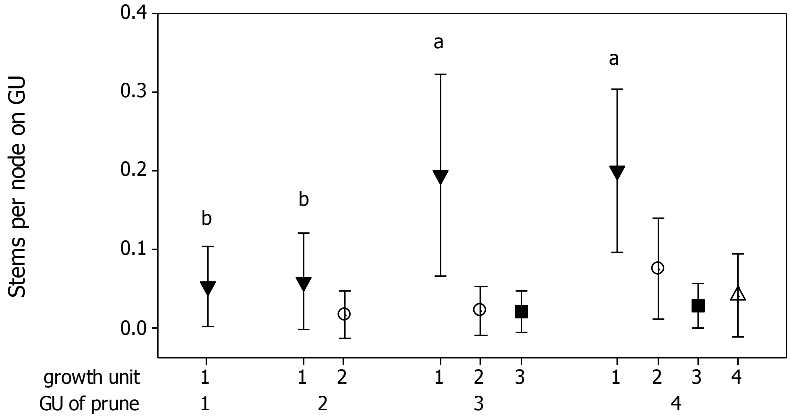

Figure 6-13. Effect of Growth Unit (GU) of pruning, on density of stems emerging on each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different letters had significantly different numbers of stems per node on the GU.

The number of inflorescences produced by GU1was higher after pruning in GU1 or GU2 than in GU3 or GU4 (Figure 6-14). More inflorescences emerged from GU2 when the stem was pruned at that GU than when it was pruned at GU4. Among those axes pruned at GU4, fewer new inflorescences emerged from GUs close to the base (r_s= 0.188, P= 0.014).

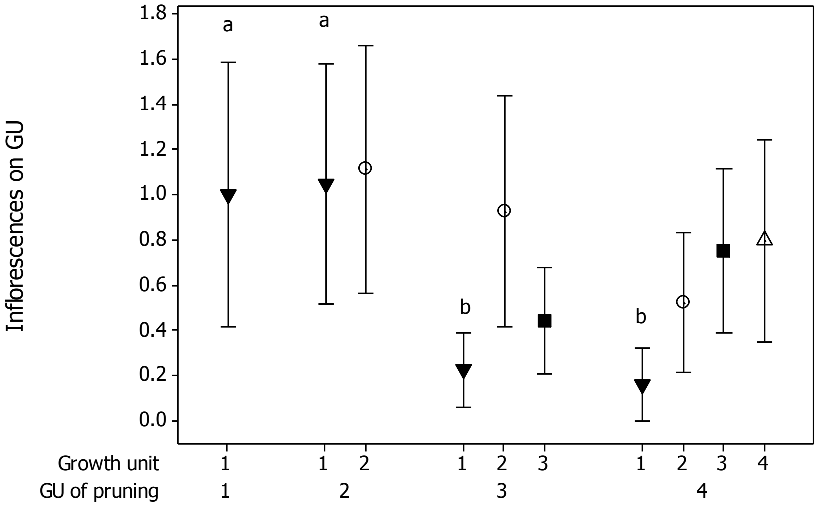

Figure 6-14. Effect of Growth Unit (GU) from base of pruning, on number of inflorescences emerging from each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different letters had significantly different numbers of inflorescences on the GU.

Pruning in GU1 or 2 resulted in a higher density of inflorescences than pruning in GU3 or 4 (Figure 6-15). The density of inflorescences on GU2 was also higher after pruning in GU2 than after pruning in GU4. In the tip-pruned stems more inflorescences emerged the closer the GU was located to the tip (r_s= 0.181 *P*= 0.018).

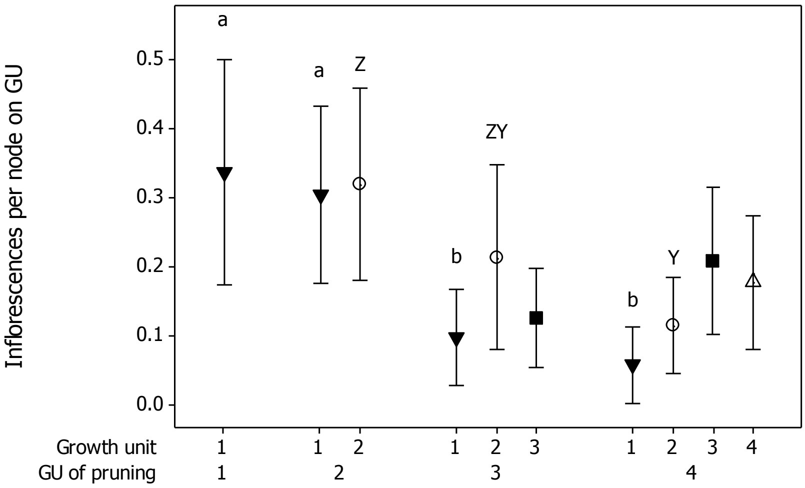

Figure 6-15. Effect of Growth Unit (GU) of pruning, on density of inflorescences emerging from each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different lower-case letters had significantly different numbers of inflorescences per node on GU1. Treatments labelled with different upper-case letters had significantly different numbers of inflorescences per node on GU2.

The overall number of shoots, i.e. the sum of the stems and inflorescences, on GU3 was significantly lower after pruning at GU3 than pruning at GU4 (Figure 6-16). This was not just due to the reduction of nodes (potential flowering sites), as the average number of shoots per node was also lower on this GU under these conditions (Figure 6-17).

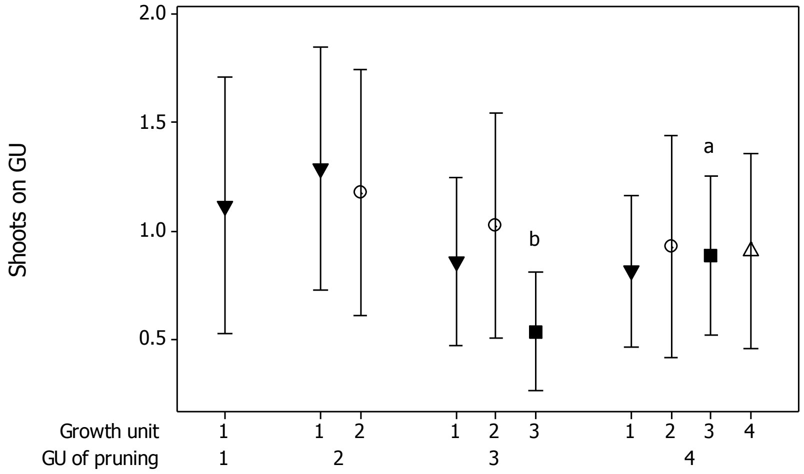

Figure 6-16. Effect of Growth Unit (GU) of pruning, on total number of axillary shoots emerging from each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different letters had significantly different numbers of shoots on the GU.

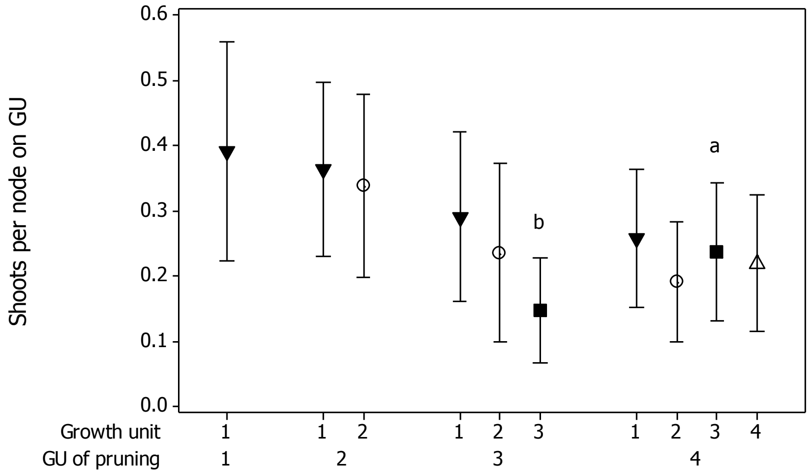

Figure 6-17. Effect of Growth Unit (GU) of pruning, on mean number of axillary shoots emerging from each GU, in thirteen year old macadamia trees of variety 741.
Bars show the confidence interval of the mean. Treatments labelled with different letters had significantly different numbers of shoots per node on the GU.

6.4. Discussion

Pruning below the top (bract) node of the middle GUs (on the three-GU axes) resulted in the emergence of stems at nodes five from the top of the middle GUs, which did not normally branch (in control axes), but did sometimes flower. This is similar to the change in branching location in eastern redbud trees (*Cercis canadensis*), which after pruning branched from buds which usually produced only inflorescences (Owens and Ewers 1997). Other increases in stem numbers with this top-node pruning were seen at only node three from the GU top, which usually bears only a moderate number of stems. This small increase was probably due to buds at this position responding to removal of the apex, removing a sugar sink and auxin source, although these sink/source roles were also played consistently (in both treatments) by new stems at node two. Node two from the top on the middle GU had the highest branching of any node on control axes, but did not branch significantly more than usual after pruning above it, nor did it flower more.

Base-node pruning left only the one node on the middle GUs, and it branched dramatically more than the control. Normally this node branched least on the GU, and did not produce more stems when the GU was pruned at the top-node. Presumably the apical dominance exerted by the new stems growing at node three after top node pruning accounted for this. This node usually flowered at moderate levels, but did not produce more inflorescences with either top- or base-node pruning.

The nodes of the base GU did not increase branching at all with base-node pruning of the middle GU. Their lack of response may be due to dominance by the base node of the middle GU. These nodes produce substantial numbers of inflorescences after pruning, so there were still buds left to respond i.e. they had not all grown out in previous years.

In both top-node and base-node pruning, significant increases in flowering were seen at individual nodes, mostly those of the base GU i.e. the GU below the pruning cuts.

The fact that the numbers of inflorescences increased after pruning on nodes that did not produce more new stems indicates that either the buds were already determined for floral growth, or that the floral buds were not sensitive to factors that held vegetative ones in dormancy e.g. auxin. Thus it may be that in macadamia, inflorescences compete with each other for fuel for emergence, but not inhibit each other once they have enough fuel to begin growth. This increase in flowering at nodes which do not branch may vary with the time of pruning. Colder weather does reduce the amount of vegetative regrowth following pruning in macadamia (McFadyen *et al.* 2008). Perhaps this pruning time of before anthesis increased fruit set and thus created a chain of physiological repercussions that led to decreased branching but increased inflorescence emergence the following winter.

The base node of the middle GU, that usually flowered more than it branched, produced many branches when pruned but not more inflorescences. It changed both the number of buds sprouting and their type.

Node two from the top of the middle GU which normally branched more than any other, did not change its flowering nor branching behaviour with pruning. Node two from the top of the base GU did not change its behaviour either. This suggests that vegetative growth is determined in all the buds of this node early in their life, while many buds at other nodes are either a mix of determined fates, with different types responding differently to pruning, or not yet determined.

In the study of effect of node of pruning, the extra inflorescences emerging with pruning at either node of middle GUs occurred not in the GU where the cut was made but in the GU below it. This increase was also seen in the GU of pruning experiment with pruning at the GU above the base (GU2) – axes cut at GU2 had more inflorescences on the base GU than did axes cut at more distal GUs. There was no similar significant increase in inflorescences on GU2 when cut at the GU3 as compared to being cut at GU1. This result is opposite to the findings in eastern redbud trees, where deeper pruning reduced the number of inflorescences per node (Owens and Ewers 1997). A possible cause of this difference is that macadamia buds may have been determined before the time of pruning, whereas eastern redbud buds were not. It may be that in macadamia proximity to the cut and flowering potential both influence the production of inflorescences on pruned axes.

When pruning was at GU1 the density of inflorescences was highest in GUs nearer the axis tip, but density of inflorescences appeared to even-out between GUs as pruning cuts were made closer to the base. The inflorescence distribution between GUs in stems pruned at the tip GU are opposite to that of unpruned four-GU axes studied in Chapter 3. Those axes, as did axes of all lengths had more inflorescences on GUs closer to the axis base, and more stems on GUs closer to the axis tip. Thus tip pruning may be changing the location of flowering. Stem density was higher on GU1 than other GUs with pruning at GU1 or GU2. This pattern also differs to unpruned axes, which tend to have fewer stems emerging from the base unit than from other units. Further investigations, using a direct comparison of tip-

GU pruning and no pruning on four unit axes, would be useful to check this unexpected reversal in distribution of inflorescences and new stems.

