## Horticulture Innovation Australia

**Final Report** 

# Maintaining and expanding the technical development of the Australian Pistachio Industry

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Project Number: PS14000

#### PS14000

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## Summary

The aim of the project was to continue the development of the Australian Pistachio Industry to achieve increased profitability. The strategy was to continue the employment of a Research Field Officer to investigate ways the industry can increase yields and improve nut quality.

With the crop losses that occurred as part of the 2011 harvest some of the trial work as part of project PS11000 had been lost so there is a need to continue the trials for further periods to gain additional results. Also with the damage caused by the fungal diseases there was a need to consider whether the past and current orchard management practices are important and influencing either the expression of the diseases or acting to control the diseases.

PS14000 has worked to further achieve very specific outputs and outcomes:

- Practical application of Calcium being undertaken
- Nitrogen application trial to determine the optimum levels applied to maximise gross returns
- Continued work on pruning, including mechanical pruning, to control biennial bearing and increase yields and reduce cost
- Continued work on winter chill hours required for Sirora pistachios
- Continued work on Australian leaf analysis standards for Sirora pistachios
- Continue to investigate the use of reflective mulch to increase crop yields
- Continue benchmarking of orchards
- Value of the use of Winter Oils

The ultimate success of the adoption of the research in past projects and project PS14000 can best be highlighted by the following:-

- The return to grower has grown from \$8.00 per kilogram in 2012 to over \$11.50 per kilogram in 2015.
- Record "off" crop yield in 2016 of over 1900 tonnes and the benchmarking of growers in relation to yields
  - 7 orchards producing above 3,000kg/ha
  - Many orchard producing 2,000 to 3,000kg/ha
  - 8 orchards below 1,500kg/ha
- Measuring of stain levels and showing a reduction in treated orchards
  - Well managed orchards, Dark Stain < 1%
  - Unmanaged orchards, Dark Stain 3% to 8%
- 🔸 Nut size average
  - ~ 92/100gms (94 in 2015)
  - Ideally below 85 nuts/100gms

The overall industry outcomes can be detailed in reviewing the current and projected growth of the Australian Pistachio Industry

- Estimated growth of 40 ha per annum since 2014
- About 100ha planted in 2016

- About 100ha pa planned for at least next few years. No known mega orchards planned
- Each 100ha will increase supply by 400 tonnes pa in a decade.
- Domestic supply could match current domestic demand in about 2025 without further consumption growth.
- Exports can easily handle surplus but at a prices below domestic prices. Single desk becomes even more important.

The further value of the research can be expressed in the assessment for the 2017 season:-

- Good bud set but maybe not achieving the record crop to 2016.
- Poor 2016 winter chill?
- New orchards continue to mature.
- Orchards recovering from 2011 anthracnose.
- Continuing better management of orchards by growers.
- Pruning model developed from the work of Dr Jianlu Zhang is mitigating alternate bearing.
- Good prices likely for 2017 but below 2016 levels.
- Processing plant expansion to successfully capacity handled 1,900 tonnes in 2016.
- Planning underway for 3,000 tonne capacity.
- Several million dollars investment by the Processor.

The continued success of the past research and development will be further reviewed over the coming future harvests particularly in 2017 and 2018.

## Keywords

Pistachio, Chill hours, mechanical pruning, winter oil, kernel, nut drop, blanks, alternate bearing, nitrogen, leaf analysis.

## Introduction

The Australian Pistachio industry, after 30 years, has reached a stage of profitability and success. The initial challenge of simply getting the trees to grow and bear fruit has been met. The industry has reached the point now that in order to help industry develop further, there needed to be a person driving the research work and communicating with growers the benefits of changing production practices. In 2003 the pistachio industry initiated a position, Research Field Officer, with the financial support of the Australian government through Horticulture Australia Ltd. The program has continued through a number of projects culminating in project PS14000.

The principal objectives of project PS14000 were to conduct and collect relevant research to achieve:

- Improved Kernel Fill
- Reduction of shell damage to nuts
- Reduction of the impact of alternate bearing
- Reaching the high yields, currently being obtained by the best Australian orchards, by most Australian orchards
- Better than world best practice for yield, quality and profitability for Australian growers.

These broad objectives cover a wide range of smaller and specific activities and these have been detailed under each of the headings later in this Report.

In determining the research requirements initial assessments needed to be undertaken through extensive literature reviews and analysis. This work is detailed in this section of the project report.

## LITERATURE REVIEW

#### **Improved Kernel Fill**

#### 1.1 Pollination

#### 1.1.1 Pollen

Pollen diameter has been recorded as  $30.5 \ \mu m$  (Erdogan et al., 1998) and  $20-24 \ \mu m$  (Davarynejad et al., 1995 Iran; Afshari et al, 2008). The general shape of all pollen grains were circular to elliptical (Davarynejad et al., 1995 Iran), spheroidal to prolate spheroidal and pantoaperturate (Belhadj et al, 2007). The exine ornamentation is reticulate (Belhadj et al, 2007). Different exine features were found on pollen from different cultivars, and from this the characteristic pollen type was classified into 3 groups by pits (Davarynejad et al., 1995 Iran). Significant differences in pollen size and number of apertures were observed between the different sites (Belhadj et al, 2007). The highest contents of B and Ca were measured in pollens with the highest percentages of germination as 4.2mg/100g and 386mg/100g respectively (Afshari et al, 2008). Protein and oil content were highest in 'Kirmizi', 'Bilgen' and 'Vahidi' (23.97 - 24.78% protein and 50.75 - 54.22% oil) but the effects of pollen on protein and oil content were inconclusive (Ak and Kaska, 1998).

The fertility of pollen from different sites was estimated using the acetocarmine staining method and showed that the mean percentage of fertile pollen was high but varied among the sites with highest value (99.7%) at the Berriane site (Algeria) and lowest value (95.9%) at the Messaad site (Belhadj et al, 2007). Germination rates were also reported averaging at 85% (Martínez-Pallé and Herrero, 1994) and 86% (Erdogan et al., 1998). The highest and lowest germination rate of fresh pollens was 85% and 53% for type R28 and N2 and, after 3 days the germination decreased to 16% and 8% respectively (Afshari et al, 2008). The amount of pollen per inflorescence averaged 173 mg and ranged from 73 to 252 mg (Martínez-Pallé and Herrero, 1994).

#### 1.1.1.1 Pollen dispersal

To understand pollen dispersal pollen motion was examined in a commercial orchard in Turkey and with wind speeds averaging 0.9-2.2 m/sec the atmospheric pollen concentrations were highest(1000 grains/m<sup>3</sup>) between 09:00 - 11:00 am. The general pattern of pollen grain motion involved direct inertial collision by windward surfaces and by sedimentation of pollen onto leeward surfaces. In addition, the behaviour of un-clumped (single) and clumped pollen grains was different in the probability of their capture. Clumped pollen rarely sedimented onto leeward surfaces. Un-clumped pollen had a higher probability of being captured by leeward surfaces. Small changes in ambient wind speed (0.5 m/sec to 1.0 m/sec) produced significant changes in the pattern of pollen motion around inflorescences and altered the number of pollen grains captured by leeward surfaces. The apparent insensitivity of pollen motion to differences in inflorescence size may ensure equitable pollination during the acropetal development of flowers (Niklas, K. J.; Buchmann, 1988). To test pollen distribution, vaseline-coated glass traps were placed 2 m above the ground to catch pollens from all directions and at increasing distances from the tree. Pollen concentration decreased markedly with increasing distance from the tree with 1138, 516, 389, 118, 54 and 19 pollen grains/cm<sup>3</sup> being measured at 5, 10, 15, 20, 40 and 80 m from the tree.

It was estimated that an average of 1.2 viable pollen grains might reach a female tree 20 m away and that this should be the maximum distance between pollinator and female trees (Erdogan et al., 1998).

#### 1.1.1.2 Pollen germination test

Maximum germination of pistachio pollen occurred in 35, 20 and 25% sucrose (Therios et al., 1985) 20% (Acar and Ak, 1998; Özeker et al, 2006) and 10% (Crane et al, 1974). There was a positive correlation between germination and the concentrations of glycine, leucine, tyrosine, phenylalanine and arginine in the media (Rashed et al., 1995). Boric acid was necessary for in vitro germination which reached a maximum at 10-30 mg/litre.  $Ca(NO_3)_2$  was also beneficial with an optimum concentration near 2.0 mM. The optimum incubation time was 24 hours and the best germination percentage was recorded at 25°C and pH 6. Higher concentrations of the respiratory inhibitors NaN<sub>3</sub>, NaCN and DNP were needed to inhibit pollen germination more than to inhibit other plant tissues and processes (Therios et al., 1985). There was also a high positive correlation between pollen germination and the concentrations of Al, Cu, Na and N (Rashed et al., 1995). An investigation on different methods (tube, saturated petri and hanging drop) showed that germination was best using the tube method with 10-15% sucrose or the petri dish method with 25 ppm boric acid (Ak et al., 1995). The optimum concentration of GA<sub>3</sub> and IAA for stimulating pollen germination and pollen tube growth were 40 mg/L and 10 mg/L, respectively. When spraying pollen powder, dotting or liquid pollination the optimum concentration of pollen concentration which could achieve the fruit set rates of 9.2%, 9.45% and 6.175% respectively were correspondingly 1%, 1% and 1.5%. In view of the pollination efficiency, spraying and liquid pollination were similar and much better than dotting (He et al, 2010).

Pollen quality (expressed as the number of well formed grains) depended on the state of the tree and on weather conditions and it varied between 60 and 99.8% over the years (Aleksandrovskii, 1977). The average percentages of pollen viability in different *Pistacia* spp. ranged between 57.51 and 85.00% by IKI and TTC tests. *P. atlantica* had the highest pollen viability percentage in both of the viability tests. The usage of different *Pistacia* spp. pollens resulted in higher fruit set than natural pollination in three years of this investigation. In general, the best fruit set was obtained in *P. vera* using pollenizers for pistachio types (\*Özeker et al, 2006).

The germination percentages of pollen of 4 *Pistacia vera* clones on a range of sucrose and agar concentrations were uniformly high 2 days after anthesis, but after one week at room temperature they ranged from 0% for 'Elk Grove' and 02-18 to 5.4% for Peters. When the week-old pollen was treated to effect gradual hydration at high humidity prior to being placed on germination medium, germination increased to >80% for 'Peters' and to 10.4, 20.6 and 63.8% for 'Elk Grove', 02-18 and 02-16, respectively. Peters pollen (when hydrated at high humidity) had a germination rate at least 50% that of fresh pollen when stored up to 18 days at ambient laboratory temperature and humidity (Polito and Luza, 1988). Viability was 86% (Erdogan et al., 1998).

Pollen was incubated at a range of controlled temperatures from 11 to 39°C, in 1 - 4°C increments, and analyzed for germination percentages. High rates of pollen germination occurred over a broad range of temperatures for all clones. Germination of pollen from the early-blooming clones showed greater tolerance

for low temperatures. Curves for pollen germination vs. temperature in the early-blooming clones showed a broader peak at the low-temperature end (Polito et al, 1988). At 20 - 26°C, germination of pistachio pollen collected from young trees was lower than that collected from older trees, but no differences were evident at higher temperatures. There was no evidence of a shift in the optimum temperature for pollen germination as a function of tree age (Polito and Weinbaum, 1988).



Figure 1.1: Germination of *Pistacia vera* pollen vs. temperature. Curves represent least-squares quartic fits to data for early ( $r^2$ =0.995), mid-season ( $r^2$ =0.963) and late ( $r^2$ =0.963) blooming clones collected on 1, 13 and 20 April, respectively (Polito et al, 1998).

Figure 1.1 shows that 50% germination occurred at 14.1°C for the earlyblooming cultivars, at 17.2°C for the mid-season collection, and at 18.6°C for the late-blooming clones. The early-blooming cultivars had consistently higher germination percentages from 11-12°C to 14-15°C. At 17-18°C, differences between the early and mid-season clones were less clear, but one of the late-blooming clones, 'Elk Grove Late', had only 17.8% germination at 18°C. This was considerably below that of the mid-season clones, which had mean germination rates ranging from 60.4 to 75.6% at 18°C, or the early clones, which had 74.4 and 85.4% germination at 17°C. Peak germination occurred at 25-29°C (Polito et al, 1998) and for 'Peter' pistachio was between 22 and 32°C (Polito and Weinbaum, 1998). A critical factor in the germination of viable pollen was the ability of the pollen grain to establish membrane integrity during hydration (Heslop-Harrison, 1979) because the membrane was insufficiently flexible at low temperatures to withstand the rigors of hydration (Hoekstra, 1984)

#### 1.1.1.3 Pollen storage

Refrigeration prolonged viability by a few days and freezing prolonged it as much as several months but the percentage pollination varied greatly (Crane et al, 1974). Slightly dried pollen kept well and retained its viability after storage in sealed glass jars at 1-3°C (Aleksandrovskii, 1977). The pollen was kept at -4°C for 7-10 days in bottles after drying using CaCl<sub>2</sub> for humidity absorption (Acar et al., 2001). Therios et al. (1985) reported that pollen stored at -19° germinated after a year. Pollen stored at -20° showed more exacting in vitro germination requirements than fresh pollen, particularly as time in storage increased. Peters pollen retained germination levels comparable to those of fresh pollen after 4 months at -20°, but, by 12 months, germination percentages had fallen sharply (Polito and Luza, 1988). Keeping wet pollen more than 4 hours or shorter under 0°C caused a reduction in percentage of germination. Long storage of pollen grains under temperatures lower than 0°C in dried conditions was possible, but 7 days after drying of pollen grains and keeping under –20°C, the percentage of germination decreased up to 30%. This descending trend continued quickly toward day 30 under –20°C and after that with lesser intensity (Afshari et al, 2008).

Conditions for successful pollen storage from one season to the next at -196°C or by freeze drying (Vithanage and Alexander, 1985). At -196°C, pollen of 'Peters' survived freezing, storage and thawing with no loss of germinability (>80%). When the relative humidity of the -20°C storage environment was maintained at or near 33%, 'Peters' pollen had a high rate of germination for up to 12 months storage. Without the control of relative humidity, 'Peters' pollen germination was high at 4 months, but declined at 12 months. Germination requirements became more exacting for pollen stored at -20°C for 12 months under suboptimal humidity conditions. Pollen of the other 3 clones did not tolerate storage at -20°C as well as Peters pollen regardless of the storage humidity environment (Polito and Luza, 1988).

Vaknin and Eisikowitch (2000) reported that weight loss as a result of desiccation was positively correlated with germinability. Pollen grains stored in room conditions only germinated after prehydration, which restored germinability even after 7 days of exposure. Refrigerated (4°C) pollen grains retained their germinability for at least a week. Frozen pollen grains irreversibly lost most of their germinability. 2-day-old pollen grains, within the anthers, retained their germinability under room conditions.

#### These findings will contribute to the improvement of pistachio pollen preservation and help to find a simple and inexpensive method for its short-term storage, while retaining its germinability (Vaknin and Eisikowitch, 2000).

#### 1.1.1.4 Effect of pollen age

Female pistachio (*Pistacia vera* cultivars 'Kerman', 'Red Aleppo' and 'Trabonella') trees were hand pollinated with pollen from 3 different male genotypes 0, 1, 2, 3 and 4 days after anthesis. Results were measured by mature fruit set and by fluorescence microscopy of pollen germination and tube growth in the pistil. Maximum pollen tube growth and fruit set of split nuts were achieved following pollination within 2 days of anthesis. Three-day-old pistils supported pollen tube growth, but fruit set was low. Four-day-old pistils supported little pollen tube growth or fruit set. Significant differences were also apparent between pollen parents, with low pollen tube growth and fruit set following inter-specific pollination with *P. atlantica* pollen. All pollen tubes were observed to penetrate chalazogamously and parthenocarpic production of blank fruits occurred in both un-pollinated and pollinated treatments.

The results indicate that for optimum fruit production in pistachio, pollen transfer must be achieved within 2 days of anthesis and pollination by *P. atlantica* should be avoided (\*Shuraki and Sedgley, 1994).

In Sirora pistachio, 43% of ovules were fertilized within 3 days after pollination compared with 25% after 2 days in Kerman. No pollen tubes were detected in up to 60% of pistils and no embryo sacs in up to 50% of un-pollinated and 29% of pollinated pistils (Shuraki and Sedgley, 1997).

#### 1.1.2 Factors relevant

#### 1.1.2.1 Ratio between male and female trees

The genus Pistacia is a dioecious species, staminate and pistillate inflorescences born on different trees and wind pollinated (Buffa et al, 2009). In Italy, for adequate pollination in pistachio plantations it is essential to have at least 10% of male trees present. Maggs (1977) suggested that it might be better to plant male trees in rows than singly among female trees.

## The recommended male:female tree ratio is 1:9 in Australia (Needs and Alexander, 1985).

However, in California, female trees of the cultivar 'Kerman' and trees of the male cultivar 'Peters' were inter-planted at a ratio of 1:24 (Vaknin, 2006) or 1:30 in Iran (Rafati et al, 2014).

#### 1.1.2.2 Bloom period matching

Protandry is often observed in pistachio. In Greece, pistachio orchards are interplanted with staminate cultivars that flower earlier than the pistillate cultivar Aiginis, but usually flowering periods do not overlap sufficiently to obtain satisfactory pollination and pollen has to be collected and stored. A study, conducted from 1985 to 1987 with the important staminate cultivar B, investigated whether anthesis could be delayed by paclobutrazol (PBZ) treatment. Foliar PBZ sprays applied in June or July of the previous season delayed anthesis; however, spraying in September was ineffective. The length of delay of flowering depended on dose (50 to 1000 mg PBZ/litre applied to incipient runoff). Using the same total amount of chemical, one spray was as effective as 2 sprays applied one month apart. Stem elongation was inhibited strongly by PBZ, but the number of nodes and flower buds/shoots were reduced only slightly. In a second experiment, 2 other staminate cultivars (A and C, early- and late-flowering, respectively) showed a response similar to that of cultivar B. Treatment by spraying was only effective for one year, whereas a soil drench was effective for 2 years. PBZ may be able to synchronize the flowering of staminate and pistillate pistachio cultivars and result in good fruit set without artificial pollination (Porlingis and Voyiatzis, 1993).

Unsatisfactory pollination in pistachio (*Pistacia vera*) was attributed to a variable flowering period in female trees as well as to the absence and/or incorrect ratio of male cultivars with different flowering times, particularly late flowering cultivars. Female trees were sprayed with 5, 10, 15 or 20% solutions of 2.5% DNOC in late January, none of which was phytotoxic. The highest concentration gave consistently better results when sprayed on female trees which had been exposed to temperatures below 10°C for 1000 -1100 h from the autumn onwards. These trees flowered 7-15 days earlier but for a shorter period than control trees. However, treatment after 1500 h at below 10° C delayed flowering. Early flowering in female trees coincided with the flowering of early-flowering male cultivars and that of the compatible and early-flowering *P. terebinthus* (Pontikis, 1975).

#### 1.1.2.3 Frost

A 2-year research project was performed in Ghazvini, Iran, to determine the critical temperatures and development of early-spring chilling injury for applications of suitable and on-time methods to inhibit the related injuries. Pistachio cultivars Ghazvini and Ohadi, 5 thermal levels (+2, 0, -2, -4 and -6°C) and three developmental stages (bud, blooming bud and flowers) were mentioned. Samples were put in a freezing chamber with precise temperature and humidity control, and the temperature was reduced at a rate of 2°C per hour. At each thermal level, 20 samples were taken out and injury was studied. Macro- and microscopic levels of injury development were mentioned. Injury levels were classified in three groups, changing the tissue colour, browning and whole necrosis. The first was identified as a reversible injury, which could negatively affect the pollination, fertilization and fruit set process. Browning was determined as the injury level at which plants received permanent and irreversible strain. Critical temperature was determined as -4°C at bud stage, -2 at blooming bud and +2 at flower. Decreasing temperature down two more degrees (e.g. -6°C at bud) would result in irreversible and serious injuries. The cultivars did not show any significant differences as the viewpoint of critical temperatures at each stage. Therefore, the only parameter affecting their injury level was the phenology of blooming (Gholipour, 2006).

#### 1.1.3 Supplemental pollination

A literature review was undertaken relating to artificial pollination for fruit trees (Pinillos and Cuevas, 2008).

The first report about artificial pollination of pistachio trees was from Turkey in 1984 utilising motorized dusters or cotton bags (Kuru and Ayfer, 1984). Acar et al. (2001) reported on the application with an atomizer connected to a tractor. Then an electrostatic pollination device was reported (Vaknin et al, 1999).

Earlier studies have shown that plants and pollinating insects poses surface electrical charges and are surrounded by electric fields. Accordingly, these electric fields should be more intense near sharp points on the edges, for example anthers and stigmas in the flowers. One mechanism for pollination involving electrostatic forces has been suggested and described as an 'electrically charged insect' visiting flowers. As the insect approaches the flower, it induces an opposite electrical charge on pollen grains located on the flower. The temporary forces of attraction created between the airborne insect and the flower could explain the detachment of some pollen grains from the flower and their deposition on the body of the insect, and the same temporary forces could explain their detachment from the body of the insect and their deposition on the flower, including the stigma. The aim was to harness these electrostatic forces and use them as a method of pollen supplementation in agriculture. Authors have devised a machine that will electrostatically charge pollen and mechanically convey it to flowers (Vaknin et al, 2001). An electrostatic system for pollination of pistachio trees was developed in order to improve penetration through the trees outer layers. The system utilizes corona charging and air streams to transport the pollen. An electrostatic ring (ESR) for reducing the amount of free ions was developed, to enable the charged pollen to penetrate more freely into the internal parts of the tree. A model of an artificial tree was constructed and laboratory experiments were conducted in order to test, optimize and refine the device. The results showed that the electrostatic charging can increase the amount of pollen deposition 18-fold compared to the uncharged treatments. Utilizing the ESR can

increase the deposition of the charged pollen by an additional 46% on the whole tree, and by 83% on the tree internal parts (Bechar et al, 2000).

Soft and durum wheat flours mixed with 1% pollen gave the best result of the blending materials and pollen rates tested (Kuru and Ayfer, 1984). The best mixtures were 1% pollen mixed with durum wheat or soft wheat flours (Kuru et al., 1995). The pollen grains were taken at full flowering stage and mixed with a mixture of wheat flour (98%) and pollen (2%) (Acar et al., 2001). The best time for artificial pollination was when female trees were between 1 and 40% in full flower (Kuru et al., 1995). However, Abu-Zahra and Al-Abbadi (2007) tested four mixtures of pollen (2, 4, 6 and 8%) with soft wheat flour have been prepared for application. Results obtained showed that:

- 2% pollen mixture could be used as an effective treatment in pistachio orchard pollination.
- 8% resulted in a lower number of fruit per cluster in comparison to 2 and 6% pollen mixture treatments, which may be due to the large increase in pollen deposition on the stigma that decreases female reproductive success by causing inferences between pollen grains on the stigma at high densities and this is similar to the previous suggestion by Young and Young (1992).

The same results were found by Vaknin et al (2002) who concluded that pollen germination on the stigma was negatively correlated with yield.

Supplementary pollen treatments significantly increased fruit set to 12% compared with 6% for the control (Çağlar et al, 1995), young female trees produced 16.2% more fruitlets per cluster (Vaknin et al, 2001) and a 11.3% higher yield (Vaknin et al, 2001). The percentage of blank nuts was 20.05% in the control trees and this was significantly reduced to 6.32% and 10.9% for supplementary pollen treatments (Cağlar et al, 1995). The blank nut rate was 47.76 for the artificially pollinated clusters and 55.32% for naturally-pollinated clusters, respectively (Acar et al., 2001). Also there was 18.6% higher split percentages and 60% lower blank percentages than those which only received drifting pollen from nearby orchards (Vaknin et al, 2001). Vaknin et al (2001) conclude that electrostatic pollination of pistachio could replace natural wind pollination and may result not only in higher crops, but also in better quality of the nuts. Supplementary pollination had positive effects on fruit number per cluster and reduction of blank percentage. Supplementary pollination not only increased quantitative traits (number of nut per cluster, kernel dry weight and yield), but also it improved some qualitative traits such as un-split nut percentage and reduced blank nut percentages in 'Qazvini' and 'Ohady' cultivars (Ebrahimi et al, 2011). The application of medium containing agar and 0.01% boric acid combined with 0.15% (w/v) pollen produced better fruit set than suspension media without boric acid. It increased fresh and dry weight of kernel. Moreover the pollination treatments affected kernel nutrient element concentrations and the highest concentrations of the kernel P, Ca, Mg, Cu and Fe were obtained with spray pollination methods (Zeraatkar et al, 2013).

