Facilitating the technical development of the Australian Pistachio industry

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Pistachio Growers of Australia

Project Number: PS06001

PS06001

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FINAL REPORT.

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PURPOSE OF PROJECT: **The project aims to "*facilitate the development of the Australian Pistachio Industry to achieve increased profitability through the continued employment of a Research Officer to investigate ways the industry can increase yields and improve nut quality.***

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Final Report:

This Horticulture Australia research report details results of research done to facilitate the development of the Australian pistachio industry by addressing major issues such as nut quality, nutrition, biennial bearing and chilling requirements.

Funding Sources:

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SECTION 1:

1 MEDIA SUMMARY

In general the Australian Pistachio Industry is a relatively young industry and the information being used to establish the industry was based very much on material from overseas.

The Australian growers saw a need to collect local information for the industry to expand and produce high quality nuts.

The Pistachio Growers Association Incorporated, with the assistance of the Australian Government through Horticulture Australia Limited, established a position of a Research Field Officer.

During the period from October 2006 to October 2011 the Research Field Officer, Dr Jianlu Zhang has undertaken significant field research in areas including:

- Chill Hours
- Stylar and Lesion
- Kernel Fill
- Winter Oil Application
- Nut Size Prediction
- Pruning and Thinning
- Reflective Mulch

In each year, trials were undertaken and the results collated. From the 5 years, significant data has been collected, collated and analysed. The results have been regularly transferred to the growers. There have been significant advances made as a result of this project. The establishment of the dynamic model for predicting chill hours has been established as the 'tool' for the Pistachio industry. The research has been translated into yearly calculations which are delivered each season to the growers for their use.

Winter Oil applications, use of reflective mulch and the importance of calcium are but just a few of the other practical results from this work.

With the crop losses in 2010/11, the project was modified to allow for additional work on fungal diseases. As a result it was established that with the right environmental conditions Anthracnose would express itself in a manner never seen before.
Work by Scholefield Robinson Horticultural Services, SARDI and a number of Pistachio growers resulted in significant information being collected, collated and distributed to the growers. This will go some way in assisting growers to manage the problem in the future.

Both components of the project will continue into the future.

2 TECHNICAL SUMMARY

The Nitrogen trial was established in September 2008 and N application is on target. In the first growing season, leaf analysis showed a little difference of total N but significant difference of NO_3-N between treatments. In the second growing season, leaf analysis showed a difference of total N between treatments from beginning to end of this growing season. In the second season, soil analysis showed high contents of N in soil depth between 45 and 60 cm indicating leaching. Trial harvest did not show any clear difference in the first season but in the second season the trial harvest showed that a treatment of 75 kg N/ha had significantly lower yield than other treatments.

The Flower counting trial provides information about the bloom process and the percentages of flower bud break. Relation from numbers of flower clusters to final yield also shows that 1600 buds seems to be maximum requirement for CMV Farms production capacity. Any more buds than those may not provide more crop. Nut quality test showed that the trees with oil application had higher percentages of blank, pick out, dark stain and other stains.

Trial 2 for reflective mulch in pistachio fields in a randomized plot design showed that trees in reflective mulch area had significantly higher crop and return than control in an 'on' year and an 'off' year. In 'on' year, it should create $9420 extra return per hectare based on price in 2004. Cost of setting up reflective mulch is around $8600 for 3 seasons. In the 'off' year, from eye test, trees above the mulch had more nuts at bottom of the canopy showing positive effect from the mulch. To test the influence of reflective light from the mulch on trees in different distances, Positions 2, 3 and 4 get the maximum benefit from the mulch. Position 1 and 5 provided limited benefit. Position 6 also showed little benefit. From position 7, up to position 19, no significant difference was found from this measurement with low values. This indicates that beyond the mulch, 2 trees for protection should be reliable for trials. Trial 3 for reflective mulch with treatments of Extenday between rows, of Extenday under canopy, of Sun Brite powder or white plastic and of control was established in season 2007-2008, before a big 'off' year. The mulch did not show any effect on yields the first season due to the shortage of flower buds. In the second season, both Extenday treatments showed significantly higher yields than control. Extenday between rows had significantly higher percentages of Jumbo nuts than others. Performance of white plastic was between Extenday treatments and control but close to Extenday side. Economic analysis showed that one-year extra return should be enough for Extenday fitting cost. In the third season, Treatments of Extenday under canopy showed significantly higher yields than control and white plastic. Investigation in the third season also showed that trees with the mulch under canopy had more nuts per cluster than control. Material test showed that white plastic, used for grape, at least does not have less reflection than Extenday. White trunk painting did not show any advantage for reflection. From reflection test, the reflection order is Extenday under canopy, Extenday between rows, Sun-Brite or white plastic (1.25 m wide) and control. Differences between them reached $p \leq 0.01$ level. Hourly measurement showed a clear picture for light changing. Soil temperature investigation shows that soil temperature in control had significantly higher than that under Extenday. Soil moisture test was not successful in season 3.

Foliar sprays showed that doubled concentrations led to leaf damage and black-side nuts. Early foliar sprays showed less leaf damage than later applications. Overall, calcium nitrate looks better than calcium chlorite. Harvest showed that Ca spray increase yield at $p=0.07$

level compared with the control. Relatively higher percentages of dark stain nuts with Ca spray should be considered in the future. Tissue analysis shows that black-side nuts have lower Ca level. The future use of K/Ca or Ca/Mg was suggested but this is in contradiction to conclusion in 2005/06, which showed black-side nuts had high Ca levels.

In our trial, strong pruning (took ¾ flower buds away) did not show significant crop reduction especially in later years. Early thinning treatments showed highest yield on average in the 2nd, 3rd and 4th year trials. It also led the accumulated yield in 4 years. However, these differences did not reach a statistical level. Also, hand thinning did not stop the big off-year showing limited crop adjustment capacity.

A mechanical-pruning trial on 1-side prior to the 'on' year or 'off' year was conducted at stage 2B at CMV Farms in winter 2009. This time only treatment prior off-year was conducted. Thus, this report actually compares the differences between pruning prior to an 'off' year and non-pruning. Trial harvest on row basis was carried out in harvest 2010. The harvest this year did not show significant difference for yield and count size between treatments, or say that pruned trees on average reduced merchantable yield in 0.4 kg or return in $2.80. In the quality test, pruning produced less damaged shells and more non-split nuts.

A mechanical-pruning trial on 1-side or on 2-sides prior an on-year was conducted at stage 2 at Kyalite Pistachios in winter 2008. In winter 2009, mechanical-pruning on 2-side prior an 'off' year was also conducted. Trial harvest on row basis was carried out in harvest 2009 and 2010. Harvest 2009 seems to show that pruning on on-year 1 side had a little higher yields and larger nuts than 'on' year 2 sides. However, harvest 2010 shows that pruning on 'on' year 2 sides had a higher yield than 'on' year 1 side but there is no difference of accumulated yield between treatments. Harvest also noticed that pruning in 'on' year 1 side showed higher percentage of blank nut than pruning in 'on' year 2 sides while pruning in 'on' year 2 sides showed higher percentage of light stain nuts than pruning in 'on' year 1-side. Pruning on 'off' year 2 sides seems to produce large nuts.

Determining the chilling requirement for 'Sirora' pistachio production in Australia and monitoring winter chill accumulation is desirable to allow growers to take timely mitigating action in years of insufficient chill. Based on greenhouse work, historical data analysis and field validation, the Chilling Hour, Utah and Dynamic Models were compared. The Dynamic Model produced the best determination for fulfillment of chilling requirement with 59 Chill Portions. The required number of growing degree hours above 4.4°C from chill fulfillment to 50% bloom were 9633. The relations between the level of fulfillment of chilling requirement, bloom delay and yield are discussed.

Treatment of 6% oil application led to earlier flowering in the spring of 2008 and 2009. In the harvest of 2009 with good chill winter, there was no significant difference between yields, counts and returns. However, percentages of shake 1 of treatment 3% were significantly higher than 6%. Treatment 3% showed higher percentages for stains while treatment 6% showed influences on physiological properties such as damaged shells, loose kernel and non-split nuts. In harvest 2010 with low chill winter, treatment of 6% oil application led early maturity, higher yield and higher return in harvest 2010 with low chill winter. Treatment at 6% also showed lower percentages of stains, pick out and blank nuts.

Pistachio nut size is an important parameter for production. Some factors influencing nut size such as variety, pollination, irrigation and crop load are known, however, different seasons still produce

different size nuts. Effects from climate factors are considered. A study on influence of temperatures and winter chilling on nut size was carried out from 1998 to 2009. The results show that the key period of maximum temperatures influencing nut size was found in spring during hull enlargement from an r-contour map. A multiple regression study shows that maximum temperatures during hull enlargement and winter chill have significant influences on nut size. When maximum temperatures increase during hull enlargement, nut size increases. Under the same maximum temperature during that period, trees with good winter chill produce larger nuts than in poor chill years.

3 INTRODUCTION

The Australian Pistachio industry, after 20 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. This is the second project from 2006 to 2011.

The principal objectives of the position 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 objectives cover a wide range of activities and are detailed under each of the headings later in this Report.

The absence of any support work, other than in PGAI/HAL sponsored and financed projects, by the Departments of Agriculture for more than 15 years has left a major gap in the detailed agronomy of pistachio growing under Australian conditions. It has been necessary in this project to conduct what may be regarded as basic to more established industries.

The implications for success of this project are significant for the existing 50 pistachio growers but of greater significance in the development of a new horticulture option for the River Murray Valley. By improving the economic performance of existing pistachio orchards, proof of viability will be shown to other farmers for an expansion of the Australian pistachio industry.

The current orchards, even at full maturity and optimal yields can only supply less than half of current Australian demand. Australian and world pistachio consumption is growing at above 5% per annum.

By proving the financial viability of pistachio orchards it is likely that there will be an expansion of plantings as there has been in almonds. Almonds developed only slowly for the first 30 years – from the early 1970s when the first irrigated orchards were planted. In the last decade, after the proving of the economics by improving yields, almond plantings have increased 10 fold.

3.1 Improved Kernel Fill

3.1.1 Research on Nutrition Status and Fertilizer Application

Nutrition is an important base for nut production. Nitrogen application is one of the key operations for crop production in the Murray River area. How to efficiently apply nitrogen is an important topic. At the same time, influences of nitrogen application on other elements are also important for Industry.

A suitable site has been selected at CMV Farms, Robinvale, comprising 15 rows each of 40 trees. Modifications to the irrigation system were undertaken converting the site to drip irrigation in September 2007. A pre-trial operation ran in the season 2007-2008 to allow the trees to adjust to drip irrigation. The trial started at the beginning of season 2008-2009 for the following 4 seasons at least, to collect yield data over 2 'off-crops' and 2 'on-crops'.

In the first 3 seasons, there was leaf analysis, soil analysis and individual tree harvest tests undertaken. Sentek SoluSAMPLERs were installed at the end of season 2009-2010. Soil solutions will be collected and analyzed in this coming season.

3.1.2 Nut Number Monitoring

From spring 2004 to 2006, flowering process monitor work was undertaken. This work lasted 3 seasons and results delivered flower process understanding. This work also recorded flowering starting dates, full bloom date and completion dates for those years.

To test oil application effect on flowering, between springs 2007 and 2009, one of the 2 monitored trees was covered by plastic during oil application. This allowed us to compare flowering process between a tree with oil application and a tree without oil application. This work lasted 3 seasons.

3.1.3 Reflective Mulch Application

Reflective mulch has a strong effect on light reflection, which shows clear benefit for fruit colouring. Trial 1 in seasons 2004/05 and 2005/06 reflective mulch strongly showed the mulch's effect. Due to all the trees mulched being together in one location it was decided to further confirm the effect of the mulch. In seasons 2006/07 and 2007/08, trial 2 was designed in a randomized plot design with 6 replicates being established to test the influence on crop.

Small plots were arranged in alternate patterns between the mulch and control. What was of interest was if control trees would get reflective light from the mulch as long distance reflection. Influence of reflective light from the mulch on trees in different distance was tested. Photosynthetically active radiation (PAR) represents the portion of the spectrum (400 – 700 nanometer waveband) which plants use for photosynthesis. Units of measurement for the instrument are given in $\mu mol/m^2s$. To compare light conditions between reflective mulch and control, PAR measurements were used in the reflective mulch trial. In the measurements, not only were percentages of PAR recorded but percentages of reflective light were also recorded.

In reflective mulch trials 1 and 2, over a 4-year period, showed yield increases compared with the control. Reflective light tests showed that the mulch under the canopy had stronger light reflection than the mulch between rows. To further explore the best mulch application, a new trial, trial 3, was established with treatments of the Extenday under canopy, Extenday between rows, Sun-Brite under canopy and control in growing season 2007/08. Poor reflection from Sun-Brite resulted in the removal of it in our trial. To show the value of this technique, further extension tests for this material was considered necessary. This may reveal some practical information for future application. David

Crawford suggested trying plastic mulch normally used for grapes. Thus, white plastic was used to replace Sun-Brite in trial season 2008/09 for further 3 seasons.

Also, there is a possibility that white trunk painting may reflect light. Maybe white trunk is more useful above reflective mulch. This may lead to secondary reflection between the mulch and white trunk. This was also tested. Light reflection, soil temperatures and soil moisture was also tested in this trial.

3.2 Reduction of Shell Damage to Nuts

3.2.1 Stylar End Lesion

Photo 3.2.1 Nuts with stylar end lesion after packing process

Stylar end lesion (SEL) was first reported in California in 1984 (Bolkan et al, 1984). This symptom only occurred in a few counties in California (Rice et al, 1985). Iran has this symptom due to high Mg in irrigation water (Hokmabadi, 2009). In seasons 1999/00, 2003/04 and 2005/06, SEL occurred in many Australian pistachio orchards causing significant damage and losses. At the beginning in Australia, it was called 'chocolate nuts'. There are 2 characteristics for SEL in Australia: one is that SEL only appears in off-years; the other is that SEL appears on trees with rootstocks or *P. terebinthus* or *P. atlantica* but not on Pioneer Gold.

We investigated this symptom during season 2003/04, analyzed historical data and tissue analysis data finding that Ca is the key element for this symptom. We tested the range of Ca foliar application in season 2004/05 ('on' years do not have SEL problems and therefore a real trial could not be set up). A trial in season 2005/06 proved Ca effect.

From this point, we need to further understand:

- If current concentration and chemical are the best choice?
- If there is another choice for chemical application to make the work easier?

A trial on a big scale with harvest results was required.

Season 2006/07 was an on-year without SEL nuts problem so we could not test the nuts. However, we still could test the concentrations on leaves. To test the tolerance of pistachio leaves to chemicals, as well as to test different concentrations, was the major work for that season. The concentrations used in the previous year were doubled but half concentrations were used in this trial.

In season 2007/08, we set up a production scale trial in stage 3T (4 ha) to test this symptom control. At the same time, Ca application timing was also tested in stage 1.

Trials showed that early Ca application gave better results than late ones. The earliest Ca foliar application should be at the time for boron application. It is well known that boric acid can also help calcium absorption. Thus, in season 2009, we tried Ca application during B application.

3.2.2 Other Symptom Related to SEL Nut

During investigation on SEL nuts, other symptoms were also noticed. The findings have been also documented in case it is useful in the future.

Something close to SEL nuts appeared a little earlier than the time SEL nuts usually appear.
In late November 2007, before we found SEL nuts, many nuts showed similar symptom. Normal SEL nuts have a transparent hull at the tip, but this symptom has the transparent part on the middle of the nuts (Photo 1). This was temporarily called 'black-side'. The process of black-side was quick. The nuts with black-side can only survive a short time, and then drop off. We also found black-tip nuts, which is sunburned black point on the tip. To understand black-side nuts and black-tip nuts, nut samples were taken for tissue analysis.

Photo1: Nuts with new symptom on our pistachio trees in late November 2007

3.3 Reduction of the Impact of Alternate Bearing

3.3.1 Pruning Access

Pistachios are known to strongly exhibit alternate bearing so pruning may be a way of solving biennial bearing. Beede et al (1991) reported that pruning as much as 50% of the fruit buds off in some treatments did not significantly alter the nut load from an unpruned tree. The effects of heavy pruning (removal of ⅔ vegetative shoots and ⅓ mixed branches) and light pruning (removing ⅓ all branches) on yield were compared with the traditional method (cutting out dead branches). The different pruning methods had no significant effect on yield. However, heavy pruning gave the lowest kernel/shell ratio (43.5%), the heaviest 100 nut weight (105.13 g) and the highest split percentage (84.7%). Both experimental pruning techniques gave better shoot growth than the local method (Arpaci et al, 1995). Evaluation of the cluster collected before harvest explains why pistachios can be severely pruned without significant yield reduction. Fruit buds lost to pruning are compensated by increasing nut numbers per cluster. This implies that more flower buds removed usually do not reduce crop. In on-year, to prune many flower shoots off may reduce extra nutrition costs and benefit flower initiation and remaining for the crop in the following season.

In many measures to control biennial bearing, Ferguson et al (1995) listed pruning as number 1. In our hand thinning and hand pruning (major in thinning out) trials, pruning showed more potential. Severely mechanical pruning in CMV Farms in winter 2000, 2005 and 2006 also manifested this possibility. In winter 2007, CMV Farms used slant mechanical pruning orchard-widely; Kyalite Pistachios maintained tree shape by mechanical pruning.

In California, mechanical pruning treatments had significantly greater nut numbers per cluster. The data suggested pistachio's compensatory capacity is less for topping than side hedging (Beede et al, 1998). Hedging or topping at 100% significantly increased the percentage of edible closed shell nuts compared to hand pruned trees. Side hedging increased nut numbers per cluster by 71% compared to about 31% for topping (Beede et al, 1998). This implies that removing some flower buds usually does not reduce the crop. In the on-year, to remove many flower shoots by pruning may reduce extra nutrition costs and benefit bud initiation and retention for the crop in the following season. To control biennial bearing, Crane and Iwakiri (1980) concluded that "It should be done, therefore, in the winter prior to the off-year of production in the alternative bearing cycle to minimize loss in yield".

To further prove this effect and to further understand the mechanism, a scientific trial with quantitative data collection was required. On 19 May 2008, a meeting of the research committee members and technical staff in the major pistachio farms was held at DPI, Mildura. It was decided that a slant topping trial be conducted at Kyalite Pistachios starting in the winter of 2008 (prior an 'on' year and at CMV Farms started in the winter of 2009 (prior an 'off' year).

This trial was to use grower pruning to test:-

- the practice of mechanical slant topping;
- which year has better results, prior to an 'on' year or prior to an 'off' year.

3.3.2 Thinning

Nut thinning may be another way to control crop and overcome alternate bearing. Late flowers seem to produce small nuts with low quality in pistachio production (Zhang, 2005b). In Australia, 'Sirora' also has a long bloom period. Late flowers may result in late maturing and maturity variation on a whole tree basis. Efficiently removing later flowers could be very important for pistachio production. Chemical thinning, burning flowers, may be one of the methods. However, dioccism provides another

way for nut setting control, i.e. pollen control, or removing all male flowers in a certain stage to stop late pollination and late nut setting.

In New Zealand, growers investigated fruit number per tree and distributions of fruitlet diameters. Based on the investigation, "size" hand thinning can be undertaken to reach a target crop load in whole blocks or orchards (Zhang, 1997). In orchards in New Zealand, tree structures of Royal Gala apple trees were transformed and only 7 – 8 cuts per tree are required for winter pruning (Zhang and Dine, 2003). All these experiences could be translated to pistachio production.

3.4 Reaching the high yields currently being obtained by the best Australian Orchards for most Australian Orchards

3.4.1 Group Visiting

Besides research on particular topics, contact with pistachio growers and improvement on their production capacity were carried out in previous 3-year project. Based on voluntary participation, an 18 member grower group was organized. This work also analysed historical data for the 13 orchards. Comparison of yields based on per tree or per hectare; comparison of nut size etc was also provided for the growers. Fertilizer applications between the orchards analysis is under way. From fertilizer analysis, the maximum application of N was about 10 times higher than the minimum application. Generally the higher the N application the higher the yield. This observation shows an obvious opportunity for improvement. These results have been communicated to all growers. This work will help growers to identify production problems and improve their yields and quality.