Pruning at either the top or base node of the middle GU increased the proportion of axes flowering as well as the inflorescences per node on the axis. In the pruned axes the total number of inflorescences produced was also higher. An interesting and potentially useful follow-up investigation would be to assess the effects of pruning on the stunted axes found to already flower prolifically on young trees (Chapter 5). If pruning increases flowering on these, and if this results in substantial yield improvements, it may be an alternative means of increasing young orchard returns to the dense plantings that later create over-shading problems.

Pruning at either the top node or bottom node of the middle GU did not change the proportion, number or density of stems produced on an axis – just their location along it. Pruning at different growth units also did not change stem density on an axis, so deeper pruning resulted in fewer stems emerging. In the GU-of-pruning experiment there was lower total stem emergence from axes pruned at GU2 relative to those pruned in GU3, as a result of a drop in stem density on GU1 as well as a loss of nodes on GU2. The lack of increased branching with pruning is unexpected, as research into macadamia canopies (Huett and Smith 2008) tell us that removal of the axis apex by hedging increases emergence of lateral stems. However, Huett and Smith may have assessed this by visual examination of the canopy surface after hedging, and so visible new stems at the canopy edge may have been offset by reduced branching towards the base of axes without their realising. Additionally, the temperature at time of hedging effects regrowth stem numbers, with colder weather resulting in fewer stems (McFadyen *et al.* 2008), so this pruning occurring in winter may account for lower numbers of stems emerging here, but probably not a complete lack of difference between pruned and unpruned axes.

The number of inflorescences on an axis also depended upon the GU of pruning, but not in the same way. Pruning in GUs closer to the base increased the density of inflorescences, so the trend in absolute number of inflorescences was not linear. The biggest changes occurred when the pruning cut was made in GU2 (just below the middle of the axis). Thus the ratio of inflorescences to stems varied with pruning location – the highest ratio was found with pruning at GU2, and the lowest with pruning at GU4. The ability to change the ratio of floral to vegetative growth may have practical applications in commercial orchards, due to yield improvements. Although such precise pruning is likely to be manageable only on small trees, it presents the possibility of yield increases at the stage of an orchards life where they represent a relatively high economic gain.

The mechanisms that could account for the flowering changes with pruning depend upon whether inflorescences are determined at the time of pruning (and are merely awaiting the trigger to emerge), or whether pruning can effect determination as well as emergence. By pruning away large proportions of an axis, far less tissue remains to be supplied by the same capacity of vessels, which may increase the concentration of resources and signal molecules arriving from the rest of the tree. These higher concentrations may stimulate greater emergence of dormant already-determined inflorescences.

However if floral determination can occur later in the life of a bud than the season formation, then pruning could result in increased evocation of undetermined meristems. In the node of pruning experiment, as there is no significant decrease in stem density, extra inflorescences resulting from pruning are probably not arising by changing bud fate from vegetative to floral, but by promoting floral growth in otherwise dormant buds.

The difference in inflorescence : new-stem ratios with location of pruning may enable tree size control with more open canopies as well as increased flowering. Increased flowering could be especially relevant to young orchards as a means of increasing early yields. This merits further investigation, by examining the longer term implications of these types of pruning. If pruning is bringing forward emergence of buds that would otherwise be staggered over several years, a longer term effect may be a drop in inflorescences in years further on than the single year examined here. Long term implications for vegetative structure and light capture would also be important to examine.

6.5. References

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Chapter 7. Effect of temperature sequence on flowering and branching

7.1. Introduction

Macadamia nut trees (*Macadamia integrifolia and M. tetraphylla*, and hybrids of the two) grow mostly during synchronised bursts of stem elongation and axillary bud release, termed flushes (Stephenson and Trochoulias 1994). New vegetative stems and inflorescences, collectively termed shoots, arise directly from axillary buds. Immature inflorescences often become visible as pale swellings behind the bud bracts in autumn or winter (Bennel 1984; Moncur *et al.* 1985). In mid- to late-winter inflorescences elongate, emerging from behind the bracts, and grow to lengths of 15 cm or more.

Inflorescence emergence occurs only if the beginning of a flush coincides with cool temperatures (Olesen 2005). Other sub-tropical perennials - including lychee (*Litchi chinensis*), mango (*Mangifera indica*) and avocado (*Persea americana*) – also respond to cool temperature (10 – 13 ºC nights and 15 – 20 ºC days) as a cue for emergence of inflorescences (Buttrose and Alexander 1978; Shu and Sheen 1987; Menzel and Simpson 1988). "*Washington Navel"* cultivar oranges *(Citrus sinensis)* differ, in that floral emergence responds to water availability as well as temperature (Lenz 1969; Moss 1969), and lemon *(Citrus limonum)* is rare among perennial evergreen trees in that day length is a trigger for floral emergence (Sedgley and Griffin 1989; Chaikiattiyos *et al.* 1994; Wilkie *et al.* 2008).

In trees the emergence of visible inflorescences or flowers or floral buds has been studied far more than determination of the meristem, probably because the latter requires labour-intensive microscopic examination of a large number of buds throughout the year (Sedgley and Griffin 1989). However distinction between the two processes is important when seeking to understand development triggers, as determination can occur months or years before floral growth becomes macroscopically visible (Sedgley and Griffin 1989; Garcia-Luis *et al.* 1992). Formation of microscopic floral organs (floral primordia) such as floral bracts, sepals and petals, is the morphological change most closely associated with determination. Initiation of floral primordia has been observed in only a few other members of the Proteaceae family. In *Banksia coccinea* and *B. menziesii* the first floral primordia form in late spring, with the inflorescence becoming visible to the un-aided eye in the following summer or autumn (Fuss and Sedgley 1990). Waratahs (*Telopea speciosissima*) initiate microscopic floral growth in early summer, with inflorescence buds becoming visible in February, but not reaching anthesis until winter or spring (Dupee and Goodwin 1990). Microscopic examination of glasshouse-grown *Leucospermum patersonii*, also of the Proteaceae family, found that this species requires long days (night length falling to under a required threshold) to bring about floral determination (Wallerstein 1989), making a spring or early summer determination likely. After long-day-determination the plants required short days for formation of primordia and development of mature inflorescences within a normal (natural) time span.

In macadamia only one study has observed the beginning of floral primordia formation along with environmental conditions affecting this. Sakai *et al.* (1982) investigated the effect of temperature on the number of inflorescences produced and the speed of their emergence. Trees were grown for 2 months at over 21 ºC, then moved to night temperatures of 12, 15, 18 or 21 ºC (all with ambient day temperatures). No statistical analysis of their data was published, but they noted a number of interesting trends. Inflorescences emerged sooner at lower night temperatures, but the total number of inflorescences emerging in the 40-week-long experiment increased with night temperature up to 18 ºC and then declined again. Microscopic observations found faster microscopic floral differentiation at cooler night temperatures, and slower differentiation at higher night temperatures. The authors put forth two linked hypotheses; i) floral determination of the meristem is greater at warmer night temperatures, while ii) growth and development of floral buds is greater at cool night temperatures.

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 five times the numbers of inflorescences than the other treatments. This difference was statistically significant within seven weeks of transfer.

The number of new stems forming in macadamia has also been shown to be affected by temperature. Between mean daily maximums of 16 ºC to 26 ºC, the number of stems emerging per day increased with temperature on pruned macadamia axes (Wilkie *et al.*

2009). Effects of temperature on branching have also been studied in other species. Poinsettia (*Euphorbia pulcherrima*) and beans (*Phaseolus vulgaris*) respond to increased temperature similarly to macadamia, with increased branching (Hagen and Moe 1981; Kigel *et al.* 1991). However 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).

A more detailed understanding of the relationships between temperature and macadamia branching and flowering would assist both commercial growers and managers of threatened wild populations. In particular, knowledge of the conditions triggering floral determination could improve efficiency of orchard management efforts to optimise ratios of floral growth to vegetative growth and, ultimately, consistent and sustainable yield. Such knowledge would also help plan changes to management under warmer temperatures expected with climate change. This study set out to make a direct comparison of the effects on macadamia flowering and branching of warm temperatures, cool temperatures and different sequences of the two, partly in order to investigate Sakai *et al.*'s two-temperature flowering theory. Thus the first hypothesis is that the sequence of exposure to warm and cool temperatures will affect the number of inflorescences emerging. The second is that the sequence of temperature treatments will affect the number of new stems emerging.

7.2. Materials and methods

Fifty-six *Macadamia integrifolia* × *tetraphylla* trees were grown from cuttings for 3 years in an outdoor nursery in Beerwah (26.86º S, 152.96º E), south-east Queensland . Half of the trees were cultivar A38 and half were cultivar A4. At the end of anthesis in early spring 2009 the trees were about 150 cm tall. They were transplanted into pots, 33 cm in diameter by 35 cm deep, of sandy-loam pot media, and were moved into a temperature-controlled glasshouse in Nambour (26.63º S, 152.96º E). Three walls of each glasshouse 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. "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. Trees 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. (See appendix D for appraisal of alternative method.)

Initial tree sizes were approximated by summing the length in centimetres of the most central (leader) axis and the second-order axes arising from this. Mann-Whitney tests were used to check for differences in initial size. There were no size differences between temperature sequence groups, but A4 trees had greater initial stem lengths than A38 trees, with means of 645 and 421 cm respectively ($P < 0.001$).

A two-month pre-treatment was set up with day / night temperatures of 26 / 23 ºC to prevent floral determination (Sakai *et al.* 1982). Inflorescences and young fruit were removed at the beginning of this time – after anthesis, in September - to prevent their inhibition of any new growth. The few new inflorescences emerging during this time were also removed. (see Appendix C for appraisal of this method.)

After the pre-treatment, in November, the 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). 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. Each regime was maintained in its own chamber of the glasshouse. 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 (to promote axillary bud outgrowth) at week five of the first exposure period, removing one growth unit from the leader axis and every second axis arising from the leader (secondary axes). The term growth unit (GU) is used here to describe the section of a stem created over one continuous growth period by its apical meristem, as defined by Hallé *et al.* (1978). This pruning removed any green stem and new leaves on these major axes, both of which contribute auxin to maintain apical dominance and prevent outgrowth of vegetative axillary buds (Cline 1991; Warner and Gitlin 1971) and possibly of floral buds also (Batten and McConchie 1995; Jacobs 1985).

At the end of the first two-month period, every inflorescence on each tree was counted, and new stems on the main leader and all second-order axes were counted. 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. Trees were again pruned at five weeks into the treatment period (to promote axillary bud outgrowth), with one growth unit removed from all second-order axes.

Each chamber’s temperatures were monitored with a pair of temperature recorders (“Tiny Tag” brand), accurate to 0.5 ºC and recording every 15 min. Eight instances of departure from set temperatures reduced the temperature differences between treatments by 2 to 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, until these shoots could be identified as stems or inflorescences, at which time new inflorescences and stems were counted as at the end of the first period. Outside temperatures in Nambour averaged 24.6 / 14.7 ºC during these six weeks. The second count included only shoots that had emerged since the end of the first period.

Differences in the proportion of trees producing new shoots under different regimes or sequences were analysed using Pearson chi-squared tests. Normality of datasets was investigated using Anderson-Darling tests. Some trees in each treatment did not flower at all, some probably because they were still flowering-incompetent, and this resulted in high variation in shoot numbers which was skewed due to the presence of many zeros. Differences in new shoot numbers between cultivars were analysed using Kruskal-Wallis tests. Differences in 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 Spearman’s rank correlations (r_s). $P < 0.05$

was used as the indicator of a significant relationship in all analyses. (Fowler *et al.* 1998; Minitab 2007)

7.3. Results

7.3.1. Inflorescences

First period

During the first two-month period, more trees flowered in the warm (W) regime than in the cool (C) regime, and a greater number of inflorescences per tree emerged from trees under the W regime (Table 7-1). There was no statistically significant difference between cultivars in the number of inflorescences, and no correlation between tree size and number of inflorescences (data not shown), so their data was pooled for subsequent analyses.