Rahemi and Abdollahi (2004) tested double pollination with pollens from different species *Pistacia mutica, P. atlantica* and Soltani (*P. vera*). In the double pollination experiments, the nut, kernel and fruit set were more affected by the first than by the second pollen. The pollen from wild pistachio species reduced kernel weight and number of split nuts but increased the percentage of deformity and blank nuts in 'Owhadi'. Pollen from *P. vera* was the best pollen source.

Abu-Zahra and Al-Abbadi (2007) tested four mixtures of pollen (2, 4, 6 and 8%) with soft wheat flour that had been prepared for application. Results obtained showed that the 2% pollen mixture could be used as an effective treatment in pistachio orchard pollination. 8% resulted in a lower number of fruits per cluster in comparison to 2 and

6% pollen mixture treatments. Acar and Eti (2008) further investigated the effect of pistil receptivity, pollen mixtures and pollen application distances on fruit set using artificial pollination in pistachios. To determine the receptivity of female flowers, anthesis was accepted at day 0, and then one application was carried out on 0, 2, and 4 days after anthesis; and repeated applications were done on 0+2, 0+4, 2+4, and 0+2+4 days, separately. Pollen and wheat flour was mixed to obtain 1%, 5%, 10%, 50%, and 100% pollen mixture, and these pollen mixtures were sprayed from 25, 50, and 100 cm distances from the inflorescence clusters with a small hand spraver. The results indicate that for optimum fruit production of pistachio, 5% pollen mixtures should be sprayed at a distance of 50 cm and 100 cm, and 50% pollen mixtures should be sprayed at a distance of 100 cm, at 0 and 4 days after anthesis. Two pollen applications on 0+4 days resulted in the lowest fruit set. The same results were found by Vaknin et al (2002) who concluded that pollen germination on the stigma was negatively correlated with yield. The large increase in pollen deposition on the stigma decreases female reproductive success by causing inferences between pollen grains on the stigma at high densities (Young and Young, 1992).

In pistachio, final fruit set varied between 9.40% and 16.50% of flowers (Ayfer et al. 1990; Kuru & Ayfer 1990). 33.3% of flowers died before reaching receptive maturation. Such flowers did not have mother cells and were located within clusters at the full bloom stage. During anthesis of pistachio, 26.7% and 23.3% of flowers were at sporogenesis and gametogenesis stages, respectively (Ayfer et al. 1990). According to Kaska et al. (1989), the average stigma surface of a pistachio flower was calculated as 3  $\text{mm}^2$  and the number of pollen grains per mm<sup>2</sup> was on average 4.60 at 5% pollen mixture. This is about 14 – 15 pollen per stigma, which is considered the adequate number of pollen grains per flower in pistachio (Acar and Et, 2008). Acar (2004) reported that an average of 15 pollen grains germinated on stigma, 6.2 pollen tubes reached to the base of style among them, 1.2 pollen tubes found the funiculus, and on average 0.5 pollen tubes reached the embryo sac in 'Siirt'. Increased numbers of pollen per stigma area would cause excessive flower abscission after a certain point (Acar and Eti, 2008). In pure pollen at 25 cm distance there was 67 pollens per stigma. It is hypothesized that germinated pollen grains and pollen tubes absorb water and nutrients from the flowers and weaken the flowers (Acar 2004; Acar & Eti, 2007). If too much pollen came to the stigma surface and simultaneously germinated on the stigma it may result in competition between pollen tubes in the pollen tube pathway (McGranahan et al., 1994) and the flowers may abscise (Acar 2004; Acar & Ed 2007). Fewer pollen grains on the stigma, which may be related to less competition between pollen tubes in the pollen tube pathway, provide higher fertilisation and lower fruit abscission (Shuraki & Sedgley 1997).

In California, applying smaller amounts of pollen (1.5 g per tree in early application only in 1996 or 0.5 g in two applications in 1997) resulted in higher yields than with open pollination or supplementary pollination using larger amounts of pollen (1.5 g per tree in four applications in 1996 or 1.0 g in two applications in 1997). Applying 1.0 g pollen in two applications in 1998 resulted in decreased yields compared with open pollination or non-electrostatic pollination.

Vaknin et al, (2002) concluded when pollination was a limiting factor, electrostatic pollination of pistachio can increase yield and may even increase fruit quality. However, when pollination was not a limiting factor, electrostatic pollination may even reduce yield.

#### 1.2 Nut Setting

#### 1.2.1 Normal fruit set and development

At the time of fertilisation the egg is a highly vacuolated cell 1.9  $\mu$ m in length with a broad chalazal end and the synergids were 1.7  $\mu$ m in length. Shortly after pollen tube penetration of the embryo sac, the penetrated synergid underwent cytoplasmic changes, including darkened shrunken cytoplasm and digestion of starch granules. The occurrence of fertilisation took place two days after pollination. Under observation using a microscope, one or more sperms or a zygote and polar fusion nucleus appeared. During fertilisation the cell wall of the central cell disappeared. The antipodals persisted up to one week after pollination. Soon after fertilisation the zygote shrank and only one of the synergids was visible. A week following pollination, some ovules had free nuclear endosperm and a one cell pro-embryo (Shuraki, 1995).

11 days after full bloom, many embryo sac contents had degenerated because of lack of fertilisation (as shown by the absence of pollen tubes in any of their tissue). Several endosperm nuclei were present in some seeds. Relatively few seeds contained a zygote and the endosperm appeared normal. The cause of degeneration was not apparent but competition for nutrient substances may be considered as one of the reasons. These results explain why so many flowers and young fruit abscise (Bradley and Crane, 1975).

One-cell embryos were 2.5-3.7 µm in diameter, contained a large vacuole, had a nucleus close to the micropylar end and had a PAS positive cell wall. At this stage less than 10 free endosperm nuclei were present in a cytoplasmic ribbon at the embryo sac periphery (Shuraki, 1995). The zygote entered a guiescent period and did not initiate its first division until 5 to 6 weeks after full bloom. The zygote became a 2-celled pro-embryo after transverse division and subsequent cell division followed rapidly. One of the 2 cells divided forming a 3celled pro-embryo. The pro-embryo at this stage remained about the same size as the zvgote from which it originated. Because of these divisions, the size of each cell decreased (Lin et al, 1984). Shuraki (1995) observed a stage earlier, 1 to 4 cell pro-embryos were observed in up to 19 % of 4-week pollinated pistils. After 8 weeks of pollination early embryos only consisted of 12-20 cells, with about 20-35 free endosperm nuclei (Shuraki, 1995). The embryo developed from a single-celled zygote to a multi-celled globular structure through horizontal, vertical, and obligue cell divisions between 1 and 15 June in California (Lin et al, 1984) and corresponded to the period of rapid sugar accumulation reported by Crane and Al-Shalan (1974). The globular embryo at the micropylar end of the embryo sac had become heart-shaped during the last half of December in California, with cotyledon primordia present. The cotyledons, apical meristem, procambium, and radicle could be discerned clearly by the end of June. From mid-June, the embryo grew rapidly and reached its final size by the beginning of August. During this period size increase was due mainly to cell enlargement in the cotyledons (Lin et al, 1984). In this period sugar was markedly decreased and crude fat content rapidly increased (Crane and Al-Slialan, 1974).

In the weeks following pollination there was significant development of the funicle, integuments and nucellar tissue. Fruit growth commenced soon after pollination with rapid growth up to week 4. The endosperm and pro-embryo grew quickly from 4 to 6 weeks after pollination when about one-third of the

fruit cavity was filled by the large funicle. The greatest size increase was between weeks 4 and 16 with funicleis from 10.47 to 21.17 mm on average, embryo sacs from 0.28 to 12.39 mm and embryos from 0.04 to 11.72 mm. The funicle was still clearly recognizable at fruit maturity (Shuraki, 1995).

The whole embryo sac at 2 weeks after full bloom was filled with free-nuclear endosperm having small vacuoles. The free-nuclear endosperm moved to the periphery of the expanding embryo sac surrounding a large vacuole during the zvgote and early pro-embryo stages. Variations were observed in the stage of embryo development at the time cellular endosperm development began. In general, the free-nuclear endosperm at the micropylar end began to differentiate into the cellular state when the embryo had 16-32 Cells. Some ovules remained in the free-nuclear state when the embryos contained 64 to 128 cells. The formation of cell plates in the free-nuclear endosperm progressed basipetally and centripetally, filling the whole embryo sac by the time the embryo had become globular or heart-shaped (Lin et al, 1984). The majority of 16 week embryo sacs had a fully cellular endosperm, some embryos with cotyledons and a testa. Around 60% of nuts had an embryo with cotyledons at 16 weeks after pollination. Endosperm wall formation started from the periphery of the embryo sac, first at the micropylar area, and it was observed near the globular embryo at 16 weeks after pollination (Shuraki, 1995). Most endosperm was digested as the cotyledons reached the chalazal end of the embryo sac. Remnants of the endosperm and nucellus became crushed against the rim of the integuments and the funiculus was flattened against the endocarp.

The mature *Pistacia* fruit is a bilaterally symmetrical structure consisting of the embryo enclosed in the pericarp which comprises a woody endocarp and fleshy mesocarp and exocarp. The endocarp forms the shell, with a dorsal and ventral suture such that at maturity of the *P. vera* fruit spontaneous split along the distal portion of both sutures (Crane and Iwakiri, 1982). Shell and hull thickness reached maximum size between week 8 (1.03 mm) and 16 (1.10 mm, finally 1.13 mm) after pollination which coincided with the commencement of shell hardening. Shell splitting in some fruits started before ripening and progressed during hull softening and coloration. The inner epidermis of the P. vera endocarp had developed thickened walls by 16 weeks, except at the suture join. Splitting of the shell commenced from the inner epidermis by separation of the thin walled cells and progressed by separation of the cells of subsequent layers. Splitting commenced at the mid points of the ventral and dorsal distal sutures and extended in both directions, with complete separation of the distal portions but only partial separation of the proximal areas (Shuraki, 1995). Liginfication of the endocarp begins about the 11 November in Australia and partial dehiscence along the suture was first noted in the last week of January.

The endocarp structure of sutures was similar up to 8 weeks after pollination, with irregularly-shaped cells with interlocking projections and thick walls (Shuraki, 1995). Early in December in California, a number of seeds had a small necrotic spot on one side of the funiculus close to the ovule. Microscopic examination revealed that the injury was usually confined to the epidermis and outer 1 or 2 layers of cortical tissue. Necrotic tissue was more extensive both in the seeds and in funiculi of some of the seeds 2 weeks later. Considerable cell proliferation had occurred in the vascular bundle in the upper part of the funiculus. This increased the diameter of the bundle and eliminated the resin ducts that typically run through the main bundle and the branches which

surround the base of the embryo sac. Necrotic tissue developing in the chalaza probably blocked the flow of nutrients to the embryo sac and these nutrients were then used in cell proliferation in the vascular bundle. Tissue surrounding several brown spots was examined carefully to determine whether fungal hyphac or bacteria were present but neither type of organism was seen. The cause of the brown spots might be a physiological disturbance. Their characteristic position suggested that the region affected was particularly vulnerable (Bradley and Crane, 1975).

In July and August in California, the embryos in some seeds without necrotic spots had also failed to develop normally. The immediate cause seemed to be a deficiency in the quantity of endosperm but the probable underlying cause of abnormal development of the nucellus. Instead of nucellus gradually diminishing in quantity the cells became hypertrophied and crowded the endosperm and embryo. In several embryo sacs, regions of enlarged nucellar cells were separated by regions of proliferating nucellar cells. Such aberrations of nucellus and endosperm may account for the subnormal size of some seeds at maturity (Bradley and Crane, 1975).

#### 1.2.2 Nutrition transport within nuts

To observe nutrition transport within fruit, the fluorescent dye disodium fluorescein, is a useful indicator of vascular continuity. It is readily transported in functional vascular tissue and is highly fluorescent, and can be easily detected in tissues at very low levels (Mogensen, 1975, 1981). As a result, presence or absence of fluorescein fluorescence is a reliable indication that transport is or is not occurring (Polito, 1999).

Movement of the fluorochrome solution to the fruit wall occurred in all samples examined, and in many cases transport through the funicular vascular bundle to the chalaza was clearly evident. When blockage of dye movement occurred, it was apparent at the juncture of the funiculus and the placenta, or in the funiculus itself. In the latter cases, the fluorochrome solution moved beyond the point of attachment of the funiculus to the placenta, into the funicular vascular strand, but failed to move through the funiculus to the chalaza. The transport of the fluorochrome was apparent in the symplast as well as the apoplast, as it could be seen in the embryo sac some distance distal to differentiated tracheary elements of the funicular xylem trace (Polito, 1999).

Transport was apparent for the full length of the funiculus in all ovules at full bloom, and in 78 % of the ovules 1 week after bloom. A complete or near complete cessation of fluorescein transport at about the time the pericarp reached full size and endocarp lignification began. Transport resumed in a large fraction of the ovules by 9 to 11 weeks after bloom, the approximate time that embryo growth begins and the ovule begins to grow to fill the empty pericarp. At this time, full vascular movement to the chalaza occurred in 78 to 90 % of the ovules. This will develop into the seeds of filled nuts, but not in those that fail to grow and result in blank nuts. Ovules from samples that show full transport were significantly larger than those from samples where transport did not proceed beyond the placenta or the funiculus (Polito, 1999).

Through observation there are 2 kinds of fruit, one has full transport to the chalaza, in the other transport was blocked basal to the chalaza. In the early season there was little difference between those fruits. By 6 weeks after bloom a marked disparity between the two classes became apparent: 100% of the

fruits that had complete vascular movement through to the chalaza had endosperm, whereas endosperm was present in only 41 % of those with transport blocked at a point basal to the chalaza. 10 days later, only 7.7 % of fruits exhibit full transport to the ovule. This indicated that this collection was taken at the time fluorescein transport had begun to decline to very low levels in all fruits. Thus, the pattern of cessation of transport that occurred during the period from 6 to 10 weeks after bloom appeared to begin preferentially in those fruits with ovules lacking endosperm, presumably unfertilized ovules (Polito, 1999).

#### **Reduction of Shell Damage to Nuts:**

#### 1.1 Nut Drop after Pollination

Flower and fruit abscission occurred primarily during the flowering and small-fruit period. In June and pre-harvest in Turkey abscissions were low. More than 80% of the flowers and fruits of Kirmizi and Ohadi pistachio cultivar abscised mainly during the initial 50 days after full blooming and Siirt cultivar abscised during the initial 35 days after full blooming (Acar and Eti, 2007). Shuraki (2006) used Pistacia muticaf for further detailed observation. Pistacia mutica had low seed set even in on-years. Flower and fruit samples were harvested from open pollinated trees then fixed and processed for assessing the pollen tube pathway, flower and fruit abnormality and abortions. In each cluster, two forms of small (abnormal) and normal flowers were observed. Most of the degenerated samples dropped in the first four weeks after pollination. Degenerated early nucellus tissue and lack of megaspore mother cells were dominant in abnormal samples. Degenerated samples of normal florets consisted of samples with no embryo sac, deformed embryo sac, an early degenerated embryo, deformed endosperm in the early stages of development and embryo destruction during the globular stage. Embryo sacs without any endosperm tissue, embryo and endosperm growth interruption or delay at the late growth stage before fruit cavity filling were other examples of fruit abnormalities. Thus, flower and fruit abnormality and abortions caused by unknown factors occurred at pre- or post-fertilisation durations. Abortion of small florets may be related to lack of nutrition and fruit growth substances.

#### 1.2 Blank Pistachio Development

A proportion of the *P. vera* crop always fails to split, related to the seedless (blank) or partially seeded (semi-blank) condition and close shell with a good kernel. Abnormal fruits have been identified by slower growth. These fruits often showed external signs of degeneration and were shed prematurely. Not all were shed and the retained fruits were observed microscopically and all showed degeneration of one or more tissues (Shuraki, 1995).

There are two phenomena associated with seedlessness. One is parthenocarpy, i.e. the production of fruit without fertilisation. The other is by undergoing post-fertilisation embryo abortion (Polito, 1999). Crane (1975) thought the latter was responsible for the major portion. There are 5 patterns of ovule degeneration that caused blank fruits, i.e. funicle degeneration, embryo sac absence, embryo sac degeneration, lack of pollen tube penetration of the embryo sac, and failure of endosperm cellularisation.

The majority of degeneration in the pistachio appeared to be related to funicle degeneration. Blank pistachio fruits contain only funicular and degenerated ovule tissue at maturity. Degeneration of the funicle occurred during anthesis with necrosis of the funicular epidermis. Degeneration of the funicle was observed at the site of pollen tube penetration or the epidermis of the chalazal end and necrosis extended towards the vascular tissue of the funicle. This early funicle degeneration was observed in both pollinated and un-pollinated abnormal pistils (Shuraki, 1995).

Ovules lacking embryo sacs and with degenerated or un-penetrated embryo sacs were observed at anthesis and nucellar degeneration commenced one week after pollination (Shuraki, 1995). Embryo abortion in pistachio has been variously attributed to lack of pollination, poor nutrition, rainfall during anthesis and water deficit during seed development (Crane and Iwakiri, 1981).

Lack of cellularisation of the endosperm and degeneration of the nucellus both resulted ultimately in the death of the pro-embryo at between 4 and 20 weeks after pollination. From 16 weeks after pollination (27 January) degeneration of the developing cotyledons arrested embryo growth (Shuraki, 1995)

Polito (1999) did not agree that embryo abortion was the major cause as suggested by Crane (1975). Polito's data suggested that parthenocarpic fruit set may be an important factor in the failure of `Kerman' pistachio fruits to develop seeds.

# If an early failure to support normal transport into an ovule is associated with needlessness, and if that phenomenon is preferentially seen in those ovules that lack endosperm, one can assume at least some fraction of the blank nuts result from parthenocarpic fruits.

A possible role for parthenocarpy contributing to blanking was further supported by the results of experiments with gamma-irradiated pollen. The intention of these experiments were to induce parthenocarpic fruit set by supplying a pollination stimulus using pollen capable of germinating at high levels, but damaged sufficiently that it was incapable of effecting normal fertilisation (Vardi *et al.*, 1988). When pistillate flowers were pollinated using pollen irradiated at this level both fruit set and the percentage of blank fruits increased. Fruit set increased by 30 % from 14:2 to 18:5 %; the percentage of blank fruits increased from 15:7 to 44.6, almost a three-fold increase. Shuraki and Sedgley (1996) found a high proportion of blank fruit from unpollinated flowers or from flowers pollinated late in their receptive period, a finding consistent with the hypothesis that parthenocarpy is a factor in pistachio blanking (Polito, 1999).

#### 1.3 Parthenocarpy

The movement of disodium fluorescein was used to study vascular transport into fruits and ovules of pistachio cv. Kerman, a cultivar very prone to producing seedless fruits. By about 2 weeks after full bloom blockages became apparent in the placenta or funiculus. At 6-9 weeks after anthesis 90-100% of fruits exhibited vascular blockages. Subsequent to this vascular conductivity was re-established in 83.3% (3-year mean) of ovules which correlated well with the percentage of seeded nuts at harvest (77.5%). A high percentage of ovules with blocked vasculature lacked endosperm and seemed to be unfertilised. Pollination using labelled pollen resulted in a 3-fold increase in seedless nut production.

## It is concluded that parthenocarpic fruit set plays a major role in the production of seedless nuts (Polito, 1999).

In a study of a natural population of the wind-pollinated essential oil plant *P. lentiscus* in Huelva province, Spain, fruits with empty seeds resulted from either parthenocarpy or embryo abortion. In both cases, such fruit rarely matured and was retained on the plant. Viable seeds occurred at a frequency of 19.89% in 1981 (a drought year) and 43.38% in 1982. The number of viable seeds produced was positively correlated with plant size. The role of parthenocarpy and seed abortion, which were not influenced by plant size, in regulating fruit number will be discussed (Jordano, 1988).

#### 1.4 Semi-Blank Pistachio Development

**Semi-blank fruit result from cotyledon breakdown during the latter stages of fruit development.** Breakdown of tissue was observed in the growing cotyledon of the pistachio seed, and appeared to start from the vascular tissue or from the epidermis late in development (Shuraki, 1995). Deficiency or imbalance of nutrient elements such as calcium, potassium, boron or nitrogen may be responsible (Goode and Ingram, 1971; Lewis and Martin, 1973; Shear, 1975).

#### 1.5 Nut Splitting and Close Shell with a Good Kernel

Shell thickness and suture angle may be significant factors in the splitting of *Pistacia vera* fruit at maturity. Shell splitting does not occur in any other species in the *Pistacia* genus although these endocarps undergo dehiscence along the longitudinal ridges as the seed germinates. Shells of the small seeded *P. atlantica* never split even following *P. vera* pollination. *P. vera* had lower proportions of full seeds and split shells following *P. atlantica* pollination. The ovary wall and shell thickness at both the dorsal and ventral sutures of *P. vera* were lower. However, there was no consistent effect of pollination on *P. atlantica* ovary wall and shell thickness (Shuraki, 1995).

Nevo et al. (1974) suggested the possibility that physical forces, exerted as the drying shell shrinks and encounters resistance from the kernel, may be responsible for inducing endocarp dehiscence. Crane and Iwakiri (1982) and Crane (1986) disagreed with this. They viewed endocarp dehiscence as resulting from unspecified biochemical factors produced by the growing embryo that activated a dehiscence response in the endocarp (Polito and Pinney, 1999).

There are two kinds of dehiscence events that occur: longitudinal shell split and apical dehiscence (Polito and Pinney, 1999).

Longitudinal shell split is driven by physical forces exerted on the endocarp by the seed. In the pistachio ovary, the longitudinal ridges at the dorsal and ventral faces of the ovary define the sites of longitudinal dehiscence (Hormaza and Polito, 1996). Cells at the line of longitudinal separation are somewhat smaller than those found in the rest of the endocarp; however, other than their smaller sizes and somewhat elongate aspect, these cells do not differ in any apparent ways from those that comprise the rest of the endocarp: they are irregular in outline with interlocking calls; they possess thick, fully lignified walls at maturity (Sexton and Roberts, 1982).

The dehiscence zone in the cortical region of the endocarp in November and January is subtly differentiated from other tissue of the endocarp. The line of dehiscence is distinguishable from adjacent tissue by the presence of somewhat smaller cells that are slightly elongated in the radial plane. There are no distinctions in cell type differentiating the dehiscence zone from the rest of the endocarp. By January 14 both the dehiscence region and the adjacent cortical tissue of the endocarp are composed of thick-walled, interlocking cells with completely lignified walls. These cells have lost their cytoplasmic contents in a fashion consistent with sclerenchyma tissue (Polito and Pinney, 1999). Nevo et at (1974) examined split and un-split shells for endocarp anatomy, shell thickness at the longitudinal dehiscence regions and cell microfibril alignments. None differed in ways apparent between dehiscent and indehiscent endocarps. They noted some shrinkage in the cells of the endocarp and concluded that the seed may exert physical force on the endocarp as it dries. In fruits with relatively larger seeds, these forces could be sufficient to induce dehiscence. This was supported by the observation that fruits with dehisced endocarps contained more massive seeds than those with un-split endocarps. Mean kernel dry mass of un-split nuts was 5%-14% less than that of dehisced nuts (Crane et al, 1982). In nuts that had split longitudinally, the shell: kernel size ratios were consistently lower than those in un-split nuts. Moreover, in the plane perpendicular to the lines of longitudinal dehiscence, where physical forces will act to drive the shell halves apart, un-dried kernels from split nuts were

larger than the inner diameter of the shells.