3.4.2 Pruning Changing in Pistachio Industry

Pruning is an important measure in pistachio production. Compared with almond wood, pistachio wood is soft. 'Sirora' pistachio used in Australia also has softer wood than 'Kerman' pistachio, which is used in California, USA. When a new shoot grows over 1 metre or even half a metre, it can bend. This damages tree structure. Thus, tree training in early stage is essential. "Cup" shape is recommended for pistachio training shape. Three base skeleton shoots above trunks provide a central open "cup" shape. From each base skeleton shoot, 2 middle skeleton shoots are produced, or say 6 middle skeletons, then 12 top skeletons and finally 24 extension skeletons. This is an idea tree structure. If 24 extension skeleton shoots are on the top, it is about every 0.5 metres for one. This allows trees to occupy good space for bearing. If the training process is very successful, in 4th winter 12 tops should be produced. Obtaining this tree shape with a certain height, normally it should be in the 7th winter. In winter pruning, to ensure strong skeleton structure, length of skeleton shoot is suggested to be around 40 cm. If longer than that, it may induce soft structure. This also influences mechanical harvesting.

Beyond skeleton shoot pruning, growers do not want too long shoots for bearing shoots. In "standard" pruning (Kallsen, 2008) "branches in the upper canopy of pistachio trees is to make the pruning cut at a point on the branches located 2 or 3 vegetative buds above the last flower bud (or call tip). These vegetative buds will provide locations for new growth in the following year." This was mature tree pruning in major pistachio orchards in Australia before 2005. In this kind of yearly pruning, it normally tips around 30 shoots for each female tree and 70 shoots for each male tree. This not only involves in a big labour input but also depresses spur development in lower parts because pruning stimulates strong growth on the top.

In winter 2005, major pistachio orchards stopped tipping on male trees. Without tipping, male trees still produce enough male flowers. In this process, growers noticed good growth of spurs in lower parts on male trees in that growing season. In the winter of 2006, major orchards stopped tipping on female trees. Crop did not have any influence but this operation reduced labour input. At the same time, a small trial for mechanical pruning was started in the winter of 2006.

Mechanical pruning was not a new technique in the pistachio industry in Australia. In 1990s, after California scientists proved hedging did not reduce the pistachio crop, hedging was introduced into Australia pistachio orchards. In one way, hedging provides room for sunlight between rows; in the other way, hedging produces many non-vertical shoots, some were even below than horizontal lines like Figure 1 showing at C area. This made the tree structure more horizontally. With a spread tree structure it is not only easy to damage heavy branches but also reduces efficiency for mechanical harvesters. This pruning also resulted in tall vertical shoots growing in the top middle area (A area), while varies angles shoots grown in middle parts of side of trees (C area). A weak shoot development was found in tree shoulder (B area).

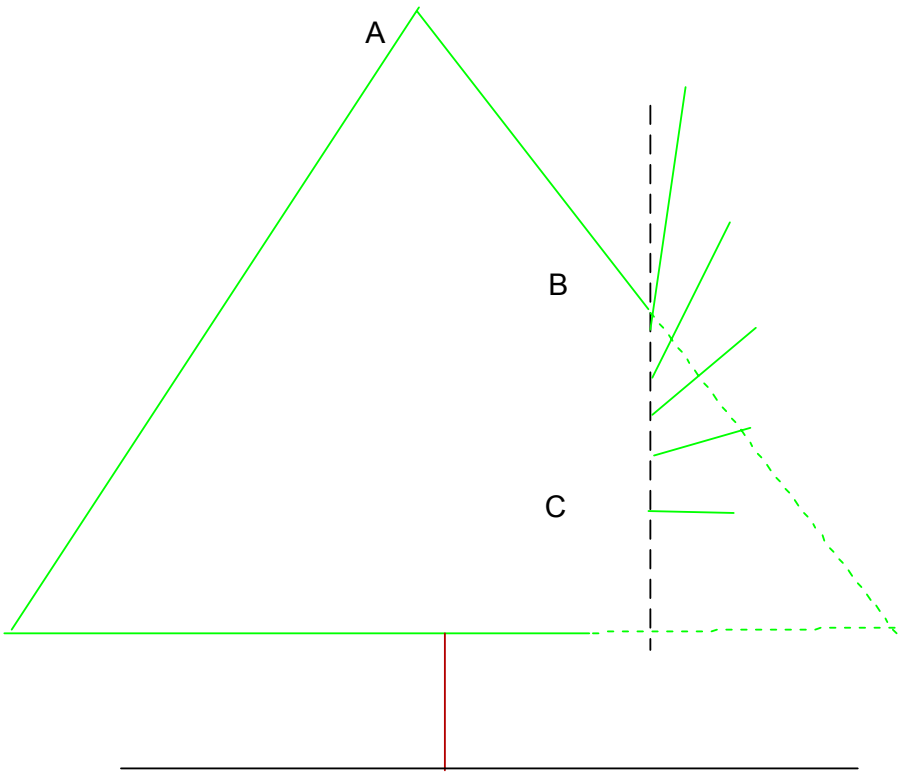


Figure 1: Diagraph showing response after hedging

To transfer tree shape from spread to vertical and develop new growth in tree shoulder area, a slant topping pruning trial was held in the winter of 2006 in 1 row in CMV Farms. After 1-year growth, more vertical shoots appeared. This was considered as a good measure for tree training transfer from spread to vertical.

In the winter of 2007, after ‘on’ year harvest 2006, (before an ‘off’ year), slant pruning was carried out on 1 side on almost all mature trees in CMV Farms as shown in Figure 2. While the paper by Kallsen (2008) detailed this type of treatment the Australian industry had recognized this before reading this publication.

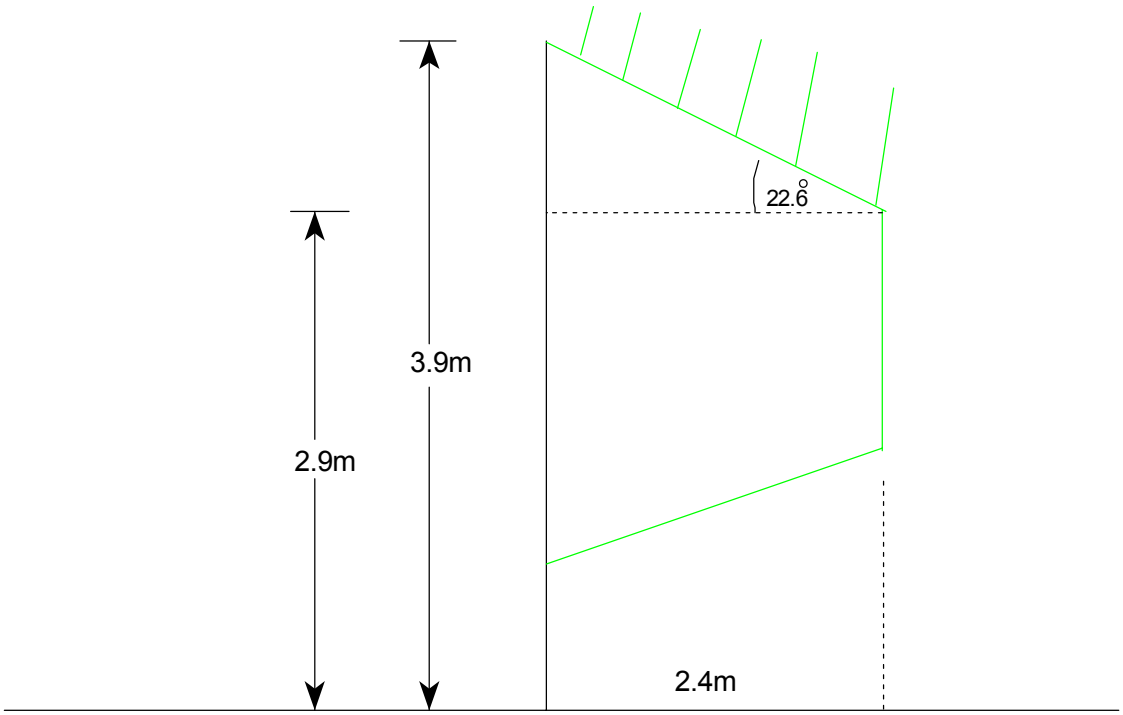


Figure 2: Diagraph of slant pruning

Figure 2 shows the tree shape after the slant pruning. Many vertical shoots grow on the top of the cut. This is useful for transfer tree shape from spread to vertical. You can document mechanical pruning on tree height at centre and on machine angle from horizontal line. After recording these 2 numbers, you can repeat your pruning exactly in the future. Here a machine was also used to prune bottom part.

In one way, heavy mechanical pruning before an 'off' year should reduce less crop than before an 'on' year. In the other way, strong cuttings prior an 'off' year will benefit crop in the following 'off' year. In the winter of 2007, many shoots were cut down to 2 or 3 years-old woods at shown by the dash line in Figure 3. In the growing season of 2007/08, many new shoots grew on the tree. Among them, many of those strong new shoots were from adventitious buds due to mechanical pruning. It should be emphasized that new shoots from adventitious buds do not have flower bud initiation in season 2007/08 ('off' year). No pruning was conducted in winter 2008 on those shoots. In growing season 2008/09 ('on' year), many flower buds (brown, small circles in Figure 3) were initiated on those shoots. No pruning was conducted in winter 2009 and flowers and nuts (red ellipse in Figure 3) were carried on season 2009/10 ('off' year). This pruning technique shows us that after a heavy pruning, at least, we should not prune the same place again within 3 years. Those cuts will grow shoots in growing season 1, initiate flower buds in growing season 2, and bear nuts in growing season 3. If you cut the shoots again before growing season 3, you will not get any crop from this pruning.

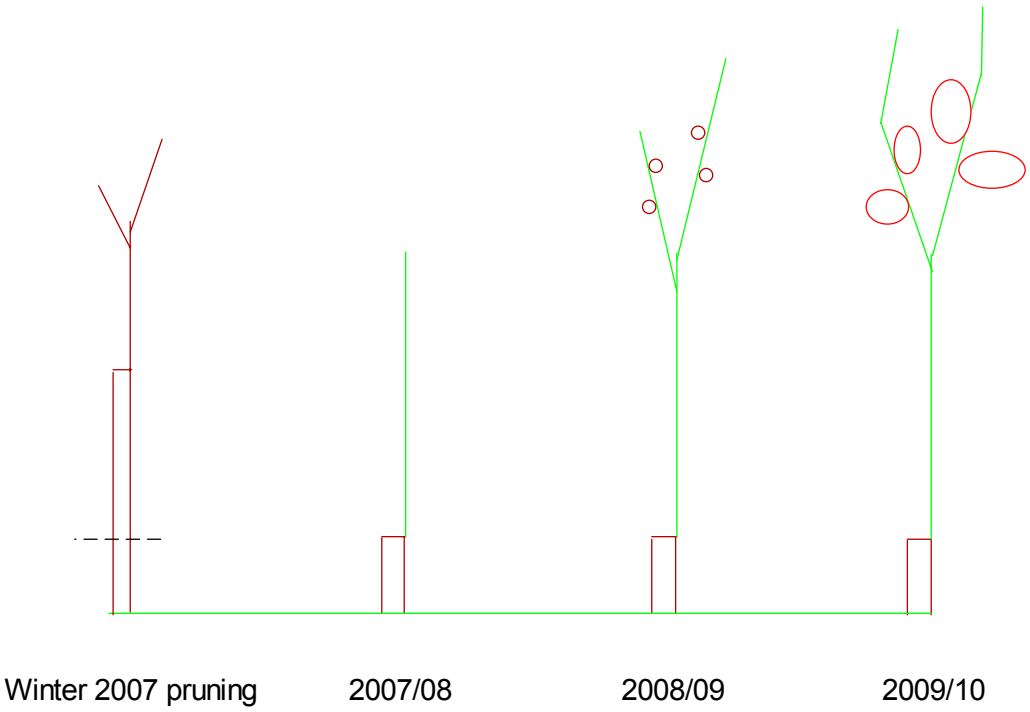


Figure 3: Diagraph showing flower bud initiation process after a severe cutting

Practice showed us that although season 2009/10 was an 'off' year; on 1 side of the tree top still have reasonable crop on. This skill, at least, allows us to keep a certain amount crop for next 'off' year. This pruning was carried out in winter 2009 and will be in winter 2011.

In pruning, another discussion was when heavy pruning should be taken, prior an 'on' year or prior an 'off' year?
In the winter of 2009, a slant pruning trial prior 'on' year or 'off' year was established in CMV Farms. There was a totally of 16 rows with 45 trees per row. 8 rows were prior to an 'on' year and the other 8 rows were prior to an 'off' year in a randomized plot design. In the winters of 2009 and 2010, 1 side slant pruning was conducted. Nuts were harvested and yields recorded on a row basis. In winter 2013, we will have a chance to clearly observe responses of shoot growing after 1-, 2-, 3- and 4-years severe pruning. This will provide more confidence for us in the future.

There was a trial in Kyalite Pistachios to compare slant pruning in 1 side and in 2 sides in the same time. In the next a few years, we will provide more reliable pruning information for the growers. Besides mature tree pruning, young tree training was also used machines after 4 years.

3.5 Achieve better than World Best Practice for yield, quality and profitability for Australian Pistachio Growers

3.5.1 Chill Requirements

Cool winter temperatures are required by many fruit and nut trees that originate from temperate or cool subtropical regions to overcome winter dormancy (Samish, 1954; Saure, 1985; Erez, 2000). There are

many unseen physiological processes happening within the tree during the period between leaf fall to budbreak (Figure 1).

Figure 1.1: Physiological processes from fruit ripening to bud break

From Figure 1.1, we know that the chilling process should start at a particular date, and then chilling accumulates to the amount required. This is another particular date called dormancy completion. In this period, cold temperatures benefit the process. After this period, the plant needs higher temperatures to initiate bud break.

Sufficient winter chill results in homogeneous and simultaneous flowering (Luedeling et al 2009c). When fruit and nut crops are grown outside their traditional growing regions, estimating the amount of chill required and that which is available is important (Luedeling et al, 2009a). "In order to select appropriate fruit and nut species and cultivars for the climate of a given site, researchers have developed chilling models, which convert temperature records into a metric of coldness" (Luedeling et al 2009c).

The chilling process in trees is not completely understood and it is difficult to produce a model that perfectly explains the accumulation of winter chill (Dennis, 2003; Erez, 2000; Saure, 1985). Chilling models can be used to determine the chilling requirement of a cultivar and the chill available at a particular location however they are not completely accurate (Luedeling et al 2009a). Quantitative measurements of winter chill are required to determine the suitability of a cultivar to a particular growing region, to determine the timing of management practices such as applying rest-breaking chemicals and to predict yield potential. Understanding the effect of dormancy completion level on interannual variation can also be facilitated by the use of chilling models (Luedeling et al 2009c).

The Chilling Hour Model was one of the earliest, developed in the 1930s (Chandler and Tufts, 1934) and 1940s (Yarnell, 1940) and is the simplest. Bennett (1949) quantified winter chill as the number of hours 0-7.2°C (32-45°F), while Weinberger (1950) suggested using the number of hours ≤ 7.2°C (45°F) during the winter season. Luedeling et al (2009c) proposed that "freezing temperatures did not contribute to winter chill accumulation" and used 0-7.2°C and denoted it the Chilling Hour Model. However it has been found that high temperatures have a negative chill-contribution (Richardson et al, 1974). Erez and Lavee (1971) reported that 10°C was about half as efficient in breaking dormancy as 6°C. The Utah Model, a weighted Chilling Hour Model with high temperatures having a negative effect on chilling accumulation, was developed in the 1970s (Richardson et al, 1974). This model has been adapted to adjust to varying climatic conditions. Norvell and Moore (1982) extended effective temperature ranges compared with the Utah Model. Shaltout and Unrath (1983) adjusted the relationship between Chill Units and temperatures by assigning greater chill contribution to lower temperatures and more negative effect to temperatures >21°C (North Carolina Model). Disregarding

Chill Units accumulated on days where there is a negative total has been found to be more suitable in marginal areas in South Africa (Allan et al 1995). This model is called the Positive Utah Model (Linsley-Noakes et al, 1995).

All the models above treat the effect of hourly temperatures on chilling accumulation, in mathematical terms, as a time-homogeneous stationery process. In other words, they assume that two hours at the same temperature recorded at different times in a long-term observation contribute equally to the breaking of dormancy (Fishman et al 1987a). Results obtained by Erez et al (1979) in experiments with periodically changed temperatures contradicted this assumption. 'Redskin' peach plants were exposed to differing temperatures for cycles of 1, 3, 6 and 9 days with temperatures of 4-6^{o}C for 2/3 of the cycle length and 24^{o}C for the remaining 1/3 of the cycle. Each cycle was repeated until all plants were exposed to 1150 Chilling Hours, or 575 Chill Units based on the Utah Model. However, plants showed significantly different responses; when plants were exposed to cycles of greater length a higher percentage of bud break was observed. The same amount of high temperature when applied in long cycles resulted in less chilling negation. Since stationary processes do not sufficiently explain these results, non-stationary and time-inhomogeneous processes should be introduced to chilling models. This is implemented in the Dynamic Model.

The Dynamic Model (Fishman et al, 1987a; Fishman et al, 1987b) was developed in the 1980s and defined a new concept for the negation process. Winter chill is assumed to accumulate in a two-step process. Cold temperatures initially result in the formation of an intermediate chilling product; high temperatures can destroy this product. Once a critical amount of this chilling product has accumulated, it converts to a Chill Portion which cannot be destroyed. A certain Chill Portion accumulation indicates fulfillment of chilling requirement. Although a few papers support the Dynamic Model on peach (Erez, et al, 1990; Allan et al, 1995), apricot (Ruiz et al, 2007) and walnut (Luedeling et al, 2009c), the Dynamic Model has not been widely adopted (Luedeling et al, 2009a). Studies of chill requirements in pistachio have mainly used the Chilling Hour and Utah Models (Rahemi and Pakkish 2009, Afshari et al 2009). The amount of chill required for 'Sirora' pistachio grown in Australia had not been established.

After chill fulfillment, temperatures above some base level will result in growth and bud development (Richardson et al, 1975). Growing degree hours (GDH) are the number of hours of heat required for bud break or bloom after the chilling requirement is fulfilled. A few models for GDH have been proposed in this area (Richardson et al, 1974, 1975; Anderson and Richardson, 1987).

The Australian pistachio industry was established in south-eastern Australia in the early 1980s and nut bearing began in the early 1990s with full yield production reached in the early 2000s. In the spring of 2005, bud break was clearly delayed, for most orchards, by one month, with some buds breaking two months, even four months late. This suggested a lack of winter chill. Chill requirement is a production problem for our pistachio trees. In winter 2006 we started laboratory work to test chill completion.

Experimental design was based on other researchers experience as listed below:

- Opening of leaf buds was examined after a period at 23°C or 25°C in a 16- or 24-hr photoperiod. 21 days (Erez and Lavee, 1971).
- Physiological determinations were made by regularly collecting shoots and bringing them into a greenhouse where they were held at 18°C. If the buds on the shoots developed within 2 weeks, the trees were classed as having completed dormancy at the time the samples were collected (Ashcroft et al, 1977).

- Bud break was determined after 30 days for forcing at 24°C in continuous light (Erez et al, 1979).
- Twigs were taken into the greenhouse at 18 to 21°C and placed in containers containing a solution consisting of 3.8 (w/v) 8-hydroxyquineline citrate and 0.033% (w/v) aluminum sulphate. Data on bud development was obtained every other day and percentage of budbreak calculated after 21 days of forcing (Shaltout and Unrath, 1983).
- To measure response to these treatments, shoots were forced in a growth chamber at 27°C day/23°C night with 350 μmol/s•m^2 of cool-white fluorescent light for 16 hours daily. Cherry bud development stages are divided into closed bud, green tip, half green, ¾ green, popcorn and full bloom. Data expressed in percentage of budbreak after 30 days of forcing and number of forcing days required reaching the half green stage for those buds on each shoot that did break (Felker and Robitaille, 1985)
- Trees were moved to a growth chamber at 20±1°C and 18-h photoperiod for forcing. After 21 days, trees were harvested (Young, 1992).
- Shoots were held for 3 weeks with their basal ends in glass jars containing distilled water at 22 to 24°C so that budbreak and bud growth could be observed (Wang and Faust, 1994).
- The cut shoots were placed in the greenhouse under forcing conditions (24°C day/19°C night) with their cut ends in distilled water. Water was changed and shoot ends were cut weekly to prevent contamination. Rest was considered completed when 50% or more of the buds had reached at least the green tip stage in out of the 3 shoots at the end of four weeks (Ghariani and Stebbins, 1994).
- The degree-hours required for 50 percent of the buds on these shoots to break are deemed to be the chilling requirement for the particular cultivars (Ryugo, 1988 (textbook)).
- Regarding your protocol: bud break of 50% in 2 weeks is just an arbitrary measure. You probably will do better with 3 week period (Erez, e-mail 24/4/2006).
- For good chill completion we looked for 50% bloom within 21days of placement in the chamber (Ferguson, e-mail 13/10/2006)
- After chilling treatments, plants were forced in a growth chamber adjusted to 25°C. Flowering counts of all flower buds were taken after 30 forcing days, to judge completion of endodormancy. Endodormancy was regarded as completed when the percentages of flowering buds exceeded 30% (Sugiura and Sugiura, 2006).