Table 7-1. Effect of the first period of exposure to either cool or warm temperatures, on the number of shoots emerging from 3 year old potted macadamia trees of varieties A38 and A4, and percent of those trees shooting, in that period. *Within a column, means followed by the same letters are not significantly different (percent of trees flowering/branching tested using Chi-Square $P < 0.05$; numbers of new shoots tested with logistic regression, carried out in conjunction with tree size, $P < 0.05$).*

Temperature regime	**Trees flowering**	**Trees branching**	**Inflorescences emerging per tree**	**Stems emerging per tree**
	(%)		(mean)	
Cool	0.04 *a*	0.78 *a*	0.04 *a*	7.29 *a*
Warm	0.28 *b*	0.88 *b*	0.58 *b*	11.19 *a*

Second period

The proportion of trees that flowered in this period depended on the previous period's temperature, as well as the current period's. When the groups with the same first-period treatment were combined (W-C together with W-W, and C-W with C-C), those exposed to warm temperatures in the first period were more likely to flower in this second period than those exposed to cool (*P*< 0.01) (data not shown). Second period temperatures had the opposite effect on flowering in the second period, with trees experiencing cool temperatures in this period (W-C combined with C-C) more likely to flower than those experiencing warm temperatures in this period (W-W with C-W) (*P*< 0.04) (data not shown).

The number of inflorescences emerging during the second period (Table 7-2) 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) (*P*< 0.03). 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) (*P*< 0.05).

Table 7-2. Effect of the exposure to different temperature regime sequences, on flowering and branching of 3 year old potted macadamia trees of varieties A38 and A4, during the second period of the sequence.
Within a column, means followed by the same letters are not significantly different (percent of trees flowering/branching tested using Chi-Square P< 0.05; numbers of new shoots tested with logistic regression, carried out in conjunction with tree size, P< 0.05).

Temperature regime sequence	**Trees flowering** (%)		**Trees branching**		**Inflorescences emerging per tree** (mean)		**Stems emerging per tree**	
Cool-Cool	38.5	*b*	100.0	*a*	1.08	*b*	10.08	*b*
Cool-Warm	6.3	*a*	81.3	*a*	0.06	*a*	8.13	*b*
Warm-Cool	64.3	*b*	92.3	*a*	3.31	*c*	10.79	*c*
Warm-Warm	46.2	*b*	92.3	*a*	0.92	*b*	7.08	*a*

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

Combination of periods

The proportion of trees flowering in total i.e. over the combination of both periods, was greater in W-C than C-W (Table 7-3). The number of inflorescences emerging in total also differed with sequence; ($P < 0.05$) for every pair of sequences except C-C and W-W (Table 7-3). There was no correlation between tree size and number of inflorescences emerging in any sequence. There was no significant difference between cultivars in the number of inflorescences emerging (Figure 7-1).

Table 7-3. Effect of exposure to different temperature regime sequences, on total flowering and branching of 3 year old potted macadamia trees of varieties A38 and A4, over both periods of the sequence.
Within a column, means followed by the same letters are not significantly different(percent of trees flowering/branching tested with Chi-Square $P < 0.05$*; numbers of new shoots tested with logistic regression, carried out in conjunction with tree size,* $P < 0.05$*).*

Temperature regime sequence	**Trees flowering** (%)		**Trees branching** (%)		**Inflorescences emerging per tree** (mean)		**Stems emerging per tree** (mean)	
Cool-Cool	38.5	*ab*	100.0	*a*	1.15	*b*	20.5	*c*
Cool-Warm	7.1	*a*	100.0	*a*	0.07	*a*	12.8	*a*
Warm-Cool	69.2	*b*	92.3	*a*	4.08	*c*	19.0	*b*
Warm-Warm	50.0	*b*	100.0	*a*	0.92	*b*	21.2	*c*

Relationships between first and second period inflorescence emergence

No correlation was detected between the number of inflorescences emerging from a tree in the first period and that emerging in the second period, over all temperature sequences or within any one sequence.

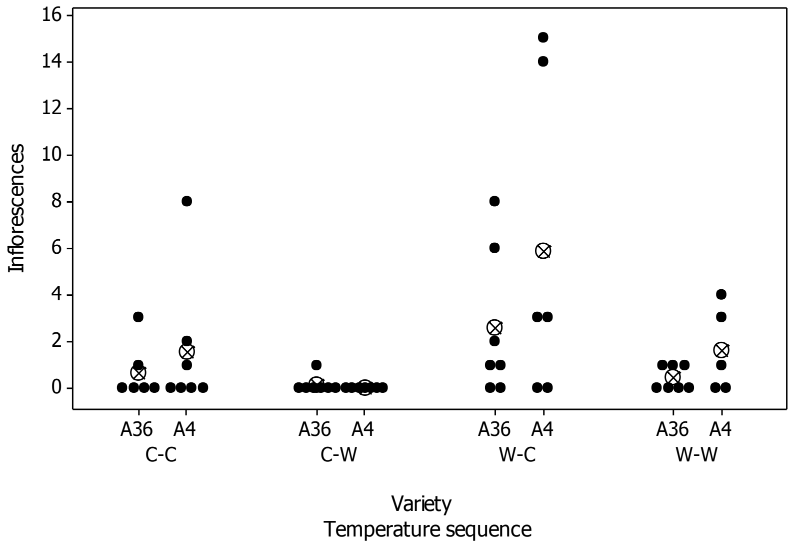

Figure 7-1. Effect of temperature sequence and cultivar on number of inflorescences emerging in total over both exposure periods, in potted three year old macadamia trees.
Dots are individual values (trees), crossed circles are means.

7.3.2. Stems

First period

During the first period, a smaller proportion of trees branched under the cold regime than under the warm regime (Table 7-1). However there was 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 regimes. Cultivar made no significant difference to the numbers of new stems produced, so their data was pooled for subsequent analyses.

Second period

The proportion of trees branching in the second period was similar for all groups. Among those trees that did branch there were differences in the number of new stems between the temperature treatments (Table 7-2). In this period there was also a difference between numbers of stems emerging from trees of different cultivars ($P= 0.04$), with a median of ten emerging from A38 and of six emerging from A4.

Combination of periods

When data for both periods was combined there was no significant difference between temperature sequences in the proportion of trees branching (Table 7-3). However temperature sequence did affect the number of new stems emerging from those trees that did branch ($P < 0.01$) (Table 7-3). The temperature sequence effect interacted with tree size - there was a negative correlation between size and number of stems emerging only in C-W ($P = 0.01$, $r_s = -0.685$). There was also a positive trend towards emergence of more new stems in larger trees in group W-C, but the correlation test result was not quite significant ($P = 0.05$, $r_s = 0.553$). Fewer stems emerged from C-W than any other treatment (Table 7-3).

There was no significant difference between the numbers of stems emerging from trees of different cultivars over the two periods combined (Figure 7-2).

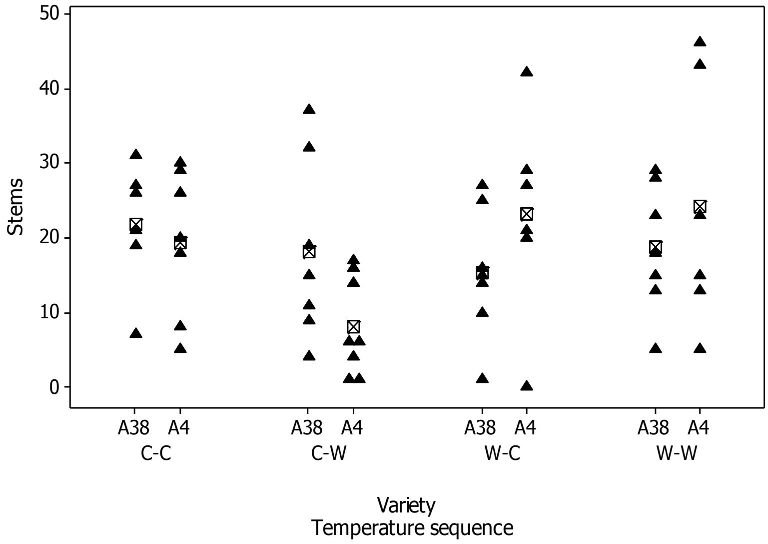

Figure 7-2. Effect of temperature sequence and cultivar on number of stems emerging in total over both periods, in potted three year old macadamia trees.
Filled triangles are individual values (trees), crossed squares are means.

Relationships between first and second period stem emergence

The number of stems emerging in the first period from a tree under any temperature sequence was not correlated with the number in the second period, over all sequences or in any one sequence (data not shown).

7.3.3. 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. There was also no correlation within any of the temperature sequences. 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.

7.4. Discussion

Flowering and branching

The order of exposure to warm and cool temperature regimes did affect 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 trees of the W-C sequence and the C-W sequence receiving the same total degree days, over the same duration of exposure to the warm regime and the cool regimes. The number of inflorescences emerging during the second period was greater when the trees had been exposed to warm temperatures in the first period. The number of inflorescences emerging during the second period was also greater when exposed to cooler temperatures in this second period. As there was far more inflorescence emergence in the second period than the first, these two observations added together logically support the overall difference between W-C and C-W.

The difference in flowering between C-W and W-C (Figure 7-1) fits with the hypothesis that an early step of inflorescence formation is favoured by warm temperatures and a later step by cool temperatures. As suggested by Sakai *et al.* (1982), the first step could be floral evocation or determination of the axillary meristem. However their suggestion that cool temperature promotes all subsequent growth may not be the whole story, as inflorescence development to the stage of floral bracts seems to occur before the cool temperatures that result in the floral flush. Moncur *et al.* (1985) found through scanning electron microscope (SEM) studies that there is a pause in the growth of inflorescences around 200 µm long, for around a month at the beginning of winter when they become visible (to the unaided eye) behind the axillary bud bracts, in cultivar 246. Many other varieties also show a similar pause in growth when just visible behind the bud bracts (pers. com. Stephenson 2009), and it is possible others pause growth before becoming visible. Cool temperatures appear to be a trigger for resumption of growth and subsequent emergence of these partly-formed inflorescences. If this determination / dormancy / emergence scenario is correct, when cool temperatures preceded warm in this experiment, there were few buds determined in the first period, and a relatively small proportion of these determined buds emerged in the second period. In the W-C sequence, warm temperatures favouring determination would have started many buds along the first, microscopic and hidden stages of floral development, and subsequent cool temperatures would have resulted in a large proportion of those many determined buds emerging.

An alternative explanation of these results is that warm temperatures enabled trees to build more reserves during the first period than did cool temperatures, and cool temperatures triggered the beginning of a one-step flowering process. This flowering would then be able to occur in more buds of W-C trees than of C-W, due to them storing more fuel (for any sort of growth) and then being exposed to the flowering trigger. However this does not fit well with the appearance behind bracts of floral buds in many macadamia varieties long before winter, when inflorescences emerge and elongate (Olesen 2005). Nor does it fit with the results of Stephenson and Gallagher (1986), where only the warmest treatment before the cool exposure produced a sizeable number of inflorescences and the remaining range of temperatures produced close to no inflorescences. If warm temperatures only enabled more energy production and were not a trigger for induction and evocation, a trend of increasing inflorescence production with increasing first stage temperature would be expected.

Over both treatment periods, trees in the W-W sequence produced a similar number of macroscopic inflorescences to those in the C-C sequence. The two-step hypothesis also accounts well for this finding. If it is correct, among C-C trees small numbers of axillary meristems would have become florally determined in the first period, and a large proportion of these would have emerged in the second. In the W-W group a large number of meristems would have been determined in the first period, and a small proportion of these would have emerged in the second.

