Horticultural Research and Development Corporation
Research Project Ma/0012/Rl:

Limitations to fruit set in macadamia
FINAL REPORT

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Research Summary
This project aimed to pin point some of the factors that may contribute to low yields in macadamias grown in Australia. The basic premise was that when compared with productivity of the same varieties in Hawaii, Australian plantings on average fare poorly.

The research spanned two years and covered crop development and tree performance from pre-flowering to nut maturity Factors studied were: flower number per raceme; supplementary pollination; influence of cross pollination; pollen tube growth; nut set and nut drop patterns;: leaf, nut and whole tree carbon demand and assimilation; influence of girdling; applied plant growth regulators; patterns of endogenous plant hormones.

Virtually all of these had some effects on the pro ress of crop development, but only a few factors appeared likely to have a major influence on final yield. In particular, supplementary cross pollination, in certain variety combinations only, dramatically increased nut set and nut retention to maturity. There were also significant pollen parent effects on quality: nuts from cross treatments had higher nut in shell weights, kernel weights and kernel recoveries.

The second major factor appears to be carbohydrate availability: on girdled branches, the number of nuts produced was either increased or decreased compared with untreated branches, depending on the number of leaves on the branch. Nut size could be decreased by low numbers but not increased by high leaf numbers, compared with ungirdled branches. Clearly if the tree can be manipulated to supply more carbohydrate to the nuts, there is potential to increase yields. A figure of approximately 50 leaves required to support each nut was calculated.

It is concluded that there is substantial scope for yield and quality improvement in macadamia, and this is likely to be achieved through alterations in orchard design and management, and through breeding and selection of new varieties.

Research findings, conclusions and achievements

This project was conducted over two years from July 1990 to June 1992, and was linked to related work funded by CSIRO, New South Wales Agriculture, the University of Queensland and the Australian National University.

The objectives set at the start of the project were:

(1) to determine energy/carbohydrate demand of fruits throughout development
(2) to determines sources of photosynthate and routes of transport in relation to fruit set and development
(3) to determine limitations to initial and final fruit set on per flower, per raceme and per tree basis
(4) to establish patterns of plant growth substance (PGS) levels in fruits and responses of fruits to applied PGSs during development.

These objectives were addressed by posing a series of questions, the answers to which are given below. More detailed information on some of the research findings is given in the two appended draft research papers. Paper 1 relates to objective 3 and Paper 2 relates to objectives 1, 2 and 3.

Ql. Does flower number affect final fruit set?

Poor flowering potentially limits the number of nuts a tree can bear, but in species such as macadamia, the number of individual flowers is extremely high and this is less likely to be a cause of low yields. The relationship between flowering and yield was tested by removing different numbers of flowers from hand-pollinated racemes, and then measuring initial nut set and final nut numbers. Racemes thus had between 10 and 250 flowers. Generally, low flower numbers tended to increase the chances of an individual nut surviving to maturity, but because the total number of flowers was low, the number of nuts per raceme was always reduced. Hand cross-pollination always increased initial nut sets and sometimes increased final nut numbers. It is concluded that yields can be influenced by the numbers of flowers on a tree, but this is unlikely to be important unless flowering is very poor Good pollination efficiency can compensate for low flower numbers, and sometimes actually increases nut numbers, particularly if cross pollen is used.

Q2. ***What proportion of flowers are fertile?***

This was tested by hand pollinating all the flowers on racemes of 3 commercial varieties (H2, 333, 660). Success of pollination was checked microscopically and, because pollen grains were visible on every stigma, was assumed to be virtually 100%. Two measures of fertility were used: proportion of nuts that survived to at least 2 weeks after flowering and proportion of flowers that had pollen tubes penetrating to the base of the style. Initial nut sets ranged from 16 to 40 % compared with 9 to 22 % on open racemes with no hand pollination. These figures were always less than the values found for pollen tubes: 42 to 68% for cross-pollinated compared with 21 to 35 % for untreated. This means that up to half the apparently pollinated flowers in every experiment did not survive even to two weeks. The cause may have been infertility, but even the lowest fertilisation rates gave more than enough initial nut set to support the heaviest of crops. The conclusion here is that potential initial nut sets are very high and that poor flower fertility does not seem to be a major problem. However, there may be instances, for example if very cold temperatures are experienced during flower development, where infertility may be significant.

Q3. Does pollination efficiency affect final fruit set?