According to the literature above, 50% flowering within 3 weeks is used to describe flowering/bloom.

There are many chill models that could have been used for our work. They were
- Numbers of hours ≤7.2°C (used by California pistachio industry);
- Numbers of hours between 0 - 7.2°C (used by California pistachio industry);
- Numbers of hours ≤7.5°C (Hobman and Bass, 1986 for Australia pistachio);

For convenience, these 3 models are sometimes called temperature models;
- Utah model (Richardson et al, 1974)
- Dynamic model (Fishman et al, 1987a).

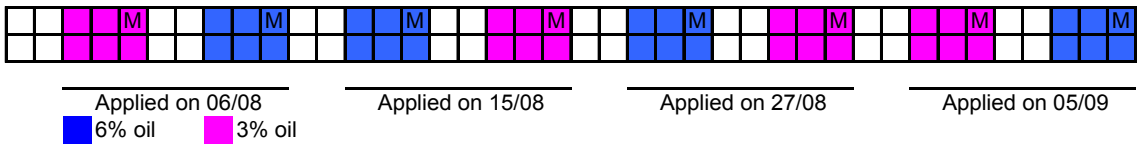
Winter oil application is a useful agent to overcome low-chill winters (Beede and Ferguson, 2002) and is used by growers to promote even and timely bud break. The decision to apply oil to alleviate low chill, needs to be made before 31 August. Mid-late August is the best time for winter oil application in Australia, or mid-late February in USA (northern hemisphere) (Beede and Ferguson, 2002). Thus, by mid-August growers need a prediction of chill accumulation to decide whether or not to apply oil.

3.5.2 Winter Oil Application

Winter chill problems threatened pistachio production in Australia. Winter oil application shows benefit to solving this problem. In the winter of 2005, CMV Farms used 6% winter oil and obtained better results for the harvest in 2006. Growers paid more attention to oil application. Thus, 6% winter oil has been used in the Australian pistachio industry.

From Californian papers, they mainly use 6 GPA oil in 200 GPA water. That is 3% oil in 1870 L water per hectare. However, the best results that we obtained in winter 2006 were 6% oil in 1800 L water per hectare. Concentrations of 3% and 6% have a significant difference in costs. It was necessary to test the difference of application between 3% and 6% oil.

In the winter of 2006 and 2007, we used 5 female trees as a plot to test differences between 3% and 6% winter oil and 4 different application dates as shown in the following map. This observation clearly showed that late application (5 September) led to late bud break. The second and the third applications (15 and 27 August) showed earlier bud break and blooming than the first application (6 August) either.



Winter oil application is a key measure for us in low chill years. We needed to further understand this for our future production. In 2008 winter, we decided to use whole stage-3 block (8.6 ha) for this trial to get more accurate results.

To test the effect of concentrations of winter oil, in the other way, Peter Weir suggested using shoot dipping to test the oil concentration effect. In the winter of 2008 we tried dipping oil using shoots on trees at the pistachio field in DPI, Mildura.

3.5.3 Nut Size Prediction

Pistachio nut size directly links up with price and income. It is of concern to both growers and traders. Some factors influencing nut size are known such as variety, pollination (Abu-Zahra and Al-Abbadi, 2007), irrigation (Ak and Agackesen, 2005) and crop load (Boler, 1998). Most of those factors can be managed in orchard planning and management. In good conditions pistachio trees still produce different size nuts in different years. Further studies could determine what influences nut size. Accurate prediction of nut size prior to harvest is of benefit to commercial producer.

Major pistachio production areas in Australia are along the Murray River. Due to low chill in this area, 'Sirora' is the major variety in Australia pistachio production. 'Sirora' pistachio nuts are relatively smaller compared with 'Kerman' (Maggs, 1990). Hence, nut size becomes more important in Australia pistachio production.

Limited papers describe how to find the key periods of climate influencing on fruit size or other production parameters. Goldwin (1982) calculated all the r-values between days for apple growing and temperatures among relevant dates and their combinations. Zhang and Thiele (1992) used r-contour map showing the r-values and found the key environmental factor influencing on 'Royal Gala' apple size, then made accurate national crop estimations for the New Zealand Apple and Pear Market Board

(Zhang and Robson, 2002). Zhang et al. (2001) also used this technique for harvest starting date prediction for 'Royal Gala' apple.

In this study, harvest records in 10 years plus local meteorological station data provide a chance for us to test climate effect on pistachio nut size. Historical data showed that extremely low N application and irrigation led to small nuts produced in some orchards. The most productive Australian pistachio orchards apply 200 – 300 kg N/ha and 600 – 800 mm water. These well managed orchards have been the focus of much of the past and current research.

4 MATERIAL & METHODS

4.1 Nitrogen Trial

4.1.1 Trial Design

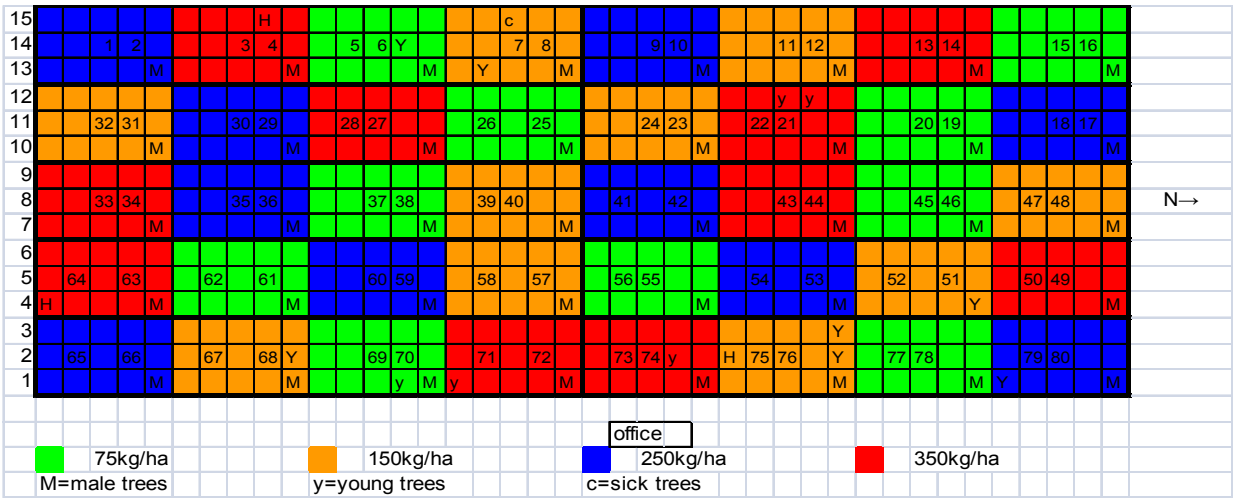


Figure1: Map of trial design

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 randomised replicates (Figure 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 flow measured and logged, and records kept on the amounts of fertiliser added to each treatment's supply tank.

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

For leaf analysis and nut quality measurements, only 3 replicates were taken and termed as 'leaf replicates'. In this way, replicates 1-4 are pooled into leaf replicate 1; replicates 5-7 into leaf replicate 2 and replicates 8-10 into leaf replicate 3. 12 leaf samples (3 replicates for each treatment) were taken on 16 February 2009 and on 1 February 2010 for full nutrition analysis. 12 leaf samples (3 replicates for each treatment) were taken from 26 October 2009 to 15 March 2010 fortnightly for nitrogen analysis. Soil samples were also taken before the treatments start and 8 soil samples (2 depths (0-15cm and 30-45 cm) for each treatment) were taken on 24 February 2009, and 16 soil samples (4 depths (0-15cm, 15-30 cm, 30-45 cm and 45-60 cm) for each treatment) were taken on 11 February 2010 just after fertigation.

4.1.2 Operation

In season 2008-2009, fertigation was started on 14 October 2008 and completed on 16 February 2009. It had one more fertigation than the rest of the farm to reach the trial target. In season 2009-2010, fertigation was started on 19 October 2009 and completed on 5 February 2010. It was the same as

CMV Farm fertigation. In this area, row distance is 6.86 m and tree distance is 4.39 m. Each treatment-plot has 15 trees and each treatment (10 replicates) has 150 trees, i.e. 0.452 hectare.

Liquid UAN is used for fertigation, which contain 42.2% N. Table 1 shows the design amount of UAN application in the 0.452 hectare and fertilizer completion in season 2008-2009 and 2009-2010.

Table 1: Amount of N application in design and in actual application

Treatments	Designed application of UAN for 0.452 ha (L)	Application of UAN for 0.452 ha (L) in season 2008-2009	Application of UAN for 0.452 ha (L) in season 2009-2010
75 kg N/ha	80.3	80.3	81.5
150 kg N/ha	160.6	161.4	162.4
250 kg N/ha	267.6	268.4	267.1
350 kg N/ha	374.7	375.2	374.0

4.1.3 Leaf Analysis

10 leaf analyses for nitrogen only were tested fortnightly for 4 treatments with 3 replicates from late October to mid March in season 2009/10 and 2010/11. In late January or early February, a basic leaf analysis test was taken for 4 treatments with 3 replicates.

4.1.4 Soil Analysis

On 14 May 2008, before nitrogen trial started, CMV Farms had a soil analysis conducted including stage 2. Our nitrogen trial was at rows 1 – 15, stage 2A. The sample was taken from between trees 26 and 27 in row 18 in stage 2A. This sample site is very close to our nitrogen trial and it could be taken as a soil sample before the trial. On 24 February 2009 and on 16 February 2010, at replicate 6 of the nitrogen trial, soil samples of each treatment were taken. In different years, depths for soil samples were different. Table 4 listed the depth for soil sampling.

Table 4: Depths for soil sampling in different years

Year	0 – 15 cm	15 – 30 cm	30 – 45 cm	45 – 60 cm
2008	V	V		
2009	V		V	
2010	V	V	V	V

4.1.5 Trial Harvest

For the 80 trial-recording trees, each tree was harvested and recorded individually. In season 2008-2009, machine harvesting of the trial trees on a single trees basis on 12 and 13 March for shake 1 and 28 March 2009 for shake 2 were undertaken. In season 2009-2010, machine harvesting of the trial trees on a single trees basis on 11 March for shake 1 and 23 March 2010 for shake 2 were undertaken. During harvest, about a 2 kg sample in each shake were collected for each tree. Then 4 samples in the same treatments were pooled together for further test according to Table 6. The pooled samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as per the normal process. Return per tree is based on prices in Table 7.

Table 6: 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)5,6,15,16	(5)18,20,25,26	(9)37,38,45,46	(13)55,56,61,62	(17)69,70,77,78
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)53,54,59,60	(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

Table 7: 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 were analyzed by 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:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

4.2 Pistachio Flower Number Monitoring

4.2.1 Flower Counting Trial

From the spring of 2004 to 2008, two mature 'Sirora' pistachio female trees named tree 4 and tree 5 were used in row 32, stage 3 of CMV Farm. The planting density is 4.39 m x 6.86 m and both trees were in an 'on' year. From the beginning of bloom, every flower at the full bloom stage was marked. Thus total marked flower numbers were recorded. This allows us to know how many flowers open for a tree.
In the spring of 2009, due to attending 5th international symposium on pistachios and Almonds, daily flower marking was not carried out. Instead, flower marking was started at a late stage of flower open. While this cannot provide an accurate process of flower opening, it still can provide information about the accurate numbers of total flower clusters open in these 2 trees.

Between the winters of 2006 and 2009, numbers of flower buds on these 2 trees were counted before bud break.

4.2.2 Oil Application Trial

Between the winters of 2007 and 2009, one tree was covered by plastic before winter oil application in that stage. This operation was on 14 August 2007, 19 August 2008. 21 August 2009. After oil application, the plastic was removed immediately.

4.2.3 Trial Harvest

In seasons 2007/08, 2008/09 and 2009/10, these 2 trees were individually harvested and yields were recorded on tree basis on 3 and 25 March 2008, 16 and 28 March 2009, 10 and 23 March 2010. In seasons 2008/09 and 2009/10, during harvest, 10 kg samples in the shake 1 and 5 kg samples in the shake 2 for each tree were collected and analysed.

4.3 Reflective Mulch Trial 2

4.3.1 Harvest

On 3rd October 2006, 1000 m² reflective mulch was fixed at stage 2 in CMV in a randomized plot design (Figure 1) until 22 February 2006 (just before harvest). On 25th September 2007, the reflective mulch was fixed again in the same location until 22 February 2008 (just before harvest).

A machine harvested the trial trees on a single trees basis on 27 February and 16 March 2007 in season 1and harvested the trial trees on a single trees basis on 29 February and 25 March 2008 in season 2. During harvest, about a 2 kg sample in each shake was collected from each tree. Then 4 samples in the same treatments were pooled together for further test according to Table 1. The pooled samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Return per tree is based on prices in Table 2.

Table 1: Pooled samples from the original trees

Reflective mulch trial	Sample 1	Sample 2	Sample 3
The mulch	①Trees 3, 4, 5, 6	②Trees 11, 12, 13, 14	③Trees 19, 20, 21, 22
Control	④Trees 1, 2, 7, 8	⑤Trees 9, 10, 15, 16	⑥Trees 17, 18, 23, 24

Table 2: 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 analyzed by 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:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

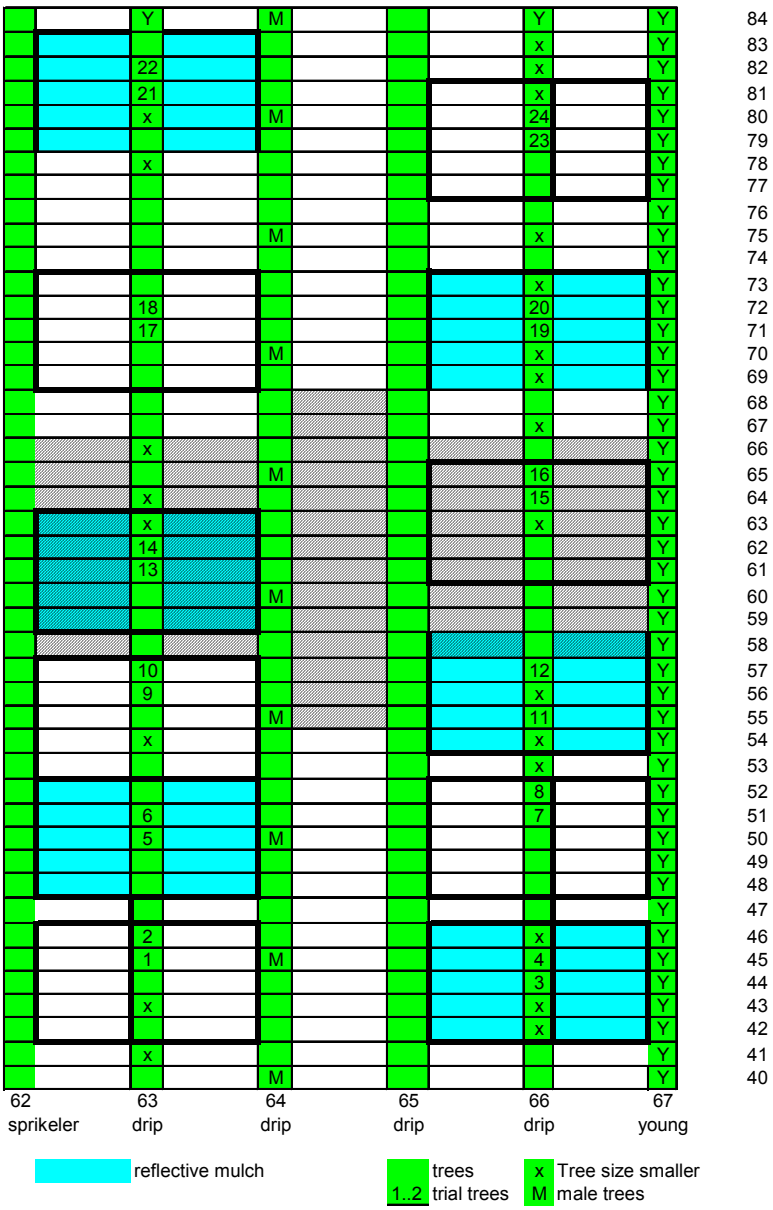


Figure 1: Trial design

4.3.2 Influence of Reflective Light from the Mulch on Trees in Different Distance

Although reflection distance was calculated based on solar angles, practical measurement will be more reliable. The November measurement was done at the mid-day and afternoon of 8 November and morning of 9 November 2006. The December measurement was in the morning and mid-day of 12 December and afternoon 16 December 2006. The January measurement was in the morning of 14 February, and mid-day and afternoon of 16 February 2007. Table 3 listed exact time range for each set of measurements.

Table 3: Times of a day for the measurement

Time	8 ,9 November	12, 16 December	14, 16 February
Morning	9:41-10:13	9:56-10:31	9:51-10:23
Mid-day	13:03-14:00	12:58-13:31	12:49-13:24
Afternoon	15:15-15:48	15:10-15:40	14:49-15:45

In Figure 1, in the left side of the mulch trees (in cyan box, from bottom to the top), from the 1st tree above the mulch (2 trees down from tree 5) to the last tree above the mulch (at the top), every tree has 4 measurements for reflective light from 4 directions (2 between rows and 2 within rows). Then an average was calculated for each tree.

To compare effects of the mulch, the 1st tree above the mulch (from bottom) was called position 1, then the 2nd tree above the mulch was called position 2, up to position 5, then keeps this order going for position 6 … . Until the next mulch, position 1 is counted again but in replicate 2, then in replicate 3.

4.3.3 Comparison of Reflective Capacity between the Mulch under Canopies and Between-Rows

In this comparison trial, only the bottom 5 trial trees in row 63 are used for the mulch between rows (Figure 2). In the corresponded position in row 59, the same length (16 m) of mulch is placed under the 5 trees.

The first measurements were conducted between 8 am to 5 pm on 18 December 2006 for each hour. The second measurement was conducted between 8 am to 4 pm on 16 February, and 5 pm measurement on 17 February. Exact times for the measurement are listed in Table 4. For measurement each hour, measurements started from tree "1" in row 63 on the map, then tree "2" in row 59, then trees "3", "4", … according to the numbers in the map. This order is to reduce variation between the comparisons.