Seed growth is the primary force-generating factor. Endocarp separation first occurs at the time the growing kernels are reaching their final size and are filling the fully sized pericarp before endocarp drying and accompanying cell shrinkage have begun. Shell dehiscence percentages do increase with time, thus it is likely that endocarp drying may be a contributing factor in dehiscence that occurs later in fruit development. Structural observations of the longitudinal ridges are inconsistent with a model of hormonally or biochemically mediated dehiscence. Shell split occurs at 2 weeks or more before the exomesocarp (hull) dehisces (Polito and Pinney, 1999).

Apical dehiscence occurs at the site of intrusion of the transmitting tissue tract into the ovary (Polito and Pinney, 1999). The transmitting tissue tract is radially elongate along the major diameter of the ovary. At the apex of the pistachio pericarp the carpel margins are ontogenetically separate (Hormaza and Polito 1996). At ovary maturity, this tissue will differentiate to define the apical dehiscence zone of the endocarp. The extent of transmitting tissue at the apex of the ovary varies among flowers. A large fraction has a more or less extensive, radially elongate region. Some flowers present an extensive, radially elongate core of transmitting tissue extending in the transverse plane from the ventral to the dorsal regions of the future endocarp. Others have a relatively small strand of transmitting tissue that is roughly elliptical in the transverse section. In extreme cases, the transmitting tract is reduced to a small number of cells forming a tissue that has the form of an only slightly elongate ellipse in the transverse plane. This variation may correlate with the variation that occurs in extent of sterile carpel development such that in flowers with more extensively developed sterile carpels the styles contribute greater amounts of transmitting tissue to the stylar-ovarian transmitting tissue tract, leading to the presence of a relatively greater extent of transmitting tissue at the apex of the ovary. Typically, the cells that differentiate from the transmitting tissue that intrudes into the ovary wall remain thin walled and un-lignified. In rare cases these cells differentiate similarly to the rest of the endocarp to become lignified sclerenchyma. If the transmitting-tissue cells become lignified, the apical zone usually does not dehisce. Calcofluor white staining highlights the cellulosic walls of transmitting tissue cells that define the site of potential dehiscence as it appears in early November before the onset of endocarp lignification. For nut size there are no differences between tip-split nuts and un-split nuts (Polito and Pinney, 1999).

Suture notch angle is also a factor for nut split. At 8 weeks (2 December) the ventral distal suture angle of *P. vera* with *P. vera* pollination was narrow in contrast to the wide angle of *P. atlantica with P. atlantica* pollination. The angle of the ventral distal suture of *P. vera* was significantly higher following *P. atlantica* pollination than following *P. vera* pollination. Conversely, the angle of the ventral distal suture of *P. atlantica was* lower following *P. vera* pollination than *P. atlantica* pollination. Similarly, the higher ventral distal shell suture angle of *P. atlantica* appeared to be imposed on the inter-specific pollination increased the dorsal proximal angle of Kerman and reduced the ventral proximal angle of *P. atlantica* (Shuraki, 1995). It is possible that notch angle serves to focus forces at the endocarp and variations in the angle will result in differences in patterns of force generation sufficient to affect endocarp separation (Polito and Pinney, 1999).

#### 1.6 Inter-Specific Pollination

*P. atlantica* had lower fruit set and a higher proportion of blank fruits than *P. vera*. This may be due to the higher incidence of ovules lacking an embryo sac in *P. atlantica*. When *P. vera* following *P. atlantica* pollination, it had more ovules that lacked an embryo sac than *P. vera* pollination. Kerman had a smaller fruit following *P. atlantica* pollination. The embryo sac of *P. vera* also tended to be smaller following *P. atlantica* pollination. Fruit set has partial compatibility between *P. vera* and *P. atlantica*. For *P. vera*, embryo sac length is greater

following *P. vera* than *P. atlantica* pollination. *P. vera* pollination has been shown to stimulate embryo sac maturation in *P. vera*, and the diminished stimulation observed following *P. atlantica* pollination may be another possible cause of reduced inter-specific fertility. Also, degeneration of endosperm was particularly high in Kerman pollinated with *P. atlantica* (Shuraki, 1995).

At 2 days after *P. atlantica* pollination, a protuberance had developed from the *P. atlantica* funicle. This connected with a corresponding invagination at the base of the style at the junction with the ovary wall, to form a bridge or ponticulus along which the pollen tube grew. In some samples, up to five protuberances were seen on the funicle at the site of pollen tube penetration, but only one of them joined with the ovary wall. Pollen tubes either passed the free space between the ovary and funicle and penetrated the funicle ponticulus or passed directly through the ponticulus from the ovary wall. Following *P. vera* pollination of *P. atlantica*, ponticulus development was similar to that of *P. vera* (Shuraki, 1995).

Ovule development following intra-specific pollination was similar for the two species. There was no difference in nucellus length, in embryo sac or nucellus length at 8 weeks (2 December) following intra- or inter-specific pollination. The difference in ponticulus structure between *P. vera* and *P. atlantica* may be one cause of the observed reduction in inter-specific as compared with intra-specific fertility. In *P. vera* the ponticulus develops from both the ovary wall and from the funicle, whereas only the funicle produced the ponticulus in *P. atlantica*. The reason for continued proliferation of the *P. atlantica* ponticulus following pollination is not clear, and it is noteworthy that this proliferation did not occur following inter-specific pollination. The ponticulus of *P. atlantica* continued to grow after *P. atlantica* pollination (Shuraki, 1995).

#### 1.7 Xenia

The effects of the pollen parent (xenia) on seed and fruit characters are known to occur in several nut crops (Gouta et al., 2002). Pollen from wild pistachio species slow down nut development (Riazi et al., 1995), reduces kernel weight (Crane and Iwakiri, 1980; Riazi et al., 1995; Riazi et al., 1996), kernel length (Crane and Iwakiri, 1980), percentages of split shells (Crane and Iwakiri, 1980; Riazi et al., 1995; Riazi et al., 1996) and increased the percentage of blank nuts (Riazi et al., 1996). Overall, pollen from *P. vera* gave the best results (Ak et al., 1998). There were no clear differences when pollen from the different *P. vera* genotypes was used (Hormaza and Herrero, 1998).

#### 1.8 Early-Split

A small proportion of the crop may have early split shells and hulls. It starts 60 days prior to harvest (Hadavi et al, 2008). At this stage, the hull is attached to the shell so that it splits with the shell, exposing the kernel to moulds and insects (Tajabadipour et al, 2011) including Aflatoxins contamination (Doster and Michailides, 1995a).

Field investigations showed that early split nuts account for 1 to 4% of total nuts at harvest (Hadavi et al, 2008) or 1 to 4% with a maximum of 10% (Doster et al, 2001), 3.5% of 'Sirora' and 4.3% of 'Kerman' (Shuraki, 1995). Early split pistachios have significantly lower weight in all examined cultivar (Amani et al, 2012; Tajabadipour and Tezerji, 2012).

Although the reason for early split, reported at cultivation, such as cultivar and rootstock type, age, quantity of crop, irrigation, nutrition, soil, temperature, and humidity (Hokmabadi et al, 2007), and reported by chemical elements (Hartung et al., 1981; Ober and Sharp, 2003; Rahemi and Pakkish, 2005; Fooladi and Tafti, 2006; Hosseinifard and Panahi, 2006; Afshari and Hokmabadi,2008;Hadavi et al, 2008), irrigation trials gave more confirmation and repeated information. **In California, irrigation skipped in May, resulted in more early split than trees with irrigation (Doster and Michailides, 1995b; Doster et al, 2001).** 

Shell size mainly grows in stage I and the kernel grows in Stage III (Crane et al, 1971). In the same nut and although the shell and kernel should have similar internal resources and a similar environment the different stages can make their development relatively independent. They may have different irrigation and fertigation; they may have different nutrition competition in the different stages. This leads to different development of the shell and kernel within the same nut. Each nut, at its origin, has its potential size. Finally, some nuts drop, some reach 100% potential, but most only reach 80% potential and some just 60%. In this development process, some shells may be large (account for 20%), some medium (60%) and some small (20%). The kernel may have the same pattern. However the shell and kernel are independent. In this way there are 9 scenarios and they are detailed in Table 1.

			shell	
		Small (20%)	Medium (60%)	Large (20%)
	Small (20%)	Normal (4%)	Narrow open (12%)	Close shell (4%)
	Medium (60%)	Big open (12%)	Normal (36%)	Narrow open (12%)
kernel	Large (20%)	Early split (4%)	Big open (12%)	Normal (4%)

Table 1 Combinations between shell and kernel in different size development.

When the shell and kernel develops in the same size categories, i.e. large vs. large, medium vs. medium and small vs. small, they are 'normal open' (orange cells). Percentages in each case are noted in the table. When the kernel develops as a larger category than the shell, nuts show 'big open' (red cells); when the kernel develops as a smaller category than the shell, nuts show 'narrow open' (yellow cells).

Most importantly all these ranges are acceptable.

When kernel develops by 2 larger categories than the shell (blue cell) the quick developing kernel breaks the shell in an early stage and is defined as 'early split'. In contrast, when the kernel develops by 2 smaller categories than the shell (green cell), this is a 'close-shell' nut.

To summarise each percentage, 'normal' is 44%; 'big open' is 24%; 'narrow open' is 24%; 'close-shell' is 4%; and 'early split' is 4%. Although the actual percentages may be a little different from Table 1, they are similar to the percentage of early split nuts in field investigation by Hadavi et al (2008) and Doster et al (2001). The results are similar for small nuts as investigated by Amani et al (2012) and Tajabadipour and Tezerji (2012).

This interference does not negate the need to investigate the influence of chemical elements. Balanced elements will always benefit nut development. However, unbalanced elements cannot cause early split for a big shell with a small kernel.

This interference does not negate the need to investigate the influence of cultivation. However, any cultivation benefitting shell size development will reduce early split nuts, and vice versa.

#### **Reduction of the Impact of Alternate Bearing**

Fruit and nut trees such as apple, mango, olive, pecan, citrus and avocado display alternate bearing (Monselise and Goldschmidt, 1982). The Pistachio is also an alternate bearing species (Crane and Iwakiri, 1981). Alternate bearing of a significant magnitude disrupts marketing and precipitates strong fluctuations in net returns to the grower and processor. Further, the nut quality decreases when the crop load is heavy. Heavy crops have significantly higher percentages of nuts with non-split shells than light crops (Ferguson et at., 1995). Shell deformation of unknown etiology has been positively correlated with crop load (Niven et al., 1994). MacDonald and Bolkan (1991) observed increased tree death due to the soil-borne fungus *Venicillium dahliae* Kreb., following heavy crop years. Zhang (2004) reported that stylar end lesion (SEL) nuts only occur in off-crop years.

#### Mitigation of alternate bearing, therefore, would be desirable.

Two hypotheses for alternate bearing are proposed. The carbohydrate competition hypothesis states that the carbohydrate competition between fruits and buds causes lower flower bud formation. In contrast to this, the hormone hypothesis states that hormones from the fruit cause bud reduction (Goldschmidt and Golomb, 1982). In most alternate bearing species, the presence of fruit is the key factor controlling flower initiation, either by producing hormones which inhibit flower initiation (Marino and Greene, 1981; Hoad, 1978) or by acting as strong sinks for assimilates and competing with the meristems initiating inflorescent buds (Monselise and Goldschmidt, 1982). To overcome biennial bearing, many methods were tested using one or other of the hypothesis above.

Researchers worked on dry matter and carbohydrate (Crane et al., 1976; Crane and Al-Shalan, 1977; Barone et al., 1995; Nzima et al., 1997; Stevenson and Shackel, 1998; Nzima et al., 1999; Arzani et al., 2002; Baninasab and Rahemi, 2006), hormone (Lovatt et al., 2006) and polyamines (Roussos et al., 2004) as well as mineral requirements (Brown et al., 1995; Rosecrance et al., 1996). To indirectly control carbohydrate, scientists worked on de-budding and de-fruiting (Wolpert and Ferguson, 1990; Caruso et al., 1992; Boler, 1998; Vemmos, 1999; Rahemi and Ramezanian, 2007) and shoot distribution (Stevenson et al, 2000). Ferguson et al. (1995) listed pruning as the number one process to control biennial bearing. Beede et al. (1991) reported that pruning as much as 50% of the flower buds off in some treatments did not significantly alter the nut load from an un-pruned tree. Fruit buds lost through pruning are compensated by an increased nut of numbers per cluster. This implies that if more flower buds are removed it usually does not reduce crop.

Pruning is an important management tool in pistachio production. Before the winter of 2006, the Australian pistachio industry usually tipped branches in the upper canopy at a point located 2 or 3 vegetative buds above the last flower buds (Zhang, 2011). The California industry also used this pruning as "standard" pruning (Kallsen, 2008). The vegetative buds would provide locations for new growth in the following year. In this kind of annual pruning the tips are usually around 30 shoots for each female tree and 70 shoots for each male tree (Zhang, 2011). Such pruning stimulates strong growth at the top and represses growth in the lower parts of the tree. In addition, labour limitations also restricts detailed hand pruning in pistachio production in Australia.

Mechanical pruning is an alternative method. To overcome this problem, '1-side mechanical slant topping' technique was tried in the winter of 2006.

The main goal of pruning is to reach a useful balance between yield and growth and to reduce cultivation costs (Intrigliolo et al, 2011). Among the labour costings, in cultural operations, pruning ranks second, just below harvesting (Giametta and Zimbalatti, 1994). Of the total management costs (Aletà and Ninot, 2000) pruning done by hand has very high costs: 10-15% (Aletà and Ninot, 2000), 23% (Fontanazza and Romiti, 1995) or 25% (Camerini et al, 1999). Mechanical pruning can greatly reduce those costs. Sonnati and Ughini (2009) reported on hazelnut pruning. For manual pruning the number of cuts per tree was around 31.8 cuts and required about 39 min/tree compared with

mechanical pruning being less than 30 s/tree. The estimated cost for the hand pruning in California was \$1,468/ha. The dormant pole saw and ground lopper pruning ranged from \$494 to \$914/ha. The mechanical pruning was calculated to cost about \$99/ha (Krueger et al, 2013). Many fruit/nut trees used mechanical pruning in production such as pistachio (Zhang, 2014), hazelnut (Malvicini et al, 2014), walnut (Ninot et al, 2006), Pecan (Wood and Stahmann, 2004), macadamia (Wilkie et al, 2011), olive (Camerini et al, 1999), apple (Mokan, 1991), pear (Montevecchi et al, 1986), peach, apricot (Neri et at, 2011), cherry (Charlot and Millan, 2010), plum (Krueger et al, 2013), grape (Smart, 2014), citrus (Intrigliolo, 1986), litchi (Lu et al, 1995), longan (Wu et al, 2012) and mango (Medina-Urrutia et al, 1997).

To mitigate the effect of alternate bearing, this project tested

- Effect of de-budding on alternate bearing
- Effect of hand pruning on alternate bearing
- Effect of mechanical pruning on alternate bearing
- Effect on alternate bearing on accumulated yield

## Most Australian Orchards Reaching the High Yields Currently Being Obtained by the Best Orchards

#### 1.1 Group Visiting

Besides research on particular topics, contact with pistachio growers and improvement of their production capacity was carried out in the previous 3-year project. Based on voluntary participation a 24 member grower group was established. This work also analyzed historical data for 13 orchards. Comparisons of yields based on per tree or per hectare and comparisons of nut size were also provided to the growers. Fertiliser applications between the orchards were also analyised and it has been found that the maximum application of N was about 10 times higher than the minimum application. Generally the higher the N applied the higher the yield. This observation showed an obvious opportunity for improvement. These results have been communicated to all growers and this work has helped growers identify production problems and improve their yields and quality.

#### 1.2 Existed Model Maintenance

In the past 10 years' work models have been created for chill, nut size and maturity. These models are still maintained each year and provide valuable information and data for production and marketing.

#### 1.2.1 Chill prediction

The Australian Pistachio industry experienced low chill winters in 2005, 2009 and 2013. After the winter of 2005, Dr. Zhang and Cathy Taylor, DPI Victoria commenced laboratory work to test the chill requirements of pistachios. After the winter of 2009 the results indicated that Sirora required 59 chill portions. The industry then commenced the collection of weekly data and the calculation of chill accumulation in every winter.

Chill accumulation reports have been prepared and distributed to growers at the end of June, July and the 16 August each year since 2009 to provide information for growers to mitigate the effect of low winter chill. In the winter of 2013, most pistachio growers took action against low chill resulting in less damage in production. In the winters of 2014 and 2015 the predictions were highly reliable and this has resulted in good production.

## Assisting the Australia Industry to Achieve Better than World Best Practice for Yield, Quality and Profitability

#### 1.1 Results of Chill Prediction from Different Stations

California pistachio research is leading the world. However their chill requirement studies have not yet determined a threshold because of the different temperature readings in different stations within their growing regions.

Figure 1 shows the pistachio production area in south-east Australia. The place names with red horizontal bars are the locations of pistachio production. Among these locations, Wagga Wagga and Kaniva did not have historical low chill records and did not have any records of symptoms resulting from chill. The rest of the areas usually utilise chill portions from Mildura and Renmark meteorological stations.

To obtain more local results the Pistachio Grower's Association Inc commenced studying chill predictions utilising local weather stations.



Figure 1: Map for pistachio production area

#### **1.2** Temperature Conversion from Daily to Hourly

The calculation of hourly temperatures from daily maximum and minimum temperatures was posed in the original Utah model (Richardson et al, 1974). This method assumed that a daily temperature curve can be approximated by plotting maximum and minimum temperature at 12 hour intervals and connecting these points with straight lines. The "straight line" method worked fairly well in Utah State (Seeley, 1996) but it certainly did not work in coastal areas with onshore afternoon breezes (Aron, 1975). Richardson considered day-length in this conversion (Linvill, 1990). McFarland et al. (1987) posed the sine function to describe heating during daytime hours. However, the nighttime cooling curve is not as simple. Cooling depends on many factors, including moisture content of the air, cloud cover and soil heat flow. Linvill (1982) used a logarithmic night time cooling curve. In 1990, Linvill developed a set of formulae for this conversion, and more recently many authors (Darbyshire, 2011; Luedeling and Brown, 2011) have used this conversion.

## Methodology

#### 1 Mechanical Pruning Trial 1 at CMV Farms



Figure 1.1: Map of mechanical pruning trial 1 at CMV Farms.

16 rows were used for a mechanical pruning trial at Block 2A at CMV Farms. The purpose of this trial was to test in which winter (prior to on- or off-year) mechanical pruning showed maximum benefit. CMV Farms started mechanical pruning their whole orchard in the winter of 2007 (prior to off-year) using a '1-side mechanical slant topping' method.

The mechanical pruning trial prior to an 'off-year' commenced in the winter of 2009 while the mechanical pruning prior to an 'on-year' commenced in the winter of 2010. The other sides of the trees were pruned 2 years later. As shown in Figure 1.2, the key parameters of '1-side mechanical slant topping' are the centre height and the cutting angle. All centre heights used have been 3.9 m. For the cutting angles, winters 2009 and 2010 used 22°; winters 2011 and 2012 used 30°; and winters 2013, 2014 and 2015 used 27°. Besides the '1-side mechanical slant topping', in each winter mechanical pruning was also conducted on the bottom portion of the trees with 1 m inside, 1.5 m outside and hedging at 1.5 m from trunk.



Figure 1.2: Diagram of 1-side mechanical slant topping'

Eight trial plots were arranged in neighbouring rows and a 2 x 8 randomized plot design was used in this trial. These rows were individually harvested and recorded in harvests 2010 - 2015 except 2011 due to the severe anthracnose problem. After row harvest the yields were divided by the number of female trees to obtain a 'fresh yield in hull' per tree for comparison.

#### 1.1. Flower bud numbers after mechanical pruning

From the winter of 2011, 5 trees were selected for flower bud number counting and both sides of the tree were recorded separately for each tree.

#### 1.2. Mechanical tipping on 1-year-old wood

In the winter of 2011, trees in 2 rows in Block 6 were pruned using the '1-side mechanical slant topping' method. In the winter of 2012, long shoots grew on the tops of trees so to reduce shoot length '1-side mechanical slant tipping' was implemented to remain most 1-year-old shoots with a 30 cm length. Due to cutting of 1-year-old wood this operation is defined as 'tipping'.

#### 1.3 Effect of alternate bearing on accumulated yield

CMV Farms has 7 blocks with historical yield records as shown in Table 1.3. Further analysis was conducted to ascertain the effect of alternate bearing on accumulated yield.

Block	Year of planted	Tree spacing (m)	2002	2003	2004	2005	2006	2007
1	1984	7.30x7.00	0.68	2.60	1.43	3.42	1.80	3.64
2	1986	6.86x4.39	3.50	4.09	1.79	4.66	2.72	4.13
3	1987	6.86x4.39	2.83	4.01	1.03	4.61	3.24	4.05
4	1988	6.86x4.39	2.98	3.83	1.22	4.64	2.14	4.15
5	1989	6.86x4.39	3.67	3.39	1.89	4.11	3.01	5.25
6	1991	6.86x4.39	3.00	1.84	2.01	3.34	2.24	4.53
7	1991	6.86x4.39	3.48	2.53	1.77	3.53	2.15	3.47

Table 1.3: Basic status and yield/ha of 7 blocks of CMV Farms

Table 1.3: (continued)

Block	2008	2009	2010	2011	2012	2013	2014	2015
1	1.18	3.17	1.49	0.91	0.64	3.15	1.84	2.66
2	1.00	4.72	1.26	1.21	0.72	4.66	3.51	3.58
3	0.52	4.92	1.31	1.47	0.74	3.33	3.83	2.96
4	0.79	5.32	0.66	0.52	0.67	4.27	3.44	3.41
5	0.98	4.29	0.82	0.49	0.70	4.92	3.22	4.03
6	1.14	4.77	1.20	3.11	1.29	4.11	3.31	2.88
7	1.29	4.97	1.41	0.45	0.2	4.51	4.33	2.96

#### 1.4 Trial harvest

The trial rows were harvested separately and yields recorded on a row basis. Harvest dates are listed in Table 1.4. During harvest, about 10 kg samples in each shake were collected for each row. The samples were weighed accurately, then de-hulled and dried on the second day. After drying, the nuts were delivered to the APPC laboratory for analysis as part of their normal process. Return calculations per tree are based on prices in 2004 and are detailed in Table 1.5.

Harvest	1 <sup>st</sup> shake	2 <sup>nd</sup> shake	
2010	11 <sup>th</sup> March	23 <sup>rd</sup> March	
2011	No trial harvest due to anthracnose		
2012	12 <sup>th</sup> March	26 <sup>th</sup> March	
2013	11 <sup>th</sup> March	24 <sup>th</sup> March	
2014	11 <sup>th</sup> March	22 <sup>nd</sup> March	
2015	13 <sup>th</sup> March	24 <sup>th</sup> March	
2016	1 <sup>st</sup> March	15 <sup>th</sup> March	

Table 1.4: Harvest dates in different years

Table 1.5: Price for different nuts (Price in 2004)

Nut type	No 1 grade small	No 1 grade medium	No 1 grade jumbo	light stain	narrow split	Pick out	Loose kernel	Non split	Floater
Price (\$/kg)	4.66	7.25	8.07	7.25	5.20	3.44	9.93	4.50	4.20

Data was analysed by a two-way analysis of variance (treatment x replicate). Analysis of variance for percentages, p-values, were calculated based on transformed data

according the following formula:  $\arcsin\sqrt{\frac{percentage}{100}}$ .

## Mechanical Pruning Trial 2 at CMV Farms

#### 2.1 Trial Design

2

Mechanical Pruning Trial 2 at CMV Farms was a continuation of the Mechanical Pruning Trial 1. Trial 1 had 2 treatments as described in section 1 above.

Trial 2 had 4 treatments as shown in Figure 2.1. Two treatments were the same as trial 1, but in 2 other treatments topping in winters 2015 and 2016 was stopped to see if it was possible to stop pruning or not.



Figure 2.1: Map of mechanical pruning trial 2 at CMV Farms.

#### 2.2 Harvest

The trial rows were harvested separately and yields recorded on a row basis. Harvest dates are listed in Table 2.1. During harvest 10 kg samples, from each shake, were collected from each row. The samples were weighed accurately, then de-hulled and dried on the second day. After drying, the nuts were delivered to the APPC laboratory for analysis as part of their normal process. Returns were calculated on a per tree and based on prices in 2004 and are detailed in Table 1.5.