Macadamia crops are dependent on insects, particularly native and introduced bee species, for efficient pollination, although some pollen transfer may occur due to wind. However, if pollinators are excluded by enclosing racemes or whole trees in bee-proof mesh, resultant yields are very low indeed. Pollination efficiency is dependent on the size and behaviour of bee populations, and will be influenced by alternative foraging sources and by the layout of the orchard.

What has not been determined is just how variable pollination efficiency is, and whether it can have negative effects on yields. In this experiment, racemes were either saturated with cross-pollen by hand or left open and untreated, and therefore dependent on natural pollination. The supplementary pollen always resulted in an increased initial nut set, usually by a factor of two. In two (cv. 660 x 333 and cv. 660 x 246) out of four occasions this translated into higher final nut numbers. The other combinations (cv.333 x 246 and cv. 660 x 344) showed no benefits from cross-pollination. It is not clear whether any beneficial effects are due to the increased pollen *quantity* or due to the pollen *source* (i.e. cross instead of self). Other work by CSIRO indicates that the latter is more likely. In fact the parent combinations appear to be critical to nut numbers and nut quality parameters. With the "wrong" combination, negative effects are sometimes found. The finding here is that unless pollination efficiency is extremely low, orchard pollination rates appear to be adequate, but the overriding influence may be the source, rather than the absolute quantity of pollen. Although not examined in this project, variety mixes and tree spacing need to be optimised as part of any orchard planting design.

Q4. *What is the total energy demand of developing fruit and what are the sources of carbon to meet this energy demand?*

Measurements were made of nut growth, nut drop, nut respiration and dry matter accumulation from flowering through to nut maturity. Macadamia nuts drop in three distinct phases approximately 2, 6 and 10 weeks after flowering. From 10 weeks to maturity, there is almost no drop except due to pest and disease problems. The nuts increase in diameter fastest in the first twelve weeks, slowing down dramatically when shell hardening occurs at 12 to 15 weeks. Maximum dry matter demand by the nuts occurs late in development due mainly to oil accumulation and shell deposition. This period of maximum demand, however, does not result in any significant nut drop, as might be expected if the tree entered a phase of carbohydrate stress or deficiency. There is therefore no simple relationship between total stored carbohydrate and nut drop.

Nut drop may nevertheless be influenced by carbon availability: in I metre girdled defoliated branches all nuts dropped within 8 weeks of flowering. Clearly, here there were insufficient reserves to support nut development and no carbon replenishment from photosynthesis. If leaves were left on the branch, the number of leaves influenced nut drop. With low leaf numbers, nut drop was greater and final nut size was reduced. With high leaf numbers, nut numbers were increased substantially, but nut size was no greater than in ungirdled branches.

In ungirdled branches, nut drop and nut growth were unaffected by leaf numbers, indicating that nut development can be supported entirely from stored reserves and current photosynthesis on adjacent branches. That does not mean that a whole defoliated tree could bear a heavy crop. It appears that even when the total carbohydrate in the tree is more than adequate to support a large crop, limitations may be due to the "plumbing" system between source (leaf or wood) and the nut, or due to limited ability to mobilise stored carbohydrate at a sufficient rate. Alternatively, some nuts may themselves be predestined to drop long before they actually fall, or they may be inferior competitors for available carbohydrate and other resources. These factors may vary between varieties and merit further investigation in terms of manipulation of tree and nut behaviour. Ultimately such characters could be used in breeding and selection programs.

Respiration accounts for about 20% of total energy demand of the developing nut, and is strongly temperature dependent over the range 15^0 to 35^0C. The number of flowers and hence potential nuts even on a modest sized tree is of the order of 300,000, but most (usually >99%) of these are shed during nut drop. Most of this drop is probably essential to prevent rapid carbohydrate depletion and it also makes good sense to self-thin the crop as early as possible to reduce energy wasted in producing dropped immature nuts.

Energy requirement per nut actually increases exponentially as the nut grows, but only up to 10 weeks. At this point, the relative rate of nut growth slows dramatically, but does not cease completely, and this coincides with the third and final phase of nut drop. It is suggested that there is a temporary energy crisis in the developing crop: the rapidly increasing energy demand cannot be sustained. The problem is solved by shedding a proportion of the crop and by reducing the nut growth rate. Energies thereafter are directed almost entirely into nuts that survive to maturity. The overall conclusion here is that restriction or enhancement of carbohydrate supply can influence nut growth and nut drop, and therefore is a factor influencing yield and quality.