Table 4: Exact time for the measurement each hour

Date	Time	8 am	9 am	10 am	11 am	12 pm	1 pm	2 pm	3 pm	4 pm	5 pm
18/12	Start	7:56	8:50	9:50	10:50	11:49	12:52	1:48	2:47	3:48	4:48
	End	8:07	9:01	10:00	11:01	12:00	1:10	1:58	2:58	3:59	4:58
16/02	Start	7:52	8:51	9:50	10:47	11:50	12:49	13:43	14:50	16:13	16;49
	End	8:02	9:01	10:01	10:57	11:59	12:59	14:02	14:58	16:22	16:59

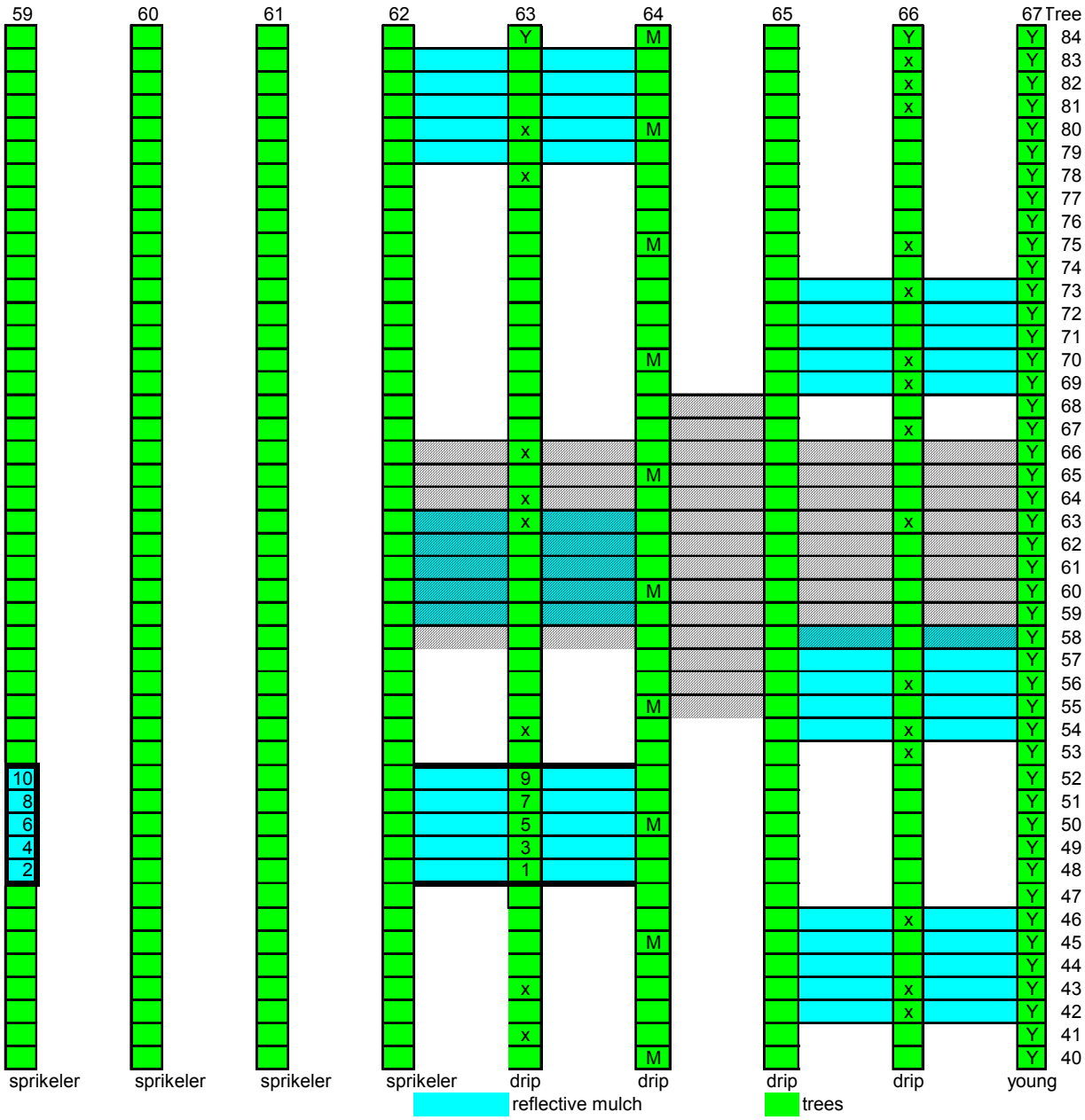


Figure 2 Map for mulch under canopy test

4.3.4 Reflection Capacity Test of Sun-Brite

Free samples of Sun-Brite were provided by Ultimate Fertilizers. Four bottles of Sun-Brite should be spray about 16 m^2. However, this liquid chemical was very thick and was extremely difficult to spray at the recommended concentration or at half of the concentration by a hand sprayer. Finally it was poured on the ground about 2 – 3 m^2 in an area without weed in stage 1, CMV Farms.

Three measurements were taken on 10 November, 18 December 2006 and 16 February 2007 for each measurement, 9 tests were carried out: 3 for Sun-Brite, 3 for Extenday mulch and 3 for common fields. In the first measurement, the order was mulch, Sun-Brite, mulch, Sun-Brite, mulch, Sun-Brite, then 3 fields. Before each test of reflection open sunlight was measured. The aim of this order is to reduce variations between comparisons. However, differences were clear from the first measurement.
In the second measurement, the order was 3 Sun-Brite, 3 fields and 3 Extendays. In the third measurement, the order was 3 Extendays, 3 Sun-Brite and 3 fields. Open sunlight was only tested once

for each of the 3 treatments. Due to different row directions for trees in this comparison, measurement was not under tree canopies. Instead, measurements were taken above good sunlight area between rows. To avoid variation between row directions, all measurements were tested in the direction of the meter to the Sun. All measurements were taken around mid-day.

4.4 Reflective Mulch Trial 3

4.4.1 Trial Harvest

This trial design was 4 treatments by 6 replicate plots by 2 trial trees within each plot in season 2007/08 as shown in Figure 1. The treatments are

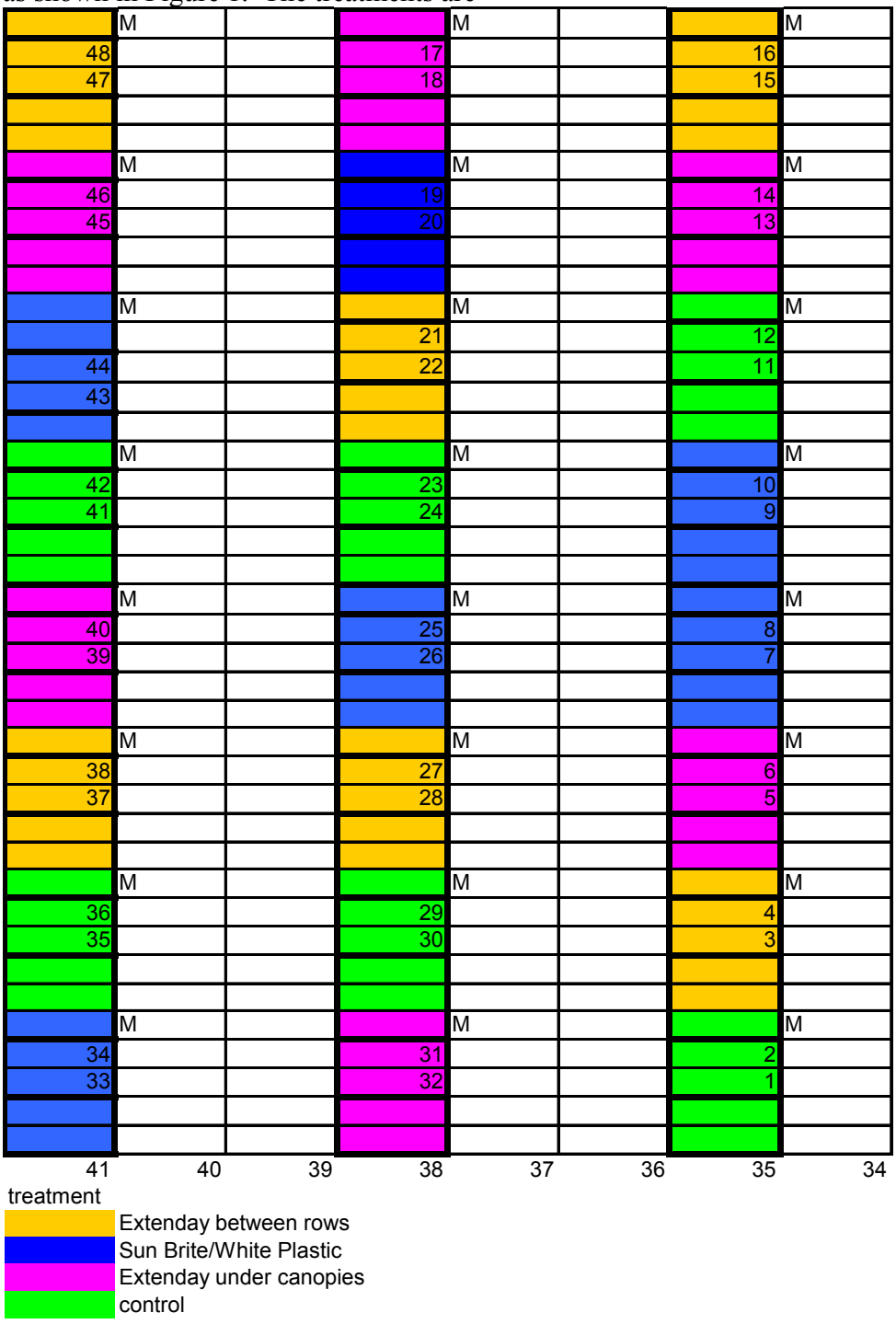


Figure 1: Trial design

- 4 metres wide Extenday between rows in each side;
- 2 metres wide Extenday under tree canopies in each side;
- 2 metres wide Sun Brite powders spray under tree canopies in each side;
- control.

Due to the bad results from Sun Brite in season 1, white plastic, which is used for grapes, was used to replace for Sun Brite in seasons 2008/09, 2009/10 and 2010/11. Also, to get more reliable statistical results, from season 2, all 3 middle trees used in each plot for trial harvest instead of 2 trial trees in season 1. The dates for mulch fixed and removed are listed in Table 1.

Table 1: Dates for mulch fixed and removed

Season	Dates for fixing mulches	Dates for removal of mulches
2007/08	2 and 15 October 2007	25 and 26 February 2008
2008/09	15 and 23 October 2008	3 and 4 March 2009
2009/10	17 and 22 September 2009	3 and 4 March 2010
2010/11	20 and 21 September 2010	

Table 2: Dates for trial harvests

Season	Shake 1	Shake 2
2007/08	3 March	25 March
2008/09	9 and 11 March	28 March
2009/10	10 March	23 March
2010/11		

Harvest dates are listed in Table 2 for each growing season. In season 2008/09, due to rainfall, the first shake was operated in 2 separate days. During harvest, about 2 kg sample in the each shake were collected for each trees. In season 2007/08 4 samples in the same treatments were pooled together for further tests according to Table 3. In the other seasons, samples from 6 trees in the same treatments were pooled together for a sample (Table 4). Totally 12 samples were taken for further test according to Table 2, 3 and 4 in each shake. The pooled samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Return per tree is based on prices in 2004 listed in Table 5.

Table 3: Pooled samples from the original trees in season 2007/08

Hand thinning trial	Rep 1-2	Rep 3-4	Rep 5-6
Extenday between rows	①3, 4, 15, 16	⑤21, 22, 27, 28	⑨37, 38, 47, 48
Extenday under trees	②5, 6, 13, 14	⑥17, 18, 31, 32	⑩39, 40, 45, 46
Sun-Brite	③7, 8, 9, 10	⑦19, 20, 25, 26	⑪33, 34, 43, 44
Control	④1, 2, 11, 12	⑧23, 24, 29, 30	⑫35, 36, 41,42

Table 4: Pooled samples from the original trees for seasons 2008/09, 2009/10 and 2010/11

Hand thinning trial	Rep 1-2	Rep 3-4	Rep 5-6
Extenday between rows	① 4,5,6,22,23,24	⑤ 31,32,33,40,41,42	⑨ 55,56,57,70,71,72
Extenday under trees	② 7,8,9,19,20,21	⑥ 25,26,27,46,47,48	⑩58,59,60,67,68,69
White plastic	③ 10,11,12,13,14,15	⑦ 28,29,30,37,38,39	⑪49,50,51,64,65,66
Control	④ 1,2,3,16,17,18	⑧ 34,35,36,43,44,45	⑫52,53,54,61,62,63

Table 5: Price (of 2004) for different nuts

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 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:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

Trunk circumference was used for comparing tree vigor (Westwood and Roberts, 1970). Before this trial started in winter 2007, trunk circumference for each trial tree was measured at 75 cm above ground. Then trunk circumference of those trees was measured each winter.

4.4.2 Nut Number per Cluster

To understand reason for yield increase from reflective mulch, on 7th March 2010, 3 trees in control and 3 trees with the mulch under tree in plot 1 were hand harvested on cluster basis. Then nut numbers per cluster were counted on cluster basis and calculated on tree basis. After shake 2, the remainder of nuts on 3 trees in control and 3 trees with the Extenday under tree in plot 2 were hand harvested for harvested yield estimation of trees in plot 1.

4.4.3 Material Tests for Reflective Light

Figure 2: Map of the light test

In row 35, there were existing mulch layouts for this trial. In row 32, there were 3 new layouts as mentioned in legend of Figure 2. 'White trunk' is the trunk with white painting while 'grape mulch' is while plastic sheet, which is usually used for grape, is under the canopy.

Light tests were in order as numbers labelled on the map. In this way, the measurements started from tree 1, then tree 2, tree 3, …, and finally for tree 18.

This test was done hourly on 17 December 2007 from 8 am to 5 pm. The exact time for the test is listed in Table 6. It should be mentioned that for measurement around 4 pm and 5 pm, the sky around the sun was not exact clear as there was very light cloud but the readings look acceptable.

Table 6: Start and end time for each clock test in December

O'clock	8	9	10	11	12	13	14	15	16	17
Start	8:10	9:03	10:01	11:00	12:01	13:02	14:00	15:01	16:01	17:03
End	8:30	9:23	10:21	11:19	12:20	13:21	14:18	15:20	16:19	17:20

To check the results tested on December, 12 and 13 February another set of measurements around 10 am, 12 pm and 3 pm were taken. Details are in Table 7.

Table 7: Start and end time for each clock test in February

O'clock	9	12	10
Start	9:57	11:57	14:54
End	10:15	12:14	15:13

4.4.4 Comparison of Reflective Capacity in Different Mulch Layout

Reflection measurements for the mulch trial were conducted in October and November 2007, and January and February 2008. Unfortunately no clear days were found in mid December 2007. Thus, no measurement was taken in December 2007. This measurement was taken in December 2008.

4.4.4.1 Measurements in October 2007, January and February 2008

A Decagon's AccuPAR model LP-80 PAR/LAI Ceptometer was used for this measurement. For photosynthetically active radiation (PAR) measurement, the Ceptometer was held horizontally at 1 m above the ground surface, one reading was taken in an open, unshaded area and 8 readings were taken under the canopy in different angle directions. Percentages of PAR were calculated by average readings under the canopy divided by readings in the open area. Reflective light measurements were also tested by the Ceptometer. In this test, the Ceptometer was facing downwards to the ground. An additional spirit level was added above the Ceptometer to control horizontal balance.

In Figure 1, 3 rows were used for the trial trees, i.e. rows 35, 38 and 41. It was considered unnecessary to test every row and every tree as they should have very similar responses between rows. Also, powder of Sun-Brite is sensitive to objectives such as feet. During reflective light testing, the feet of the machine holder had to be on the area. To reduce this kind of damage, only row 41 is used for this test.

In row 41, there are 40 trees for the test. Four readings from 4 directions of each of the trees tested were recorded. This process took about 35 minutes. To reduce difference of the strength of sunlight, reducing measurement time is necessary.

Under each treatment, there are 5 trees defined as tree 1, tree2 … tree 5 (from bottom to top in Figure 1). Both tree 1 and tree 5 are boundary trees. In production with long mulches, boundary trees do not really show treatment value in the test. Thus measurements in January and February 2008 only tested the middle trees, i.e. trees 2, 3 and 4. This also reduced variation within treatments for analysis of variance.

On 7 Feb 2008, this measurement was for morning and mid-day measurement. Cloud blocked the measurement in the afternoon. Afternoon measurements were taken on 13 February. Table 8 listed the exact starting time and ending time for each set of measurements.

Table 8: Exact time for measurements

Time	Oct			Nov			Jan			Feb		
O'clock	10am	12pm	3pm	10am	12pm	3pm	10am	12pm	3pm	10am	12pm	3pm
Start	10:00	11:58	2:55	10:00	12:00	2:58	10:00	11:57	2:56	9:56	11:54	2:54
End	10:34	12:33	3:31	10:32	12:30	3:28	10:21	12:18	3:15	10:19	12:13	3:13

4.4.4.2 Day-length measurements in November 2007

To understand continuous change between hours each treatment, this test measured reflective light hourly from 8 am to 5 pm on 15 November 2007. The timetable for the 10 tests is in Table 9.

Table 9: Measurement time

Around clock	8	9	10	11	12	13	14	15	16	17
Start	8:00	9:01	10:00	10:58	12:00	12:59	13:58	14:58	15:57	16:57
Complete	8:31	9:36	10:32	11:29	12:30	13:28	14:28	15:28	16:27	17:27

4.4.4.3 Measurements in December 2008

Measurements were taken around 10 am, 1 pm and 3 pm on 8 December 2008. Table 10 listed exact starting time and ending time for each set of measurements.

Table 10: Exact time for measurements

Time	8 Dec 2008		
Around time	10 am	1 pm	3 pm
Start	10:23	12:58	2:55
End	10:49	13:17	3:31

4.4.5 Soil Temperature Measurements

Temperature data loggers were installed on 17 September 2009 as shown in Figure 3 before the reflective mulch was fixed. 4 loggers were installed 20 cm outside and 4 loggers were installed 20 cm inside the drip lines for each treatment between trees 2 and 3 as graph above. All are in row 38 (middle trial row). To bury data loggers, 8 pits were dug. The bottom of the pits were 20 cm below the ground surface.

Data loggers were numbered (Table 11) and recorded for each location before burying. All data logger were removed out in the afternoon of 3rd March 2010. Unfortunately one data logger among the total of 8 data loggers did not work. This led to the loss of readings from inside of treatment of Extenday between trees.

Table 11: for data loggers numbered

Treatment	Under	Between	Plastic	Control
Logger code out	51	53	55	57
Logger code in	52	54	56	2P

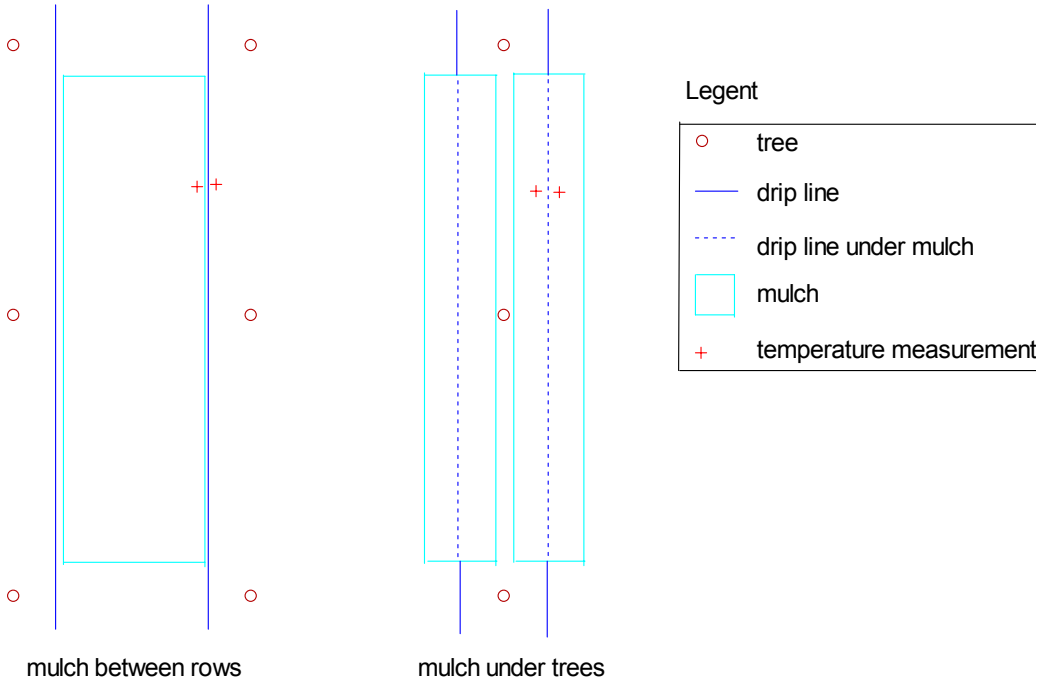


Figure 3: Showing graph for the mulch trial

4.5 Stylar End Lesion

4.5.1 Trial in 2006/07

5 foliar applications according to trial design were applied as shown in Figure 1 on 23 and 30 October, 7, 13 and 18 November.