Thus the best fitting explanation at the moment for these flowering results is a two-step, warm-then-cool, determination / growth resumption theory. The combination of SEM analyses of dormant inflorescences (Bennel 1984; Moncur *et al.* 1985) suggest that the first stage of flowering includes both determination and formation of the rachis and initiation of floral bracts, and the second stage includes resumption of growth of the rachis and then formation of the florets. A two-step / two-trigger flowering process also occurs in the only other member of the Proteaceae family to be studied in this way - *Leucospermum patersonii* requires long days for floral primordia initiation and short days or cool temperatures for macroscopic growth and emergence (Wallerstein 1989).

The number of stems emerging over the combination of the two periods was dependent on temperature as well as temperature sequence, however the proportion of trees branching did not differ. This is not surprising given that all trees were tip-pruned. Thus temperature is probably only affecting vegetative growth through the degree of response to pruning – i.e. the number of new shoots per tree. Such types of interaction between a number of dormancy controls are regarded as common (McSteen and Leyser 2005). In macadamia the extent of vegetative flushes is thought to be affected by water supply and light levels (Stephenson and Trochoulias 1994; Olesen *et al.* 2011), while timing of latter flushes is related to the growth rate of the previous flush (Olesen *et al.* 2006).

W-C trees produced more new stems than C-W trees. This difference suggests that branching also is a multi-stage process, with different stages reacting to temperature differently. Stafstrom and Sussex (1988) showed that pea axillary buds are released from dormancy into a transition stage, from which they can either grow to new stems or be re-suppressed by larger buds around them. If macadamia has a similar process, it may be that release from dormancy and progression from transition to un-suppressible new stem respond differently to the cool and warm temperature used here.

The finding that there was no difference in the number of stems emerging between trees exposed to the W-W temperature regime sequence and trees exposed to the C-C sequence is unexpected, given Wilkie *et al.*'s (2009) finding that stem emergence increases with temperature, and given the general positive relationship of plant vegetative growth and temperature. Possibly differences in branching occurred predominantly in the most distal growth units of the oldest axes (as seen in Chapter 3), and the pruning conducted here would have removed much of the difference between temperature treatments. The depth of pruning may have been the difference between this study and Wilkie's that lead to different results. Removing only the apical bud may be a more useful method of growth stimulation for future studies of branching. In addition, the pruning used in this study varied slightly between the first and second study periods – in the first one the central leader and half of the second-order axes were tip-pruned, in the second the leader no longer had a tip to prune and every second-order axis was tip-pruned. This could lead to more inflorescences emerging in the second period (Chapters 4 and 6 of this thesis). A follow-up study to look for an interaction between extent of pruning and temperature on their effects on inflorescence emergence is necessary to be sure the results here are due only to temperature.

No consistent relationship was found between growth in the first period - either floral or vegetative - and growth in the second period. The lack of a relationship is an indication that inflorescences may not be produced at the expense of new stem production. This may be because buds becoming florally determined are found at different locations to those which produce new stems. This has been observed to occur in apricot (*Prunus americana),* peach (*Prunus persica*), and apple (*Malus domestica*), in which flowering peaks at different distances from the axis base to branching (Costes and Guedon 1996; Fournier *et al.* 1998; Renton *et al.* 2006). The possibility of this occurring in macadamia is examined in Chapter 5 of this thesis.

Implications

While this experiment is the third piece of work that points to a multi-step flowering process in macadamia - along with Sakai *et al.* (1982) and Stephenson and Gallagher (1986) - none of these experiments have been replicated, in this case due to the cost of temperature controlled work with trees of a flowering age. Sensibly, further research in this area would incorporate replication of this work to confirm the findings before follow-up topics were investigated. The addition of microscopic observations of axillary meristem activity throughout temperature-sequence studies would provide clear evidence of whether temperature is affecting flowering through triggering floral primordia formation and resumption of growth after dormancy.

While further investigations are also necessary to clarify exactly which time of year practices benefiting flowering should be carried out, this study suggests that an early stage of flowering is favoured by temperatures found in macadamia’s natural geographic range during late spring or late summer. However, this is at odds with the late autumn to winter flowering time commonly accepted by the macadamia industry and scientific community (Trueman 2013). Spring and summer are common times of vegetative flushing in macadamia, suggesting that floral determination may occur concurrently with vegetative growth, either at the time of axillary bud formation (as part of elongation growth), at the time of axillary bud release, or both. A spring determination is not unlikely given that this would be similar to macadamia’s close relatives *Banksia coccinea* and *B. menziesii* (Fuss and Sedgley 1990).

A two-step flowering process in macadamias which requires two temperature triggers may have implications for methods of climate change mitigation, for both commercial macadamia production and the threatened wild *M. jansenii* population. As warmer temperatures are known to increase the rate of vegetative flush development in macadamia (Stephenson and Cull 1986; Wilkie *et al.* 2009), the time of the winter flush may move with climate change, and then no longer coincide with the cool temperature trigger as required for good inflorescence emergence. Pruning to reset the flushing cycle and move the time of the winter flush by Olesen (2005) caused drastic declines to flowering . Bringing the flush forward by 3 weeks or delaying it by 3 to 6 weeks roughly halved the number of stems producing inflorescences. Such a decline in flowering may also reduce crop yield or seed production, although fruit set and nut retention can complicate the relationship between numbers of inflorescences and numbers of mature nuts (Wilkie *et al.* 2009).

In Honolulu macadamia orchards, where monthly average night temperatures do not fall below 19 ºC, flowering is spread over six months, with the subsequent drawn- out nut maturation resulting in far higher costs of harvest (Nagao *et al.* 1994).

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Chapter 8. Axillary bud formation and microscopic floral differentiation

8.1. Introduction

The formation of the first uniquely floral primordia by a meristem is a clear, microscopically visible sign that meristem evocation has occurred. Although in flowering literature the macroscopic appearance of floral structures is sometimes referred to as floral initiation, in many plants including those of the family Proteaceae, macroscopic floral structures can appear months or years after evocation and microscopic floral differentiation (Sedgley and Griffin 1989; Garcia-Luis *et al.* 1992). *Banksia coccinea, B. menziesii* and waratah (*Telopea speciosissima*) are the only Australian plants of the Proteaceae family for which microscopic floral differentiation has been sighted in plants growing through natural seasonal cycles. Evocation occurs in late spring and early summer for *Banksia* species and waratah respectively, with inflorescences becoming visible to the unaided eye in the following autumn, winter or spring (Dupee and Goodwin 1990; Fuss and Sedgley 1990).

The times of floral induction of macadamia trees and floral evocation of macadamia axillary buds are not known, but this information could be very valuable for optimising the effects of management practises in orchards or natural reserves. Just one study has observed the beginning of microscopic floral differentiation of macadamia buds in tandem with environmental conditions, but not over natural seasonal cycles. Potted macadamia trees were treated with a range of night temperatures in a controlled glasshouse environment, and axillary bud development observed with a light microscope (Sakai *et al.* 1982). Three types of buds and their associated behaviour were described. Those with a bulging, slightly convex meristem with a top at the same level as the single whorl of primordia went on to produce vegetative stems. Those with meristems forming a high 'peaked' dome with a top far above that of the primordia produced inflorescences. Those with a flat meristem below the level of the primordia remained dormant. "Floral apices" – microscopic inflorescences, but at an un-specified stage of development, so perhaps high domed meristems or perhaps further developed inflorescences - were seen earliest in trees from the coldest treatments. However the authors concluded that 'floral initiation' is greatest at higher temperatures in the range used of 12 to 21°C, because total emergence over the 40 weeks was greatest at the intermediate temperature of 18°C, but speed of emergence was lowest at the highest temperatures. This seems a contradiction, and may result from small numbers of buds examined at infrequent intervals, but the method is too brief to judge this. Consequently the only information available on effects of temperature of microscopic floral differentiation in macadamia is unclear.

However the same meristem shapes described by Sakai *et al.* (1982) i.e. flat, bulging and domed, were also reported as dormant, floral and vegetative respectively by two other studies of meristem morphology using scanning electron microscopy (SEM) (Bennel 1984; Moncur *et al.* 1985). These studies described details of the first few whorls of primordia forming as well as later primordia, but without recording environmental conditions. Bennel's study showed clearly that vegetative meristems are triangular when viewed from above, with new leaf primordia forming at the corners. He noted that in dormant buds one whorl of primordia was present. Floral meristems were round when viewed from above. The combined works of Bennel (1984) and Moncur *et al.* (1985) reveal that a second whorl of 3 involucral bracts - resembling broad, thin flaps - forms at the base of the meristem. After this, swellings of common bracts become visible under the meristem (on the flanks of the young rachis) in a helical phyllotaxy. Moncur *et al.* (1985) also found the common bract primordia appeared at the same time as the inflorescence first became visible to the naked eye behind the bud bracts and entered dormancy, in the variety "246". The first part of this chapter's work aimed to find the time of microscopic floral differentiation in macadamia buds, by determining the time of year when the microscopic floral morphology described above first appears.

The second part of this chapter aimed to identify any microscopic differences in morphology of buds in different positions along growth units (GUs), during the growth flush of their creation i.e. before they entered dormancy. Chapter 3 found non-linear relationships in bud probability of branching with differences in bud location along GUs. Visible differences between buds (such as size, colour, or texture), and the time of appearance of any difference, may provide clues to why some are receptive to florigen later in their life and some not. In decapitated pea seedlings, the buds which are most likely to form branches are the largest ones (Stafstrom and Sussex 1988), known to have formed a greater number of nodes and primordia while the parent GU was growing (Gould and Cutter 1987). In *Juglans regia* (Persian walnut) trees, buds in the middle of GUs were larger in length and breadth, and contained more pre-formed leaf primordia. Buds in these positions on intact GUs were more likely to branch than the smaller ones at either end of the GU (Sabatier and Barthelemy 2001). However little information has been published on the relationship between flowering patterns along a GU and bud morphology, in any species. While the process of formation of buds along a GU with different flowering behaviour has been observed before (Larkin *et al.* 1990), no differences in morphology were recorded.

8.2. Materials and methods

8.2.1. Time of microscopic differentiation

It was hypothesised that there was no difference in the number of microscopic floral buds forming around the year. Over the period February to April 2013, axillary buds from 13 year old *Macadamia integrifolia* × *tetraphylla* variety 842 trees were collected once a month, from an orchard near the Glasshouse Mountains township in south east Queensland. The study was designed to begin before the summer flush when temperature studies suggested that evocation may occur, and continue throughout the year in months when inflorescences were not visible to the unaided eye, which was until April.

Each month, 8 buds were taken from each of 10 undamaged axes, each axis from a different tree. Each axis was mature and hardened wood, and above the 1.5 m skirting height. Buds from nodes on the base half of GUs around four nodes long were selected, those units being on the base half of axes around three GUs long. These nodes were used because analyses of location of flowering indicated they would have the highest rates of flowering. Nodes were cut with at least 5 mm of wood on either side, wrapped in plastic and then newspaper, and transported on ice to the laboratory. The highest bud at each axil was excised from the woody stem and immersed in 0.05 M phosphate buffer pH 6.9 with 3% glutaraldehyde for 24 hrs at 4°C. Buds were then rinsed in phosphate buffer without glutaraldehyde for 15 minutes, and then stored in fresh phosphate buffer at 4°C.

In preparation for microscopy, 40 buds from each month’s sampling were dehydrated in an ethanol series. This involved submerging the buds in ethanol and then microwaving them at 240 W for 40 seconds, one time each using 30%, 50%, 70% ethanol, in that order, and then three times in 100%. Buds were then critical-point-dried with CO_2 and then dissected, as per Bennel (1984), removing outer bracts, hair and one or more primordia. Dissected buds were then sputter-coated with gold, and viewed using a Jeol Neoscope JCM 5000 scanning electron microscope. Buds with meristems not damaged by the preparation were identified as vegetative, floral, or undifferentiated, by the shape of their meristems and number and shape of primordia, based on the descriptions and photographs in Bennel (1984), and photographs in Moncur *et al.* (1985). Flat meristems covered by one whorl of primordia under the bud bracts were identified as undifferentiated (Figure 8-1). Flat or slightly bulging triangular meristems with swellings at each corner, forming a second whorl of primordia (not counting the bud bracts), were identified as vegetative (Figure 8-2). Highly domed, round meristems with thin flaps on their flanks forming involucral bracts were identified as floral (Figure 8-3). Differences between sampling times (months) in the proportions of buds in different categories were analysed with Chi Square tests.