Table 2.1: Harvest dates in different years

Harvest	1 <sup>st</sup> shake	2 <sup>nd</sup> shake
2016	1 <sup>st</sup> March	15 <sup>th</sup> March

Data was analysed by a two-way analysis of variance (treatment x replicate). Analysis of variance for percentages, p-values, were calculated based on transformed data

according the following formula:  $\arcsin \sqrt{\frac{percentage}{100}}$ 

#### 3 Winter Oil Application Study

#### 3.1 Introduction

After a 6-year study comparing winter oil at 3% and 6% the trials were modified to a water/volume study.

#### 3.2 Trial Design

Stage 3 had 63 rows with 45 trees per row in 8.6 hectares and the trials were based on the 2 the following treatments:-

- If chill portion < 57 on 15 August
  - o 6% winter oil in 2000 L water
  - $\circ~~$  6% winter oil in 4000 L water
- If chill portion ≥ 57 on 15 August
  - 3% winter oil in 2000 L water
  - 3% winter oil in 4000 L water

with 5 replicates. Each replicate consisted of 2 neighbouring rows. Thus 20 rows were used for the trial and the rest of the rows were used for protection rows (Figure 3.2.1).



Figure 3.2.1: Map for trial design

#### 3.3 Winter Oil Application

Winter oil was applied between 19 and 26 August each year and the details are listed in Table 3.3.1. In the bloom period the flower dates were compared between treatments.

Winter	Portion on 15 <sup>th</sup> Aug.	concentration of oil	Application date	Application time	Portions on application
2014	54	6%	28 <sup>th</sup> August	Day time	59
2015	64	3%	28 <sup>th</sup> – 31 <sup>st</sup> Aug.	Day time	69 - 71

Table 3.3.1: Winter oil application dates and chill portions in different years

#### 3.4 Trial Harvest

The trial rows were harvested separately and yields recorded on a row basis. Harvest dates are listed in Table 3.4.1. During harvest 5 - 10 kg samples from each shake were collected from each row. The samples were weighed accurately, then de-hulled and dried on the second day. After drying, the nuts were delivered to the APPC laboratory for analysis as part of their normal process. Returns were calculated per tree based on prices in 2004 and are detailed in Table 1.5.

Table 3.4.1: Harvest dates for each shake each year

Year for harvest	1 <sup>st</sup> shake	2 <sup>nd</sup> Shake
2015	13 <sup>th</sup> March	24 <sup>th</sup> March
2016	1 <sup>st</sup> March	15 <sup>th</sup> March

Data was analysed by a two-way analysis of variance (treatment x replicate). Analysis of variance for percentages, p-values, were calculated based on

transformed data according the following formula:  $\arcsin \sqrt{\frac{percentage}{100}}$ .

#### 4 Chill Prediction Study

#### 1.1 Results of Chill Prediction from Different Stations

#### 1.1.1 Degree of Agreement between Prediction and plant Response

Collection of hourly temperature data from 9 Bureau of Meteorology (BOM) meteorological stations was undertaken and chill portion accumulation from 1 March to 31 August (or later to achieve 59 portions) was calculated. Bud break status of the pistachio trees was recorded to identify if chill was enough.

#### 1.1.2 Multiple Meteorological Stations Comparison

Data was downloaded at 15-minute intervals between 2007 and 2015 from stations at Lindsay Point, Merbein, Red Cliffs, Nangiloc, Robinvale, Lake Powell and Piangil from Lower Murray Water (LMW) weather stations and stations at Swan Reach, Waikerie and Peebinga from the SA Murray-Darling Basin Natural Resource Management Board (SAMDBNRM) weather stations.

Excess data was deleted and only 'sharp-hour' data was maintained in the database. If there was 1 or 2 missing data samples between the available data averages were used to fill the gaps. If there were more than 2-continuous-hours of missing data then that year's data was not used for this study or it was called a missing-data year.

In assessing the temperature, at the same time between LMW weather stations and Mildura meteorological station (BOM), from 1 March 0:00 to 31 August 23:00, there was 4416 hours difference. Temperature data from each LMW Weather station minus that from Mildura meteorological station (BOM) at the same time was then used. Graphs were plotted to show the variation from zero (no difference).

To find the difference of temperature at the same time between Renmark meteorological station (BOM) and the Mildura meteorological station (BOM), due to 0.5 hour difference in the time zones, data at 23:30 at Renmark minus data at 0:00 at Mildura was used for each of the 4416 hours. Graphs were plotted to show the variation from zero (no difference).

The difference of temperature at the same time between the Waikerie SA Murray-Darling Basin NRM Weather stations and the Renmark meteorological station (BOM), from 1 March 0:00 to 31 August 23:00 was 4416 hours. Temperature data from each LMW Weather stations minus Renmark meteorological station (BOM) at the same time was then used. Graphs were plotted to show the variation from zero (no difference). Stations at Swan Reach and Peebinga were not compared with the Renmark station because they were too far away from Renmark.

#### **1.2** Temperature Conversion from Daily to hourly

The formula for conversion (Linvill, 1990) for daytime is

$$T_{t_D} = T_{min} + (T_{max} - T_{min}) \times \sin\frac{\pi \times t_D}{DL + 4}$$
<sup>[1]</sup>

Here  $T_{t_p}$  is daytime temperature at  $t_p$  hours after sunrise;

T<sub>max</sub> is maximum temperature

T<sub>min</sub> is minimum temperature

DL is daylength in hours

The formula for after sunset is

$$T_{t_N} = T_s - \ln(t_N) \times \frac{T_s - T_{min}}{\ln(24 - DL)}$$
 [2]

35
Here  $T_{t_N}$  is night-time temperature (°C) at  $t_N > 1$  h after sunset

 $T_s$  is the sunset temperature obtained from Eq. [1]

The calculation for day length utilises climatological day number (CD) and latitude (LA). Climatological day numbers are the same for a given calendar day every year. Days are numbered from 1<sup>st</sup> March rather than the 1<sup>st</sup> January to avoid the 29<sup>th</sup> February numbering problem in leap years (Stuff and Dale, 1973).

If the latitude is  $\leq 40^{\circ}$ , then

$$DL = 12.14 + 3.34 \text{ x} \tan(LA) \times \cos(0.0172 \times CD - 1.95).$$
 [3]

If the latitude is  $> 40^{\circ}$ , then

$$DL = 12.25 + [(1.6164 + 1.7643 \times (tan(LA))]^2 \times (tan(LA))^2 \times (tan(LA)$$

Hourly temperatures from the Mildura meteorological station were collected from BOM office weekly.

# 5 Nitrogen Trial





Four-treatment levels of 75, 150, 250 and 350 kg nitrogen/ha were applied, using liquid urea ammonium nitrate (UAN) fertiliser. The trial design was 4 treatments x 10 randomized replicates (Figure 5.1.1). The irrigation schedule was managed by CMV farm staff in accordance with the practices for the rest of the farm. Each treatment had its on flow measured, was logged and records kept on the amounts of fertiliser added to each treatment's supply tank. In seasons 2013/14, 2014/15 and 2015/16, P and K were also applied as 4 treatments equally (at the same amount as the rest of the area in the CMV Farm).

In Figure 5.1.1, every treatment-plot had 3 rows and each row had 5 trees. For trial recording both side rows were protection rows and in the middle rows both side trees were protection trees. Thus, for trial record purpose only the 3 middle trees in the middle rows were used as trial recording trees. Within these 3 trees, 2 good trees were selected for trial recording trees and numbered in the cells from 1 to 80.

To avoid any influence of roots from neighbouring treatments a root cutting operation was undertaken in November 2014. The boundaries of every plot (3 rows x 5 trees in same colors in Figure 1) were cut (except the middle thick line) to a depth of 1 metre.

#### 5.2 Operation

In season 2014/15, fertigation commenced on the 7th October 2014 and concluded on the 6th February 2015. In season 2015/16, fertigation commenced on the 9th October 2015 and concluded on the 10 February 2016. Fertigation application met the project designs as shown in Table 5.2.1.

Season	Mid-month	Off-year %	350 kg N/ha	250 kg N/ha	150 kg N/ha	75 kg N/ha
		accumulation	Litre UAN	Litre UAN	Litre UAN	Litre UAN
	Oct to Nov	21	78.68→64.4	56.2→42.3	33.72→24.9	16.86→2.6
2014	Nov to Dec	47	176.09→185.8	125.78→129	75.47→87.5	37.73→25.4
-	Dec to Jan	73	273.50→290.8	195.36→207.7	117.21→146	58.61→55.5
2015	Jan to Feb	100	374.66→374.8	267.61→267.6	160.57→175.0	80.28→80.5
	Oct to Nov	21	78.68→95.1	56.2→81.3	33.72→64.4	16.86→21.3
2015	Nov to Dec	47	176.09→199.4	125.78→160.7	75.47→105.3	37.73→37.1
-	Dec to Jan	73	273.50→288.0	195.36→223.6	117.21→136.5	58.61→62.8
2016	Jan to Feb	100	374.66→375.1	267.61→267.6	160.57→160.6	80.28→80.3

Table 5.2.1: Fertigation progress in whole season

 $^z$  before  $\rightarrow$  is target and after  $\rightarrow$  is actual.

## 5.3 Leaf Analysis

Dates for sampling are listed in Table 5.3.1. This trial was designed with 10 replicates for harvest as shown in Figure 5.3.1. Each replicate contained all 4 treatments (75, 150, 250 and 350 kg N/ha). For leaf analysis the 10 replicates were pooled into 3 replicates as shown in the thick lines in Figure 5.3.1.

Table 5.3.1: Dates for leaf analysis

Season	1	2	3	4
2014/15	17/11	8/12	5/01	2/02*
2015/16	9/11	7/12	4/01	1/02*

NB: \* indicate leaf analysis for all elements.

250	350	75	150	250	150	350	75
150	250	350	75	150	350	75	250
350	250	75	150	250	350	75	150
350	75	250	150	75	250	150	350
250	150	75	350	350	150	75	250

Figure 5.3.1: Trial replication design

#### 5.4 Soil Analysis

Table 5.4.1: Soil sampling times, locations and depths

Year	Date for end	Date for soil sampling	location			Soil sample depths (cm)				
	fertigation	Samping .		0–15	15–30	30– 45	45–60	60–75	75-90	90-120
2015	6/02	10/02	Rep 2		V		V	Ň	V	V
2016	10/02	16/02	Rep 8		V		V	Ň	V	V

Before the nitrogen trial was set up on the 14 May 2008, CMV Farms had a soil analysis conducted in the orchard, including stage 2. The nitrogen trial was in rows 1 - 15, stage 2A. The sample was taken from between trees 26 and 27 in row 18 in stage 2A. This sample site was very close to our nitrogen trial and it was recorded as the soil sample before the trial. After the trial was established, soil samples were taken each year just after fertigation was completed. It can be seen that the soil sampling became deeper and deeper. In the results the graphs reported soil levels at 0 - 15, 15 - 30, 30 - 45, 45 - 60, 60 - 75, 75 - 90 cm. Current sampling included 90 - 120 cm. As it was difficult to put 7 levels on a graph the report in this paper uses, 0 - 30, 30 - 60, 60 - 90 and 90 - 120. Historical data with 15 cm intervals has been stored.

#### 5.5 Soil Solution from Sentek Solusampler

Sentek SoluSamplers were installed by the 31 January 2010. Among the 10 replicate plots (plots with 4 treatments), 3 replicate plots were installed with the samplers. They were placed around all trial trees in row 5 (2 replicate plots) and in the north half of row 14 (1 replicate plot). In each location, the samplers were installed at 3 different depths, i.e. 30 cm, 60 cm and 90 cm from the surface.

In season 2014/15, samples were taken on the 20<sup>th</sup> November, 23<sup>rd</sup> December, 28<sup>th</sup> January and 27<sup>th</sup> March and all samples were tested using a Merck meter. In season 2015/16 the samples were taken on 11<sup>th</sup> November, 10<sup>th</sup> December, 6<sup>th</sup> January, 8<sup>th</sup> and 17<sup>th</sup> February, and 22<sup>nd</sup> March and all samples were tested using a Merck meter.

#### 5.6 Test well

In season 2015/16 water samples were taken from the test wells 12 times. For the dates 11<sup>th</sup>, 17<sup>th</sup>, 23<sup>rd</sup>, 30<sup>th</sup> December, 7<sup>th</sup>, 13<sup>th</sup>, 17<sup>th</sup> January, water samples were obtained. For the dates, 10<sup>th</sup> November, 20<sup>th</sup> January, 4<sup>th</sup> February, 10<sup>th</sup> March and 22<sup>nd</sup> March no water was obtained.

#### 5.7 Trial Harvest

Season	Shake 1	Shake 2
2014/15	13 <sup>th</sup> March	24 <sup>th</sup> March
2015/16	29 <sup>th</sup> February	14 <sup>th</sup> -15 <sup>th</sup> March

Table 5.7.1: Harvest dates

For the 80 trial-recording trees, each tree was harvested and recorded individually. Harvest dates are listed in Table 5.7.1. During harvest approximately a 1 kg sample was collected from each shake of each tree. 4 samples in the same treatments were pooled together for further testing according to Table 5.7.2. The pooled samples were weighed accurately and then de-hulled and dried on the second day. After drying, the nuts were delivered to the APPC laboratory for analysis as per the normal process. Returns were calculated per tree based on prices in Table 1.5.

Table 5.7.2: Pooled samples from the original trees

N trial	Rep 1-2	Rep 3-4	Rep 5-6	Rep 7-8	Rep 9-10
75 kg N	(1) <b>5,6,15,16</b>	(5) <b>18,20,25,26</b>	(9)37,38,45,46	(13)55,56,61,62	(17) <b>69,70,77,78</b>
150 kg N	(2)7,8,11,12	(6)23,24,31,32	(10)39,40,47,48	(14)51,52,57,58	(18)67,68,75,76
250 kg N	(3)1,2,9,10	(7)17,18,29,30	(11)35,36,41,42	(15) <b>53,54,59,60</b>	(19)65,66,79,80
350 kg N	(4)3,4,13,14	(8)21,22,27,28	(12)33,34,43,44	(16)49,50,63,64	(20)71,72,73,74

Data was analysed by a two-way analysis of variance (treatment x replicate). Analysis of variance for percentages, p-values were calculated based on transformed data

according the following formula:  $\arcsin\sqrt{\frac{percentage}{100}}$  .

# 6 Pollination Trial

This was a special pollination trial to use nut setting from natural pollination; compare with nut setting from intensive hand pollination and compare with nut setting by avoiding pollination through the use of pollination bags.

# 6.1 2014

29 September 2014:	Set up 5 bags v	vith 5 shoots for non-pollination					
30 September 2014:	Set up 5 shoots pollination	with added pollen and 5 shoots with natural					
rom 30 September to 3 October 2014: Pollination was by single male flowers							
From 4 October to 7 Oc	tober 2014:	Pollination was within pollination bags.					
16 October 2014:	Bags were remo	oved from treatments.					

## 6.2 2015

Table 6.2.1 lists the treatments from the 2015/16 season.

treatment	number	Bagging date	Removal date		
Intensive pollination	10	Pollination between 28/09-7/10			
Open pollination	10				
Bagging	10	21 September	23 November		
Old bag	10	21 September	23 November		
Bag for observation	20	22 September	23 November		
Early bag	5	21 September	21 October		
Late bag	5	23 September	23 November		

Table 6.2.1: Treatments

# Results

# **1** Mechanical Pruning Trial **1** at CMV Farms

## 1.1. Influence of on/off-season pruning on alternate bearing



Figure 1.1.1: Yield and accumulative yield per tree between 2014 and 2016

Since the harvest in 2013 the tree yields have maintained a high level of 30 - 50 kg/tree. The following 3 years with relatively stable yields has shown the positive effect of this pruning for controlling alternate bearing. No significant difference was noticed between the 2 treatments for the yearly crops (left graph in Figure 1.1.1) and the accumulative crops (right graph in Figure 1.1.1).

# **1.2** Influences on Flower Bud Formation from Pruning Angle

As shown in Figure 1.2 (Page 28), this trial pruning had 2 key parameters being central height and pruning angle. With the 2 parameters being the same, geometrically we had a unique pruning technique. In the trial work the central height used was always 3.9 m high. In winters 2013, 2014 and 2015, 27° was used.

Figure 1.2.1 showed flower bud formation in the pruning side after slant mechanical topping. The 4 graphs on the top show responses for treatments prior to the off-years while the 3 graphs on the bottom show responses for treatments prior to the on-years. Unfortunately no flower buds were recorded for the winters of 2009 and 2010.

In the winters of 2011 and 2012 30° topping was used. In the winter of 2013 the

treatment, prior to an off-year, showed very high flower bud formation implying that crop adjustment was strong. In the winters of  $2013 - 2015 27^{\circ}$  topping was used and showed medium flower bud formation.



Figure 1.2.1: Flower bud formation in pruning side after slant mechanical pruning

#### 1.3. Mechanical Tipping on 1-Year-Old Wood

Table 1.3.1: Investigation on flower bud numbers after mechanical tipping

Pruning winter	Investigated trees		Flower bud number	
		Pruning side	Other side	Total
Control	5	639.0	140.6	779.6
Tipping	5	357.8	172.6	531.4
p-value		0.027	0.284	0.061

After mechanical tipping on 1-year-old wood, flower bud investigations were undertaken in the following winter and the results are detailed in Table 1.3.1.

Investigations on flower bud numbers showed that tipping significantly reduced flower

bud numbers on the tipped side. Although the other side showed some level of compensation the total flower bud numbers were reduced by the tipping.



#### 1.4 Effect of Alternate Bearing on Accumulated Yield

Figure 1.4.1: 1-year and 2-years average yield of CMV Farms from 2002 to 2016 Overcoming alternate bearing could result in more even yield; provide a more consistent income for growers and consistent quantity of product for processors.

Is there any extra benefit to yield itself? The CMV Farms yield data over 15 years and from 7 blocks may provide an answer.

Figure 1.4.1 shows yearly average yield per hectare with the standard error bar. Large fluctuations up and down do not show any clear patterns. Using 2-year averages plotted at 2-year intervals presents a yield pattern.

Although CMV Farms started 1-side mechanical slant topping in the winter of 2007 (prior to off-year) the weak topping angle did not shift crop very much. The comparisons were not to test the effect of 1-side mechanical slant topping but instead it was to test the effect of alternate bearing on accumulated yield. Due to the anthracnose in 2011 the yields have been divided into 2 stages:-

- a) 2002 to 2010 is alternate bearing stage,
- b) 2013 2016 is even crop stage, and

The project mainly compares the 2 stages – a) and b).

The average alternate bearing index for 2002 - 2010 was 0.414 while that for 2013 -

2015 was 0.115 highlighting a very significant difference. For average yields, 2002 – 2010 was 2.79 tonnes/ha while 2013 – 2015 was 3.57 tonnes/ha. Large variations from large alternate bearing waves make it hard to reach a level of significant difference. The 2-year average showed a clear pattern of increase. **Average comparisons** showed that an even crop produced an extra 0.78 tonnes merchantable yield per hectare every year (on- and off-years). The trial plots had 308 female trees representing an increase of 2.5 kg per tree.

What is the reason for those extra benefits? In a very large on-year, trees produced 3000 – 4000 flower buds but actually may only produce 1500 flowers. That means more than 1500 flower buds are non-functional flower buds. However the costs are the extra nutrition and energy used during flower bud initiation and development through until fruitlet setting with no real crop benefit. If the extra nutrition and energy could be used by the functional 1500 flowers there would be real production and yields benefits.

# 2 Mechanical Pruning Trial 2 at CMV Farms

Tables 2.1, 2.2 and 2.3 show 4 comparisons. The treatment 'prior to off-stop' showed significantly higher results than the treatment 'prior to on' and on average higher readings than the other two treatments. Figure 2.1 shows this comparison and also shows details about the fresh yield in-hull for the 4 replicates.

The treatment 'prior to off stop' showed the highest yield but this may not be a good result. No pruning on '1-side top' left many flower buds remaining and it was not surprising to see an increase in the yield. But what will be the influence for next year? Flower bud count in the coming winter would be the first step in observing the possible influence.

Seasons	Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Return /tree (\$)	Acc. Yield in hull/tree (kg)
	prior to Off	42.7ab	72.3	96.3	14.73b	101.9b	180.5
2015-	prior to Off stop	47.9a	69.8	95.0	17.05a	118.6a	
2016	prior to On	41.3b	67.2	94.0	14.37b	100.2b	183.3
	prior to On stop	43.3ab	67.9	95.1	14.97b	104.2b	
	p-value	0.036	0.114	0.265	0.005	0.004	0.836

Table 2.1: Yields, count size and returns



Figure 2.1: Yield comparison between 4 treatments in the 2016 harvest.

Seasons	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damag ed shell
	Off	0.46	58.7	0.30	0.42	2.21	2.59	4.80	5.99	0.37
2015-	Off stop	0.16	60.8	0.54	0.49	1.94	1.99	3.93	5.99	0.30
2016	On	0.12	60.5	0.59	0.42	2.00	1.77	3.77	6.24	0.24
	On stop	0.14	60.1	0.39	0.28	2.27	2.40	4.67	6.33	0.40
	p-value	0.104	0.765	0.601	0.608	0.710	0.338	0.097	0.950	0.685

Table 2.2: Percentages of nut qualities in physiological aspects

Seasons	Treat	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
	Off	4.50	0.52	1.41	2.61	0	0	2.80	24.3
2015-	Off stop	4.43	0.52	0.99	3.03	0	0	3.27	23.1
2016	On	4.04	0.40	0.95	2.79	0	0	2.98	24.0
	On stop	3.72	0.45	1.07	2.20	0	0.01	2.34	23.9
	p-value	0.429	0.666	0.456	0.208			0.182	0.951

Table 2.3: Percentages of nut qualities in stain aspects

There was no significant difference of quality in harvest in 2016.

# 3 Winter Oil Application Study

#### 3.1 Investigation on Flowering

In the winter of 2014 the chill finally reached 59 portions before the end of August. No bloom difference was observed during the bloom period.

In the winter of 2015 chill accumulation was very good but there was no significant differences in bud break. CMV Farms had 4 rows that did not receive a winter oil application and these trees had flowers that opened 3 days later.

## 3.2 Trial Harvest

#### 3.2.1 Yield

As detailed in Table 3.2.1.1 both seasons - 2014/15 and 2015/16 - showed yields with no significant difference. Figure 3.2.1 shows the yields and accumulated yields in both seasons.

In Figure 3.2.1.1 the 3 top graphs shows the results from 2014/15 while the 3 bottom graphs shows the results from 2015/16. For percentages of shake 1, significant difference was found in season 2014/15, however there was no significant different for shake 1 in season 2015/16. Also, from Figure 3.2.1.2 in the different replicates no significant difference was found between treatments for yield and count size.

Season	Chill portion	Treat	Yield/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Return \$/tree
2014-		2000L	28.4	82.5a	90.7b	10.03	70.40
2015	59	4000L	28.3	78.9b	94.4a	9.85	68.60
		p =	0.930	0.022	0.011	0.607	0.506
2015-		2000L	38.0	76.5	91.2	12.8	87.16
2016	71	4000L	37.4	75.1	90.5	12.4	83.96
		p =	0.578	0.066	0.464	0.341	0.305

Table 3.2.1.1: Yields, count size and returns



Figure 3.2.1.1: Yield and accumulated yield in seasons 2014-15 and 2015-16





#### 3.2.2 Quality

In Table 3.2.2.1, the treatment using 2000 L water showed significantly lower percentages of total non-split nuts in both seasons. This needs further investigation to see if this becomes a long term trend. However, in 2015/16, using 2000 L water showed a significantly higher percentage of blank nuts. This also needs further investigation and trial work.