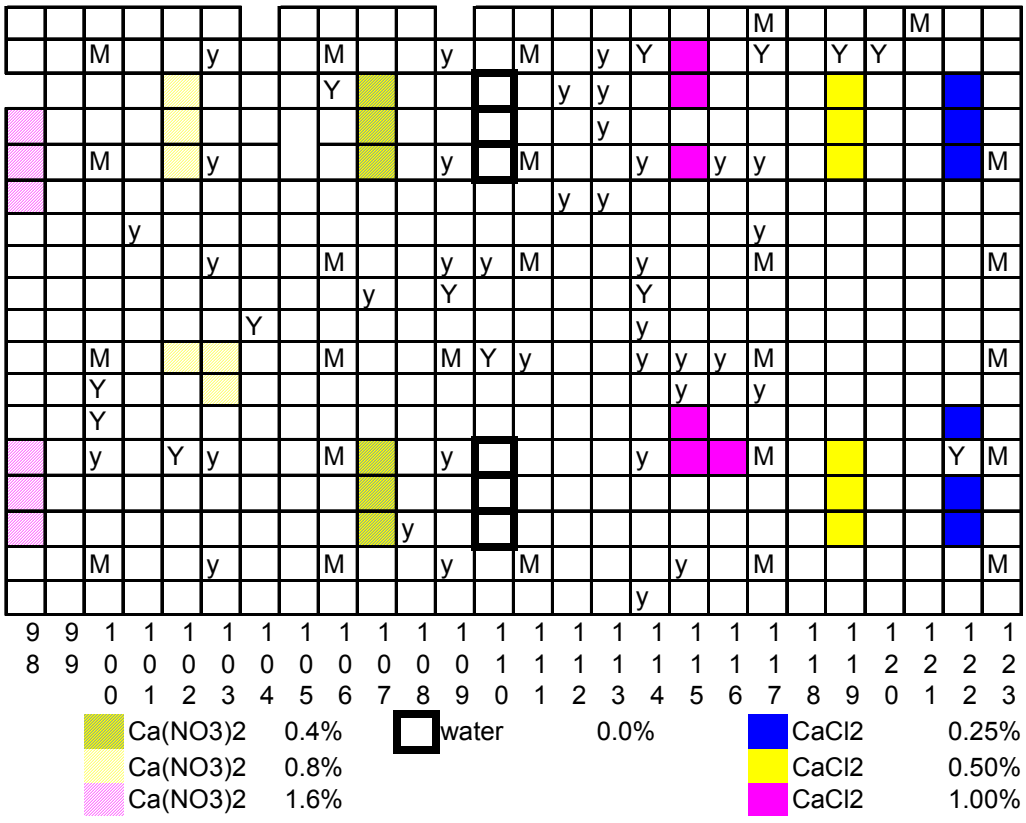


Figure 1: Trial design

NB: in 1st application, the concentrations of Calcium Nitrate were also done incorrectly, resulting in 0.4%, 1.14%, and 2.67% treatments, respectively.

4.5.2 Trial in 2007/08

4.5.2.1 Trial in Production Scale at Stage 3

Figure 2 shows the trial design in stage 3 *terebinthus*. Six rows in pink were applied with 0.8% Ca $(NO_3)_2$, 5 times on 26 October, 1, 8, 16 and 27 November with 750 L/ha. Six green rows were not applied with Ca and retained as the control. The rest rows were not applied Ca as protection rows. Between 2 different treatments, there are always 2 protection rows.

It should be recognized that although this area is called a stage 3 *terebinthus*, in the total 34 rows, there are a lot of rootstocks with Pioneer Gold. Those are marked in the map (Figure 2). In the map, young trees and male trees are also marked.

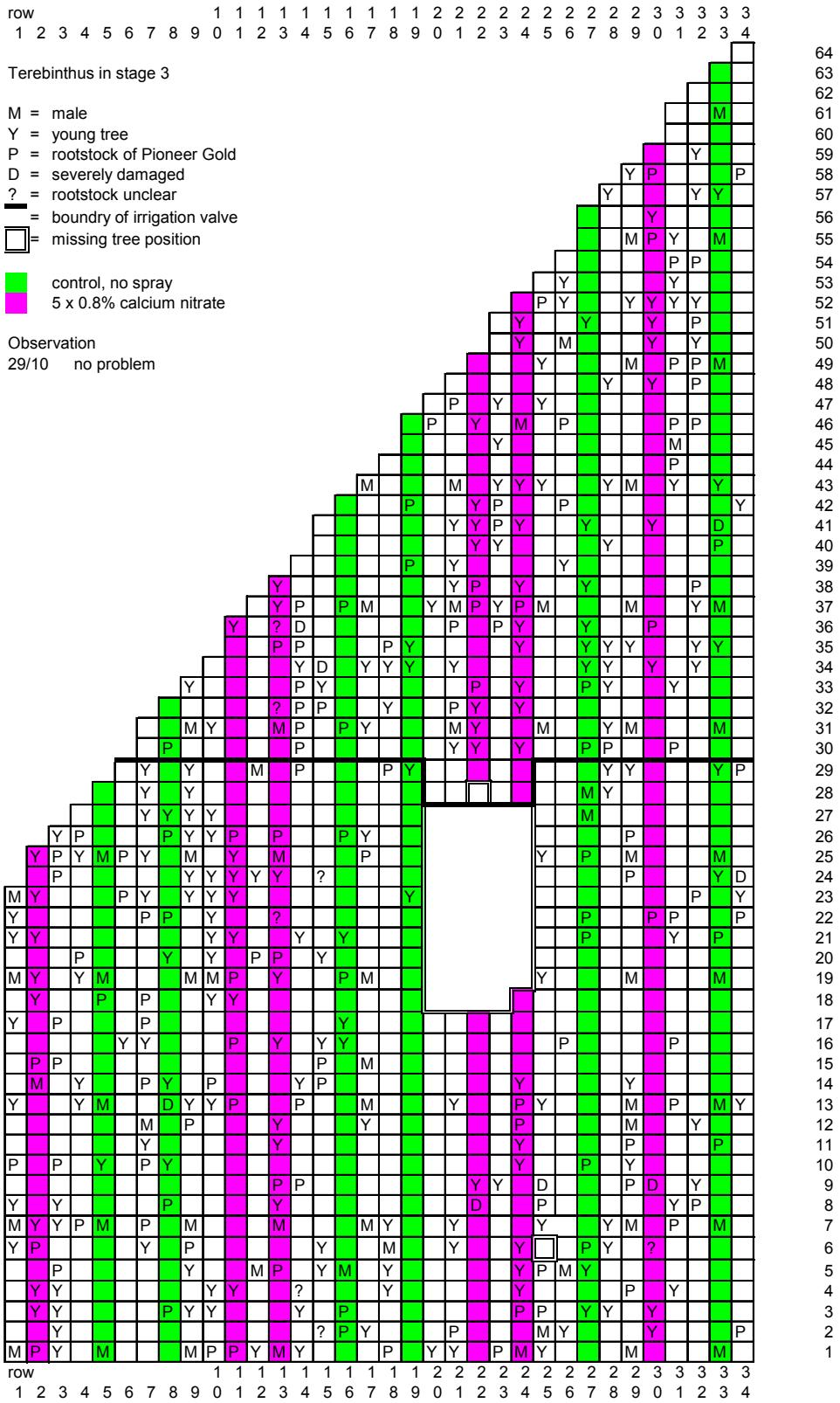


Figure 2: Trial map in stage 3

4.5.2.2 Trial in concentration and timing test at Stage 1

Figure 3 shows a map for trial in stage 1. This trial tested the effect of low concentration of Ca (red), of early application of Ca (pink), of late application of Ca (Brown), as well as control (yellow) and K (blue). The 5 application dates were 26 Oct, 1, 8, 16 and 24 Nov with 2400 L/ha. In late November, investigation was conducted on damages on leaves and nuts. Investigation by eve defined damages in 4 levels from 0 (none) to 3 (severe damage) for leaves and nuts.

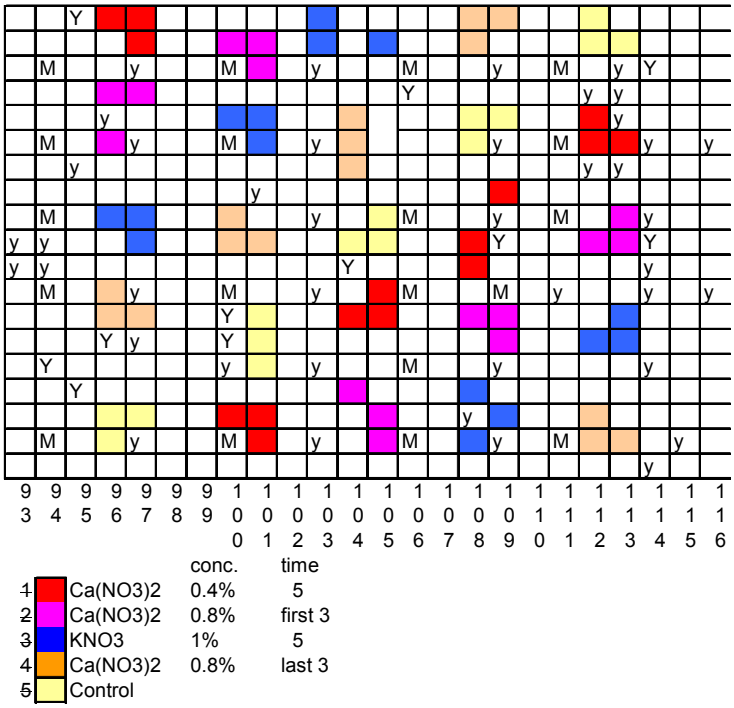


Figure 3: Map of trial at stage 1

4.5.3 Trial in 2008/09

This observation was at stage 3 terebinthus. There are a total of 34 rows and the first 30 rows were applied Ca+B. The solution was 0.8% $CaNO_3$ + 0.533% SOLUBOR (24 kg $CaNO_3$ + 16 kg SOLUBOR in 3000 L) with 750 L/ha application.

4.6 Other Symptom Related to SEL Nut

4.6.1 Trial in 2006/07

5 foliar applications according to the trial design were applied as shown in Figure 1 on 23 and 30 October, 7, 13 and 18 November. Originally 6 samples were to be taken, those are good nuts and bad nuts from control, Ca $(NO_3)_2$ and $CaCl_2$ treatments (to save funds, sampled were not taken from different concentrations). Unfortunately there were not enough samples of bad nuts from the control. Thus a total of 5 samples were analyzed.

4.6.2 Trial in 2007/08

To test black-side nuts, at the same time black-tip nuts were sampled. We also sampled black-side nuts on Ca-treated trees and on non-Ca-treated trees. Plus control, there were 5 kinds of treatments with 3 replicates each treatment.

4.6.3 Results

The results are complicated and some results may contain false conclusions. The nut sampling was around 9 am and the nuts were washed, dried, kernels removed and placed in sealed bag and posted to the laboratory office and placed in the laboratory refrigerator. This could take about 24 - 48 hours. Alive tissue still respires. For normal nut samples, we can assume that all the nuts have similar respiration. However, for the nuts with symptoms, we cannot take this assumption. Photo 1 shows, some nuts with decayed tissue and some nuts with abnormal shapes. Even inorganic elements have a similar pattern to enter those nuts with symptoms; their organic nutrition costs are higher than normal nuts. This even happens before nut sampling when the nuts show decay or abnormal shape. This leads to low dry matter in those nuts with symptoms. In test, if they have a similar inorganic elements, low dry matter may bring the results high values of the elements per unit dry matter. To overcome this problem, ratios between elements were used in the results.
In the results section, the direct results are listed first, and then the ratios are listed in the conclusions.

4.7 Hand Pruning Trial to Overcome Alternate Bearing

4.7.1 Trial design

In this trial, thirty 18 years-old 'on' year 'Sirora' pistachio female trees were used at CMV Farms, Robinvale commencing in March 2004 with pruning commencing in winter 2004. The planting density was 4.39 m x 6.86 m. This row had a total of 45 trees. To make convenience for harvesting 30 trees were within a single row. These 30 trees were divided into 10 replicates. Table 1 shows trees from 1 to 45. In Table 1, the colours represent treatments while numbers represent replicates. Besides the 30 trial trees, the other 15 trees are also listed according to the legend below. In this row, there were 22 sound trees, i.e. trees that did not lose structural branches, with ≤ 3 clusters in harvest 2004. These trees are used in the first 7 replicates arranged from left to right in the Table. The remainder of the trees had light cropping at harvest in 2004 and were used in replicates 8 – 10 arranged from right to left in Table 1.

Pruning treatments are listed in Table 2.

Table 1: Treatments in the trial row

Treat		1	1	1	2	2	2	3	3		3		L				4	4	4	10		Y	10
Tree No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Treat	5	L	5	10	9	3		6		L	9	6	9	6		Y	7	7	8	8	7	8
Tree No	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45

	<= 3 nut clusters	Y	young trees
	light crop in harvest 2004	L	Wind Damaged trees - lost 1 or 2 structural branches
	relatively normal crop		

Table 2: Treatments of the pruning trial

	Treatments	Methods
	Control	Orchard staff prune these 10 trees before other treatments
	Middle	Retain ½ of the flower shoots of the total flower shoots
	Aggressive	Retain ¼ of the flower shoots of the total flower shoots

4.7.2 Pruning process

In July 2005, experienced orchard staff pruned the 10 Control trees using the usual orchard criteria. The other 2 treatments were pruned by Dr Zhang. The numbers of cuts and flower shoots were recorded per tree and then further pruning was added until reaching the exact required level. Table 3 lists the total flower shoots per tree before and after pruning, as well as the percentages of flower shoot pruning during the first winter pruning. To describe the variation between trees, standard deviations are attached with a sign "±". In the first winter pruning, Dr Zhang pruned treatments of "strong" and "middle" removing 50% or 75% flower shoots. This process provided for the removal of horizontal shoots and improved the tree shape towards the vertical.

Table 3: Total flower shoots per tree before pruning

Treatment	Flower shoots before pruning	Flower shoots after pruning	Percentage of pruning
Control	587.5±34.7	452.1±95.0	23.3
Middle	623.5±34.6	311.8±54.8	50.0
Strong	717.0±36.5	179.3±28.9	75.0

In winter 2006 - 2008, no further aggressive pruning conducted. Zhang continued his common pruning on those 20 trees but aimed to change the relatively horizontal pattern to vertical pattern with an open centre. CMV Farms staffs prune their 10 trees again.

Trunk circumferences provide an indication of tree size. On average, trees in the treatment Middle (51.7±3.2 cm) seem smaller than the other 2 treatments (54.6±4.1 for treatment Aggressive and 54.4±4.1 for Control) but this did not reach a significant level.

4.7.3 Nut cluster investigation before harvest

20 nut clusters were randomly selected from each trial tree just before harvest in 2006. Then numbers of nuts were recorded on a per cluster basis. Average nut numbers per cluster were calculated on a tree basis. All of the nuts were harvested from each tree. Using the nut analysis by the APPC laboratory; and the percentages of the number sampled from the total crop, the total yield and quality was calculated.

4.7.4 Harvest

Trial trees were individually harvested in 2 shakes as listed in Table 4.

Table 4: Harvest dates

Harvest	2005	2006	2007	2008
1st harvest	24th March	17th March	1st March	3rd March
2nd harvest	9th April	3rd April	16th March	25th March

Trial trees were harvested a little later than commercial production trees in 2005 and 2007 but at the same time in 2006. In 2008, the first trial harvest was earlier but the second trial harvest was later than commercial production trees.

During mechanical harvesting, the machine was stopped when every-tree harvest was completed. All nuts on the machine were collected into a bin. Then baskets were used for collecting all nuts. All nuts

were weighed and yields in-hull per tree were calculated for each trial tree. Subsequently about 10 kg samples per tree in the first shake and about 5 kg samples per tree in the second shake were collected at harvest in 2005 and 2006, and 2 kg samples in each shake were collected at harvest in 2007 and 2008. Harvests in 2007 and 2008 pooled 3 or 4 trees into 1 sample for further work (Table 5). The samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Return per tree is based on prices in Table 4.

Table 5: Pooled samples from the original treesCode number vs trees

Pruning trial	Rep 1-3	Rep 4-6	Rep 7-10
Aggressive	① Trees 22, 25, 28	② Trees 7, 12, 20	③ Trees 2, 4, 8, 14
Middle	④ Trees 23, 27, 29	⑤ Trees 9, 15, 19	⑥ Trees 1, 5, 13, 18
Control	⑦ Trees 24, 26, 30	⑧ Trees 11, 16, 21	⑨ Trees 3, 6, 10, 17

Table 6: Price for different nuts

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 analyzed by 2-way (treatment x replicate) analysis of variance (ANOVA). Analysis of variance for percentages, p-values were calculated based on transformed data according the following formula: $\arcsin\sqrt{\frac{percentage}{100}}$.

4.7.5 Winter investigation

In the winter of 2005, the total flower shoots and the flower shoots remaining after pruning were recorded. In the winter of 2006, a primary branch was selected from each of the 30 trees. On average, these branches accounted for about a quarter of the flower buds for a whole tree. Flower buds per shoot were recorded for every shoot on the selected branches. This provided an opportunity to estimate the flower buds per shoot for the whole tree. All flower shoots on the remaining branches as well as the flower shoots removed by pruning were recorded at the same time.

4.8 Hand Thinning Trial to Overcome Alternate Bearing

4.8.1 Trial design

In this trial, thirty 18 years-old ‘on’ year ‘Sirora’ pistachio female trees were used at CMV Farms, Robinvale. The planting density was 4.39 m x 6.86 m. Trial design was a 3 x 10 randomized plot design. Rows in this block had 45 trees. To make it convenient for harvest, 30 trees were selected within one row. Table 1 shows treatments for each trial trees.

Table 1: Treatments in the trial row

Treat	Y	1	1	1	2	10	10		10	2	L	2		9		9	3	9		3	3	8	L
Tree No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23

Treat	8	4	4	4	5		8	5	5	L	7	6	6	7	7	Y	6						
Tree No	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	

	<= 3 nut clusters	Y	young trees
	light crop in harvest 2004	L	Wind Damaged trees - lost 1 or 2 structural branches
	relatively normal crop		

Table 2 shows trees from 1 to 45. In the table, colours represent treatments while numbers represent replicates. Besides the 30 trial trees, the other 15 trees are also listed according to legend below. Observations were made of the crop load of each tree in March 2004. In this row, there were 18 "sound" trees (had not lost structural branches to wind damage) with ≤ 3 clusters in season 2003/2004. These trees are used in the first 6 replicates arranged from left to right in the Table. The remainder of the trees with light cropping in season 2003/2004 were used in replicates 7 – 10 arranged from right to left in Table 1. These 30 trees were divided into 10 replicates and were pruned using the usual CMV Farms methods in winter 2004 - 2007.

For thinning treatments, early thinning (aggressive), late thinning and no thinning (Control) were conducted in the spring of 2004 and 2006 ('on' years only, Table 2).

Table 2: Treatments of the thinning trial

		Hand thinning dates	
	Treatments	Spring 2004	Spring 2006
	Control	No hand thinning	No hand thinning
	Late	14th October 2004	23rd September 2006
	Early	8th October 2004	27th September 2006

4.8.2 Hand thinning process

The early hand thinning was held on 8th October 2004 and 23rd September 2006. At that time, the top long shoots had about 80% flowers open. All flower clusters before or at tight cluster stage were removed. The details for the flower opening process are described by Zhang (2005b). On average, 900 flower clusters were removed on 8th October 2004. Unfortunately there was no record for removed flower clusters on 23rd September 2006. In early thinning, quite a few short shoots remained with no flower clusters at all. This may benefit flower bud retention in an 'on' year.