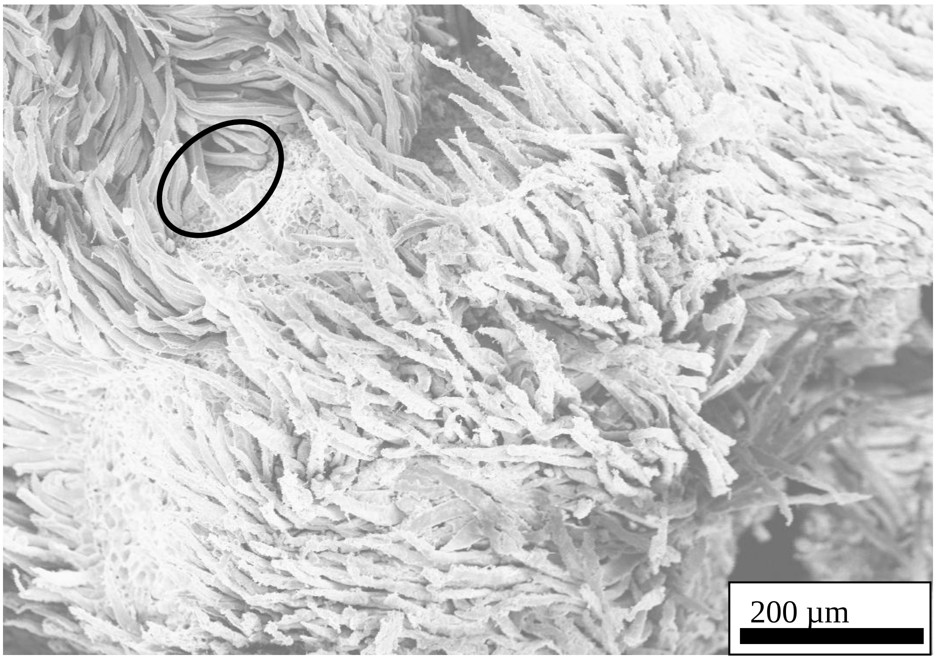

Figure 8-1. A dormant axillary meristem of *Macadamia integrifolia* variety 842.
The small, flat meristem (circled) is between the two remaining large, hairy (undetermined) primordia, and behind the snap scar where a third primordia been removed. Also removed to enable this view were the two bud bracts, the scars from which are partially hidden by surrounding hairs.

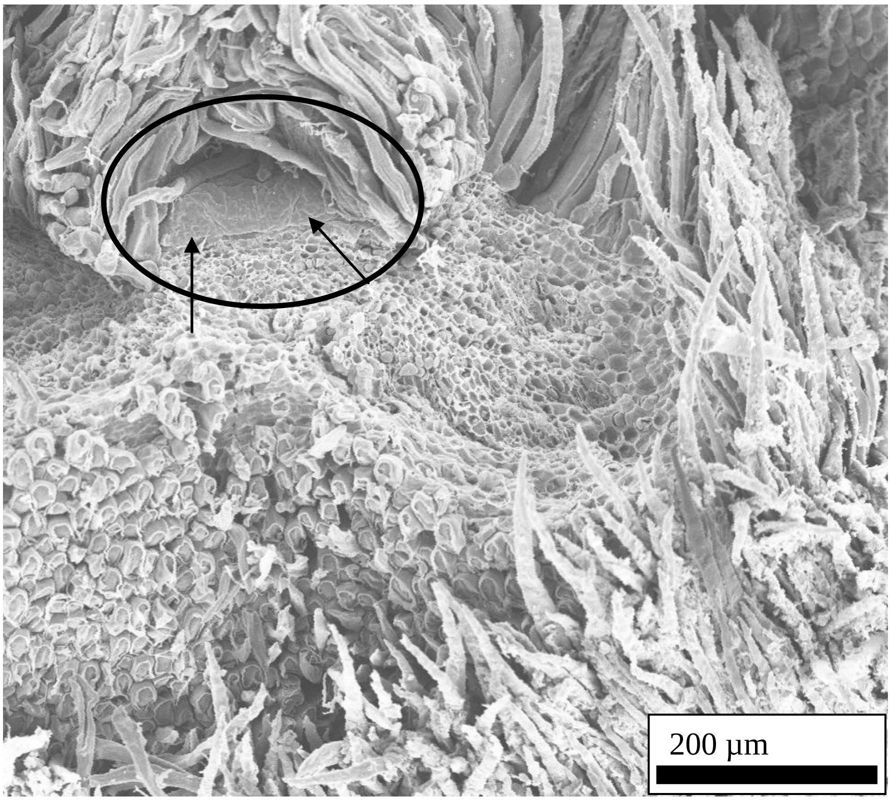

Figure 8-2. The triangular vegetative axillary meristem of *Macadamia integrifolia* variety 842, between two hairy leaf primordia.
Arrows point to the front two (of three) swellings at corners of the meristem where new leaf primordia are beginning to form. The rough hair-free surfaces are the snap-scars from removal of one of the outer whorl of leaf primordia and the two bud bracts.

8.2.2. Bud morphology at formation

During March 2014, recently pruned *Macadamia integrifolia* variety 741trees and *Macadamia integrifolia* × *tetraphylla* variety “A4” trees growing outside in pots at Nambour, south-east Queensland, began to flush. During April, towards the end of this flush, buds from GUs at various stages of formation were collected, in order to measure their size and examine the structure of buds along their lengths.

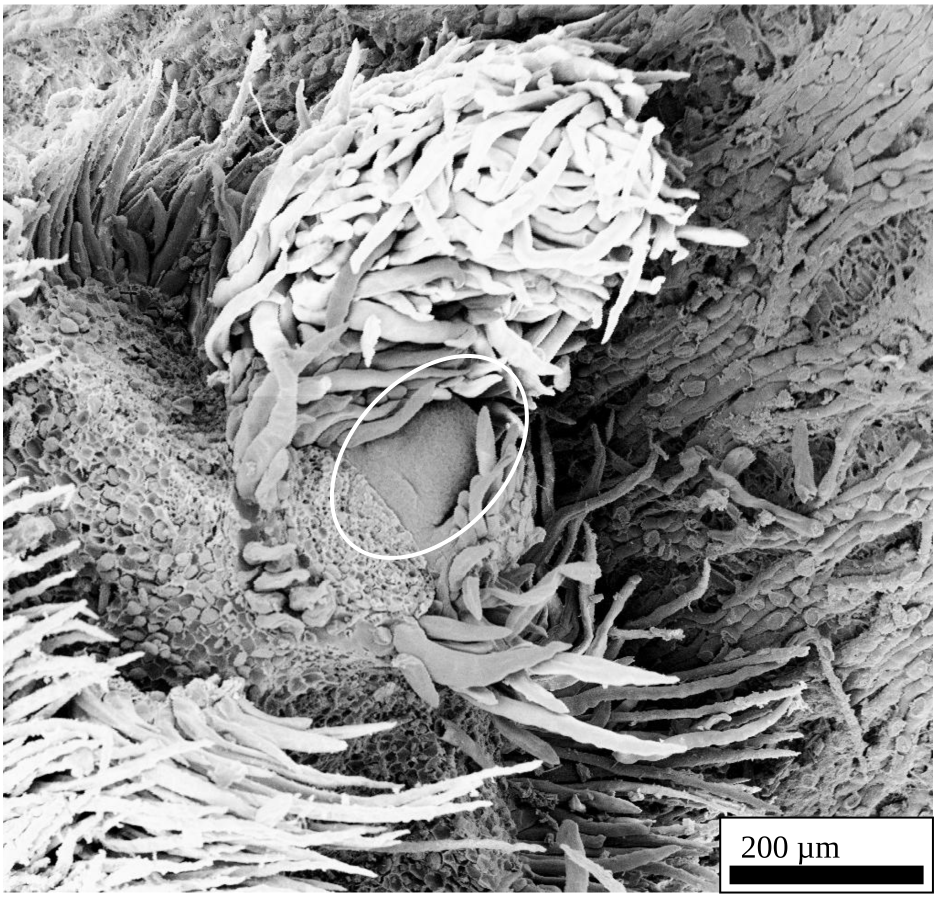

Figure 8-3. The dome-shaped floral axillary meristem of *Macadamia integrifolia* variety 842, between one large and one small hairy outer involucral bracts.
The rough hair-free surfaces are the snap-scars from removal of one of the outer whorl of involucral bract primordia and the two bud bracts. The small crease at the base of the dome is the first morphological sign of an inner involucral bract.

Twenty-two variety 741 buds were collected from a total of six GUs that had recently ceased expanding, the latter indicated by the presence of apical bracts over the axis tip and a change of colour from green to brown. The position of each bud's node along its GU was recorded, and the length of each bud's outer bracts were measured against a haemocytometer grid under a Wild Heerbrugg compound light microscope. Differences between node positions in bud sizes were analysed using Kruskal –Wallis tests (for comparing all positions to each other at once) and Mann-Whitney tests (for comparing two groups of positions).

Dissections of dozens of variety A4 buds under the light microscope were carried out to identify categories of developmental stages. The categories were based on appearance of buds from both recently extended GUs and non-extended GUs that were hardening (see results). One bud from each node of thirteen extending GUs were then dissected and classified into these categories - fifty-two buds in all. These GUs were of many different lengths i.e. composed of different numbers of nodes. The order of formation of each bud's node along its GU (the same as its position on a GU that has finished extending), and the number of nodes from the parent axis' apical meristem (SAM) each bud had gained by the time of sampling were also recorded. Differences in stage of development between different node positions and between different numbers of nodes from the SAM were analysed concurrently, using ordinal logistic regression with a logit link function.

8.3. Results

8.3.1. Time of microscopic differentiation

Some buds of each type – floral, vegetative and undifferentiated - were successfully prepared and viewed. Among buds for which clear images were obtained, there was no significant difference between months in the proportion that were floral as opposed to vegetative ($P = 0.093$). Nor was there any significant difference in the proportion of floral buds as opposed to non-floral (vegetative or undifferentiated) ($P = 0.055$). While this last figure was close to significant, there was no trend over time apparent.

The proportion of meristems that could not be classified, due to unclear results from damage to meristems by the preparation/dissection process or due to debris obscuring vision, was very high – between a half and four-fifths. The use of brittle material did not facilitate precise dissection, despite this method of drying before dissection to take advantage of brittle tissues being recommended (Bennel 1984). As a result this technique proved to be too time consuming to process the number of buds planned for this survey.

Table 8-1. Number and percent of buds identified as undifferentiated, floral or vegetative, on 13 year old macadamia trees variety 741, during February, March or April 2013.

	Feb		Mar		Apr	
Bud type	Count	%	Count	%	Count	%
Undifferentiated	7	41	9	69	3	43
Vegetative	4	24	3	23	0	0
Floral	6	35	1	8	4	57

8.3.2. Bud morphology at formation

On GUs that had finished expanding, buds of the most distal leafy node were larger than other nodes on the GU, averaging 2.2 mm ($P = 0.0225$). Many GUs which were not extensions of already existing axes - they were the first GU of a new axis - had leafless bracts at their base.

Axillary bud development stages in variety A4 were defined after dissection of around 50 buds. These definitions are presented in Table 8-2 and some in Figure 8-4 to Figure 8-7. External differences between buds were able to be seen, as were (unexpectedly) differences in bud structure inside the bud bracts.

Table 8-2. Axillary bud development stages of macadamia variety A4.