Table 3.2.2.1: Percentages of nut qualities in physiological aspects in seasons 2014/15 and 2015/16

season	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
2014-	2000L	0.42	48.1	12.51	0.30	2.37	1.34	3.70b	6.95	0.12
2015	4000L	0.54	51.9	10.78	0.47	2.62	1.77	4.39a	7.28	0.12
	p-value	0.103	0.127	0.075	0.070	0.269	0.225	0.042	0.472	0.781
2015-	2000L	0.17	59.3	0.52	0.44	2.07	2.92	4.99b	7.40a	0.33
2016	4000L	0.12	58.1	1.24	0.61	2.76	3.74	6.50a	6.73b	0.41
	p-value	0.255	0.750	0.082	0.129	0.076	0.073	0.024	0.038	0.531

In Table 3.2.2.2, although some significant differences were noticed in season 2014/15, there was no any significant difference in season 2015/16.

season	Treat	%	% loose	%	% dark	% gold	%SEL	% other	% light
		pickout	kernel	adhere	stain	stain	nut	stain	stain
2014-	2000L	5.97	0.85	0.34b	5.58	0	0.00	0	21.2a
2015	4000L	6.06	0.99	0.86a	5.10	0	0.01	0	17.5b
	p-value	0.996	0.348	0.018	0.391		0.334		0.028
2015-	2000L	7.31	0.60	2.20	4.75	0	0	4.71	19.3
2016	4000L	7.10	0.60	1.77	4.80	0	0	4.81	19.0
	p-value	0.942	0.923	0.307	0.769			0.820	0.939

Table 3.2.2.2: Percentages of nut qualities in stain aspects in seasons 2014/15 and 2015/16

# 4 Chill Prediction Study

# 4.1 Results of Chill Prediction from Different Stations

## 4.1.1 Degree of agreement between prediction and plant response

Table 4.1.1 shows chill portion accumulation and actually plant response. Those marked in yellow show winters with low-chill-response by the trees and the dates in red highlight the incorrect predictions. All predictions for Mildura were correct, i.e. the 59 chill portions were reached before the 31 August and the plant response was good; 59 chill portions were reached after the 31 August the plant responded accordingly to the insufficient chill. With the Renmark prediction there was 2 incorrect predictions, in 2011 and 2014, chill portions reached 59 at 1 or 6 days after the 31 August but the plant bud break was good. Although this prediction was not 100% accurate from a biological point of view the results were acceptable.

Table 4.1.1: Calculation chill completion dates (59 portions)

Winter	Chill comp	letion date
	Mildura	Renmark
2004	10 August	18 August
<mark>2005</mark>	3 September	11 September
2006	1 August	6 August
2007	24 August	13 October
2008	14 August	19 August
<mark>2009</mark>	1 September	15 September
2010	16 August	21 August
2011	8 August	1 September
2012	4 August	11 August
2013	19 September	25 October
2014	26 August	6 September
2015	5 August	8 August

#### 4.1.2 Multiple meteorological stations comparison

Table 4.1.2 shows portions from the 1 March to the 31 August from different meteorological stations. In the table those marked in yellow are Bureau of Meteorological (BOM) stations. The station order is displayed geographically from west to east. Any empty cells were due to too much missing data and therefore no calculations.

Stations around Mildura showed different readings. Winter 2009 was a low chill season and the Mildura reading was 58 and the Renmark reading was 55 by the 31 August. While Robinvale was 62 portions and Swan Reach was 61 portions these area clearly showed low chill symptoms within the pistachio trees. Winter 2015 was a good chill season, however Swan Reach only had 32 portions and Piangil only had 44 portions. Around these areas the pistachio trees showed good bud break.

These results make it difficult for industry to decide the value of using these additional stations.

Winter	2008	2009	2010	2011	2012	2013	2014	2015
Swan Reach	75	61	71				61	32
Peebinga	97	95	92		80		68	82
Loxton	<mark>69</mark>	<mark>55</mark>	<mark>66</mark>	<mark>59</mark>	<mark>71</mark>	<mark>59</mark>	<mark>58</mark>	<mark>69</mark>
Waikerie	69	44	66				63	71
Renmark	<mark>65</mark>	<mark>55</mark>	<mark>65</mark>	<mark>58</mark>	<mark>68</mark>	<mark>55</mark>	<mark>55</mark>	<mark>70</mark>
Lindsay point		51		54			57	69
<mark>Mildura</mark>	<mark>68</mark>	<mark>58</mark>	<mark>69</mark>	<mark>67</mark>	<mark>73</mark>	<mark>57</mark>	<mark>59</mark>	<mark>71</mark>
Red Cliffs	70	57	71	66	74		61	77
Merbein							58	70
Nangiloc							64	65
Robinvale	70	62	72	67	70		60	72
Lake Powell				70	73		64	79
Piangil					78		52	44
<mark>Swan Hill</mark>	<mark>76</mark>	<mark>73</mark>	<mark>79</mark>	<mark>76</mark>	<mark>81</mark>	<mark>69</mark>	<mark>69</mark>	<mark>80</mark>

Table 4.1.2: Portions in different meteorological stations

#### 4.1.2.1 Direct comparison

To understand the difference we viewed the different readings at the same hours from  $0:00 \ 1^{st}$  March to  $23:00 \ 31^{st}$  August. Figure 4.1.2.1 shows this relationship.

As the 2 lines in the graph were so close together it was difficult to see any significant differences.



2009



#### 4.1.2.2 Zero comparison

To view the differences it was necessary to utilise another method. This was to use zero comparison which plotted the differences directly. Figures 4.1.2.2 to 4.1.2.6 show these results.

In Figure 4.1.2.2, there are 7 sub-graphs. The top four are for Lake Powell, the bottom three are for Piangil. In the Lake Powell graphs the values in the y-axis = hourly temperature of Lake Powell minus that of Mildura in each same hour. Positive values at that hour mean that Lake Powell had a higher temperature than Mildura. Negative values at that hour mean that Lake Powell had a lower temperature than Mildura. A reference line at 0 was also plotted on the graph.

The title of each graph shows the station and year (winter) and in the brackets are the portion readings. The first reading is Lake Powell

reading (or any other station marked on the title), after ":", the second reading is Mildura reading for that particular winter.

For very large readings (big difference from Mildura reading at that particular hour) text with the date, hour and temperature were attached. For example, on the top-left of top-left graph in Figure 4.1.2.3, 13/03 15:00 shows that particular hour in that year (see title). 32 - 22.4 = 9.6 means at that hour the Lake Powell temperature was 32° while Mildura temperature was 22.4° and the difference was 9.6°. At the bottom it means that at 10 am on 7<sup>th</sup> March the Lake Powell temperature was 23.5° while the Mildura temperature was 28.9° and the difference was -5.4°. All Other graphs are like this. All stations and all years were plotted if there was no missing data during the chilling period



Figure 4.1.2.2: Difference of hourly temperature of Lake Powell and Piangil from Mildura hour by hour.



Figure 4.1.2.3: Difference of hourly temperature of Red Cliffs from Mildura hour by hour.



Figure 4.1.2.4: Difference of hourly temperature of Robinvale from Mildura hour by hour



Figure 4.1.2.5: Difference of hourly temperature of Renmark from Mildura hour by hour.



Figure 4.1.2.6: Difference of hourly temperature of Merbein and Nangiloc from Mildura, and Waikerie from Renmark hour by hour.



Figure 4.1.2.7: Boxplots show the difference variations for each station each winter

While the 36 graphs above showed the differences in a visual form the conclusions are described statistical. Figure 4.1.2.7 shows the difference variations for each station each winter. Except for the 2-years data from Merbein the other stations did not show any advantages compared with the Renmark BOM station. To further exploit Figure 4.1.2.7, means and standard deviations of means of the differences are listed in Table 4.1.2.3 for each station. In Table 4.1.2.3, Nangiloc had the smallest mean difference, Merbein the second closest and Renmark was ranked number 3 among the stations. For standard deviation Merbein was the smallest one (but just 2 years), Renmark was the second smallest one with 7 years' data, among all the stations. This indicated that the Renmark readings were not only close to the Mildura readings but also kept stable variations, year by year, compared with other stations although all Lower Murray Water (LMW) stations were closer to Mildura than Renmark. From this comparison it seemed to show that the differences between BOM Renmark and Mildura stations were less than most of LMW stations, although the latter are geographically much closer to Mildura.

Station	Ν	Mean	Standard deviation
Nangiloc	2	0.033	0.901
Merbein	2	-0.080	0.025
Renmark	7	-0.180	0.165
Red Cliffs	7	-0.307	0.211
Lake Powell	4	-0.350	0.441
Piangil	3	0.389	1.349
Robinvale	7	-0.412	0.319
Waikerie	4	-0.635	0.197

Table 4.1.2.3: Means and standard deviation of mean from each station each winter

#### 4.1.3 An exploration of a special station

In the winter of 2014 when the Mildura station had 59 portions the Piangil station had 52 portions and in the winter of 2015 when Mildura had 71 portions Piangil had 44 portions. Why?

Figure 4.1.3.1 shows the geographic status as observed through a visit to the station. The observation machine was located on the north side of a very small hill (around 3 metres). On the south side of the machine there were tree shelters, then a small hill and on the other side of hill there were peach trees. When cold air blew from the south the trees and hills prevented the cold air from reaching the observation machine. This led to warm temperatures being recorded in the machine and the Piangil station showing lower chill than expected.

Meteorological observation machine have multiple functions and aims, however for the purpose of recording chill accumulation they may be unsuitable.



Figure 4.1.3.1: Geographically status of Piangil meteorological observation station.

#### 4.2 Temperature Conversion from Daily to hourly

To test the reliability for chill assessment the Mildura daily maximum/minimum temperatures

against hourly temperatures from 1 March to 31 August, between 2000 and 2014, were used. Sunrise and sunset time was calculated according to Zhang et al (2003).

From Table 4.2 the results of the Dynamic portions, between hourly temperature assessment and daily temperature assessment, were relatively close, while the hours between  $0 - 7.2^{\circ}$ C showed that daily converted temperatures had much more hours between  $0 - 7.2^{\circ}$ C than the real hourly tempeature assessment. Using the Utah model, most results showed that the daily converted temperatures had much more Utah units than the real hourly tempeature assessment. This indicated that converted temperatures had more low temperatures than the really hourly records.

Winter	Dynamic			Utah			Hours between 0 - 7.2°C			
	hourly	daily	% var	hourly	daily	% var	hourly	Daily	% var	
2000	75	71	-5.33	1294	1338	3.40	715	915	27.97	
2001	63	64	1.59	1062	1122	5.65	635	780	22.83	
2002	59	60	1.69	941	996	5.84	640	790	23.44	
2003	64	64	0.00	1077	1130	4.92	629	750	19.24	
2004	65	71	9.23	1097	1218	11.03	625	826	32.16	
2005	57	56	-1.75	857	942	9.92	574	742	29.27	
2006	72	72	0.00	1137	1257	10.55	773	977	26.39	
2007	59	57	-3.39	1020	1015	-0.49	688	854	24.13	
2008	68	71	4.41	1152	1278	10.94	662	821	24.02	
2009	58	61	5.17	823	956	16.16	488	589	20.70	
2010	69	71	2.90	1198	1254	4.67	644	790	22.67	
2011	67	64	-4.48	1101	1119	1.63	646	781	20.90	
2012	73	71	-2.74	1152	1207	4.77	754	906	20.16	
2013	57	58	1.75	816	869	6.50	575	665	15.65	
2014	59	59	0.00	858	892	3.96	569	719	26.36	

Table 4.2:Chill accumulation between 1 March and 31 August in different winter, model and<br/>temperature source.



Figure 4.2: Comparison between hourly temperatures and converted hourly temperatures between 1 July and 16 July 2014.

Figure 4.2 shows the diffrence between hourly temperatures and converted hourly temperatures between 1 July and 16 July 2014. Continuously 16-days of data were chosen to avoid any distortion of the figures.

In the figure the red dash lines represent hourly records while the green lines represent converted temperatures.

It can be seen that daily temperatures were relatively close however the night temperatures were quite different. Converted night temperatures were going down smoothly while real hourly records usually kept higher temperatures just after sunset then sharply went down in certain stages. This produced more low temperatures, from converted temperature, than the real records and led to high estimations for Utah units and hours between  $0 - 7.2^{\circ}$ C.

Linvill (1990) developed his model in Clemson, South Carolina, USA and produced different results than those from the research through this project. It is possible that the model was suitable for the USA. Because night temperature change can be affected by the moisture content of the air, cloud cover and soil heat flow, Mildura may be totally different from Clemson. The Utah conversion methodology showed it was unsuitable for Mildura area, at least in winter months and may lead to an over estimate of chill accumulation.

# 5 Nitrogen Trial

#### 5.1 Leaf Analysis

#### 5.1.1 N analysis

Figure 5.1.1 shows the change in total N in the leaf, measured fortnightly in first 3 seasons and then for season 2012/13 leaf sampling time was reduced to 4 and for season 2013/14 was reduced to 3.



Figure 5.1.1: Fortnight leaf analysis for N only in 3 growing seasons

In general the N content showed a reducing pattern from the early growing season through to harvest time. This pattern did not change when reducing the sampling from 11 leaf-sampling times to 4 or 3 sampling times. Also, treatments with more N application showed higher readings. In Figure 5.1.1, different letters on the same date and same year showed significant difference at p=0.05 level (Fisher test). After root cutting in late 2014 the last 2 seasons showed the differences between treatments becoming larger.

#### 5.1.2 Yearly leaf analysis for all elements

#### 5.1.2.1 Major elements

Figures 5.1.2.1 and 5.1.2.2 show the element changes across 2009 to 2016 with the different colour lines for different treatments. Standard error bars highlight the variations and different letters reflect the

significant differences at the  $p \le 0.05$  level. The horizontal lines show the suggested ranges used in California for each element and the dash horizontal lines show the critical values. In some graphs where the actually readings are far different from the suggested ranges the suggested ranges were listed on the graphs as text.

Total N had higher readings in 2010 and 2011 but this was possibly due to the sampling. In 2013, which was a large on-season, except for the highest N treatment all means were below the suggested range. 2014 was still an on-year on 1 side of the trees. While 2015 was an on-year, for the whole tree, it was not a very heavy crop but the N content went up. Leaf N readings reached the suggested range in 2015 and 2016 however, the treatment of 75 kg N/ha showed a significant lower level in leaf N content. Based on personal observation, since 9<sup>th</sup> January 2016, the leaves close to nut clusters became yellow under the 75 kg N/ha treatment while the other treatments had yellow leaves starting in early February.

In contrast, for K only the lowest N treatment had K readings above the suggested range in 2013. However through the continuous on-years K was lower for all treatments in 2014 but had reasonable K contents in 2015 and 2016 due to K application. When the treatment of 75 kg N/ha showed a significant lower level in leaf N content it also showed a significant high level in leaf K content.



Figure 5.1.2.1: Major element changing pattern

For all major elements, except the significant differences found for N and K, they did not show any clear differences within years. Values for the treatment of 75kg N/ha were lower for N, and P, whereas other nutrients were about the same. Why lower P? The soil data would suggest that there had been movement of P deeper in the soil under the drip line. Anyway P application in season 2013/14 changed the status and in 2014/15 and 2015/16 the P readings went up.

Among the elements, Ca and Mg demonstrated high readings in onyears and low readings in off-years. In contrast, K showed higher readings in the off-years and low readings in the on-years. S showed a similar pattern as K except in year 2012 where S did not show higher readings than K.

5.1.2.2 Minor elements and Na + Cl



Figure 5.1.2.2: Minor element changing pattern

Figure 5.1.2.2 shows that most CI readings were above the suggested range. In off-seasons some of the B readings were below the suggested ranges including 2015 however the B reading in 2016 went back to a more normal range.  $NO_3$  showed significant differences in 4 years out of the 6 years. Treatments of 75 kg N/ha always showed low readings. Other elements showed variable readings but were higher than the treatment of 75 kg N/ha.

## 5.2 Soil Analysis

In some graphs the standards for "marginal", "optimum" or "high" etc are highlighted and are based on the standards according to Orr (2006).



#### 5.2.1 Organic matter, conductivity and pH values

Figure 5.2.1: Organic carbon, conductivity and pH values

#### 5.2.1.1 pH values

All pH values seemed to be reduced in 2012 however, most pH values showed an increasing trend. At least there was no reducing trend from 2012 to 2013. The treatment using 350 kg N/ha decreased extremely quickly in 2012 with the pH value of the treatment reaching 4.7 in water and 3.7 in CaCl<sub>2</sub> in the 0 - 30 cm zone. This has been determined as an undesirable side effect of high level nitrogen fertiliser application as the values like this can be toxic to roots. Lime was applied on the 2<sup>nd</sup> January 2013 and in the winter of 2013 to negate this problem. In the soil sampling in 2013 the pH values showed a large increase. Then 1 year later the 350 kg N/ha treatment, at the top soil level, reduced the pH value again. However, it was still an acceptable level in 2014, reached moderately acid in 2015 and below the acceptable level again in 2016.

#### 5.2.1.2 Organic carbon

It was noticed that organic carbon was going up in 2014 but it might be as a result of a sampling error.

5.2.1.3 Conductivity

All conductivity readings were at satisfactory levels.

#### 5.2.2 N, P, K and S

#### 5.2.2.1 Nitrate-N

In most cases treatments at 350 kg N/ha usually showed the highest nitrate-N content. In soil between 30 and 60 cm depth this treatment showed high levels in 2009 and 2010 however, it returned to a more normal level in 2011 and 2012. The results were high again in 2013 and 2014, especially in deep soil. However, in 2015 and 2016 the treatment at 350 kg N/ha did not show very high readings. Except for the 350 kg N/ha treatment no other treatments showed high readings at 30 cm or deeper.

#### 5.2.2.2 Ammonium-N

Ammonium-N produced unusual results in that the treatment at 75 kg N/ha had extremely high readings compared with other treatments in 2013.

#### 5.2.2.3 Phosphate

P amounts went up in 2012 and then down in 2013. After phosphate fertiliser application in seasons 2014, 2015 and 2016 the readings again went up.

#### 5.2.2.4 Potassium

The results seemed to show that potassium as total content declined but there were a lot of readings at marginal levels. Treatments at 350 kg N/ha showed the quickest decline in K. In depths of 0 - 60 cm the readings were in the "low" levels in February 2013.

Drip irrigation was used in this trial and roots were concentrated under/around drip lines. K consumption occurs around drip lines and consumption occurs much quicker. In California (Zeng et al, 1999) it was seen that K application increased pistachio yield when the soil K content was between 97 and 156K-Colwell mg/kg. Our K-Colwell mg/kg reading for the 350 kg N/ha treatment reached 111 in February 2013 and showed a continuous declining pattern.

K became a restriction factor and this indicated that potassium application needed to be considered. K was applied in seasons 2014

and 2015 and the level of K showed an upward tendency. In 2016 K seemed to go down again as the pH value also went down.

#### 5.2.2.5 Sulphur

This element was in very low levels in the top soil.



Figure 5.2.2: N, P, K and S

#### 5.2.3 Percentages of exchangeable elements

Exchangeable elements can be expressed in absolute readings and percentages but the percentages of Cations in Cation Exchange Capacity (CEC) looks more important.

#### 5.2.3.1 % Exchangeable K

According to desirable levels the exchangeable K seemed to be a little lower, especially in February 2013. Since September 2013, K was applied in the soil a number of times resulting in the percentages going up.

#### 5.2.3.2 % Exchangeable Ca

According to desirable levels and in most cases the exchangeable Ca was enough. However exchangeable Ca under at the 350 kg N/ha

treatment suddenly decreased in the top soil in 2012 but the reading was back up in 2013. This seemed due to lime application on the  $2^{nd}$  January 2013. In 2015 and 2016, Ca decreased again as did the pH value.

#### 5.2.3.3 %Exchangeable Mg

Most of readings for exchangeable Mg were in the desirable levels. However most readings from the 350 kg N/ha treatment were the lowest in each of the years.

#### 5.2.3.4 % Exchangeable Na

Exchangeable Na has a clear standard of no more than 5% CEC. Almost all readings, especially in the last 2 years were < 5%; however since 2014 the readings for Na had been going up.



Figure 5.2.3: Percentages of exchangeable elements

#### 5.2.3.5 % Exchangeable Al

In other standards exchangeable AI readings are not more than 5% CEC. However in the trials the percentages for top soil at the 350 kg N/ha treatment, in 2012, were significantly higher than the standard. In the 2013 soil samples, AI readings reduced to be within the standards but this may have been as a result of lime application. In 2015 the AI reading for the 350 kg N/ha treatment reached 5.2%

indicating lime application may well be again required. In 2016 the Al readings for treatment 350 kg N/ha reached 23%.

#### 5.2.4 Zn, Mn, Cu, Fe and B

## 5.2.4.1 Zn, Mn, Cu and Fe

Zn, Cu and Fe were all in optimal levels, while Mn was in an optimal to high level. These elements showed a tendency to be up in 2012, showed a downward tendency in 2013 and then up in 2014. This seemed to correlate with the biennial bearing cycle. In the off seasons of 2012 and 2014 the elements were high while in the on-season of 2013 the elements were low. It was also noticed that Mn in the leaf analysis showed an increase pattern in 2012 and decrease pattern in 2013. Fe was up in 2016 and this seemed because of the low soil pH value.

5.2.4.2 Boron

The top soil showed a stable low level of boron; however the deep soil seemed to show an optimal level.



Figure 5.2.4: Zn, Mn, Cu, Fe and B

## 5.2.5 Conclusion

- With the nitrate test, except for the 350 kg N/ha treatment, there were no high readings in the deep soil.
- Total potassium content decreased especially for the 350 kg N/ha treatment. After potassium applications the content increased.
- After lime applications, pH went up and Al readings went down while the Ca reading went up.
- The 350 kg N/ha treatment leads to a lowering of pH value and low K content which the results in high Al content.

#### 5.3 Soil Solution from Sentek SoluSampler

#### 5.3.1 Solution collection

Table 5.3.1 lists sample collections in seasons 2014/15 and 2015/16. Generally speaking, due to the daily irrigation with less irrigation each day, sampling at 90 cm became difficult. Samplers established at 60 cm were in a soil layer that was a very rocky layer resulting in it being difficult to keep water in the layer. It was therefore very difficult to get water from these samplers during many years of the trial.

# Table 5.3.1:List of successful soil solution sampling collection in dates, treatments and<br/>depths in seasons 2014/15

Season	Treat	rep	350					250	150					75	Total
	Depth		3	6	9	3	6	9	3	6	9	3	6	9	
			0	0	0	0	0	0	0	0	0	0	0	0	
	20 Nov	2	V			V	V		V			V		V	18
		7	V		V	V		V	۷	V	V		V		
		8	V			V		V			V				
	23 Dec	2	V			V	V		V			V		V	14
2014		7	V			V			V	V		V			
-		8	V			V		V							
2015	28 Jan	2	V			V	V		V			V			12
		7	V			V				V		V			

		8	V			V		V							
	27 Mar	2	V			V			V			V			8
		7	V			V	V								
		8				۷									
	10 Nov	2	V			V	V	V	V			V	V	V	26
		7	V	V		V	V	V	V	V	V	V			
		8	V	V	V	V	V	V			V		V	V	
	10 Dec	2	V	V	V	V	V	۷	V	V	V		V	V	32
		7	V	V		V	V	۷	V	V		V	V		
		8	V	۷	۷	V	V	۷	V	V	۷	V	V	V	
	6 Jan	2	V			V	V		V	V		V			19
2015		7	V			V		۷	V	V		V			
-		8		۷		۷	V		V	V		V	V		
2016	8 Feb	2	V			V			V			V			9
		7	V			V					V				
		8										V		V	
	17 Feb	2	V			V			V			V			6
		7	V			V									
		8													
	22 Mar	2	V			V			V			V			6
		7	V			V									
		8													
			I												

#### 5.3.2 Measurement results



Figure 5.3.2.1: Nitrate-N from samplers in season 2014/15



Figure 5.3.2.2: Nitrate-N from samplers in season 2013/14 to 2015/16

When utilising a Merck meter the program could only test for nitrate resulting in no ammonium tests in this report.

Figure 5.3.2.1 shows the nitrate-N changing pattern in the seasons from 2010/11 to 2012/13. Figure 5.3.2.2 shows the nitrate-N changing pattern in the seasons from 2013/14 to 2015/16. Generally speaking the treatments with more fertiliser showed high concentrations. Due to missing data it was difficult to present a clear picture, especially within the deep soil.