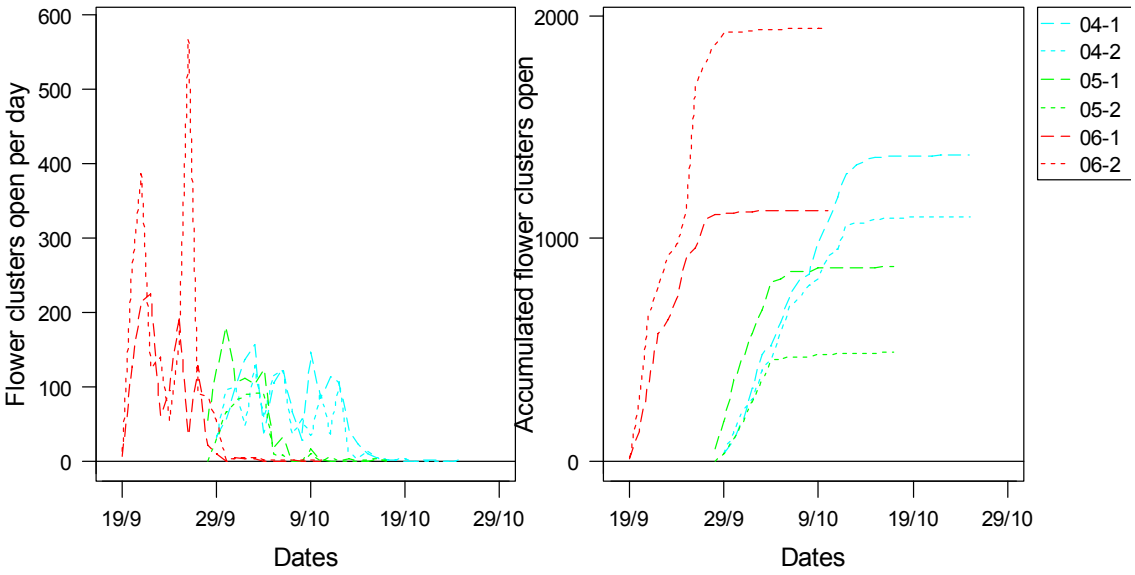


Figure 1: Flower number opening and accumulated numbers in spring 2004 - 2006

The late hand thinning was conducted on the 14th October 2004 and 27th September 2006. At that time, the top long shoots had about 90-95% flowers open. On average, 510 flower clusters or buds were removed in 2004 but with no record in 2006. Figure 1 records flower open process from 2 marked trees (see section flower open monitoring). It can be seen that after early thinning in both years there were big peaks of flower opening, but after late thinning the peak almost disappeared although flower opening lasted. In late thinning, about a half of the removal of flower buds were unopened buds (these may or may not ultimately open). Late thinning did not retain many short shoots without flower clusters as did early thinning. It should be emphasized that early treatment made a big difference from the control while the difference between late thinning and the control were not large.

4.8.3 Nut cluster investigation before harvest

20 nut clusters were randomly selected from each trial tree just before harvest in 2006. The numbers of nuts were recorded per cluster. Average nut numbers per cluster were calculated per tree. All of the nuts were harvested from each tree. Using the nut analysis by the APPC laboratory, and the percentages of the number sampled from the total crop, the total yield and quality calculated.

4.8.4 Harvest

Trial trees were individually harvested in 2 shakes. Each harvest date was listed in Table 3.
The trial trees were harvested a little later than the commercial production trees in 2005 and 2007 but at the same time in 2006. In 2008, the first trial harvest was earlier but the second trial harvest was later than commercial production trees.

Table 3: Harvest dates

Harvest	2005	2006	2007	2008
1st harvest	24th March	16th March	1st March	3rd March
2nd harvest	9th April	31st March	16th March	25th March

At harvest, the machine was stopped when every-tree harvest was completed. All nuts on the machine were collected into a bin and then baskets were used to collect all nuts. All nuts were weighed and yields in-hull per tree were measured for each trial tree. Subsequently about a 10 kg random sample from the first shake and about a 5 kg sample from the second shake were collected in harvest 2004 and 2005. In harvest 2006 and 2007, to save laboratory labour input, about a 2 kg sample was collected per tree. Then 3 or 4 samples in the same treatments were pooled together for further test according to Table 4. The samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Returns per tree were based on prices in Table 5.

Table 4: Pooled samples from the original trees

Hand thinning trial	Rep 1-3	Rep 4-6	Rep 7-10
Early	①trees 19, 27, 30	② trees 1, 10, 12	③ trees 3, 9, 18, 24
Late	④ trees 16, 22, 29	⑤ trees 4, 8, 11	⑥ trees 2, 15, 21, 26
none	⑦ trees 17, 23, 28	⑧ trees 5,7, 13	⑨ trees 6, 14, 20, 25

Table 5: Price for different nuts

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 2-way (treatment x replicate) analysis of variance. Analysis of variance for percentages, p-values were calculated based on transformed data according the following formula:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

4.9 Mechanical Pruning Trial at CMV Farms

This trial was located at rows 1 – 16 at stage 2B at CMV Farms. Trees at stage 2 were on rootstock of Pioneer Gold. This trial should, at least, be conducted across 4 growing seasons. Normally pruning in production should be taken as a control however pruning in production is changing quickly. The same pattern pruning it is difficult to keep consistency across 4 winters. Thus, no control was established in this trial.

This pruning operation was completed in early July 2009. At stage 2, there were 2 treatments as shown in Figure 1, i.e. slant topping, single side prior to an 'off' year (pruned in winter 2009) and slant topping, single side prior to an 'on' year (pruned in winter 2010, no pruning in winter 2009). In these 16 rows, 2 neighbouring rows made up a trial plot. These 2 treatments were arranged in an 8 x 2 randomized complete-block design. In stage 2 of CMV Farms, distances between rows were 6.86 m. After the slant hedging, the height to the top was 3.9 m and to the shoulder was 2.9 m. From the calculation, the angle from the top to the shoulder was 22.6°.

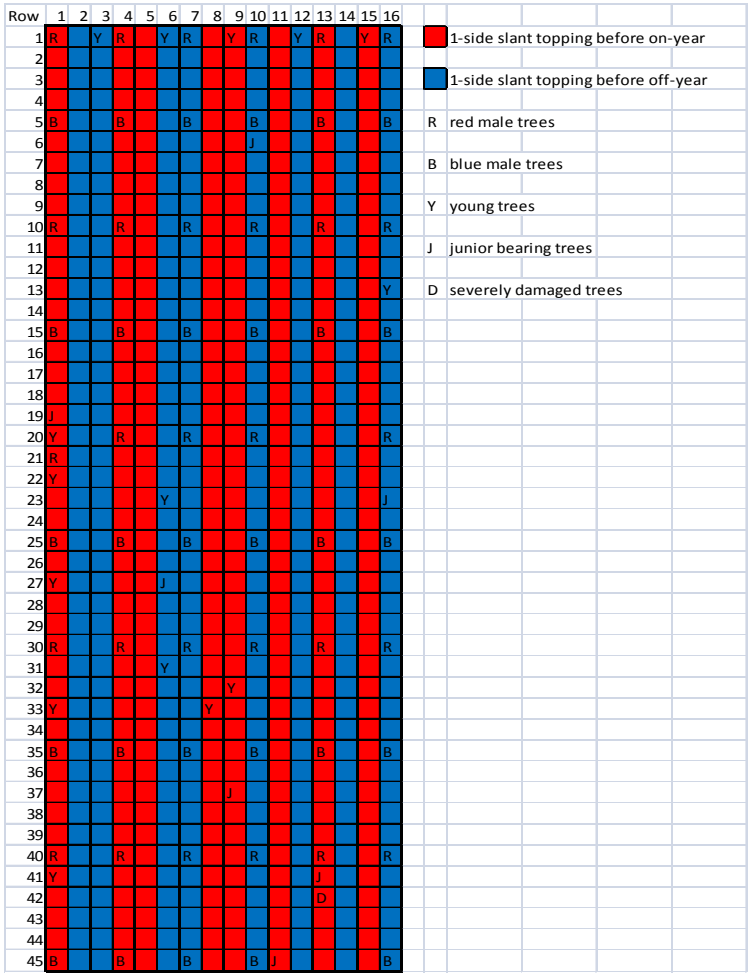


Figure 1: Trial design map

Two mechanical harvests of the trial trees on a single row basis were conducted on 11 and 23 March 2010. During harvest, about 10 kg sample in each shake were collected for each row. The samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Returns per tree were based on prices in Table 1.

Table 1: 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 were analysed by two-way (treatment x replicate) analysis of variance. Analysis of variance for percentages, p-values were calculated based on transformed data according the following formula:

$$\arcsin\sqrt{\frac{percentage}{100}}$$

4.10 Mechanical Pruning Trial at Kyalite Pistachios

This trial was located at rows 86 – 97 at stage 2 at Kyalite Pistachios. Trees at stage 2 were on rootstock of *terebinthus*. This trial should, at least, be conducted across 4 growing seasons. Normally pruning in production should be taken as a control. However, pruning in production is changing quickly. A same pattern pruning is difficult to keep for 4 winters with no change. Thus, no control was established in this trial.

In the winter of 2008, this pruning operation was completed by end of May. At stage 2, there were 2 treatments, i.e. topping both sides (rows 92-97) called 'on' year 2 sides and topping single sides (rows 86-91) called 'on' year 1 side in the winter of 2008. In Kyalite Pistachios the distances between rows were 5m. After slant hedging, the height to the top was 4.8 m and to the shoulder was 3.7 m. From the calculation, the angle from top to shoulder was 32°.

In the winter of 2009, another 6 rows, rows 99-104, were topped on both sides in the same manner called 'off' year 2 sides. For existing rows, rows 92 – 97 which were topped both sides last winter, were topped again about 30 – 40 cm above the top in the previous year. For rows 86 – 91, which were topped 1 side last winter, in winter 2009, the topped sides were topped again about 30 – 40 cm above the top in previous year; for the other side, it was topped at the same as the new topping as showing in Figure 1.

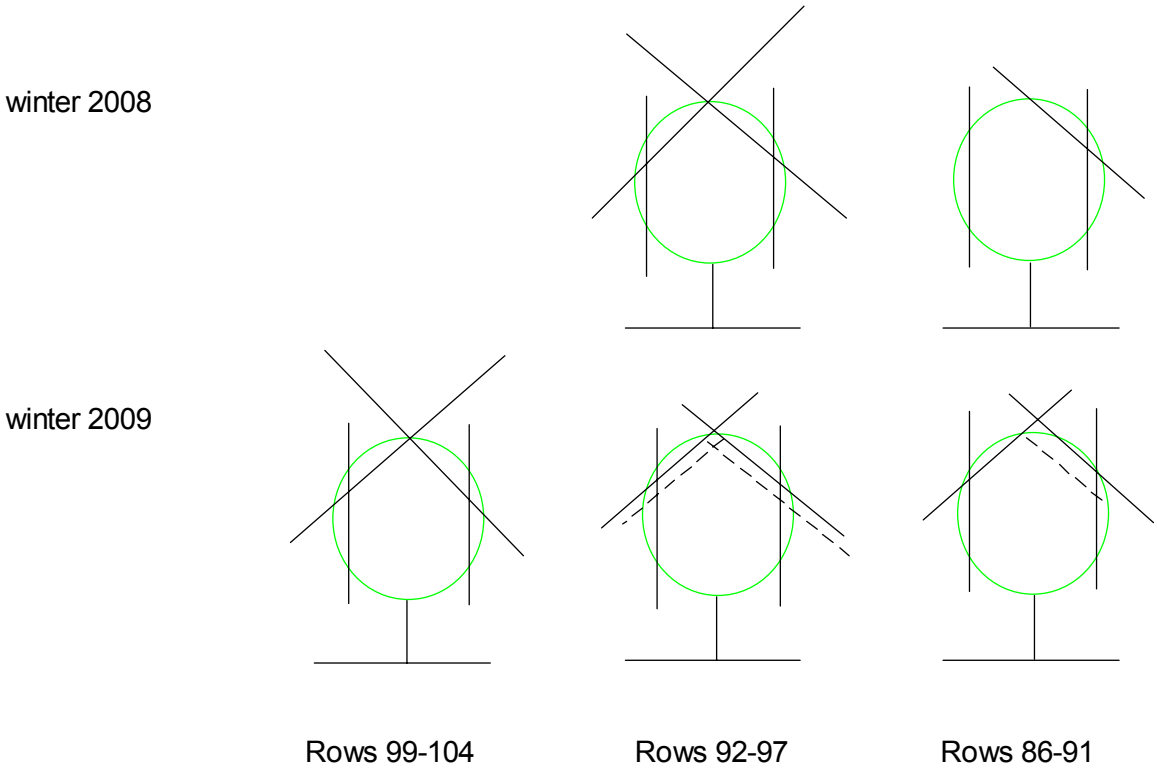


Figure 1: Diagram of pruning treatments in different years

All trial trees were as the same as other trees in stage 2 being those hedged on both sides.

Two machines harvested the trial trees on a single row basis on 1 and 14 March. During harvest, about 10 kg sample from each shake were collected for each row. The samples were weighed accurately,

then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Returns per tree were based on prices in Table 1.

Table 1: 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 analyzed by 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:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

4.11 Research on Chilling Requirement

4.11.1 Greenhouse Test

4.11.1.1 2006

To test chilling completion, five pistachio trees on rootstock of *P. terebinthus* were selected at DPI-NSW, Dareton. Shoots were collected from each tree 10 times as listed in Table 1. On each date, 2 shoots, 1 from the east side and the other from the west side of the tree, were taken from each of the selected trees. In total, 593 flower buds were collected.

Table 1: Shoots collecting dates

Collection Time	1	2	3	4	5	6	7	8	9	10
Date	6/07	13/07	20/07	27/07	3/08	10/08	17/08	24/08	31/08	07/09

It should be noted that the shoot sampling aimed to take shoots with at least 3 flower buds as part of the trial design. Actually the shoot sampling normally took shoots with 5-6 flower buds.

Immediately after being cut, shoot ends were placed in water. In the laboratory, 2 shoots from the same trees were held in a vase with water. All the vases were placed in a greenhouse with 23±1.5°C with light day and night. Bud development stages as listed in Table 2 plus the date buds dropped for each flower bud were recorded daily.

Table 2. Flower open stages

				
Loose scale	Cluster appearing	Cluster extension	Tight cluster	Loose cluster

4.11.1.2 2007 & 2008

The same procedure as section 2.1.1 was continued in winters 2007 and 2008. In the winter of 2008, 2 male clone tests were started.

4.11.1.3 2009 & 2010

The same procedure as section 2.1.1 was continued in winters 2009 and 2010, however, this work was not in the greenhouse in DPI, Victoria; it was in a warm box in CMV Farms. In winter 2010, 'Kerman' tests were started.

4.11.2 Hourly Temperatures from Meteorological Stations

Hourly temperature readings were obtained from 6 meteorological stations; they are Mildura, Renmark, Swan Hill, Nhill, Lameroo and Wagga Wagga. Hourly temperature readings from Dareton Agricultural Station were obtained from that station.

4.11.3 Models

There are 5 chill models that may influence the work. They include

- Numbers of hours ≤7.5°C (Hobman and Bass, 1986)
- Numbers of hours ≤7.2°C (Weinberger, 1950)
- Chill Hours Model: numbers of hours between 0 - 7.2°C (Bennett, 1949)
- Utah model (Richardson et al, 1974)
- Dynamic model (Fishman et al, 1987a).

A model for growing degree hour (GDH) from Shaltout and Unrath (1983) was used. It is

$$GDH = \Sigma(Tm - 4.4°C)$$

Where Tm is temperature at a given hour in the day; 4.4°C is the base temperature, or, if any hour has a temperature ≤ 4.4°C it was recorded as 0.

4.11.4 Orchard Visit

Since spring 2007, between late October and early November, the Research Field Officer visited all growers in our group work to decide chill progress each growing season.

4.12 Winter Oil Application Study

4.12.1 Trial Design

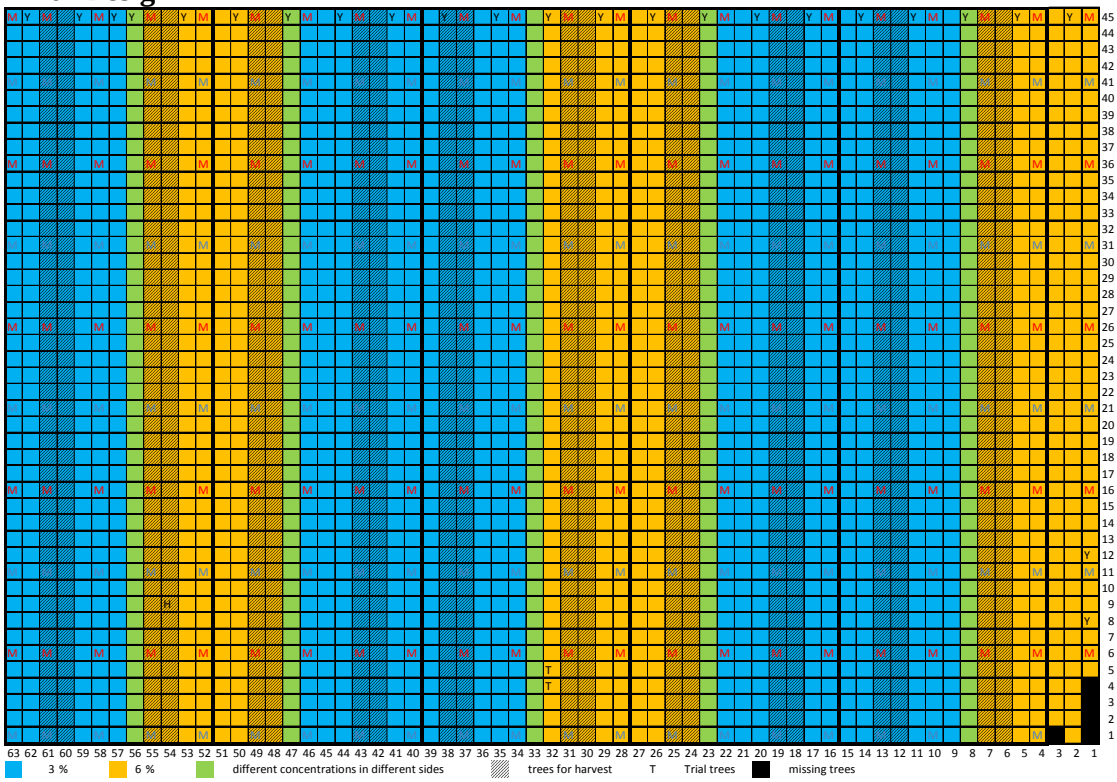


Figure 1: Map for trial design

Stage 3 has 63 rows with 40 trees per row. The trial applied 2 treatments, 3% and 6% winter oil, with 5 replicates. Each replicate consisted of 2 neighbour rows. Thus 20 rows were used for the trial and the rest rows were used as protection rows (Figure 1).

4.12.2 Bloom Observation

In bloom period, flower dates were compared between treatments.

4.12.3 Trial Harvest

In the season of 2008/09, Oil was applied between 8 am and 11 am on 19 August 2008 in order from row 1 to row 63. In the season of 2009/10, Oil was applied in the night of 21 August 2009. In the season of 2010/11, oil was applied in the night of 24 August 2010. In all 3 seasons, 2 tractors applied at the same time, one for 3% oil and the other for 6% oil.

The trial harvest on single row basis was on 16 and 28 March 2009 and on 10 and 23 March 2010. During harvest, about 10 kg samples in each shake were collected for each row. The samples were weighed accurately, then dehulled and dried in the second day. After drying, the nuts were delivered to APPC laboratory for analysis as normal process. Return per tree is based on prices in 2004 in Table 1.

Table 1: Price (of 2004) for different nuts

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 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:

$$\arcsin\sqrt{\frac{percentage}{100}}.$$

4.13 Winter oil dipping trial

An observation of shoot oil dipping was held at pistachio fields of DPI, Mildura. Oil dipping was conducted on 3 dates, i.e. 13, 20 and 27 August. Each date 2 trees were selected, one for 3% winter oil dipping and the other for 6% winter oil dipping. On each tree, 10 shoots were selected for this tipping.

4.14 A study on climate factors on 'Sirora' pistachio nut size

Ten orchards around Swan Hill, Mildura and Renmark along the Murray River were selected, which accounted for 76% of production in 2009 in Australia. All of these orchards apply around 200 – 300 kg N/ha and 600 – 800 mm water yearly. Tree numbers, planting areas, yields and nut count size of those orchards from 2000 to 2009 were recorded. Yields per tree and per hectare were calculated for each orchard each year. Average nut weights in grams for each orchard each year were calculated from nut count sizes. Fertilizer applications and leaf analyses of some of those orchards were recorded in corresponding years. In this area, 2000, 2002, 2004, 2006 and 2008 were recorded as 'off' years while 2001, 2003, 2005, 2007 and 2009 were 'on' years.

Daily maximum and minimum temperature data from meteorological stations of Swan Hill, Mildura and Renmark were collected from 1998 to 2009. Thus in this study, the period starts in October in the previous growing season before flower bud initiation, through flower bud opening in the second October, until nut harvest in March. The total is 18 months. In those 18 months, 12 months from April (after previous harvest) to March (current harvest) are termed *current* season, and 6 months (from previous October to previous March) are termed *previous* season. In tables below, months in previous season are marked as Oct-, Nov- etc while those for current season are marked as Oct, Nov etc. This study also included any combinations between months, e.g. from October to December. There was a total of 171 month-combinations used. In combination calculations, all the combinations used represent averages for the period. Correlation and regression analyses were used with the Minitab packages.