Development stage	Description of bud bracts	Description of bud inside the bud bracts
a	No visible sign of bud	-
b	Smooth patch – 0.0 5 mm high	-
c	Bract primordia visible, 2, no hair – bracts 0.1 mm high	-
d	Bract hair forming (appears clear) but can still see lobes of the two bract primordia – bracts 0.2 mm high	-
e	Thick bract hair (appears white), obscures lobe forms – bracts 0.4 mm high	-
f	Some pigmentation of bract hair (copper among white)- bracts 0.6 mm high;	Whorl of 3 (or 4) primordia just visible on meristem. Either green hairless primordia around flat or slightly bulging meristem, OR smooth domed meristem pale in colour
g	Fully pigmented bud bract hair (copper) – bract 0.8 mm high;	Either white hair on green leaf primordia and sometimes second whorl forming, OR rachis forming under the domed meristem with bumpy common bract primordia visible on rachis flanks, and sparse feathery hairs on tips of inner involucral bract primordia

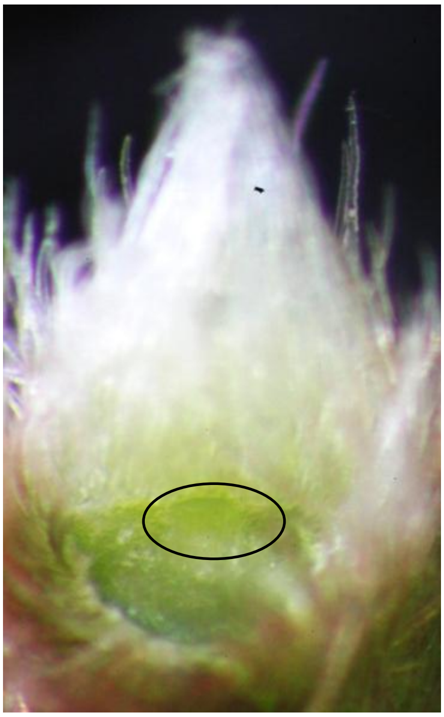

Figure 8-4. Bud development stage “b”, in macadamia hybrid variety A4.
Bud appearance at stage “b” is a smooth green patch, above the line of the rough green scar from the removed leaf primordium, just encroaching on the hair line of the younger whorl of leaf primordia above it (x75 actual size).

Figure 8-5. Bud development stage “d”, in macadamia hybrid variety A4.
Visible at stage “d” are bud bract primordia with hair (x40 actual size).

Figure 8-6. Bud development stage “e”, in macadamia hybrid variety A4.
At stage “e” thick hair on the bud bracts obscures their individual shapes completely, making them appear a single growth. Here subtending leaves and the axis apex have been removed (leaving a green scar visible under the left bud) (x30 actual size).

Figure 8-7. Bud development stage “g”, in an apparently floral bud, of macadamia hybrid variety A4.
At stage “g” a rachis appears to be forming, above the dashed line which shows the top of the dissection slice surface that goes diagonally down through the parent axis. This slice has revealed the face of the bud normally hidden by the parent axis. On the other side of this structure, bud bracts have also been removed. Bumpy common bract primordia are just visible on the sides of this ‘rachis’, and feathery hairs at the top right of the tip are probably the tip of an involucral bract, attached at the base of the rachis on its far side (x30 magnification).

The stage of development of buds on green, still extending GUs was related to both the order of bud formation by the meristem ($P = 0.010$), and the number of nodes from the SAM ($P < 0.001$). Development stage had a strong positive linear relationship with the number of nodes from SAM (Figure 8-8), and a strong negative linear relationship with order of formation (when allowing for number of nodes from the SAM, Figure 8-9) . r_s values for correlations could not be calculated due to the small set of non-normally distributed data. There was no interaction detected between these two variables. There was no morphological difference detected between buds from nodes just under the top of the GU - which were far more likely to branch than those at other positions – and any other buds on the GU.

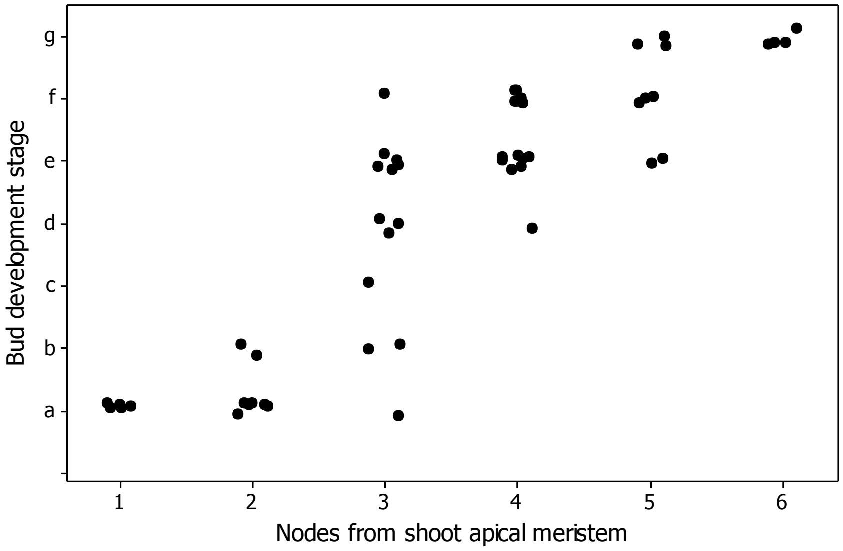

Figure 8-8. Relationship between axillary bud development and the number of nodes from the shoot apical meristem at which buds are located, on extending growth units of various sizes, in macadamia hybrid variety A4.
Stages of development are defined in Table 8-2. Data symbols at identical points are offset to show relative frequency.

In the course of examining the very beginning of axillary bud formation, apical buds were also dissected. Two whorls of primordia were usually found inside the leaflets with tips still touching that formed the outer whorl of the apical bud. Axillary buds of nodes outside / below this apical bud outer whorl - i.e. 4 or more nodes from the apical meristem) - had usually reached stage “e”, meaning that they had already formed their own whorl of primordia, before entering dormancy. Buds one or two nodes older, which would be two or three nodes outside the apical bud outer whorl and five or six nodes from the SAM, usually reached stage “f” or “g”, and so may already be determined as either floral or vegetative buds. Of the 52 buds dissected for development stage categorisation, six appeared to contain a domed meristem or rachis and thus appeared floral. The small number of these buds prevented any analysis for patterns in their location.

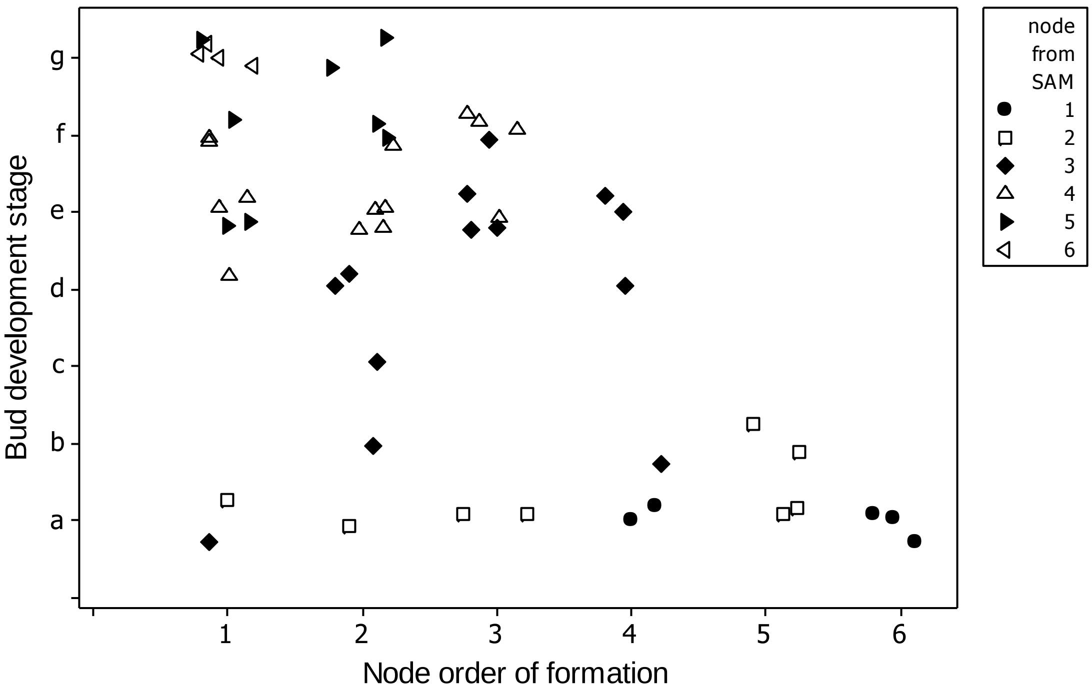

Figure 8-9. Relationship between axillary bud development and the order of formation of a bud's node, on extending growth units of various sizes, in macadamia hybrid variety A4.
The number of nodes from the shoot apical meristem (SAM) of each bud is also shown, to illustrate the lack of interaction between this and order of formation. Stages of development are defined in Table 8-2. Data symbols at identical points are offset to show relative frequency.

8.4. Discussion

In the study of timing of microscopic floral differentiation, the null hypothesis was not disproved: no clear difference between the months of sampling in the number of meristems initiating floral primordia was found. This may have been because there is no peak in differentiation in the months studies, or in any months - differentiation may instead occur over much of the year. Finding no peak in differentiation may also have been because insufficient numbers of buds were examined, and so by chance population differences were not reflected in the buds sampled.

The examination of buds from green GUs unexpectedly found domed organs resembling early stages of inflorescence growth as photographed and described by Sakai, *et al.* (1982), Bennel (1984), and Moncur *et al.* (1985). A small number of such organs were viewed, and at much lower magnification and lower resolution than with electron microscopy, so the results should be confirmed with further studies. If these structures are indeed floral, the time of evocation then would be directly after bud formation, during axis extension in the growth flush period, before the buds enter dormancy. This finding then would help answer the question of the first investigation of this chapter – evocation may occur during the late summer vegetative flush. However, as this flushing occurred a month or two later than the normal flush season (due to pruning), even with confirmation of floral bud sightings on green stems it still cannot be firmly stated that bud evocation occurs during such flushes in macadamia’s normal phenological cycle. Nor would it exclude evocation happening at other times of the bud’s life.

The investigation into morphology of newly formed buds at different positions along GUs did also find differences in bud size and in degree of development. Preformed nodes appeared to be present in all of the axillary buds formed first or second in a GU i.e. those at the base of the GU, but only some of the third and fourth nodes formed. These differences were related to duration of development available before the end of the flush period, so the first buds formed reached a higher stage of development by the end of the flush season than the last buds formed. These larger and more developed buds are thus located in the same position as most inflorescences (Chapter 6). It may be that the more mature buds are most able to respond to the presence of florigen at times of induction. This would differ to the situation in *Ipomea nil* (Japanese morning glory), in which axillary buds appear to have the ability to respond to florigen at only one node removed from the meristem, when they are just a patch of tissue with no bract formations and long before forming their own whorls (Larkin *et al.* 1990). However *I. nil* is a herbaceous creeping species, and so differences in growth strategies between it and macadamia may be widespread.

Larger buds were also found in the axils of the leafy nodes closest to the base of the hardening GUs, but on these GUs no linear correlation of size with bud position existed. Thus bud size on GUs that have finished extending did not match the gradient of flowering probability, while size and development stages in still expanding GUs did. This is probably due growth of axillary bud bracts continuing later in the flush season than growth of axillary meristems.

Two whorls of primordia were usually found inside the apical bud leaflets of axes that were extending. This suggests that the most proximal bud or two on a GU formed by axis extension – about 50% of GUs (Chapter 3) - may be created at the end of the preceding flush, but held within the apical bud until the next flushing period. This pre-formation of lower nodes on the parent GU may be a head-start in growth that then enables the pre-formation of whorls within their buds. Pre-formation of nodes may also change the behaviour of the axillary buds on those nodes because of the different range of conditions they are exposed to while contained in the apical bud. If, like the axillary meristems of *I. nil,* macadamia axillary buds are determined when barely visible with a microscope, it may be that the axillary buds within the apical bud are the only ones receptive to flowering signals that may be circulating between flushes.

To detect the formation of the first floral primordia, light microscopy with clear magnification at x100 would give sufficient detail while retaining the ability to manipulate the sample while viewing. The latter is not possible with electron microscopy, but very useful in determining three-dimensional shape which helps in differentiating between floral and vegetative meristems and primordia. Together with the large amount of time required for sample preparation for electron microscopy, this makes light microscopy the preferred option for at least initial stages of future studies of this type. This of course relies on there being fairly similar sized meristems in any other varieties of macadamia used.