#### 5.4 Test well

During the 2015/16 season 12 water samples were collected from the test wells. For the dates 11<sup>th</sup>, 17<sup>th</sup>, 23<sup>rd</sup>, 30<sup>th</sup> December, 7<sup>th</sup>, 13<sup>th</sup> January water samples from the 250 kg N/ha treatment was obtained. On the 7 January 2016 some water was collected from the 150 kg N/ha treatment, but not enough for test but water samples were collected on the 17<sup>th</sup> February. For the dates, 10<sup>th</sup> November, 17<sup>th</sup> and 20<sup>th</sup> January, 4<sup>th</sup> February, 10<sup>th</sup> March and 22<sup>nd</sup> March all test well had no available water.



Figure 5.4.1: Test well records from season 2015/16

Most treatments had no water samples in the test wells indicating that the irrigation did not push water deep enough and kept the nitrate in the soil. For the treatment 250 kg N/ha treatment the concentration of nitrate appeared to decrease due to the irrigation solution concentration decreasing. However the resulted seemed to indicate nitrate leaching. Treatment at 150 kg N/ha had no nitrate in the water.
#### 5.5 Trial Harvest

#### 5.5.1 Yield

Seasons 2014/15 and 2015/16 did not show a significant difference in yield between the relevant treatments (Table 5.5.1.1). The 75 kg N/ha treatment showed a significantly higher percentage in shake 1 compared with others in both seasons. There was no significant difference in count size between the treatments.

Before the commencement of the growing seasons total flower buds on 20 trees, in the 350 kg N/ha and 75 kg N/ha treatments were counted. After harvest, a plot showed the relationship between flower buds and yields under the different treatments and the results are in Figure 5.5.1.1. The figure shows crops in 5 seasons under 2 treatments:-

- Points for off-season 2012 are at the left-bottom indicating an off-season.
- Points for on-season 2013 are at the middle of the graphs and above the regression line indicating more nuts borne per flower bud. This probably was due to tree capacity recovering after the anthracnose infection.
- Points for off-season 2014 are at the right side and most of them are below the regression line. This seemed to indicate that tree capacity became weak after a strong bearing season.
- Points for on-season 2015 showed to be closer to the regression line. After a 'wave' of bearing most of the trees returned to the normal pattern.
- Points for off-season 2016 also showed the return to a normal pattern. In 2016 under the 350 kg N/ha treatment, there were 2 points with high numbers of flower buds but low yield. In checking those two trees the low yields were due to machine shaking and a lot of nuts being left on the trees.

Seasons	Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Total return/ tree (\$)	Acc. Yield in hull/tree (kg)	Sum yield in continuous years
2008-	75	55.0	73.5a	91.6	18.3	118.7	55.0	
2009	150	53.5	64.6b	89.5	17.6	114.0	53.5	

Table 5.5.1.1: Tree status	, yields,	count size	and	returns
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On-	250	54.3	69.7a b	90.7	18.2	118.1	54.3	
Season	350	57.3	70.9a	89.6	19.7	128.1	57.3	
	p-value	0.559	0.037	0.303	0.145	0.108	0.559	
2009-	75	9.8B	86.3	82.1	3.55	24.3	64.8b	64.8b
2010	150	15.1A	85.9	83.8	5.73	38.7	68.6ab	68.6ab
Off-	250	15.6A	88.3	82.8	5.81	39.7	69.9ab	69.9ab
Season	350	17.5A	87.1	82.8	6.72	45.9	74.8a	74.8a
	p-value	0.001	0.496	0.166	0.079	0.088	0.044	0.044
	20:	10-2011 h	as no trial	harvest o	due to anthrach	ose		
2011-	75	7.5	94.4a	83.9	2.16	13.4	72.0	
2012	150	8.5	85.6b	83.1	2.74	16.4	77.2	
Of-	250	10.4	80.2b	90.2	3.05	17.4	80.4	
Season	350	9.2	78.9b	87.7	2.87	17.2	84.0	
	p-value	0.442	0.001	0.639	0.393	0.512	0.060	
2012-	75	57.3	93.2a	89.2	17.53	118.8	129.3	64.7
2013	150	56.1	91.1b	90.4	16.65	111.7	133.3	64.7
On-	250	53.6	89.6bc	88.1	16.18	108.4	134.0	64.0
Season	350	56.7	88.8c	89.4	16.71	108.3	140.8	65.9
	p-value	0.638	0.000	0.065	0.703	0.510	0.318	0.956
2013-	75	30.0b	85.6a	87.4	12.6	76.1	159.0	94.4
2014	150	35.6a	84.5a	87.1	14.2	87.3	168.9	100.3
Off-	250	31.8b	80.3b	88.0	11.9	73.7	165.7	95.8
Season	350	34.3ab	79.4b	87.5	12.7	77.9	175.1	100.2
	p-value	0.017	0.000	0.822	0.101	0.113	0.101	0.294
2014-	75	26.9	81.0a	88.0	11.1	78.4	185.9	121.3
2015	150	26.4	73.5b	88.8	9.6	68.1	195.3	126.7

On-	250	26.0	73.6b	87.8	9.7	67.7	191.7	121.8
Season	350	28.3	74.5b	89.0	10.4	72.4	203.3	128.5
	p-value	0.768	0.014	0.956	0.620	0.585	0.106	0.345
2015-	75	33.6	83.4a	92.2	12.9	91.4	219.5	154.9
2016	150	35.4	74.6b	92.7	13.2	93.3	224.0	162.1
Off-	250	32.3	70.2bc	94.9	12.0	85.0	230.8	154.1
Season	350	33.4	65.5c	97.9	12.5	87.9	236.7	161.9
	p-value	0.657	0.000	0.217	0.239	0.277	0.171	0.411

The left graph in Figure 5.5.1.1 showed that the yearly fresh yield in hull changed. On/off year made it difficult to identify the effects. The right graph showed the change in the 2-year average. It can be seen that in the first 2 years, treatment 350 kg N/ha had a significantly higher yield than treatment 75 kg N/ha. However, after anthracnose infection this effect disappeared.



Figure 5.5.1.1: Yearly fresh yield in hull changing and 2-year average changing.

Before the growing seasons total flower buds on 20 trees, in treatments of 350 kg N/ha and of 75 kg N/ha were counted. After harvest the relationship

between the flower buds and yield, under different treatments, were plotted and detailed in Figure 5.5.1.2.

The figure 5.5.1.2 shows crops in 5 seasons under 2 treatments:-

- Points for the 2012 off-season are at the left-bottom indicating an offseason.
- Points for the 2013 on-season are at the middle of the graph and above the regression line indicating more nuts borne per flower bud. This probably was due to the trees capacity to recovery after the anthracnose infection.
- Points for the 2014 off-season 2014 are at right side and most of them are below the regression line. This seemed to indicate that the tree capacity become weak after a strong bearing season.
- Points for the 2015 on-season were closer to the regression line. After a 'wave' of bearing, most trees appeared to return to the normal pattern.
- Points for 2016 off-season showed the trees returning to a normal pattern. In 2016 under 350 kg N/ha treatment, there were 2 points with high numbers of flower buds but low yield. On checking these two trees had low yields due to the machine shaking leaving a lot of nuts on the trees.

**In all harvests from 2012 to 2016 the 350 kg N/ha treatment showed more flower buds than the 75 kg N/ha treatment.** At the 2012 harvest both treatments seem to show a similar pattern of nut bearing. At the 2013 harvest the 75 kg N/ha treatment seemed to show the bearing of more nuts per flower bud than the 350 kg N/ha treatment. On average, the former produced 35.8 nuts per rachis while the latter only produced 25.8 nuts per rachis. The variance between the 350 kg N/ha and 75 kg N/ha treatments cannot be fully explained particularly in relation to the higher yield from the 75 kg N/ha treatment. What was obvious was that the 56.74 kg per tree, on average, for the 350 kg N/ha treatment was not a low yield. However, the 75 kg N/ha treatment really had a high yield, 57.28 kg per tree. At harvest in 2014, 2015 and 2016 the 350 kg N/ha treatment seemed to show more flowers buds and a little higher yields than the 75 kg N/ha treatment but the nuts per buds were much lower than that in 2013.



Figure 5.5.1.2: Relationship between yield and flower buds

In the N trial there was a discrepancy in the treatments in that in January 2010 the 75 kg N/ha treatment, in replication 9 - trees 69 & 70 - received a large amount of nitrogen fertiliser applied by mistake. Figure 5.5.1.3 defines a green area (treatment of 75 kg N/ha) with a thick line box in the bottom 2 rows. In the bottom row it includes 5 green cells and 1 brown cell. All these trees, except the cell with "y" (young tree) died after this event. In the middle row no trees died due to this event but they were severely damaged. The 2 trial trees (69, 70) survived with crop in that off-year (season 2009/10).

In the final report the results of these two trees are always removed for the ANOVA analysis.

The exact yields for these 2 trees against the treatment average are detailed in Table 5.5.1.3. 2009 was before the event and 2010 have crop before the event in January. 2011 was the first year after the event but without yield record. Table 5.5.1.3 showed the clear advantage of including the 2 trees in the 2012 and 2013 harvest. From 2014 this advantage was not clear. However, in 2014 and 2016 the 2 trees were below the treatment averages and if those 2 trees were added the treatment average may have gone down a little bit but does not significant change the results.

			67	68	Y		69	70			71	72	
		М			М			y	М	у			М

Figure 5.5.1.3: Damaged trees in replication 9

Table 5.5.1.2: Yield records for trees 69 and 70

Tree		Fresh yield with hall (kg)/tree								
	2009	2010	2011	2012	2013	2014	2015	2016		
69	52.7	10.1	N/A	17.4	53.2	28.7	25.9	22.0		
70	57.1	6.3	N/A	9.1	28.7	26.7	31.0	14.8		
Treatment average without 69 & 70	55.0	9.8	N/A	7.5	57.3	30.0	26.8	34.5		

#### 5.5.2 Quality

In this seasons nut quality test significant differences were found in hull nuts and dark stain. These may be due to harvest time not being exactly correct for different treatments. There were no significant differences found between treatments for the 2015/16 season.

Table 5.5.2.1: Percentages of nut qualities in physiological aspects

Seasons	Treat (kg N/ha)	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
					opire	opiic				onen
2008-	75	1.43	58.7	0.14	3.96	4.95	2.52	7.46	7.9	1.79
2009	150	0.90	56.3	0.47	5.19	6.12	2.70	8.82	10.3	2.03
On-	250	1.41	59.9	0.32	5.07	5.90	2.60	8.50	7.2	2.07
Season	350	0.97	61.2	0.29	5.12	6.18	2.40	8.58	6.2	2.02
	p-value	0.271	0.218	0.341	0.125	0.663	0.924	0.606	0.088	0.690
2009-	75	0.36	56.4	2.00	1.26	1.25	2.08	3.34	9.77a	1.12
2010	150	0.41	54.2	2.10	1.78	1.38	1.53	2.91	8.40ab	1.86
Off-	250	0.46	55.0	2.74	1.39	1.80	1.84	3.64	7.34b	1.37
Season	350	0.43	56.9	2.20	1.37	2.19	2.32	4.51	7.10b	1.88

	p-value	0.952	0.973	0.287	0.406	0.064	0.562	0.266	0.024	0.509
2010-201	1 has no tria	al harvest d	ue to anth	iracnose						
2011-	75	1.44	43.9	1.68	0.47	0.83	5.18	6.01	13.13	0.94
2012	150	1.00	37.8	1.86	0.36	1.56	8.40	9.96	10.28	1.17
Off-	250	0.48	35.3	1.04	0.59	1.17	6.56	7.72	12.50	1.79
Season	350	0.51	39.6	2.32	0.68	1.08	7.24	8.33	11.15	1.64
	p-value	0.077	0.338	0.216	0.704	0.099	0.621	0.451	0.657	0.253
2012-	75	1.12	61.2	1.00	0.88	5.50b	1.06	6.6b	8.8	2.14
2013	150	0.58	60.4	1.22	1.15	6.71b	1.69	8.4b	9.4	2.31
On-	250	0.67	60.5	1.39	1.19	7.20b	1.32	8.5b	7.8	1.73
Season	350	1.35	55.1	0.49	2.63	12.11	1.05	13.2a	8.9	2.98
						а				
	p-value	0.296	0.227	0.606	0.166	0.008	0.710	0.022	0.788	0.349
2013-	75	0.17	29.3	5.62	0.44	4.22b	3.41b	7.63b	0.834	0.87
2014	150	0.17	40.5	3.19	0.47	5.90b	3.41b	9.30b	0.922	0.58
Off-	250	0.18	33.7	6.17	0.42	7.58a b	4.11ab	11.69ab	0.765	0.32
Season	350	0.26	37.6	5.62	0.48	8.75a	4.47a	13.22a	0.850	0.65
	p-value	0.863	0.154	0.181	0.976	0.022	0.045	0.007	0.632	0.209
2014-	75	0.27b	59.7	8.1	0.15	1.84	0.87	2.71	5.10	0.14
2015	150	0.32b	57.7	10.4	0.39	1.64	1.13	2.77	6.22	0.13
On-	250	0.63a b	60.6	8.5	0.30	2.01	1.27	3.29	5.31	0.07
Season	350	1.30a	56.2	7.7	0.28	1.80	1.69	3.49	6.67	0.09
	p-value	0.043	0.224	0.600	0.070	0.876	0.387	0.505	0.296	0.621
2015-	75	0.25	62.6	1.59	0.28	1.19	1.18	2.37	5.15	0.31
2016	150	0.13	60.1	2.63	0.44	2.03	1.67	3.69	5.09	0.29
On-	250	0.13	56.2	2.42	0.48	1.91	1.60	3.51	3.91	0.30
Season	350	0.39	56.9	0.44	0.20	1.78	1.66	3.44	3.92	0.24
	p-value	0.657	0.446	0.340	0.413	0.692	0.566	0.522	0.675	0.890

Seasons	Treat	% pickout	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2008-	75	10.19	0.19	1.56	6.14	1.44	N/A	4.76	10.0
2009	150	8.47	0.24	0.83	5.19	1.48	N.A	3.53	9.3
On-	250	8.81	0.19	1.08	5.21	1.25	N/A	4.03	8.5
Season	350	8.47	0.30	0.79	5.16	1.43	N/A	3.65	8.9
	p-value	0.095	0.416	0.055	0.592	0.902		0.367	0.818
2009-	75	7.10	0.57	0.40	5.43	1.91	0.11	3.12	19.2
2010	150	9.26	0.29	0.21	7.09	2.43	0.25	4.21	20.6
Off-	250	7.21	0.49	0.39	5.29	1.34	0.05	3.76	21.7
Season	350	6.87	0.39	0.22	4.68	1.41	0.00	3.00	20.2
	p-value	0.670	0.212	0.786	0.772	0.138	0.180	0.910	0.954
2010-201	.1 has no t	rial harvest	due to anth	racnose					
2011-	75	19.12	0.12b	12.8	5.36	0.08	0.00	4.94	14.15
2012	150	21.75	0.26ab	14.0	6.61	0.07	0.03	5.81	16.72
Off-	250	27.66	0.44a	19.5	6.46	0.07	0.01	5.60	14.27
Season	350	22.37	0.40a	16.8	3.87	1.43	0.00	3.47	14.63
	p-value	0.072	0.036	0.093	0.269	0.748	0.611	0.371	0.271
2012-	75	6.12	0.53	0.48	3.48	0.004	0.00	3.12	13.8
2013	150	6.19	0.61	0.28	3.54	0.000	0.00	3.27	12.0
On-	250	6.79	0.57	0.33	4.70	0.000	0.00	4.33	12.6
Season	350	7.10	0.72	0.42	3.69	1.000	0.00	3.52	10.6
	p-value	0.836	0.948	0.504	0.645	0.455		0.607	0.684
2013-	75	24.68	0.41	0.67b	23.1	N/A	N/A	N/A	25.49a
2014	150	20.66	0.54	1.52a	18.5	N.A	N.A	N.A	18.95b
Off-	250	18.63	0.38	2.14a	16.1	N/A	N/A	N/A	18.86b

Table 5.5.2.2: Percentages of nut qualities in stain aspects

Season	350	18.48	0.31	1.86a	16.0	N/A	N/A	N/A	17.63b
	p-value	0.574	0.284	0.003	0.452				0.005
2014-	75	4.09b	0.70	0.11	3.75c	0	0.00	0	19.2
2015	150	4.96b	0.93	0.17	4.79b	0	0.38	0	16.2
On-	250	6.16a	1.11	0.14	5.89a	0	0.00	0	14.1
Season	350	6.03a	1.34	0.28	5.60a	0	0.62	0	17.0
	p-value	0.006	0.125	0.455	0.030		0.631		0.223
2015-	75	1.90	0.34	0.37	1.20	0	0.00	1.21	25.5
2016	150	2.54	0.48	0.38	1.82	0	0.04	1.83	24.9
On-	250	2.36	0.46	0.39	1.59	0	0.00	1.70	30.5
Season	350	2.63	0.43	0.53	1.76	0	0.00	1.93	31.7
	p-value	0.567	0.612	0.722	0.602		0.455	0.464	0.309

#### 5.5.3 Trunk increment between treatments

Table 5.5.3.1 and 5.5.3.2 lists yearly trunk increase in different units. In the 2010-11 season the 75 kg N/ha treatment had significant increases compared with all other treatments. This implied that there was a very low crop for the 75 kg N/ha treatment compared with those in other years. Unfortunately due to anthracnose no records for yield were kept. Except for 2010-11, all the other seasons did not show a significant difference.

Table 5.5.5.1.							
Treatment	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15
75 kg N/ha	0.45	0.52	1.19a	1.02	2.02	1.09	0.77
150 kg N/ha	0.19	0.81	0.81ab	1.01	2.31	0.85	1.23
250 kg N/ha	0.38	0.79	0.84ab	0.66	2.30	1.13	1.25
350 kg N/ha	0.51	0.58	0.68b	0.93	1.81	0.96	0.78
p-value	0.704	0.518	0.008	0.202	0.158	0.655	0.148

Table 5.5.3.1: Trunk circumference increase between treatments

Treatment	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15
75 kg N/ha	4.1	5.0	12.2a	10.4	22.1	12.2	7.8
150 kg N/ha	1.9	8.0	8.3b	10.1	26.6	9.2	13.5
250 kg N/ha	3.7	7.7	8.2b	6.6	24.9	11.9	13.5
350 kg N/ha	5.1	5.8	6.8b	9.3	23.5	10.3	8.5
p-value	0.742	0.529	0.009	0.207	0.623	0.708	0.120

Table 5.5.3.2: Trunk cross sectional area increase between treatments

### 6 Pollination Trial

#### 6.1 Nut setting investigation for intensive pollination and natural pollination

On the 16 October 2014 initial single flower numbers were counted. At that time, single flowers were relatively larger before dropping. However, counting was difficult and accuracy was not easy to obtain.

On the 16 January 2015 a second investigation was conducted. The final clusters were harvested on the 27 February 2015.

No	Date	1		2		3		4		5	
Intensive pollination	16/10/14	101		158		201		112		131	
	16/01/15	24		33		59		36		28	
	27/02/15	24		34		56		29		28	
Good nuts			23		28		45		25		25
Half nuts			0		0		1		0		0
Blank nuts			1		6		10		4		3
Natural pollination	16/10/14	75		119		58		126		119	
	16/01/15	0		41		26		46		46	
	27/02/15			41		28		51		52	
Good nuts					33		9		48		34
Half nuts					2		19		0		13
Blank nuts					6		0		3		5

Table 6.1: Nut setting for treatments of intensive pollination and natural pollination

#### 6.2 Bagged clusters

Clusters were bagged at the stage shown in the photo



However, there were many nuts that set even though they were bagged

No	1-1	1-2	1-3	2-1	2-2	2-3	4	5
Good nuts	23	28	34	26	21	5	42	11
Half nuts	2	7	2	6	6	0	5	0
Blank nuts	5	12	9	2	6	0	6	0

Table 6.2: Harvest nuts from bagged clusters

#### 6.3 Blank nut investigation

A branch was broken during the N trial containing a total of 179 clusters with 4916 nuts.

In the 2015/16 season weekly nut cutting was undertaken on a total of 6229 nuts of which 330 were 'close shell' nuts and 730 were blank nuts. In the early stages (before 20 December) a total of 283 nuts were cut and there were only 3 blank nuts. This indicated that most blank nuts appeared after kernel development.

Figure 6.1 shows the relationship between close + blank nuts against total nuts per cluster. The curve regression showed that there was a tendency that close + blank nuts increased as the total nuts per cluster increased. In other words, larger clusters may produce more close + blank nuts.

The photos taken on the 27 January and the 3-4 February further highlight the assumptions. Big clusters had so many undeveloped kernels while small clusters only had 1 or 2.



Figure 6.1. Relationship between close + blank nuts against total nuts per cluster.



Photo 6.1: Nut cutting on different dates.

### **Outputs**

### 1 Existed model maintenance

#### 1.1 Chill Requirement Modeling

The paper on the chill requirement model of 'Sirora' pistachio trees was published as

Zhang, J.; C. Taylor. 2011. The dynamic model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. HortScience 46(3):420-425.

Yearly chill accumulation calculations were collected weekly across June, July and August using 4 different models. **Each winter, 3 chill accumulation reports utilising the Dynamic Model have been distributed to growers at the end of June and July, and on the 16<sup>th</sup> August.** All pistachio growers were aware of the especially low-chill winter in 2013 and most growers applied winter oil to mitigate the effect of low winter chill and minimise reduction in production. In the winters of 2014 and 2015 the predictions showed reliable results in relation to chill hours.

#### 1.2 Nut Size Prediction

The work on nut size prediction was published as

Zhang, J.; Joyce C. 2009. A study on climate factors on 'sirora' pistachio nut size. Acta Horticulturae 912:129-135.

In the paper the predictive formula was

Weight =  $0.219 + 0.0278 \text{ MaxT}_{11 \text{ Oct-}21 \text{ Nov}} + 0.000192 \text{ hours}$  (R =  $0.61^{***}$ )

Here  $MaxT_{\rm 11\ Oct-21\ Nov}$  is the mean of the maximum temperature between 11 October and 21 November

Hours is the number of hour's  $\leq$  7.2°C between the 1 May and 31 August in the previous winter

At the end of November each year nut predictions were carried out. Table 1.2.1 shows the predictive results and the actual results. This is useful information for the processing facility and the industry marketing department.

Table 1.2.1: Nut size prediction

season	Orchard	Max Temp	Hours<7.2°C	Predicted	Predicted	Actual
		between10	between May	weight	count	count
		Oct & 21	and August	(g)		5120
		Nov				
2009/10	КР	30.486	705	1.20170	83.2	81.5
	CMV	30.960	506	1.17667	85.0	83.8
2010/11	КР	24.455	864	1.06454	93.9	86.8
	CMV	24.538	655	1.02672	97.4	94.0
2011/12	КР	27.693	819	1.14593	87.3	88.4
	CMV	28.483	652	1.13583	88.0	88.0
2012/13	КР	26.229	922	1.125	88.9	90.9
	CMV	27.521	783	1.134	88.2	90.9
2013/14	КР	25.774	732	1.07587	93.9	93.9
	CMV	26.421	579	1.06448	92.9	91.5
2014/15	КР	28.9714	797	1.17725	84.9	92
	CMV	29.8581	604	1.16485	85.8	90
2015/16	КР	29.5024	826	1.21062	82.6	
	CMV	30.4333	633	1.18641	84.3	

#### 1.3 Maturity Prediction

#### 1.3.1 Model based on maximum temperature



Figure 1.3.1: Relationship between starting harvest date and weather

Harvest dates are influenced by the weather. From the calculations the maximum temperatures between 10 January and 5 February had a strong affect as shown in Figure 1.3.1.

Prediction results from 2006 to 2014 are as follows:-

- Results for 2007, 2008, 2010, 2011, 2012 and 2013 were acceptable;
- 2006, 2009 and 2014 which had high maximum temperature averages resulted in poor predictions.
- Harvest 2006 was special in that the winter of 2005 had low-chill. Kyalite Pistachio had bloom in the middle of November 2005 and this definitely led to late harvest.
- Harvest predictions for 2009 and 2014 deviated from the predictions and the reasons why need to be considered.