Q5. How does photosynthate move between leaves and fruit?

Doses of stable-isotope labelled (^{13}C) CO_2 were fed to leaves on small branches to determine the movement of carbohydrate from leaf to nut. Whole-tree experiments were also carried out using sealed tents flushed with air depleted in $^{13}CO_2$. This particular experiment was the first in the world to have been carried on this scale. The tree were 3.5 m tall - previously only crops such as wheat had been analysed in this way. Samples of leaves, nuts, bark and roots are being analysed by ANU, Canberra, but final results are not yet available. The data will indicate how much of nut growth is supported by newly synthesised carbohydrates and how much comes from storagc forms , This information, together with data from girdling, nut drop and controlled environment experiments will be incorporated into a model describing the development of the macadamia nut. This will reveal potential points of carbohydrate limitation of nut growth. Another benefit may be in prediction of behaviour of macadamia trees under Greenhouse Effect conditions of higher temperatures and higher atmospheric CO_2 levels.

Q6. ***What changes in plant growth substance levels occur in relation to fruit set and fruit drop, and can applied PGSs affect initial and final fruit set?***

Although carbohydrate supply clearly influences nut growth and nut drop, internal and externally applied chemicals (plant growth substances) may also affect crop development. Two classes of growth promoting PGSs, gibberellins and cytokinins, were analysed in developing nuts from just after flowering to maturity. No clear pattern was found with gibberellin levels, but massive levels of cytokinin accumulated around 10 weeks after flowering. This is the time of late fruit drop and also coincides with rapid embryo (kernel) development. After this point cytokinin concentrations fell again.

Of the various PGSs applied to nuts before, during and after flowering (gibberellin, cytokinin, auxin, Cultar), only cytokinin had any consistent effect on nut retention. Cytokinin sprays always reduced initial drop and the greater number of nuts was still apparent at 8 weeks. However, even with follow-up sprays, no increase in final nut numbers was found. It is therefore clear that cytokinins at least are involved in nut development, but no immediate commercial uses are envisaged. Perhaps the greatest potential would be to use cytokinin treatments in conjunction with girdling to nut retention in labour intensive breeding programs. Hybrid nuts which would otherwise abort prematurely could possibly be rescued by culturing excised embryos under sterile conditions, then transferring the resulting seedlings to field conditions for evaluation.

Q7. Does temperature limit/affect initial and final fruit set, and are there cultivar differences?

Potted, grafted trees were allowed to flower in the controlled environment glasshouses at Wollongbar, NSW. This experiment was conducted on Hidden Valley A4 using HAES 344 as the pollen source. Although these trees were too small to bear a crop to maturity and therefore no final nut numbers were counted, dramatic effects of temperature on flowering were seen. Rate of progress through flowering was proportional to temperature, with flowers remaining open for only one day at the highest temperature used (35^0C). This extreme also caused all nuts to drop within a few days of flowering and had severe adverse effects on the rest of the tree. Development proceeded more slowly at the lower temperatures used (15^0, 20^0, 25^0, 30^0C) and flowers were collected 7 days after pollination for microscopic analysis of pollen tube development. This data has not yet all been processed but will indicate whether there are any temperature-related problems at this stage of crop development. Other work by CSIRO has covered the influence of temperature on pollen germination, pollen tube growth and fertilisation. The conclusion from this project is that extreme high temperatures are detrimental to fruit set but moderately low temperatures merely slow development. The low temperature limit has not yet been determined but may vary depending on variety and on the particular process being studied e.g. pollen development, stigma and ovule development, pollen germination, pollen tube growth, fertilisation, embryo development. As mentioned earlier, unusually cold weather before, during or after flowering may be severely detrimental to crop development.

Summary of conclusions

- Yield can be influenced by the numbers of flowers on a tree, but this is unlikely to be important unless flowering is very poor.
- Flower fertility does not seem to be a major problem.
- Unless pollination efficiency is extremely low, orchard pollination rates appear to be adequate, but the overriding influence may be the source, rather than the absolute quantity of pollen.
- Carbohydrate supply can influence nut growth and nut drop, and therefore is a factor influencing yield and quality.
- Both current photosynthesis and stored carbohydrate contribute to nut development
- Data on carbohydrate partitioning will indicate how much of nut growth is supported by newly synthesised carbohydrates and how much comes from storage forms.
- Applied plant growth regulators were unable to generate any increases in final nut numbers and no immediate commercial uses are envisaged.
- Cytokinin treatments in conjunction with girdling could potentially be used to maximise nut retention in labour intensive breeding programs.
- Extreme high temperatures are detrimental to fruit set but moderately low temperatures merely slow development.