Hourly temperature data from meteorological stations of Swan Hill, Mildura and Renmark were collected from 1999 to 2008. Chilling hour model was tested for each station, each winter from winters 1999 to 2008 to match harvest data in 2000 to 2009. Chill hours were calculated based on hour numbers ≤ 7.2ºC from 1 May to 31 August.

5 RESULTS

5.1 Nitrogen Trial

5.1.1 Leaf Analysis

In season 2009-2010, Leaf analysis from 26 October 2009 to 15 March 2010 showed that treatments of 350 kg N/ha had high N while the treatment of 75 kg N/ha had low N from the beginning to the end (Figure 2). The other 2 treatments were in the middle.

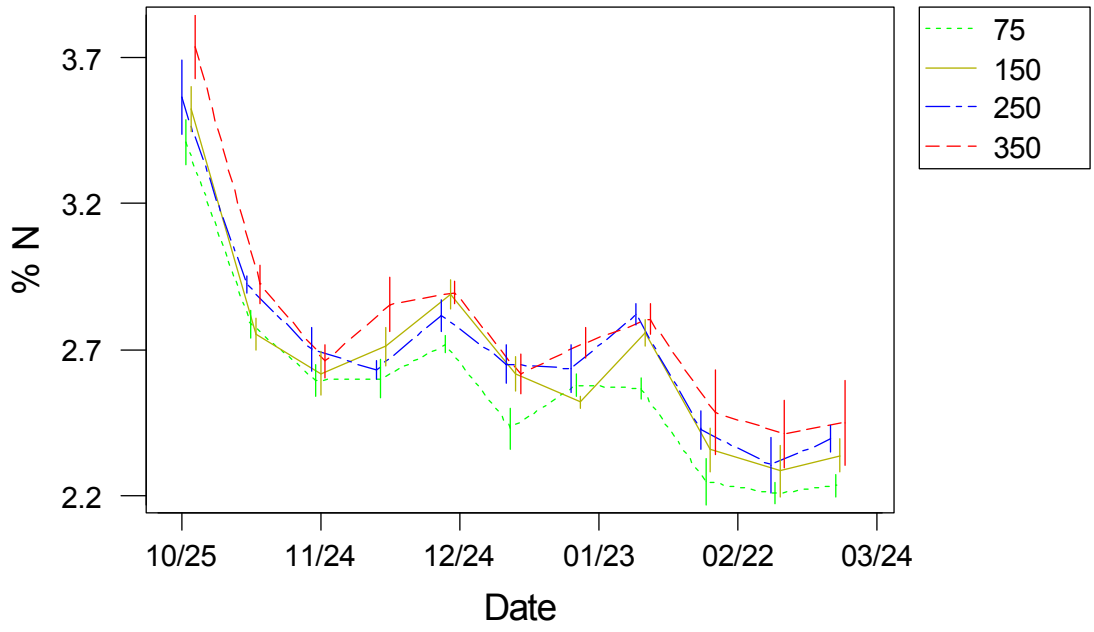


Figure 2: Nitrogen contents in leaves in different dates

Table 2: Major elements analysis season

season	Treat	N	NO_3	P	K	Ca	Mg	S
2008-2009 On-Year	75	2.08	41b	0.129	1.079	2.85	0.50	0.13
	150	2.16	57ab	0.136	1.127	2.83	0.50	0.14
	250	2.30	72ab	0.134	1.171	2.87	0.47	0.14
	350	2.26	123a	0.137	1.093	3.02	0.51	0.14
	P	0.096	0.025	0.217	0.681	0.240	0.619	0.185
2009-2010 Off-Year	75	2.57b	214b	0.130	2.36a	1.77	0.30	0.19
	150	2.76a	299a	0.137	2.42a	1.80	0.30	0.20
	250	2.82a	338a	0.137	2.22a	1.86	0.31	0.19
	350	2.80a	304a	0.153	1.94b	2.11	0.34	0.21
	P	0.023	0.047	0.350	0.009	0.255	0.507	0.461

In season 2008-2009, Leaf analysis samples were taken on 16 February 2009, the last day of fertigation. In season 2009-2010, full nutrition analysis was taken on 1 February 2010.

In season 2008-2009, from Table 2, N is close to a statistical difference with a p-value of 0.096. Averages of N are between 2.08 and 2.30. However, the variations within treatments are quite small. This can be found from the original data. If the last reading of 2.14 for 350 kg N/ha was a little larger, so a significant difference may have been reached. In season 2009-2010, Figure 2 showed that the treatment of 350 kg N/ha had high N while the treatment of 75 kg N/ha had low N from the beginning to the end. The other 2 treatments were in the middle. N contents at 75 kg N/ha was significantly lower than all other treatments.

In season 2008-2009, from Table 2, NO_3 reached a statistical difference with a p-value is 0.025. In season 2009-2010, NO_3 showed the same results. However, it is interesting to see that the lowest values of both N and NO_3 in the 'off' season of 2009-2010 were higher than the highest values of those in 'on' season 2008-2009.

In season 2008-2009, for other elements, most of results look reasonable and close to the historic records in CMV leaf analysis. K is an exception. Readings for K were extremely low. This was an 'on' season, low K is reasonable. But for this group of readings, the maximum was 1.28% and the minimum was 0.99%. These are far below the California "internal" standard of 1.4%. In 85 readings of CMV Farms historical record from 1989, there was only 1 reading in 1997 at stage 3 below 1.4%. In season 2009-2010, K contents are much higher than the previous year, most are above 2%. However, a significant difference still shows between treatments at a very high level. Treatment at 350 kg/ha showed significant lower potassium levels than the rest. This is probably due to high crop.

Table 3: Minor elements analysis season 2008-2009

	Treat	Cl	Na	Zn	Mn	Cu	Fe	B
2008-	75	0.23	0.023	52ab	145	34b	179	191
2009	150	0.35	0.024	63a	151	47a	182	207
On-	250	0.35	0.023	50b	151	39ab	185	192
Season	350	0.33	0.026	52ab	155	44ab	180	206
	P	0.417	0.250	0.034	0.724	0.017	0.990	0.643
2009-	75	0.27	0.047	264	148	130	158	152
2010	150	0.26	0.060	259	146	125	162	152
Off-	250	0.25	0.050	248	163	111	172	135
Season	350	0.28	0.063	238	170	108	161	149
	P	0.438	0.842	0.327	0.260	0.101	0.918	0.530

In season 2008-2009, for minor elements, Cu and Zn reached significant levels for some tests (Table 3). This may be due to the treatments or the sampling. Anyway, all data was in reasonable ranges. In season 2009-2010, all the minor elements were in the reasonable ranges without a significant difference.

5.1.2 Soil Analysis

Figure 3 – 7 summarised soil analysis results. According to Kenneth (2006), boundary lines for very low, low, marginal, optimal and high are used in left sides of our analysis graphs with words. This allows readers to easily understand the element level compared with the standards. In the legend the 4 different colours represent 4 different treatments: red for 350 kg N/ha, blue for 250, cyan for 150 and green for 75. In the groups of graphs, each column shows a single element. In that column, there are 4 sub-graphs showing soil depths between 0 – 15 cm, 15 – 30 cm, 30 – 45 cm and 45 – 60 cm. Although

the lines show good patterns for element content changes the markers show the actual test reading. Especially in soil depth 15 – 30 cm, there was no tested reading in 2009. Although the lines pass through 2009, there is no actual reading.

5.1.2.1 N and K.

Comparing with the original in 2008 in soil depth 0 – 30 cm, for N-NO_3, both 350 and 250 kg N/ha showed higher readings than 2008. Treatment 150 kg N/ha also showed a high value at depth 15 – 30 cm (Figure 3). In general, readings are in the low and marginal levels. For deep soil, 350 kg N/ha showed values in the high level indicating leaching. This trend needs to be investigated in the future.

There was no record for N-NH_4 in 2008. Except in the top soil in 2009, treatment 75 kg N/ha was always in the lowest level of N-NH_4 but there is no clear pattern for other treatments.

For K (Colwell), comparing with the reading in 2008, most readings showed low values. However, almost all the readings are above a marginal level and most readings are still in the optimal level. There is no big difference between soil depths. Different levels of nitrogen treatments seem to have influence on the K content.

For exchangeable K, there is no standard. Generally speaking, there is no big change between years. However, treatment effects are clear. Treatments with less N application showed higher readings for exchangeable K. There are 2 explanations: one is NH_4^+ in position to K^+ in the soil. The other is that high N treatments made heavy crops and those trees took more K^+ from soil.

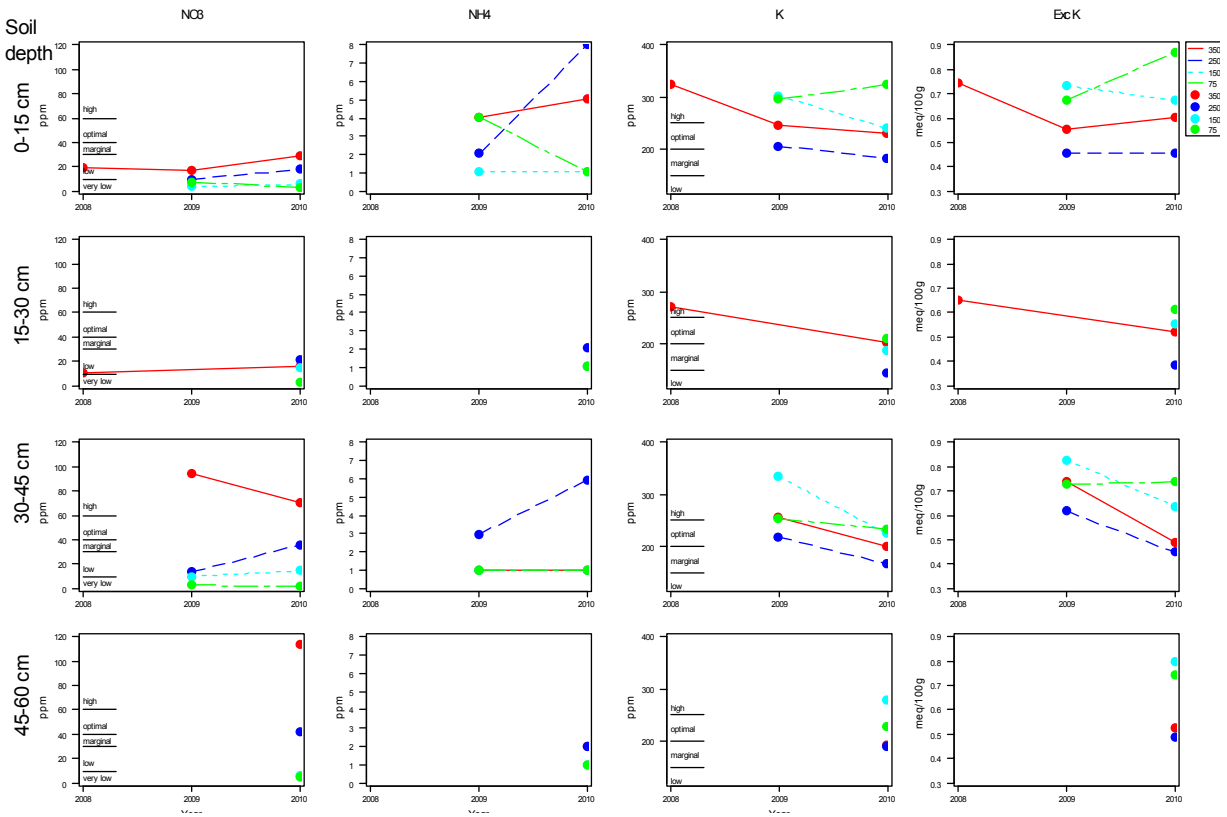


Figure 3: Contents of N and K in soil in 2008 - 2010

5.1.2.2 Ca, Mg, P and S.

For exchangeable Ca, comparing with the reading in 2008, all the readings increased (Figure 4). All readings are within an optimal level. Deep soil shows a trend for Ca increase.

For exchangeable Mg, all readings are in the optimal and high level. Deep soil shows a trend for Mg to increase. Comparing with readings in 2009, readings in 2010 reduced clearly. The reason is unclear. Further data in the future may help us to understand this.

For P (Colwell), the reading in 2008 showed the highest value in the high level range according to the standards. In 2009 and 2010, readings at depth of 0 – 15 cm still show optimal levels; at 15 – 30 cm, they show optimal and marginal levels; at 30 – 45 cm, they show marginal and low levels; at 45 – 60 cm, they show low and very low levels.

Generally speaking, S is at a very low level. Deep soil showed a little better reading. Our fertilizer is UAN. It is not linked with S. Further data is required for this explanation.

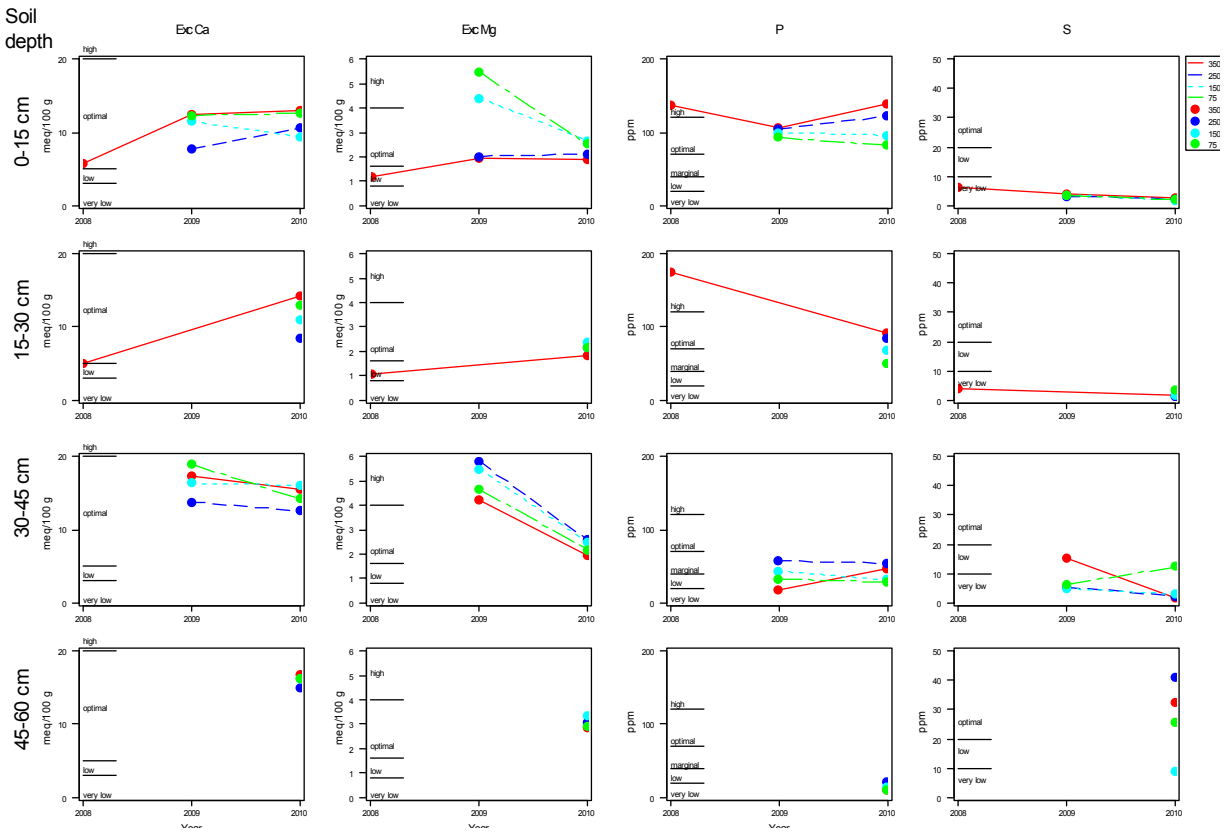


Figure 4: Contents of Ca, Mg, P and S in soil in 2008 - 2010

5.1.2.3 Zn, Mn, Cu and Fe

For DTPA Zn, the original readings were extremely high (Figure 5). Readings in 0 – 15 cm were in the optimal level range. Readings at 15 – 45 cm showed a marginal level. Only readings at 45 – 60 cm showed very low levels. Further data is required for explaining the drop from the original readings.

For DTPA Mn, top soil is in the high and optimal level and deep soil is in the optimal and marginal level. For DTPA Cu, top soil is in the optimal level and deep soil is between marginal and optimal level. For DTPA Fe, most readings are in the optimal level range.

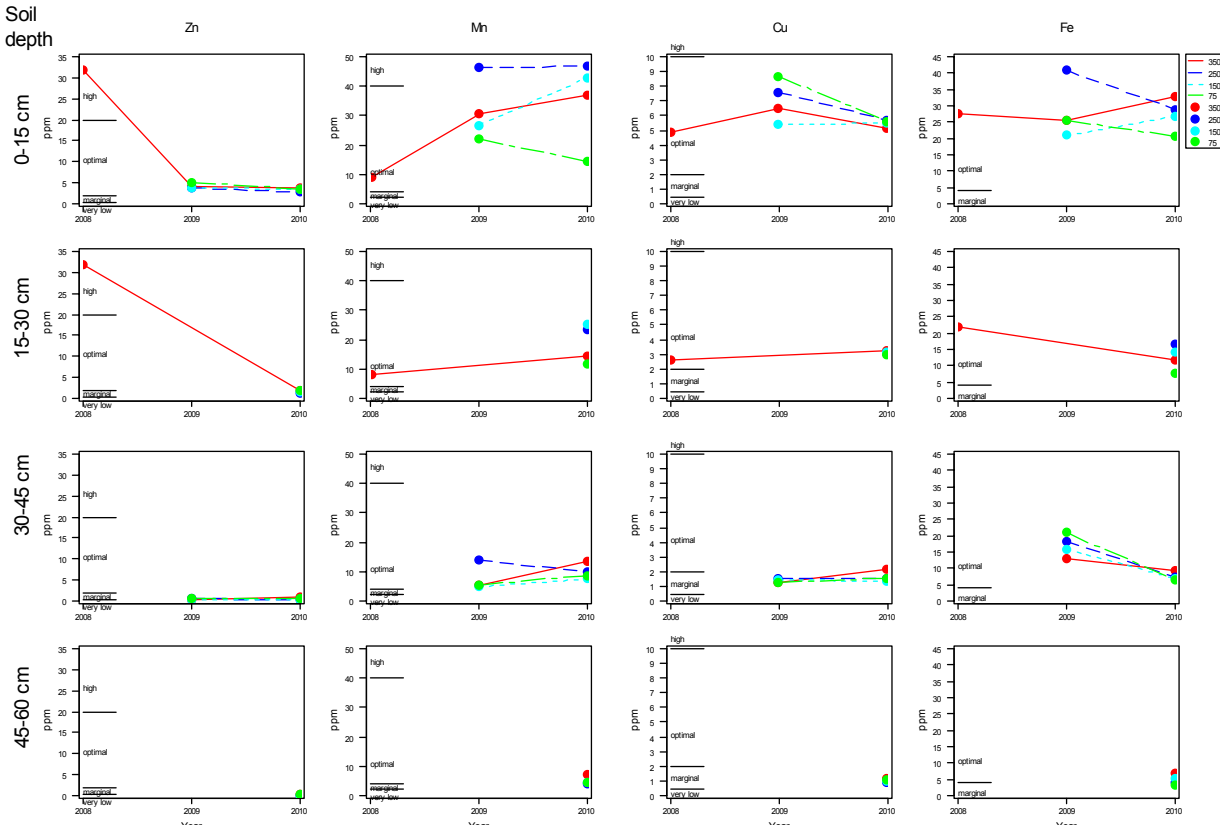


Figure 5: Contents of Zn, Mn, Cu and Fe in soil in 2008 - 2010

5.1.2.4 B, Na, Organic carbon and Conductivity.

For B in hot $CaCl_2$, most readings are at optimal levels (Figure 6). Deep soil shows a little higher reading.

There are no standards for Exchangeable Na. Comparing with readings in 2008, Na level is increasing. Deeper soil also shows higher readings.

For organic carbon, most readings are in the low level range and deep soil showed readings in the very low level range.

For electrical conductivity (EC), all readings were at a satisfactory level. However, deep soil showed higher conductivity. Electrical conductivity clearly shows influences by nitrogen fertilizer treatments.