The two simple investigations attempted here have converged, at a preliminary finding that could be the most important, of the chapter; that macadamia floral evocation may occur during vegetative flushing, many months before floral growth is visible to the naked eye. It may also occur even earlier, when the buds at the base of the newest growth unit are inside the apical bud during the period between flushing. Inflorescences can emerge on growth units one year old or younger (Nagao *et al.* 1994), but most inflorescences emerge on older wood. If evocation did occur during GU formation, the older-wood inflorescences have either remained dormant for a long time or axillary meristems can also be evoked at a later date. Clarification of these possibilities with replication and use of naturally flushing trees is important, as the effect of tree management efforts on flowering (and thus many situations on yield), is likely to be dependent on the timing of that management in relation to evocation.

8.5. References

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Chapter 9. Discussion and Conclusion

In 12 year old trees of *Macadamia integrifolia* variety 741, distribution of new stem outgrowth from axillary buds along an axis was found by this study to depend on node position along a growth unit (GU), and GU position along the axis, in that order of importance (Chapter 3). Such a relative contribution of architectural attributes to axillary bud branching behaviour has not been previously reported in any species. In non-extending axes, more new stem outgrowth was found in GUs closer to the apex than the base, but in extending axes the effect of GU position is not linear. Neither of these patterns matches that found in apple trees (Costes *et al.* 2003), in which more branching occurs at the base of a GU, but this may be because that study did not examine extending and non-extending axes seperately.In extending axes a third factor appears to influence new stem distribution: after accounting for the first two stronger factors, it appears that there is more branching on nodes further from the tip (Chapter 3). This is at least partly because of the lack of sylleptic branching, which may be due to buds not being able to grow fast enough to form a new stem within the flush period. But it also suggests that apical dominance (AD) is part of axillary bud dormancy in macadamia, despite removal of the apex by pruning not increasing the number of new stems formed in the winter-pruned axes studied here. This is different to the response to tip removal seen in many other species, in which the number of new stems is increased (Cline 1991). The lack of change in macadamias suggests the new stems are able to suppress (via apical dominance) all lower buds on the GU. Pruning did change the location of new stems, a similar result to that of Owens and Ewers' (1997) work in Eastern Redbud trees (*Cercis canadensis*). Here, after pruning macadamia new stems cluster in the node or two below the pruning point (Chapter 6), rather than on the nodes below the growth unit tops.

Around a third of the axes extended during the year of this study (Chapter 3). Axes which extended branched more than those which did not, which may indicate AD in macadamia is weak, but it is possible that extension and branching occurred in different flush periods of the year studied. The tree nutrition experiment (Chapter 4) suggests AD can vary in strength in macadamias, similar to previous findings in *Epilobitum ciliatum* (Irwin and Aarssen 1996) and *Verbascum thapsus* (Lortie and Aarssen 1997), both herbaceous plants, but contrary to results in *Triplochiton scleroxylon*, a tropical tree which did change branching habits with changes in nutrition (Leakey and Longman 1986). Axes of trees receiving no fertiliser sometimes extended without branching, but never branched without extending. The addition of a moderate amount of fertiliser but no extra water increased apical growth more than it increased branching. Thus it appears that the apex had preferential use of the extra mineral resources over axillary buds (Chapter 4).

The distribution of emerging inflorescences between architectural structures of axes was examined by this study in 12 year old trees of variety 741 (“741/12”), 13 year old trees of variety 842 (“842/13”), and 5 year old trees of variety 741 (“741/5”) (Chapter 5). In 741/12 and 842/13 trees (the ‘mature’ trees), node position within a GU, GU along the axis, and GU size in nodes determined inflorescence distribution, in that order of importance. This assessment of relative importance of architectural structures on floral axillary bud emergence has not been previously reported.

Nodes in the middle of longer GUs were the nodes most likely to flower in 741/12 trees, much like the pattern seen within the GUs of apricots (*Prunus americana*) (Costes and Guedon 1996). However on the majority of GUs, which were shorter, likelihood of flowering increased with node proximity to GU base, unlike any previously published patterns in perrenials. Nodes at the base of 842/13 GUs also flowered most, with GU size having relatively little influence in this group. In both groups of mature trees flowering was greatest in these nodes just described when they were on a GU at the base of an axis. Flowering increased with GU proximity to axis base also in the 741/5 trees (the ‘young’ trees), thus this relationship was the strongest architectural relationship with flowering that was consistent across all three groups studied. In the young trees the best model of flowering location was composed of axis size in GUs plus the same three variables relevent to mature trees – node position on GU, GU position on axis, and GU size in nodes. However the relationship between GU size and probability of node flowering was opposite to that found in the mature trees - nodes on smaller GUs flowered more often. In the longest GUs the node flowering most was second from the GU base, not the very base node, but in the majority of GUs (those closer to the average length) the likelihood of flowering increased with node proximity to the GU base. The differences in flowering location relationships with architecture between age groups was in size of GUs and axes, not position of structures within the architecture, and so they are proabably due to differences in vigour, and in the number of growth seasons since reaching floral competency.

The type of lateral shoot formed by an axillary bud – floral or vegetative - is closely associated with the position of the bud along the GU (Chapters 3 and 5), and thus with the time at which the node was formed within the flush season. The nodes formed immediately under the bract that covers the resting apex i.e. as the flush period was finishing, were by far the most likely to grow out as new stems, and they rarely formed inflorescences. Nodes formed just before this time, i.e. those located a few nodes under the bract node, have a low-to-moderate probability of branching and a moderate probability of flowering. Towards the base of the unit nodes become unlikely to branch and more likely to flower. The relationship between likelihood of flowering and node position along a GU is fairly linear, and especially so for the sizes of GU that make up the vast majority of GUs, of three or four nodes. However the relationship between a node's position and its probability of branching appears was not linear or a simple curve, and this remains the case when node order is changed to incorporate preformation of one or two nodes. This suggests nodes are predisposed to become either stems or inflorescences, according to physiological changes over the flush season of their formation, and not simply because of constant gradients of nutrients and plant growth regulators (PGRs) in the vessels along the GU. This is similar to findings in apple and peach, in which different bud fates were found to be linked to different combinations of growth rates of organs at the apex (Fulford 1965; Kervella *et al.* 1995). Such differences in bud behaviour have also been proposed to be due to different sensitivities of buds to PGRs, including florigen (Trewavas 1982) – meaning some buds are predisposed to certain courses of development. A third possibility is that some macadamia axillary buds go past predisposition and become florally determined during the flush of their formation. The finding in this study of microscopically floral appearance of some buds by the end of the flush period in which they were created (Chapter 8) makes investigation of this possibility worthy of inclusion in future studies.

Chapter 7 of this thesis suggested that flowering in *Macadamia integrifolia* × *tetraphylla* involves at least two stages which require different temperatures. More axillary meristems complete an early stage (or stages) of inflorescence formation – probably evocation and determination - at warmer night temperatures of 19°C than cooler ones of 11.5°C. This fits well with the results of Stephenson and Gallagher's 1986 study, in which only macadamias kept at 20 ºC, and not those kept at 15 ºC or below, flowered after transfer to night temperatures of around 11 ºC. They are supportive of Sakai *et al.*'s 1982 results, in which macadamias transferred from night temperatures of over 21 ºC to cooler night temperatures (12 ºC to 21 ºC) produced inflorescences faster at the coolest end of the range.

The results here suggest that, of those axillary meristems that completed the early stage to become floral buds, more floral buds completed the later stage to emerge as inflorescences in the cooler night temperatures of 11.5°C. Promotion of evocation by warm temperatures may also be why larger GUs are more likely to be floral in 741/12 trees – the warmer temperatures that increase evocation could also increase the number of nodes on a GU. The temperatures so far identified as optimum for the early stage of floral growth are very similar to those of the late summer flushing time in *Macadamia integrifolia's* natural habitat of south-east Queensland (Bureau of Meteorology 2013). However this early stage of development still occurs at lower temperatures, just at a slower rate (Sakai *et al.* 1982). Thus if evocation takes place during flushes, it may be more common in summer flushes but could occur in spring flushes as well. If an axillary bud does not need to be released from dormancy to respond to florigen, evocation may take place throughout non-flushing periods as well. Dormant axillary buds have been shown to be metabolically very active (Stafstrom and Sussex 1988), and so dormant buds may be able to react to signals such as florigen. However the change in likelihood of flowering along a growth unit (Chapter 5) may be an indication that evocation occurs during bud formation. The increased proportion of floral buds on nodes created early in the formation of a new growth unit – at the growth unit base – may be because of the warmer temperatures earlier in the summer flush period.

If floral evocation did occur in axillary buds during the flush period in which they are formed, location of flowering could also be effected by carbohydrate gradients. On a growing GU which does not yet produce enough photosynthate to support itself, carbohydrates are sourced from the rest of the tree (Newell *et al.* 2002), and would be higher in concentration at the lower nodes of the GU than the upper. This gradient probably also exists in mature GUs on extending axes, as the growing apex draws sugars from lower down the GU, and so gradients in carbohydrate concentration could also affect floral evocation taking place on extending axes during any flush period. Biochemical assays testing the potential physiological patterns behind the bud behavioural patterns proposed here would be a useful form of future research into understanding control of flowering and branching in macadamia.

This study also found that axillary buds at some of the most frequently flowering positions - those at the node or two from the base of GUs - appear to be pre-formed (Chapter 8). Apical buds contain at least one and possibly more whorls of primordia, which form the base node or nodes of any and every subsequent GU of an axis. The base node of the base GU of all axes is also pre-formed, as axillary buds have a whorl of primordia visible from the time they leave the apical bud. In *Ipomea nil*, axillary buds can be evoked when they are just visible above the microscopic leaf primordia (Larkin *et al.* 1990). If macadamia is similar, all its preformed axillary buds within dormant apical buds may be held in a state where they are receptive to florigen for far longer than buds which are formed during vegetative growth flushes, which develop relatively quickly. While there was a gradient in axillary bud morphological development that paralleled the gradient in probability of a node flowering (Chapters 5 and 8), there was no clear morphological difference between the highly branching node just under the unit top and the other nodes on the GU that branched far less frequently (Chapters 3 and 8). This does not mean that there was no non-morphological difference between buds at this and other positions. But it could also be that there is no large difference between this bud and its neighbours, and that the far higher frequency of branching on this node is due to a combination of i) an even gradient of decreasing flowering potential with proximity to the GU top, and ii) the apical dominance in later flushing periods of the top-most stem over lower buds.

A number of the findings of this study suggest that floral buds are sensitive to at least one of the components of apical dominance - either carbohydrate limitation or suppression by auxin/strigolactone production. In variety 741, apical extension inhibits inflorescence formation, as does branching (Chapter 4). Damage to the axis tip (of young 741 trees), or pruning in the middle of the axis (of older 741 trees) each increased the probability of the axis flowering and the total number of inflorescences on it (Chapters 4 and 6). Flowering was more likely on nodes of the base unit of an axis (Chapter 5), in all groups of trees studied. These nodes are closer to the main source of stored carbohydrates in the larger limbs and roots of the tree (Newell *et al.* 2002), and so possibly at locations with a higher concentration of carbohydrates. The number of inflorescences per remaining node increased as pruning took place in GUs closer to the base (Chapter 6), perhaps because the carbohydrates available from the parent axis was shared between fewer nodes. Again, biochemical assays are the logical next step to address these possibilities. The change in flowering behaviour with GU of pruning was accompanied by changes in branching behaviour, resulting in high flowering-to-branching ratios when pruned in the GU just above halfway along the axis, and lowest flowering-to-branching ratios when pruned at the base GU. With further investigations, it may be found that this pruning could be used to alter these ratios for maximum yield, at least in small trees in which pruning is more practical. However the aim of such manipulation need not be an increased proportion of growth invested in flowering - not all increases in inflorescence production will result in increased yield (McFadyen *et al.* 2008). Perhaps a reduction in excessive flowering (i.e. a reduction in inflorescence density, to that where yield begins to plateaux) may conserve resources that could be used to hold a higher proportion of the nuts on the tree to maturity.