Major research achievements

Established and validated an efficient technique for rapid mass pollination of macadamia flowers. using a donor test-tube system

Discovered that there are three distinct phases of nut drop in macadamia and that nut drop is complete by 11 weeks after flowering

Developed a predictive model by which nut dry weight can be estimated non-destructively from measurement of nut diameter

Demonstrated that pollen source can have a dramatic effect on nut yields, nut size and nut quality

Undertook first ever whole-tree photosynthesis studies in macadamia Measured respiration-temperature relationships in macadamia leaves and nuts

Industry benefits, recommendations and further research

In summary, all the initial objectives have been achieved by using a range of experimental approaches. The project has been extended and linked to related research on productivity and quality, and provides crucial information from which recommendations to industry will ultimately be made. The project in its various components and as a whole can be judged successful. The macadamia industry will undoubtedly benefit from this work in the near future if the next phase of research can be supported.

The most significant findings are that nut set and nut drop can be dramatically influenced by flower numbers, carbohydrate supply, growth regulators, cross pollination and by environmental factors. Of these, manipulation of carbohydrate supply and cross pollination are the most promising areas for improving yields and quality. Pollen parent effects (cross pollination experiments) were not a major area of study in this project, but results obtained here and independently by other work at CSIRO and previously in Hawaii, strongly indicates that optimised cross pollination is likely to bring substantial benefits in improved yield and nut quality. Further work in this area is particularly recommended. It is apparent that maximum retums on the substantial investment in the long-term Regional Variety Trials will only come if a better understanding of crop limitations is developed.

Two general goals are suggested:

(1) develop specific recommendations for the macadamia industry on:

- optimum variety combinations for maximum yield and quality
- optimum orchard design - variety layout and tree spacing
- pruning regimes to maintain productivity in mature trees

(2) instigate and continue breeding and selection programs aimed at producing improved varieties better suited to the range of macadamia growing environments in Australia. High efficiency controlled cross pollination is now feasible using CSIRO methods. Maximised benefit:cost ratio will be achieved by using

- parents from superior existing varieties (further work needed to decide which these should be)
- only parent combinations with high cross compatibility (some variety combinations already assessed, most yet to be done)
- only parent combinations which have potential to raise kernel recovery, kernel size and/or yield (some information already available on positive and negative combinations)
- emasculation/hand cross pollinatioin/bagging sequence to minimise selfing and foreign pollen
- branch girdling and flower number restriction to maximise proportion of nuts retained to maturity (e.g. girdle I metre branch pre-flowering with 600+ leaves, remove all except 20 flowers on each of 6 racemes and pollinate all of these. Under these circumstances, it can be predicted that 10% of pollinated flowers would survive to maturity compared with normal field values of 0.5%. From the 120 flowers there would therefore be approx. 12 nuts, and the 600 leaves would be able to support these at the previously measured ratio of 50 leaves per fruit.)
- isozyme or DNA fingerprinting to verify parentage early selection of superior seedlings: precocious flowering, high photosynthetic rate, dwarf habit etc.

Thus, although labour-intensive in the initial stages, the 20-fold improvement in hybridisation efficiency coupled with the total control of parents, make this a very attractive and manageable genetic improvement strategy. Because of the resources required, this program would need to be managed across several research organisations. There is ample room for industry input on characters that would form the basis of primary selection. These decisions need to be taken *before* the crosses are performed. A ten to twelve year period would realistically be required for completion of such a program, culminating in release of a range of superior performing varieties on which a more productive and higher quality industry can be founded.

Publications from project

Trueman, S.J., Turnbull, C.G.N. *Effects of cross-pollination and flower removal on fruit set in Macadamia* Submitted to Annals of Botany

Trueman, S.J., Turnbull, C.G.N. *Fruit set, abscission and dry matter accumulation on girdled branches of macadamia* Draft in preparation

Turnbull, C.G.N. (1991) *Understanding yield limitations in Macadamia* AMS News Bulletin 18(1):27

Turnbull, C.G.N., Lloyd, J.J. (1992) *Limitations to fruit set in Macadamias* AMS News Bulletin 18(6):33