Figure 1.3.2 shows a curve relationship between the starting harvest date and the average maximum temperature for that period. When the average maximum temperature increases up to 35° or 36°C the nut maturity speeds up. When the average maximum temperature is above 36°C the nut maturity process slows down.

This model was established after the harvest of 2014 and ongoing data collection and modeling is required.



### Fitted Line Plot Actual start = 38851 - 45.47 110-205 + 0.6379 110-205^2

Figure 1.3.2: Curve relationship between starting harvest date and weather

#### 1.3.2 Prediction from heat accumulation

The work on maturity prediction based on heat accumulation was published as

Zhang, J.; Ranford, T.; Taylor, C. 2015. Heat model for pistachio bloom and harvest. Scientia Horticulturae. 186:47-53.

From full bloom to nut maturity the accumulated GDH (based on 14°C and maximum temperature of 32°C) reaches 23447.

#### A copy of this paper has been supplied to Horticulture Innovation Australia Limited



Figure 1.3.2: Heat accumulation from full bloom to harvest

#### **1.3.3** Prediction from nut shell pressure

This model appears to be a new technique within the pistachio world. This work started in November 2014 and there is only limited data as shown by the two lines. Information over coming seasons will assist in further developing the new model.



Figure 1.3.3: Nut shell pressure measurements

#### 1.4 Prediction on Percentage of Blank Nuts

Blank nuts represent wasted production and effect the overall yield and production of pistachios. One model being assessed, to predict the percentage of blank nuts, is based on the average of minimum temperatures between the 12 August and the 1 September each year. Harvest 2016 will be the first year for this new predictive model.



Figure 1.4: Relationship between % blank nuts and weather

#### **1.5** Prediction on Percentage of Narrow Split Nuts

Narrow split nuts receive low prices and therefore affect the yield and profitability of pistachio production. The model to predict the percentage of narrow split nuts is based on the average maximum temperatures between 10 June and 13 December each year.



Figure 1.5: Relationship between % narrow-split nuts and weather

#### **1.6** Prediction on length of harvest season

To organise labour for harvest and processing growers need to know how long the harvest period might be. The new model to predict the length of the harvest period is based on average minimum temperatures between 30 October and 6 December each year.



Figure 1.6: Relationship between length of harvest period of Kyalite Pistachio nuts and weather

#### **1.7** Aflatoxin Statistics in Different Dates, Shakes and Orchards in 2015

There were a total of 312 lots delivered as part of the 2015 harvest. Each lot was tested for aflatoxin and recorded as pass or fail. This section tried to link those assessments to dates, growers, shakes, as well as quality parameters.

Table 1.7.1: lists results of aflatoxin tests in different dates.

Date	Pass	Fail	Total	% Fail
25/02/2015	5	0	5	0.0
26/02/2015	3	0	3	0.0
27/02/2015	7	0	7	0.0
28/02/2015	7	1	8	12.5
1/03/2015	7	0	7	0.0
2/03/2015	13	0	13	0.0
3/03/2015	16	0	16	0.0
4/03/2015	18	1	19	5.2
5/03/2015	17	1	18	5.6
6/03/2015	15	3	18	16.7
7/03/2015	1	11	12	91.7
8/03/2015	9	4	13	30.8
9/03/2015	11	0	11	0.0
10/03/2015	17	0	17	0.0
11/03/2015	15	0	15	0.0
12/03/2015	13	1	14	7.1
13/03/2015	6	3	9	33.3
14/03/2015	5	1	6	16.7
15/03/2015	5	2	7	28.6
16/03/2015	7	4	11	36.4

17/03/2015	5	5	10	50.0
18/03/2015	6	0	6	0.0
19/03/2015	8	0	8	0.0
20/03/2015	7	0	7	0.0
21/03/2015	6	0	6	0.0
22/03/2015	8	0	8	0.0
23/03/2015	8	0	8	0.0
24/03/2015	8	0	8	0.0
25/03/2015	5	0	5	0.0
26/03/2015	6	0	6	0.0
27/03/2015	4	0	4	0.0
29/03/2015	5	0	5	0.0
30/03/2015	1	1	2	50.0
All	274	38	312	12.2

Table 1.7.2 lists results of aflatoxin tests in different shakes.

Table 1.7.2: Pass and fail of aflatoxin test for different shakes

Shake	Pass	Fail	Total	% Fail
1	197	25	222	0.9
2	63	11	74	14.8
3	12	1	13	7.7
All	272	38	309	12.3

Table 1.7.3 lists the results of aflatoxin tests for each of the different growers. The order of growers in the table is based on geographical locations from west to east.

Grower	Pass	Fail	Total	% Fail
1	2	0	2	0.0
2	1	0	1	0.0
3	3	0	3	0.0
4	2	0	2	0.0
5	1	0	1	0.0
6	4	0	4	0.0
7	3	0	3	0.0
8	3	0	3	0.0
9	11	2	13	15.4
10	3	1	4	25.0
11	50	1	51	2.0
12	21	0	21	0.0
13	0	2	2	100.0
14	0	1	1	100.0
15	1	0	1	0.0
16	2	0	2	0.0
17	3	1	4	25.0
18	65	10	75	13.3
19	1	1	2	50.0
20	1	0	1	0.0
21	1	0	1	0.0
22	4	1	5	20.0

Table 1.7.3: Pass and fail of aflatoxin test for different growers

23	72	7	79	8.9
24	16	9	25	36.0
25	2	0	2	0.0
26	2	2	4	50.0
All	274	38	312	12.2

Table 1.7.4 details the relationship of harvest parameters with aflatoxin.

Table 1.7.4: Analysis of variance of aflatoxin with harvest parameters

items	%Loose	%	%	%Pick	Count	% Small	%	%
	Kernel	Dark	Damaged	Out			Medium	Jumbo
		Stains	Shells					
Pass	0.39	6.48	0.49	7.62	94.4	2.77	63.04	2.46
fail	0.56	8.90	0.61	10.17	91.9	1.93	61.09	2.75
p=	0.001	0.000	0.031	0.000	0.047	0.052	0.242	0.758

Here in Table 4, when  $p \le 0.05$ , it indicates significant difference between the items.

Table 1.7.4 (continue)

items	%Narrow	%	% light	%Floaters	%
	Splits	Non	Stain		Adhering
		Split			Hull
Pass	0.15	3.18	7.44	1.86	0.66
fail	0.16	2.73	8.49	1.53	0.66
p=	0.631	0.130	0.228	0.280	0.324

From Table 1.7.4 the incidence of dark stain showed a significant difference between pass and fail. This indicated that in any assessment the grower and processor should pay more attention to high levels of dark stain nuts. Loose kernel and damaged shell also showed significant differences between pass and fail. Although they reached a significant different at  $p \le 0.001$  level the low percentages in nut sampling may not be

presenting an accurate result.

The question is whether there is any physiological importance and cultural meaning from these results. The data from sum of the adhering hull, dark stains and damaged shells showed a difference at p=0.000. When both the dark stains and damaged shells showed significant difference, the sum of them showing significant difference was not surprised.

Count size showed significant differences but this appeared to contradict with normal industry understanding. Normally it is believed that small nuts/small shells will have earlier split so increasing the chance of aflatoxin infection. The contradiction in these results was highlighted in that Mallee Pistachio Orchard, who usually produces smaller nuts, had no aflatoxin.

#### 2. OTHER ACTIVITIES

#### 2.1 Meetings

The Research Field Officer attended a meeting with staff from the Lower Murray Water (LMW) network, SA Murray Darling Basin NRM (SAMDBNRM) and MEA to discuss data collection from the meteorological stations of LMN and SAMDBNRM to assist with predicting local chill.

#### 2. 2 PIT Activity

The Research Field Officer attended and presented information and technology transfer to the 'PIT' Group meetings held on  $13^{th} - 15^{th}$  May,  $13^{th} - 14^{th}$  July and  $17^{th} - 18^{th}$  November 2015.

(PIT Group meetings are part of project PS13003.)

#### 2.3 Field day

A research information day was held by the Pistachio Growers Association Incorporated for growers at CMV Farms, Robinvale, Victoria, on 17<sup>th</sup> September 2015. A presentation was given by the Research Field Officer and covered the trials on

- nutrition,
- pruning,
- nut size and quality, and
- harvest date prediction.

Growers had the opportunity for questions and discussion at the symposium and in the orchard.

#### 2.4 Orchard visit

One of the outputs from this project is to visit growers and have one-on-one sessions. Besides discussing relevant research topics, from the past 18 months, the contact with the pistachio growers assists them in improving their production capacity.

Based on voluntary principle, a group of 24 growers were visited. Pruning advice in the orchard was provided last winter and will continue to be provided every winter going forward. As part of industry benchmarking historical data, from the 24 orchards, has been analysed including comparison of yields based on tree or hectare, nut size, etc and the information provided to each of the individual growers.

Fertiliser analysis has commenced and will continue after the conclusion of this project. This work will help growers better define their production problem(s) and improve their yield and productivity.

#### 2.5 Chill hours.

Using the results of the Chill Hour modelling PGAI prepared annual chill hour assessments and recommendations. These were distributed to all growers on the 15<sup>th</sup> August 2015 and assist the growers in understanding what chill hours their orchard may have received and what action might need to be taken.

#### A copy of the 2015 Chill hour report is attached as Appendix A to this report.

#### 2.6 Technical Articles

The Research Field Officer has prepared and submitted a number of technical articles for use in the Australian Nutgrower and include:-

- a) "SEL Stylar End Lesion IN PISTACHIO"
- b) "CAN A COMMERCIAL PISTACHIO TREE BEAR NUTS WITHIN 1 YEAR?"
- c) "HOW TO CHOOSE SUITABLE PRODUCTION AREAS USING CLIMATE FACTORS the Pistachio experience."

Copies of these articles are attached as Appendices B, C and D.

## Outcomes

The 2016 Australian Pistachio harvest was an industry record at in excess of 1900 tonnes. One of the major reasons has been the value of the research and development undertaken by the industry.

In assessing the outcomes of the past and most research work undertaken by the Pistachio Industry Research Field Officer this report looks at the outcomes in a number of ways. Initially some specific outcomes from the work of PS14000, then how these relate to the broader industry outcomes and finally how the research work has been adopted by growers and the practical results being achieved in the orchard and through to the processing facility. The end result is strong returns to growers.

#### **SPECIFIC OUTCOMES:**

Some of the specific outcomes relevant to growers are as follows:

- Alternate bearing of a significant magnitude disrupts marketing and precipitates strong fluctuations in net returns to the grower and processor. Further, the nut quality decreases when the crop load is heavy. Therefore mitigation of alternate bearing would be desirable.
- Through the mechanical pruning trial the Average comparisons showed that an even crop produced an extra 0.78 tonnes merchantable yield per hectare every year (on- and off-years). The trial plots had 308 female trees representing an increase of 2.5 kg per tree.
- Regional Meteorological observation machines (LMV and SAMDBNRM) have multiple functions and aims; however for the purpose of recording chill accumulation they may be unsuitable.
- With the nitrate test, except for the 350 kg N/ha treatment, there were no high readings in the deep soil indicating that fertilisers are being utilised by the plant.
- Total potassium content decreased especially for the 350 kg N/ha treatment. After potassium applications the content increased.
- After lime applications, pH went up and Al readings went down while the Ca reading went up.
- The 350 kg N/ha treatment leads to a lowering of pH value and low K content which the results in high Al content
- In all harvests from 2012 to 2016 the 350 kg N/ha treatment showed more flower buds than the 75 kg N/ha treatment.
- The regression curve showed that there was a tendency that close + blank nuts increased as the total nuts per cluster increased. In other words, larger clusters may produce more close + blank nuts.
- Each winter, 3 chill accumulation reports utilising the Dynamic Model have been distributed to growers at the end of June and July, and on the 16<sup>th</sup> August.
- When the average maximum temperature increases up to 35° or 36°C the nut maturity speeds up. When the average maximum temperature is above 36°C the nut maturity process slows down.

#### **BROAD INDUSTRY OUTCOMES:**

The above outcomes have led to achieving some of the broad measurable benefits:-

- Adoption of Calcium sprays to decrease the incidence of Stylar End Lesion in 'off-crop' years from around 8-10% to less than 0.5% and resulting in an economic gain in each 'off-crop' year.
- Increase in crop yield of 5% per annum by optimising nitrogen application rates, improved pruning techniques, and use of other management techniques resulting in an economic gain.
- Reduction in the ratio of yields of 'on-crop' to 'off-crop' to less than the current value of 2:1.
- A reduction in the impact of the next warm winter where insufficient natural chill occurs

The practical outcomes have been evaluated by the industry benchmarking the growers by the Research Field Officer. Data collected from both on-property during the growing season and collected from the processing plant, has enabled evaluation of the outcomes for each grower. Individual property data has been correlated with yield improvements achieved through the adoption of recommendations of nitrogen application, calcium sprays, chill mitigation, tree pruning, orchard management practices and resulting in improved nut quality. This confidential grower information has been made to each of the growers

Overall industry production figures of yield and quality have be used to evaluate the outcomes on a whole of industry basis.

#### **BROAD INDUSTRY RESULTS:**

The value of this past and most recent research can be best detailed in the following summary of the review of the Australian Pistachio Industry production for the past years including 2016.

- a) Annual production figures with particular reference to the period 2013 to 2016:-
  - 2016 1,907 tonnes
  - 2015 1,698 tonnes
  - 2014 1,190 tonnes
  - 2013 1,662 tonnes
  - 2012 539 tonnes
  - 2010 602 tonnes
- b) Record "off" crop yield in 2016 and the benchmarking of growers in relation to yields
  - 7 orchards above 3,000kg/ha
  - Many orchard 2,000 to 3,000kg/ha
  - 8 orchards below 1,500kg/ha
- c) Measuring of stain levels and showing a reduction in treated orchards
  - Well managed orchards, Dark Stain < 1%
  - Unmanaged orchards ,Dark Stain 3% to 8%
- d) Nut size average
  - ~ 92/100gms (94 in 2015)
  - Ideally below 85 nuts/100gms

The overall industry outcomes can be detailed in reviewing the current and projected growth of the Australian Pistachio Industry

- Estimated growth of 40 ha per annum since 2014
- About 100ha planted in 2016
- About 100ha pa planned for at least next few years. No known mega orchards planned
- Each 100ha will increase supply by 400 tonnes pa in a decade.
- Domestic supply could match current domestic demand in about 2025 without further consumption growth.
- Exports can easily handle surplus but at a prices below domestic prices. Single desk becomes even more important.

The further value of the research can be expressed in the assessment for the 2017 season:-

- Good bud set but maybe not achieving the record crop to 2016.
- Poor 2016 winter chill?
- New orchards continue to mature.
- Orchards recovering from 2011 anthracnose.
- Continuing better management of orchards by growers.
- Pruning model developed from the work of Dr Jianlu Zhang is mitigating alternate bearing.
- Good prices likely for 2017 but below 2016 levels.
- Processing plant expansion to successfully capacity handled 1,900 tonnes in 2016.
- Planning underway for 3,000 tonne capacity.
- Several million dollars investment by the Processor.

# The ultimate outcome is that the return to grower has grown from \$8.00 per kilogram in 2012 to over \$11.50 per kilogram in 2015.

### **Evaluation and Discussion**

#### 1 Mechanical Pruning Control Biennial Bearing

One of the major disadvantages of alternate bearing is "half of the yield of an 'on-year' and an 'off-year' together is less than the yield of an 'on-year' of regular bearing" (Jonkers, 1979). While this statement was from a literature review there was no scientific paper to supports it. Our investigation supports this statement. **The benefit of an extra 2.5 kg per tree may not be large but this has been achieved for both on- and off-years.** Clearly the benefit came from reducing the extra flower bud formation and young nut setting in the early stages and the saving in tree nutrition and energy. While the benefit is not too larger it has offered a useful advantage.

Under traditional methods, hand thinning and hand pruning has shown an effect in reducing the 'wave' of alternate bearing. Comparing our work with Beede et al. (1991), 50% flower bud removing did not alter crop but the removal of 75% flower buds significantly reduced the crop. In the Beede et al (1991) trial, before removing flower buds the trees had more than 4000 flower buds. After removal there were still 2000 flower buds on each tree but flower bud numbers beyond 1500 seemed to result in a similar crop load. Thus, there seemed to have been no difference in crop with between 2000 and 4000 flower buds. In this trial, when around 2500 flower buds were reduced to around 630 flower buds, yield should be reduced. Although these methods showed some effects, these methods did not stop the big drop in year 4 and were therefore not further applied.

Traditional pruning methods tip around 30 1-year-old shoots at 3 buds above flower buds. According to our research the top long shoots above 25 cm should have around 10 flower buds for each shoot. That means that tipping 30 shoots should produce around 300 flower buds. Even with some other buds the crop would not reach an off-year.

In practice, pistachio production is strongly alternate bearing and in part this could be because the 'tipping' instructions are not perfect. For "normal" trees casual pruners will tip around 30 shoots. However, some trees do not have 30 long-shoots on the top with flower buds but may just have 20 so the casual pruner may only tip these 20 because there is no extra instruction for further pruning. When trees have 10 shoots they may only tip those 10. Graduate trees that had 3000 – 4000 flower buds may have no long shoots at all. This induces alternate bearing in orchards.

Pistachio trees show strong apical dominance. Thinning out shoots may not stimulate new shoots arising laterally from the remaining branches. Also some shoots may have no leaf buds at all (Crane and Iwakiri, 1981) and may actually have a terminal flower bud. When this terminal flower bud germinates there is a small shoot with leaves but there is no bud beside the leaves. After harvest these shoots become dead ends (photo 6.1). On strong alternate bearing trees these types of buds can be easily found.

When CMV Farms applied mechanical pruning it was difficult to find this type of shoot. This implied that when the whole trees had strong growth, this type of shoot were difficult to be found. This also explained that while hand thinning created balanced crops, without the strong stimulation of shoot growth, big off-year crop still happened. Mechanical pruning can maintain

this stimulation.



Photo 1. Dead end shoots after nut harvest from a terminal flower bud.

Although both Ferguson et al (1995) and Crane and Iwakiri (1980) suggested that pruning before off-years was the best time to mitigate alternate bearing, Beede et al (1992) suggested heavily pruning prior to on-years. This paper showed test results based on 6 years' data.

Logically, heavy pruning makes strong shoot growth in the first season and forms flower buds in the second season. Under this inference, heavy pruning prior to an off-year will benefit the next off-year and heavy pruning prior to on-year will benefit next on-year. This work showed these results. In 2012, pruning in 2009 (prior to off-year) showed a higher yield than pruning in 2010 (prior to on-year). However, after 2012, both treatments showed very similar yields and accumulated yields did not show any clear differences. This meant that after the first few years, continuous pruning did not only stimulate shoots just under the cut, but also stimulated inside buds/shoots. Under these conditions the trees have become more balanced. The 6-years' data did not show any clear difference but in practice heavier pruning prior to an off-year will be easier for growers to accept.

Results for 1-year-shoot mechanical tipping were unexpected. Before mechanical pruning the routine industry winter pruning was tipping 1-year-old top shoots about 3 buds above the flower buds (Zhang, 2011). This usually represented about 30 cuts in each tree. After hand tipping, flower buds formed on the new shoots in the following season. However, mechanical tipping killed all flower bud formation. Through observation some small trees with less than 5 shoots, when mechanical tipped, still resulted in the formation of flower buds. In normal circumstances, flower buds on the top shoot disappeared in trees with more than 5 tippings. This implied that mechanical cutting was different from hand cutting. When every cut was located at a similar height, even with less than 30 cuts, the stimulated response was more than that.

Pruning angle is now seen as a key point for mitigate alternate bearing. ...... In our work,

different pruning angles produced different results. Angles at 22° appeared to too weak while those at 30° appeared a little strong. Angles at 27° seemed to produce more balanced results. From Figure 1.2 (Page 28), the slash on the canopy shows the pruning angle and divides the canopy into "A" and "B" parts. Assuming the pruning is prior to an off-year, if the angle is too small the "A" parts will be too big. This leads to too much flower bud formation in on-year and leads to less flower bud formation in the following off-year. In the other way if the angle is too big the "A" parts will be too small. This leads to fewer flower bud formations in the on-year and leads to many flower bud formations in the following off-year. In an extreme case the on-year and off-year may swap over. For growers to achieve the best balance in their orchards will require more practice but this balance will be easier than the traditional 2 or 3 set shoot method.

To control alternate bearing, the most important information is to know how many flower buds there are after pruning (it is not important if pruning prior to off-year due to almost no flower bud). The grower needs to know how many at 1 year after pruning, how many at 2 years after pruning and how many each year. If growers fully control flower bud numbers in a reasonable range each year, under normal production condition, there should be no alternate bearing. This was the aim of 1side mechanical topping.

#### 2 Winter Oil Application

Although this was the first year of this specific trial some results from previous trials have been incorporated try and identify why 3% or 2000 L water treatment differs from the 6% or 4000 L water treatment.

The 2014/15 season finally obtained enough chill although not enough was achieved by the  $15^{\text{th}}$  August. The 2015/16 season obtained enough chill and under those conditions using 6% oil/4000 L, seemed to be of no value. The situation led to a high percentage of non-split nuts in both seasons. Although 6%/4000 L led to small nuts and late mature (low percentage of the first shake) in season 2014/15 this did not happen in season 2015/16.

In relation to assessing yield, the new this trial was a continuation of the previous trial(s). Treatment using 4000 L equates to the treatment of 6% while 2000 L equates to the treatment of 3%. In season 2013/14, 6% oil had a fresh yield of 35.9 kg while 3% had a fresh yield of 37.0 kg. In season 2014/15 the 6% treatment should have shown a little higher yield but it did not and in season 2015/16 the 6% treatment still showed low yields. There may be some side effects from the 6% application.

#### 3 Chill Prediction Study

BOM stations are surrounded by large open ground (both Mildura and Renmark stations are at air ports), while other weather stations are in more confined situations. The LMW Weather stations and Natural Resources SA Murray-Darling Basin Weather stations appeared to reflect more local environments and micro-climate. Did this influence chill portions?

From the portion calculations in 2014, Nangiloc had 64 portions, Merbein had 58 portions and Piangil had 52 portions but the pistachio trees did not have different responses. All had good bud break. In 2015, Piangil had 44 portions while Mildura had 71 but bud break clearly showed enough chill for trees around 30 km distance east and south.

A pistachio tree has canopy from 1 m to 5 m from the ground. Temperatures at different heights can influence trees while meteorological stations can only provide temperatures at a particular height (1.5 m). In this case weather stations on open ground may provide more reliable information because temperatures at 1.5 metres and 5 metres show less difference.

Growers emphasized the importance of microclimate observation. On a large farm, meteorological stations may record different readings in different locations. People can observe the differences in bud break, shoot growth and harvest date. However, in any large farm, there have been no reports that 1 side had enough chill and the other side showed insufficient chill. This implies that microclimate does not really influence chill accumulation. Growers need to pay more attention of the climate in large areas. It should be remembered that if the plant responses do not match the prediction results it is certainly not the mistake of the plant. The mistake may arise from the prediction model or from the temperature collection.

The Dynamic Model was proposed in 1987 (Fishman et al., 1987) and has recently received popularly acceptance. In 1980s, hourly temperatures were only obtained from large meteorological stations but in recent years, different meteorological observation equipment and even data loggers can record hourly (even less than hour) temperature data. But none of this equipment can produce reliable data for chill accumulation. **In this way, BOM stations have shown to be more reliable for the purpose of collecting data for chill calculations.** While all other stations may be suitable the users need data, over more years, to prove they are reliable before the industry can really use them.

The local station weather stations still have important functions in collecting rainfall and leaf wetness measurements and other climatic information.

## Recommendations

#### a) Mechanical pruning

The Mechanical pruning trials offered some breakthrough results.

Humans have struggled with alternate bearing for more than 2000 years and have still not developed the ultimate method of controlling the phenomena. While growers have set up a methodology of using 2 or 3 sets of shoots this has not controlled alternate bearing.