New inflorescence distribution reacts differently to pruning than does new stem distribution, and so inflorescences may not be sensitive to both components of apical dominance. Pruning at either end of the middle GU of three-GU-long axes was found by this study to result in a change in location of new stem outgrowth, from the top node of remaining GUs to the node or two immediately below the cut, but little outgrowth below that (Chapter 6). The lack of branching on any nodes lower than one or two nodes below the pruning cut is probably caused by auxin/strigolactone from the new stems formed immediately below the cut preventing successful transition from dormancy at lower nodes. But extra floral emergence occurs at nodes all along the base GU, starting two nodes below the cut. The PGRs that are controlling further vegetative bud release seem not to suppress the floral growth, at least not the later stage i.e. emergence. However as branching could happen in either of the two flush periods, and as the timing of the early stages of flowering is unknown, further investigations looking at the timing of these events along with their relative locations is necessary to be clearer about the mechanism/s by which inflorescences relate to vegetative growth.

The knowledge gained in this study about the distribution of inflorescences and new stems enables more efficient microscopy studies of the early stages of shoot formation. Among the groups of trees studied here, relatively efficient collection of floral buds can be achieved by using mature trees of variety 842, and collecting nodes at the base of GUs four nodes long, where those GUs are found at the base of an axis. Aound 21% of such nodes formed an inflorescence in the year studied here, more than three times the average rate of 7%.

This study also enabled the identification of buds most likely to branch. To select buds most likely to form new stems before the shoot is visible in variety 741 trees, buds should be taken from axes that have recently added a new GU. They should be taken from nodes second from the GU top (i.e. that below the bract node), of a GU second from its axis tip (Chapter 3). In variety 741 around 42% of these nodes formed a new stem in the year-long period studied, seven times the average of 6%.

The findings of this study about vegetative regrowth after pruning (Chapter 6) and inflorescence location (Chapter 5) can help us understand the effects of pruning, including hedging, on yield. While pruning did not increase the number of branches in this study, it did change their location so that they all formed just below the cut (Chapter 6). In whole tree hedging this would result in the dense outer layer of leaves noticed by field workers, which changes light distribution in the macadamia canopy, and possibly increases premature fruit drop. This also reveals a new aspect of how hedging may have a long-term effect on flowering. Because pruning cuts bring the location of new stems up to the nodes below the cut, new axes will be forming very close to the hedging line. Thus most new axes are very likely be removed by the next round of hedging (the next year or two), before their buds can mature to the point of substantial inflorescence production. At the axis scale studied here, this would result in medium to long term decline in inflorescence production. On the scale of a whole tree, there may be differences to regrowth location, which could mitigate or accentuate such a decline. Being clear about this is important enough to industry to warrent further investigation.

The increase in flowering with tip pruning (Chapter 6) is another area worthy of follow-up research. It may result in a method of increasing flowering and thus possibly yield in young trees, especially if pruning the highly floral ‘stunted’ axes (Chapter 5). This is a possible alternative to dense planting, which results in shading problems when the trees mature. Further investigation of the effect of fertiliser-to-water ratios on canopy openness (Chapter 4) could also provide useful techniques to industry; here in particular the transferability of the result from potted trees to trees planted in the ground needs to be tested. However the question of when floral induction and evocation takes place in macadamia may be the most important area for ongoing study. It remains unanswered by this study, as the sighting of floral buds on GUs that had not yet hardened (Chapter 8) is regarded as a tentative one. The flowering location information collected in this thesis can substantially increase the efficiency of future studies into microscopic development (Chapter 5), and thus a larger scale investigation is far more manageable than in the past. This area is an important one for future study, as the timing of floral induction and evocation has implications for further understanding of much macadamia physiology and for crop production.

This project has established that there are clear patterns in location of inflorescences and new stems within and between the architectural structures of *Macadamia integrifolia*. The results show that inflorescence location and new stem location have some similar controlling factors – most importantly node along a growth unit, and growth unit position along an axis - and that these affect the distribution of the two types of lateral shoot in different ways. The results also show that there are some controlling factors that the two types of shoot do not share. The results suggest that alternative pruning and nutrition regimes may increase sustainable productivity in young orchard macadamias. The findings of locations of high densities of flowering will enable far more efficient studies of the important process of initial microscopic development of inflorescences. The study has also demonstrated that formation of mature inflorescences is a multi-stage process with different temperature requirements for each stage, which are not just a matter of total degree days. It suggests that floral induction may take place much earlier in the phenological cycle than commonly assumed. Although a small contribution, it is hoped these findings will assist in sustainable production of macadamia nuts and protection of wild macadamia trees into our future.

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Appendices

Appendix A. Pot media

1. Mix equal quantities sand, composted sawdust, composted pine-bark

2. Pasteurise

3. Add fertiliser, per cubic metre:

    1 kg fine superphosphate

    1 kg fine lime

    1 kg fine dolomite

    3 kg 9-month slow-release NPK (18 : 2.6 : 10)

    1 kg 3- month slow release NPK (16.3 : 3.5 : 10)

    1 kg slow release micronutrients

    0.5 kg coated iron

Appendix B. Fertiliser

Osmocote Plus Trace Elements Native Gardens

Nutrient	**Proportion**
Urea	60%
Potassium sulphate	30%
Ammonium nitrate	<10%
Monoammonium phosphate	<10%
Sulphur	<10%
Iron sulphate	<10%
Calcium phosphate	<1%
Ammonium sulphate	<1%
Magnesium oxide	<1%
Magnesium sulphate	<1%
Calcium sulphate	<1%
Iron EDTA	<1%
Iron oxide	<1%
Manganese sulphate	<1%
Copper sulphate	<1%
Zinc sulphate	<1%
Sodium borate	<1%
Sodium molybdate	<1%

Appendix C. Inflorescence removal tests

Background

Low levels of inflorescence emergence (around 10%) were noted in all treatments of an experiment examining relationships between evocation, emergence and temperature. This occurred after mature inflorescences were removed from the trees, during the pre-experiment preparation. This investigation checked to see if inflorescence removal could be the cause of this late emergence.

During Stephenson and Gallagher's (1986) temperature of macadamia flowering experiment, constant low numbers of inflorescences emerging was recorded (cultivars 246, 344, 660).

Successive flowering – several cohorts of inflorescences developing at different times - in has been noted in macadamias by Nagao and Sakai (1990) and Wilkie (2009), and observed personally. Rapid replacement of removed inflorescences has been recorded by Stephenson *et al.* (1989) (cultivar "Own Choice"). This occurred for several successive rounds of removal in one season, through the fruit set season of spring and into summer. Own Choice however is known to flower sporadically anyway.

Wilkie *et al.* (2009) removed racemes from cultivar A203, but trees appeared not to replace them (pers. com.).

Are subsequent cohorts of developing inflorescences due to 'spare' dormant inflorescences, pre-evoked and awaiting detection of prolonged conditions favourable to flowering/fruiting for released?

Method

4 year old trees

In early August, near Nambour Qld, inflorescences were removed from six 4 y.o. outdoor potted macadamia trees, three of variety A4 and three of variety A38. Five weeks later new inflorescences were counted on these trees, and on three A4 and three A38 trees, also 4 y.o. potted trees, from which no inflorescences had been removed.

9 year old trees

In late August, near Beerwah Qld, eight 9 y.o. macadamia orchard trees of each the varieties A4 and A38 were identified to be of similar size and vigour. Within these groups, trees were randomly allocated to either the control or treatment group. One north facing branch with a base circumference of between 8 and 10 cm was selected from each tree.

Inflorescences were removed from the treatment trees, with those on the selected branches of those trees counted as they were removed. Inflorescences on the selected branches of control trees were marked if shorter than 2 cm, to aid in identifying old from new inflorescences later in the experiment.

[For two A38 trees, every inflorescence on the tree was counted.]

Four weeks later new inflorescences on branches in the ‘removal’ groups were counted. New inflorescences were fully developed by this time.

Results

4 year old trees

After removal, new inflorescences emerged from A4 trees but not from A38. New inflorescences also emerged from A4 control trees and from one A38 control tree.

plant	**var A**	**trtmnt**	**original infs**	**new infs**	**% new**
92	4	c	34	21	62
93	4	c	9	5	56
211	4	c	0	8	all new
44	38	c	6	1	17
61	38	c	6	0	0
51	38	c	3	0	0
207	4	r	1	8	800
88	4	r	19	5	26
209	4	r	4	1	25
55	38	r	24	0	0
8	38	r	4	0	0
43	38	r	1	0	0

9 year old trees

Both A38 and A4 trees grew a very small number of new inflorescences after removal of the initial ones. In A38 the number of new inflorescences was around 0.2% of the number previously on the branch. In A4 the number of new inflorescences was about 0.7% of the number previously on the branch.

It was estimated that around 20 inflorescences grew on each A38 tree after removal. As the average number of inflorescences on the A38 trees counted was 2937, this would give a whole-tree replacement rate estimate of 0.7%.

By the time of counting the new inflorescences, they had fully elongated. Thus those in the control group were not able to be distinguished from the old inflorescences.

tree	**variety**	**Initial inflorescences**	**New inflorescences**	**% regrowth**
B	A38	80	0	0.0
E		197	1	0.5
F		149	0	0.0
H		329	1	0.3
J	A4	858	2	0.2
L		421	2	0.5
M		284	4	1.4
P		719	5	0.7

Discussion

Among the younger (4 y.o.) trees, inflorescences emerged from only A4s over the duration of the experiment. The numbers emerging were highly variable, and this combined with the low replication prevents decisive statistical analysis. But the ranges of both absolute numbers of new inflorescences and % replacement are similar between the control and treatment group.

Among the older trees, far fewer inflorescences emerged during the duration of the experiment. This may be because of a more condensed flowering or earlier flowering, due either to age or location. While the control data was incomplete, the % new inflorescences was much lower than that occurred during the temperature experiments. Even if a difference between the control and removal treatment could be shown, this level of replacement following removal would still not explain the degree of out-of season flowering which occurred.

In both the young and the older trees, more inflorescences emerged from A4 plants than A38 plants. This may simply be a slight difference in flowering seasons.

These results indicate that inflorescence removal could result in the emergence of extra inflorescences. However the time of the season in which inflorescences are removed could change the effect dramatically. Removal at the end of the flowering season after anthesis, after much energy has gone into inflorescence production, may result in the negligible replacement, more similar to that seen in the field exercise than potted trees, although it has been shown that potted plants can behave differently to plants in the ground (Poorter *et al.* 2012).

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Appendix D. Cut branch tests

Background

The possibility of using cut stems for experiments investigating inflorescence emergence under different temperatures was investigated, as an alternative to using whole trees in a glasshouse, the latter being very energy and thus funding intensive.

Method

Eleven stems were cut from each of 20 yr old macadamia trees, variety 741, and placed immediately in buckets containing 2L of water. Each stem was at least 3 growth units long, many had side branches.

They were randomly divided into two groups. One was chilled at 12ºC overnight for four nights. The other was kept at ambient temperatures over night - 18.7 – 19.7 minimums. Both were kept at ambient temperatures - 25.2 – 26.4 maximums, out of direct light during the day (8 hours).

After four nights of treatment the stems were exposed to ambient temperatures both day and night for six weeks (April 1 to May 15). Twice a week the water in the buckets was changed, and 1 teaspoon of sugar and 150 mg of aspirin added to each bucket. Also at this time the stem-bases were washed to remove algal build up. Every fortnight the stems were examined for any axillary bud outgrowth.

Results and discussion

After four weeks (April 27) most stems had dropped most of their leaves, and were assessed to be dead or dying. Only 2 stems appeared healthy in the control and only four in the chilled treatment.

After 6 weeks no buds had emerged, either vegetative or floral, from either tipped or untipped. At this time parent trees had some bud break of both types.

Other Proteaceae do not keep well after cutting, due to a stem-end blocking response that appears to confer disease resistance upon the parent plant. Cut stems cannot be used instead of whole trees for studies on bud behaviour, unless a treatment counteracting this blocking response is found to be effective in macadamia.