Our mechanical pruning directly deals with flower buds 1 and 2 years after pruning. Also it has shown to adjust flower bud formation. The method has shown that over 4 continuous seasons of treatment we can achieve a more even yield. Statistical analysis showed that accumulated yields from even yield were higher than that from alternate bearing yields.

While controlling alternate bearing by mechanical pruning every 2 seasons has given good results the challenge now is to see how growers can minimize pruning but yet still control alternate bearing.

#### b) Chill response

The meteorological station study has revealed that chill response is not sensitive to microclimate. Information from BOM stations produced reliable chill hour results and data from more localised meteorological stations require better and more consistent data before they can be effectively used.

Temperatures from daily conversions into hourly figures are not recommended.

#### c) Nut Shell Pressure Model

The industry needs to continue to build the data base of information for at least another 4 years to better understand if there are any patterns and whether the model has any real value to the growers.

#### d) Predictive model on assessing the percentage of Blank Nuts

The industry needs to continue to build the data base of information for at least another 4 years to better understand if there are any patterns and whether the model has any real value to the growers.

#### e) Predictive model on assessing the percentage of Narrow Split Nuts

The industry needs to continue to build the data base of information for at least another 4 years to better understand if there are any patterns and whether the model has any real value to the growers.

#### f) Predictive model on assessing the length of the harvest season

The industry needs to continue to build the data base of information for at least another 4 years to better understand if there are any patterns and whether the model has any real value to the growers.
# **Scientific Refereed Publications**

- Zhang, J.; Ranford, T. 2014. Winter Oil Application in Pistachio Production in Australia. *Acta Horticulturae.* Submitted.
- Zhang, J.; Ranford, T.; Taylor, C. 2015. Heat model for pistachio bloom and harvest. *Scientia Horticulturae.* 186:47-53.
- Zhang, J.; Ranford, T. 2015. How to choose suitable production areas using climate factors the pistachio experience *Australian Nutgrower* 29(2):34-37.
- Zhang, J.; Ranford, T. 2015. Review on mechanical pruning on fruit/nut trees. *Australian Nutgrower* 29(3):32-35.

# **Intellectual Property/Commercialisation**

No commercial IP generated.

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# Appendices

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- APPENDIX B: Australian Nutgrower article: "SEL Stylar End Lesion IN PISTACHIO"
- APPENDIX C: Australian Nutgrower article: "CAN A COMMERCIAL PISTACHIO TREE BEAR NUTS WITHIN 1 YEAR?"
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#### APPENDIX A: 2015 Final Chill Newsletter



#### PGA Chill Newsletter Number 3 - 2015-16 Season

# 14<sup>th</sup> August 2015

### Winter Chill 2015

The Charts below show that this is one of the best chill years in many seasons. All areas have, at 14<sup>th</sup> August, already reached the 59 Dynamic Chill Portions considered to be required as satisfactory chill for Sirora.



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

Wagga Wagga: 73 portions

73 portions at 9 am on 14<sup>th</sup>August.



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

Swan Hill/Mildura: 64 portions at 9 am on 14<sup>th</sup> August



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

**Nhill:** 82 portions at 9 am on 14<sup>th</sup> August



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

**Renmark**: 62 portions at 9 am on 14<sup>th</sup> August



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

**Loxton**: 62 portions at 9 am on 14<sup>th</sup> August



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

**Yarrawonga**: 75 portions at 9 am on 14<sup>th</sup> August



PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

**Albury**: 73 portions at 9 am on 14<sup>th</sup> August

#### **Chill Background Information**

Pistachios are extremely sensitive to lack of winter chill.

Lack of chill will result in very uneven opening of flowering and vegetative buds; some will not open until November or December, many buds will not open at all. Harvest will be very uneven.

PGA research, supported by the Australian government through Horticulture Innovation Australia Ltd, has shown the Dynamic Chill model to be the most appropriate method to measure the chill requirement of pistachios. The research has shown that *Sirora* pistachios require 59 Dynamic Chill Portions between 1<sup>st</sup> March and 31<sup>st</sup> August. The research also shows that 57 Dynamic Chill Portions to the 15<sup>th</sup> August will, in 95% of years, accumulate to the required 59 Portions by 31<sup>st</sup> August. Unless the required Chill Portions are received, growers should take mitigating action.

#### Mitigating the effect of low winter chill

Research in California and Australia has shown that winter oil application will significantly mitigate the effect of insufficient winter chill. Correctly applied oil can increase crops with insufficient chill by up to 15%. If there has been sufficient chill, little benefit seems to result from the oil application.

Oil application may bring the trees into flower up to a week earlier. The increased risk of frost damage should be considered by growers before applying winter oil in August.

Trials over seven years in California showed limited adverse affects from annual oil application. In the single season where lower yields were recorded from the oil treated trees, the week during the flowering of the treated trees was very wet, affecting pollination. A week of rain during pollination will affect crop load.

Winter oil is registered in NSW and SA only for the treatment of scale. Growers can only apply oil for the registered purpose.

Application time:	Ideally the third week of August.	
Oil to use:	Refined, heavy, emulsifiable horticultural spray oil. Typically about 860 g/litre petroleum oil. One brand that is used is: "Vicol Winter Oil" – Winter Dormant Miscible Oil – Insecticide	
Concentration:	3% to 6%, i.e. 3 to 6 litres per 100 litres of applied spray volume. PGA research has shown that in low-chill years, the higher concentration shows better yields. Care must be taken not to over spray – excessively high rates of oil will burn trees and perhaps kill them.	
Application rate:	Spray volume is dependent on tree size, but must be applied to the point of runoff. It is critical that all bud scales are thoroughly wetted. On average size trees, the application rate is up to 1,800 litres/ha. The very warm winters of 2013 and 2014 demonstrated the benefit of well applied oils. The orchards t ensured total coverage achieved the good off-crop results. Orchards that did not spray oil had 2/3 <sup>rds</sup> of the fruit buds not opening, i.e., 2/3 <sup>rds</sup> of the crop potential was lost. The vegetative shoots that sprouted late, in November and December did not have fruit buds, i.e., the following crop was also reduced.	

A critical issue for the application is the tractor speed. Californian research shows that tractor speeds of 2mph, 3.2kph, produce significantly better results for any spray application than faster speeds.

Current PGA research is focused on the effect of spray concentration (litres/ha) on bud opening and hence yield. Results are expected in a few years.

Some growers always apply oil unless the chill is well above the required Chill Portions. They say they do this to ensure scale control and also to be conservative. In such cases, to reduce cost, they use a 3% oil spray rather than 6%. If the chill has been low, growers usually apply at 6%.

The raw data is collected from the Bureau of Meteorology sites. The data for each orchard may be different. This data and information is provided as a guide to growing pistachios in Australia. Each grower should ensure that actions taken on their orchard is appropriate for their orchard. The PGA Inc and its office bearers will not accept responsibility for the actions of individual growers on their orchard.

Chris Joyce

Chair, Research Committee Pistachio Growers' Association

14<sup>th</sup> August 2015

### APPENDIX B: AUSTRALIAN NUTGROWER ARTICLE – "SEL - Stylar End Lesion – IN PISTACHIO"

#### Stylar End Lesion – SEL – is a disorder of pistachios.

Dr Jianlu Zhang, Research Field Officer for the Pistachio Growers' Association Inc, has undertaken research in Australia on Stylar End Lesion or SEL over a ten year period.

Dr Zhang has presented technical information to the Australian Pistachio Industry across that period. This paper is a summary of that work.

A short summary of SEL in Australia

- It is called 'chocolate nut' because of the brown discolouration of the end of the nut.
- In late November or early December a "water immersion" stain can appear through the hull surface
- Nuts with SEL can
  - affect the quality of nuts for the market.
  - increased packing labour costs
- Confirmed that a pathogen is not the cause of the problem
- SEL is a calcium-deficiency disorder
- The disorder usually occurs in the 'off-year' cycle.
- The key period for absorption of Ca from post-bloom to end of November.
- Normally 5 foliar applications of Ca would be applied

### SEL - Stylar End Lesion - IN PISTACHIO

### Jianlu Zhang

Pistachio Growers Association Incorporated, 3067 Murray Valley Highway, Robinvale, VIC 3549

Stylar End Lesion (SEL) was first reported in California in 1984 (Bolkan et al, 1984). The symptom only occurred in a few counties in California (Rice et al, 1985) however, in seasons 1999/00, 2003/04 and 2005/06, SEL occurred in many Australian pistachio orchards. When wet from the huller, the nuts with SEL look as though they have been dipped in chocolate (Photo 1). Thus, Australian growers have called it "chocolate nuts". In Iran, it is called Pistachio Endocarp Lesion (PEL) and has been known to be prominent in the Kerman province (Sajadian and Hokmabadi, 2011).



Photo 1. Nuts with stylar end lesion after packing process

In late November or early December in Australia, a "water immersion" stain appears through the hull surface as shown in photo 2. It appears to be a shadow inside the hull and appears prior to shell hardening. The worst affected nuts display a classic darkening of the stylar end, as well as necrotic tissue on the underside of the stem end. From observation no new shadow symptoms occur once shell hardening has begun. Many of the worst affected nuts may drop off between shell hardening and harvest (Zhang, 2004a).



Photo 2. Appearance of nuts with stylar end lesion

Upon cutting open damaged nuts (Photo 3), the embryo appears to be developing normally. At the stem end, the tissue (nuts 2, 3 and 5 showing clearly) develops normally and has the classic green colour. At the other end, the tissue is white in colour with a black colour underneath. In mid-December, the hull and the shell at the damaged end are soft compared to the hardened undamaged part.



Photo 3. Inside of Stylar end lesion nuts

Damaged nuts remaining on the tree until harvest will have a hard shell, although the damaged end is much thinner and darkened compared to the healthy end of the shell. The embryo can develop as a normal kernel. The stylar end will remain black after the hull is removed.

Nuts with SEL can seriously affect the quality of nuts for the market.

In season 1999/00, a high percentage of nuts with SEL appeared in the packing line. This affected nut quality and increased packing labour costs. In season 2003/04, most of nuts with SEL dropped before harvest. This did not influence packing as much but reduced the crop significantly especially in an 'off-year'. It was estimated that 5% to 15% of the crop was lost due to SEL.

Pathological tests have been conducted and confirmed that a pathogen is not the cause of the problem (Wong, 2004; Adibfar et al, 2012). Observations appear to indicate that SEL nuts appear in the southern parts of the tree and have been observed only in 'off-crop' years. SEL nuts have been found on trees on rootstock *P. terebinths, P. Atlantica* and UCB1 but are uncommon on trees on rootstock Pioneer Gold (*P. integerrimia*). The orchards in which this disorder is most often observed are on alkaline soil.

SEL is a calcium-deficiency disorder (Zhang, 2004b; Zhang 2005; Sajadian and Hokmabadi, 2011; Adibfar et al, 2012). Most of the Calcium (Ca) activity is to stabilise the permeability of cell membranes. Ca is predominantly in the cell walls and at the plasma membrane and its function are the regulation of membrane permeability and related processes and the strengthening of the cell walls. Pectates behave as glue between cell walls. The degradation of pectates is mediated by polygalacturonase, which is drastically inhibited by high Ca<sup>2+</sup> concentrations.

In calcium-deficient tissue polygalacturonase activity is increased, and a typical symptom of calcium deficiency is the disintegration of cell walls and the collapse of the affected tissues. High growth rates of low-transpiring organs such as fruit and nuts increases the risk that the tissue content of Ca falls below the critical level required for the maintenance of membrane integrity, leading to typical so-called Ca-deficiency-related disorders (Marschner, 1986).

Trees on the rootstock Pioneer Gold had higher concentrations of calcium (Janick and Paull, 2008) resulting in no SEL nuts.

SEL nuts have been observed to develop under these conditions. In Australia, this disorder usually occurs in the 'off-year' cycle. In the 'on-year', heavy crop loads remove many elements including calcium. Thus, in the early stages of the 'off crop', Ca-deficiency may occur. Element transport occurs through the xylem through the transpiration process. However fruit and nuts are low-transpiring organs.

Under conditions of low transpiration, the rate of xylem volume flow from the roots to the shoots is determined by the root pressure (Marschner, 1986) and root respiration. High temperature may increase root respiration and root pressure and lead to high Ca transport. Figure 1 shows accumulated maximum temperature based on 25°C (any temperature minus 25 < 0, marked as "0"; if it  $\geq$  0, marked as the difference) in different years from 1<sup>st</sup> October to 30<sup>th</sup> November. For low accumulation years, 1999, 2003 and 2005, SEL is clearly recorded. In 2007 and 2009 SEL symptoms were not evident. Theoretically 2011 and 2013 (2 years with a little better accumulation than 1999 and 2005) could have displayed symptoms however, after the severe anthracnose infection in season 2010/11, SEL was not apparent in those years.



Figure 1: Accumulated maximum temperature based on 25°C in different years in October and November.

Between 2004 and 2006, Ca foliar application was tried with good results recorded based on individual clusters. Calcium nitrate with 0.8% concentration showed the best results. Normally 5 foliar applications would be applied from post-bloom to end of November as this period is the key period for absorption of Ca.

In 2007 the trial utilised 3 early applications and 3 late applications. However warm weather resulted in no SEL and there were no useful results from this spray pattern.

In 2007 and 2009 there was a larger scale trial in a 3.33ha block with 34 rows of trees. The purpose was to test the effect of Ca foliar applications on yield. Again due to warmer conditions in those 2 seasons no SEL occurred and no scientific conclusion can be drawn.

Over a 10 year period of trail work on SEL, weather and anthracnose affected the research in many of those years, making it very difficult to present consistent results. There is still no clear plan by the industry to undertake further studies.

This paper summarizes what we have done previously, and hopes to provide information for growers facing this problem in the future.

The SEL problem in Iran is slightly different to Australia, but the outcome is the same. Irrigation water in some areas has very high Magnesium levels, resulting in low availability of Calcium. Hence Calcium deficiencies may occur resulting in SEL. (Hokmabadi, 2005).

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## APPENDIX C: AUSTRALIAN NUTGROWER ARTICLE – "CAN A COMMERCIAL PISTACHIO TREE BEAR NUTS WITHIN 1 YEAR?"

#### CAN A COMMERCIAL PISTACHIO TREE BEAR NUTS WITHIN 1 YEAR?

Phil Hewett, Property Manger, CMV Farm, Robinvale

Jianlu Zhang, Research Field Officer, Pistachio Growers' Association Inc

Pistachios are small nut trees, normally dioecious. To increase resistance, pistachio varieties are normally budded onto rootstock. Normally after bud break from the scion flowers open in year 5 and economic harvest is usually in years 7 or 8. Full production happens between years 10 to 15.

Recently a pistachio tree in leaf 2 was noticed bearing nut cluster at CMV Farms, Robinvale, Victoria. The rootstock was "Pioneer Gold" (*Pistacia integerrima*) and the scion was 'Sirora' pistachio. The rootstock was planted in October 2014 and the scion was budded on the 15 January 2015. Bud break of the scion occurred in late January 2015, immediately after budding. After leaf fall and the dormancy period, in October 2015, the second leaf stage occurred within the same year. This tree had flower buds opening, nut cluster development and nut production (photo 1). From budding to nut set was less than 1 year.



Photo 1. Pistachio nut bearing in leaf 2.

The flower clusters looked different from the normal clusters as the axes of clusters were long (Photo 2), reaching 61 cm. This was clearly longer than the normal cluster of around 20 cm.



Photo 2. The length of pistachio nut clusters.

273 nuts were harvest on 19<sup>th</sup> February 2016 from 10 clusters. Most of the nuts were parthenocarpy nuts, however, there were 5 nuts with fully developed kernels.

Due to being in a new planting there were no male flowers in close proximity. The distance of the nearest male tree was around 250 metres. Turkey has record that pistachio pollen grains might reach a female tree from 80 metres (Erdogan et al., 1998). In this instance the distance was greater than 80 metres. However, the amount of pollen was very limiting given that there were just 5 nuts (out of the 273) that obtained enough pollen to set nuts.

During harvest the nut shell pressure was examined. For normal nut shells before harvest, they are above 7 kg against a  $1.94 \text{ mm}^2$  of head area of the plunger of a fruit pressure tester. However, for this young tree the shell pressure was 3.5 kg indicating a soft nut.

We have also checked for flower buds for next year but we did not find any.

Reference:

Erdogan-V; Kunter-B; Ayfer-M; Ferguson-L (ed.); Kester-D. 1998. Pollen dispersal in pistachio (Pistaciavera L.). *Acta-Horticulturae*. 470:286-293.

#### APPENDIX D: HOW TO CHOOSE SUITABLE PRODUCTION AREAS USING CLIMATE FACTORS - the Pistachio experience.

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The Australia nut production is expanding as new areas are developed. If you have the right soil and water the next important factor is climate. In general, climate aspects have three components to be considered: chill, heat and specific crop requirements.

#### **CHILL REQUIREMENT**

Problems clearly showed up for the pistachio industry with low chill in the winters of 2005 and 2013. Low chill, in the pistachio trees, resulted in later and uneven bud break and many buds breaking in November, December or even in February. More than 50% of the crop was lost due to low chill in those seasons. In testing chill requirements for each plant species it is necessary to test each variety.

The traditional test for chill requirement is to rely on forcing bud break in greenhouses. Between leaf fall and budbreak, there are many unseen physiological processes happening within trees. From Figure 1, we know that from the start of chilling to the completion of chilling the plants need low temperatures. After chill is completed the plants need higher temperatures for bud break. The problem is that we do not know which day the chill requirement is completed as in winter we cannot find obvious tree changes. If we knew the completion date then it would be easily to calculate out the chill requirements.

Autumn		Winter	Spring
harvest	leaf fall		bud break
ch	illing start	chilling con	nplete
	cold req	uirement	heat requirement

Figure 1. Physiological processes from fruit ripening to bud break

To find this particular date, people cut twigs on different dates during winter and then put the twigs into vases in greenhouse to force bud break (normally requires 3 weeks under temperatures around 20-25°C) and to find the date that chill is completed. They then calculate the chill accumulation for the chill requirement for a particular variety (Zhang and Taylor, 2011).

In a suitable area, most winters should reach chill requirement for the particular variety. However, some winters may be warm and will not meet the requirements. In these cases, dormancy-breaking substances can be used to mitigate the low chill problem. The Pistachio industry successfully used winter oil to overcome the low chill winters of 2009 and 2013 without any clear yield lose (Zhang and Ranford, 2015).

Can an area usually with insufficient chill accumulation be a productive growing region? Pistachios were

planted in Alice Springs, almond were planted in West Australia. Although those attempts were not successful they did offer some hope. If these areas had the correct dormancy-breaking product and appropriate application requirements such areas may be successful for production.

It is essential for such areas to have the correct dormancy-breaking products, correct application timing and correct concentrations. Achieving all of this is not easy.

In our main pistachio production area (around Mildura), we usually apply winter oil between the 16<sup>th</sup> and 31<sup>st</sup>August. This allows bud break in early October. However, in Alice Spring, pistachio bud break would normally be around the 10<sup>th</sup> August so a winter oil application between 16<sup>th</sup> and 31<sup>st</sup>August would clearly not work.

To determine new dormancy-breaking product applications it requires many trials with application dates weekly from mid-June to late July. Different dormancy-breaking products would need to be trialed at different concentrations. This could result in more than 100 treatments.

Trials for spray treatments can be extremely difficult and there is a need for many protection rows to protect each treatment from contamination. More than 100 treatments including many protection rows may seem to be impossible. Zhang and Ranford (2015) posed a new method particularly for this work. Each treatment just needs a few shoots. When you choose a particular date, you can prepare dormancy-breaking product at a certain concentration and place it in a container (Photo 1). You can put a single shoot into each container soaking it in the solution for a few seconds - this is a treatment. You need a number of replicate shoots and each shoot must be appropriately marked. On one mature tree, you may use 30 shoots for this work. For a 100 treatments just 20 trees may provide sufficient useful information. Using this methodology, you may find a few treatments that produce the best results but to provide more reliable information field trials are necessary.



Photo 1: Shoot soaked in container with dormancy-breaking substance

## **HEAT REQUIREMENT**

Although heat requirements have been published for grapes the work was undertaken nearly half a century ago. Other tree crops have been subject to very limited studies. Insufficient chill may result in more than 50% crop loss but insufficient heat normally does not have such a significant crop loss. The possible reason is that heat requirement studies are not as advanced as chill requirement studies.

Growing degree hours (GDH) is a valuable tool to estimate heat. People may use base temperatures of 4.4°C and 4.5°C and/or an optimum temperature of 25°C (Richardson et al., 1974). To produce a curvilinear increasing model, Anderson et al. (1986) posed an asymmetric curvilinear GDH model. They used a base temperature (T<sub>b</sub>) of 4°C, optimum temperature (T<sub>u</sub>) of 25 °C, and critical temperature (T<sub>c</sub>) of 36°C. At temperatures between T<sub>b</sub> and T<sub>u</sub>, the following formula was applied: Asymcur GDH =  $\frac{T_u - T_b}{2} \cdot (1 + \cos\left(\pi + \pi \cdot \frac{T_h - T_b}{T_u - T_b}\right))$ 

At temperatures between  $T_u$  and  $T_c$ , a second formula was applied: Asymcur GDH =  $(T_u - T_b) \cdot (1 + \cos\left(\frac{\pi}{2} + \frac{\pi}{2} \cdot \frac{T_h - T_u}{T_c - T_u}\right))$ 

When hourly temperature  $(T_h) < T_b$  or  $>T_c$ , Asymcur GDH = 0.

Although Anderson et al. (1986) suggested values for  $T_b$ ,  $T_u$  and  $T_c$  many researchers have tried different values. Recently Zhang, Ranford and Taylor (2015) completed a paper about their heat requirement studies using 8-years data of full bloom and harvest. They systemically calculated 'Sirora' pistachio heat requirements under different formulas and parameters to find the least variation for GDH for full bloom and harvest date accumulation. They concluded that heat requirement from chill completion to 50% full bloom was 18642 GDH at  $T_b$ = -1°C,  $T_u$  = 26°C and  $T_c$  = 26°C. For heat requirements from 50% full bloom to the first day of harvest was calculated as 23447 GDH at  $T_b$ =14°C and  $T_c$  =32°C. For major pistachio production areas (Renmark, Mildura, Robinvale, Swan Hill and Wagga Wagga) most errors between calculated date and actual date were within 10 days and the maximum was not over 20 days. However, for the Nhill area, maturity was always far below the GDH that is suitable for this area. Nuts from this area are usually a little small with more close-shell nuts. This indicates that this area can produce pistachios with the same inputs but they have less output than other areas with enough heat. This phenomenon was also observed in the Bendigo area but there is no solid data. This research has delivered useful results in relation to low heat accumulation.

This least variation method was also used for chill requirement calculations. Using at least 10-years of bud break or full bloom data plus climate data, Luedeling and his co-authors calculated chill requirements for chestnuts (Guo et al, 2013; Luedeling et al, 2009; Luedeling and Gassner, 2012; Luedeling et al, 2013) and apricots (Guo et al, 2015). Darbyshire (2013) calculated chill requirements for apples and pears. Maulión et al (2014) calculated the chill requirements for nectarines and peaches. We used existed pistachio data and tried this method and obtained chill requirements at 60 portions, which is just 1 portion more than the real chill requirements. This indicates that pure calculations can bring us close to the real result. We could only wish that greenhouse force method or the calculation method would give us a truly exact answer. If these methods can provide an answer, which is  $\pm 2$  portions from the true answer then we have achieved a good result. Growers need to test chill requirement over many years to confirm that the research results are correct. The Pistachio Growers' Association is still testing whether the current 59 portions is suitable or not.

#### **CROP SPECIAL REQUIREMENTS**

Besides requirements of chill and heat, some crop has special requirements. For example, during the harvest period rainfall may damage the pistachio quality. Rain during harvest or the lack of rain during harvest is a very special requirement for production. In the early years, areas in the Blue Mountain, close to Sydney, were considered suitable areas for pistachio production but the over a period the results showed poor quality due to rainfall during the production cycle. Currently the most easterly area of commercial pistachio production is just close to Wagga Wagga. In general terms, if the average monthly rainfall in February, March and April is above 20 - 25 mm, then an area may have trouble during harvest and affect the quality of the nuts.

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