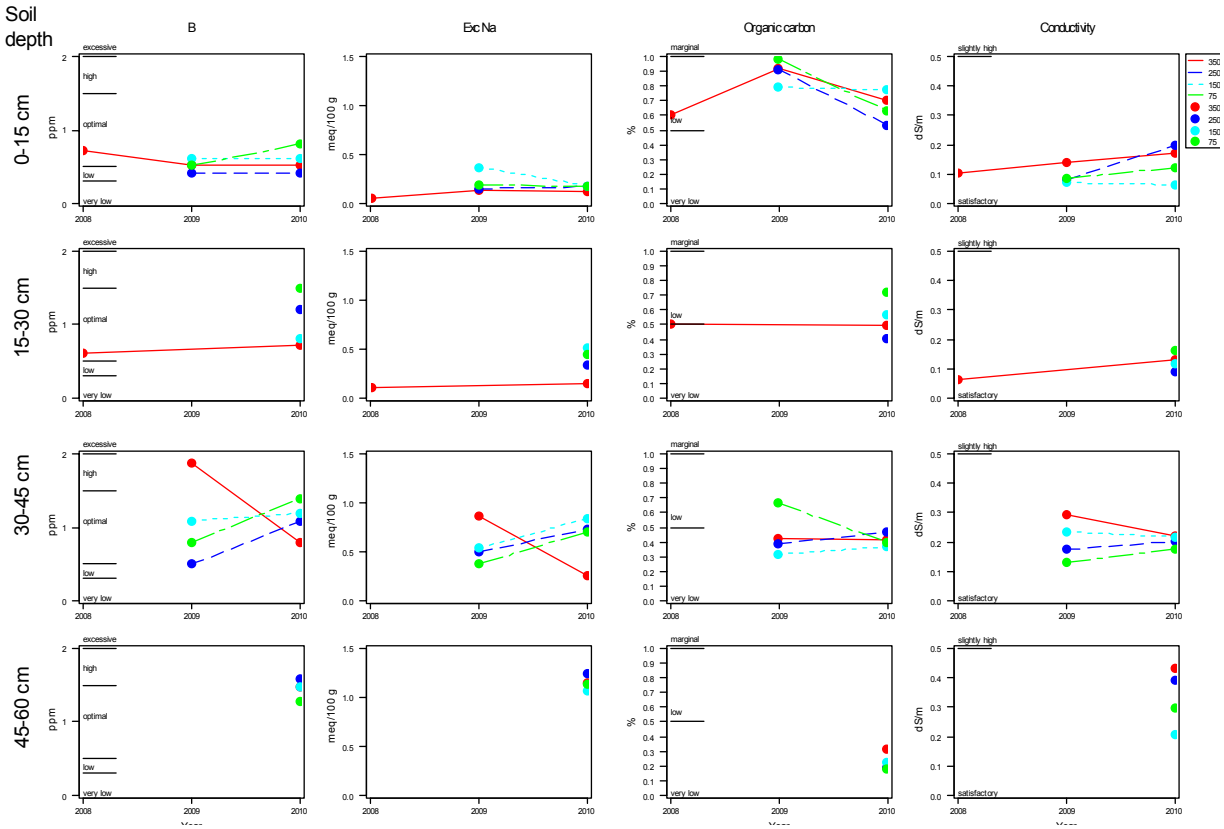


Figure 6: Contents of B, Na, organic carbon and conductivity in soil in 2008 - 2010

5.1.2.5 pH value in water, in $CaCl_2$ and the difference between those two.

Top soil pH values in both water and $CaCl_2$ solution tests show neutral to slightly alkaline while deep soil shows moderately alkaline (Figure 7).

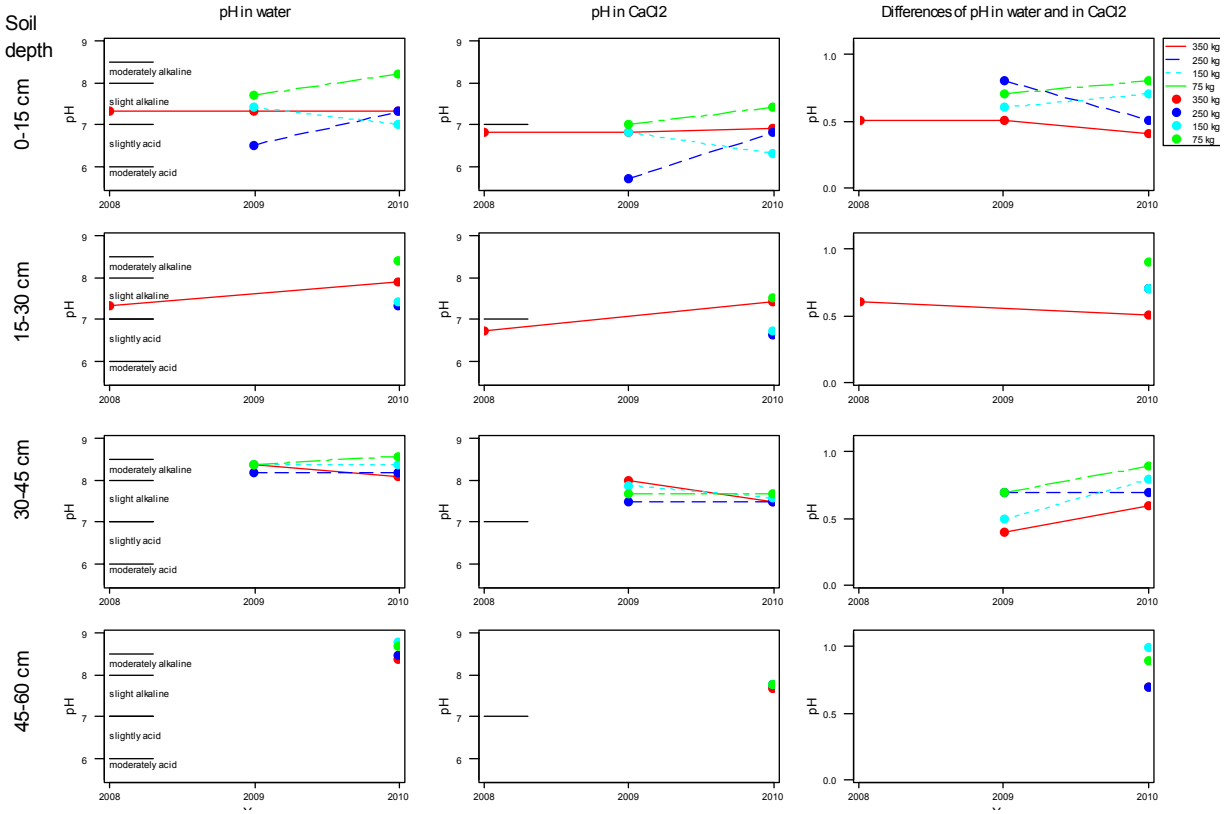


Figure 7: pH values in water, in $CaCl_2$ and their difference in soil in 2008 - 2010

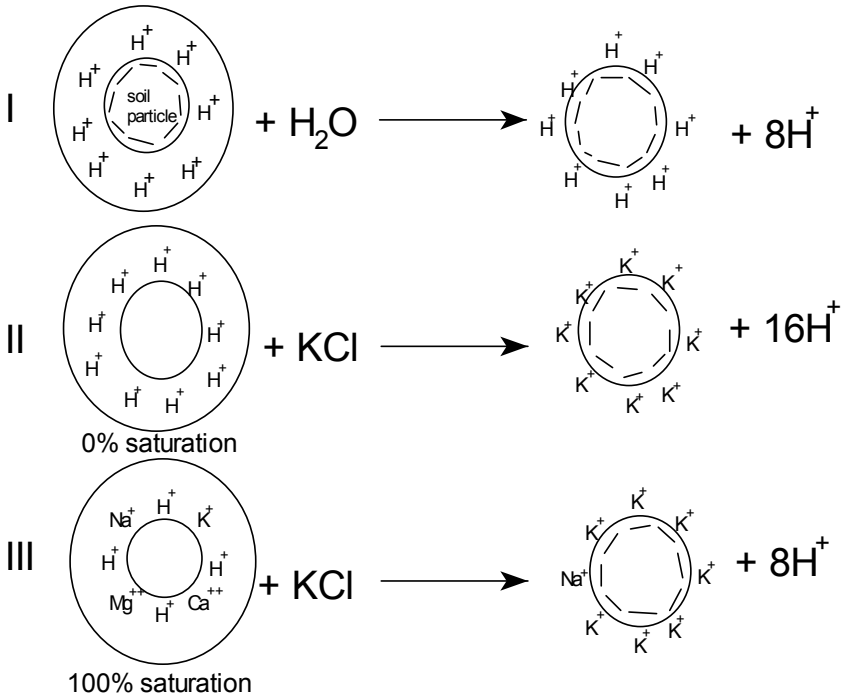


Figure 8: Soil status difference between extraction by water and by KCl solution (Hasegawa, 1982)

Difference of pH between water test and $CaCl_2$ is a new concept as shown in Figure 8. When water is used for the extraction, it only tests H^+ in the soil solution. When KCl solution is used, it tests H^+ in the soil solution plus H^+ combined in the soil particle. Thus, pH from KCl solution is always lower than pH from water. However, some soil has no pH difference between water and KCl solutions. III in Figure 3 explains this. Soil particles have negative charges and attract many cations. Due to saturation of the cations, K^+ in KCl cannot replace H^+ from the soil particle. Thus, a big amount fertilizer cannot be held in this soil. $CaCl_2$ should have the same principle.

Differences of pH value between water reading and $CaCl_2$ show strong influence from the nitrogen fertilizer treatments. High N treatments show low difference while low N treatments show high difference.

5.1.2.6 Soil Analysis Summary

Table 5 lists element level in soil for horticulture production and their response to nitrogen treatments.

Table 5: Summary of element tests

Elements	NO_3	P	K	Ca	Mg	S	B
Top soil	Low	Optimal to high	Optimal to high	Optimal	Optimal to high	Very low	Optimal
Deep soil	Marginal	Marginal	Marginal to optimal	Optimal	Optimal to high	Low to optimal	Optimal to high
Influence by treats	Yes	No	yes	No	No	Possible	Possible
Elements	Zn	Mn	Cu	Fe	Organic carbon	Electrical conductivity	pH
Top soil	Optimal to marginal	Optimal	Optimal	Optimal	Low	Satisfactory	slightly alkaline
Deep soil	Marginal to very low	Optimal to marginal	Optimal to marginal	Optimal	Very low	Satisfactory	moderately alkaline
Influence by treats	No	No	No	No	No	Yes	Possible

5.1.3 Trial Harvest

5.1.3.1 Yield

Table 8: Tree status, yields, count size and returns

Seasons	Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Total return/tree ($)	Acc. Yield in hull/tree (kg)
2008-2009 On-Season	75	55.0	73.5a	91.6	18.3	118.7	55.0
	150	53.5	64.6b	89.5	17.6	114.0	53.5
	250	54.3	69.7ab	90.7	18.2	118.1	54.3
	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
2009-2010 Off-Season	75	9.8B	86.3	82.1	3.55	24.3	64.8b
	150	15.1A	85.9	83.8	5.73	38.7	68.6ab
	250	15.6A	88.3	82.8	5.81	39.7	69.9ab
	350	17.5A	87.1	82.8	6.72	45.9	74.8a
	p-value	0.001	0.496	0.166	0.079	0.088	0.044

In the first season, no significant differences of yield in hull per tree were found between treatments. In the second season, treatment of 75 kg N/ha had significantly lower yields than the rest (Table 8). This also led to significant differences for accumulated yield. Merchantable yield and return did not reach a significant level. This is because merchantable yield needs nut sample analysis and this analysis only has 3 replicates as leaf samples. Less replicates results in a non-significant result. From this trial up to the second growing season, fertilizer program did not show a clear influence on nut size.

From Table 8, treatments of 75 kg and 350 kg N/ha showed significant higher percentages in shake 1 than the treatment at 150 kg N/ha. However, there is no significant difference of percentages in shake 1 in the second season.

5.1.3.2 Nut quality

Tables 10 and 11 showed results of quality tests. It is hard to make all the quality items different just by a treatment. Normally most quality tests are similar between treatments. A few differences will be important. In season 2008-2009, there is no significant difference between treatments. In season 2009-2010, a clearly notable difference was % blank shells & FM where treatments of 75 kg N had significantly higher percentages than treatments of 250 and 350 kg N.

Table 10: 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
2008-2009 On-Season	75	1.43	58.7	0.14	3.96	4.95	2.52	7.46	7.9	1.79
	150	0.90	56.3	0.47	5.19	6.12	2.70	8.82	10.3	2.03
	250	1.41	59.9	0.32	5.07	5.90	2.60	8.50	7.2	2.07
	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
2009-2010 Off-Season	75	0.363	56.4	2.00	1.26	1.25	2.08	3.34	9.77a	1.12
	150	0.408	54.2	2.10	1.78	1.38	1.53	2.91	8.40ab	1.86
	250	0.464	55.0	2.74	1.39	1.80	1.84	3.64	7.34b	1.37
	350	0.429	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

Table 11: Percentages of nut qualities in stain aspects

Seasons	Treat	% pickout	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2008-2009 On-Season	75	10.19	0.19	1.56	6.14	1.44	N/A	4.76	10.0
	150	8.47	0.24	0.83	5.19	1.48	N.A	3.53	9.3
	250	8.81	0.19	1.08	5.21	1.25	N/A	4.03	8.5
	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
2009-2010 Off-Season	75	7.1	0.572	0.398	5.4	1.91	0.112	3.12	19.2
	150	9.3	0.290	0.208	7.1	2.43	0.251	4.21	20.6
	250	7.2	0.489	0.390	5.3	1.34	0.046	3.76	21.7
	350	6.9	0.388	0.224	4.7	1.41	0.000	3.00	20.2
	p-value	0.670	0.212	0.786	0.772	0.138	0.180	0.910	0.954

5.1.3.3 Trunk circumference

Table 9 did not show a significant level of trunk circumferences between treatments. From the beginning, there is no significant difference recorded. In seasons 2008-2009 and 2009-2010, trunk increase did not show a significant difference between treatments.

Table 9 Tree status, yields, count size and returns 2008-2009

Treat	Trunk circumference (cm)	Increase (cm) in 2009	Increase (cm) in 2010
75	60.0	0.70	0.22
150	62.1	0.19	0.81
250	61.2	0.38	0.79
350	61.4	0.51	0.58
p-value	0.794	0.565	0.076

5.2 Pistachio Flower Number Monitoring

5.2.1 Bloom Observation between spring 2004 and 2006

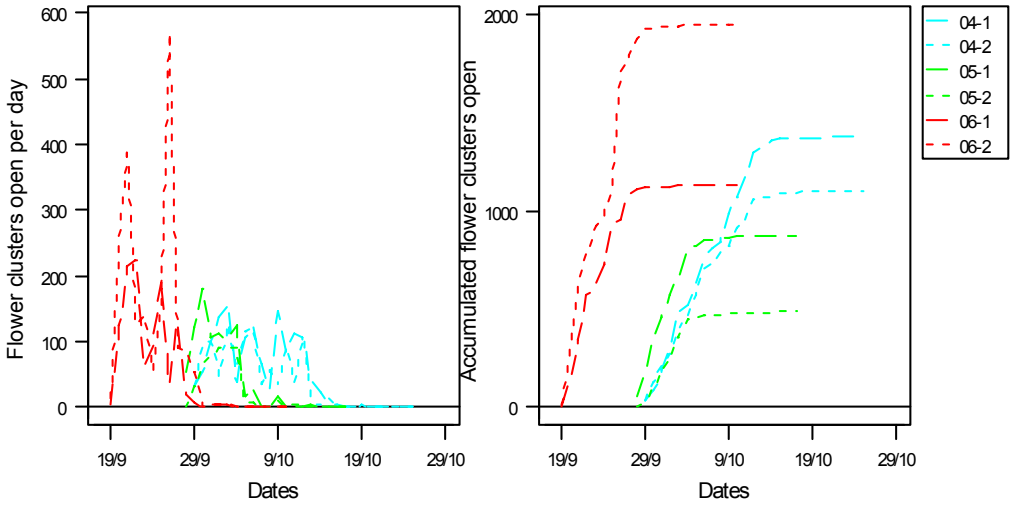


Figure 1 Distribution and accumulation of flower open between 2004 and 2006

Figure 1 shows flower process for the 2 particular trees in springs of 2004, 2005 and 2006. Table 1 shows the total bloom periods each year and durations. The table also shows how long for the opened flower numbers to reach the first quarter of the final flower clusters, and to reach the medium of the final flower clusters, and how long for the last quarter of flower opening.

Table 1: Flower opening process in 2004 - 2006

Year	Start	¼	½	¾	Complete
2004	29/09	3/10	6/10	10/10	25/10
Duration (days)	5	3	4	15	Total 27
2005	28/09	1/10	2/10	4/10	17/10
Duration (days)	4	1	2	13	Total 20
2006	19/09	21/09	23/09	26/09	10/10
Duration (days)	3	2	3	15	Total 22

Flower bud counting in the spring of 2006 for these 2 trees before bud break was 1066 and 1759, the final flower cluster counting was1126 and 1949. In other words, 60 and 190 flower buds more than the original counting. This is because terminal buds consist of a group of small buds (Photo 1). Although flower buds of pistachio trees are easily identified, correct identification of every single bud is not easy. In the winter time, terminal buds are smaller. They were counted as "1" flower bud. However, in bloom period, they become bigger with a lot of clusters open. At this time, they were counted as each individual cluster. This led to more clusters being counted than buds. This happened in the spring of2006 and 2008. In the other words, it happens in the 'on' years. In the 'off' year, in the winter of 2009, time was spent to carefully check these but terminal buds like that in the 'on' year were not found.

Photo 1: Terminal bud

5.2.2 Bloom Observation between spring 2007 and 2009

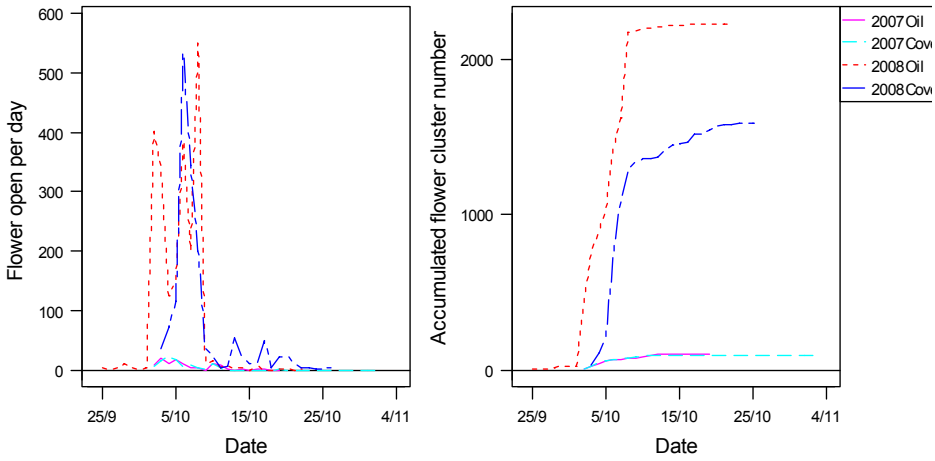


Figure 2: Flower opening process and accumulative flower number in 2007 and 2008

Figure 2 shows the flower opening process and the accumulative numbers of flower clusters in 2007 and 2008 since covering trees with plastic during oil application. In both seasons the oiled tree completed the process quickly and the non-oiled tree had a longer time for completion. Especially in 2008, the oiled tree had 3 peaks in bloom, then 90% bloom open on 8 October; non-oiled tree had only 1 big peak plus 2 or 3 small peaks, 90% bloom open on 14 October. There is a possibility that non-oiled tree should have 3 peaks, due to lack of oil stimulation, tree did not have enough stimulation, the 2nd and 3rd peaks became smaller and some buds remained un-open. The trial in 2009 showed the effect of oil application clearly.

In 2007, it was an extreme off-year with very low numbers of flower buds and flowers opening (Table 2). The percentages of flower cluster opening were 79% (non-oiled tree) and 67% (oiled tree). In 2008, it was the first time that flower bud numbers were similar (0.5% difference). However, oiled tree 4 had flower opening > 100% while non-oiled tree 5 had flower opening at 92%. In 2009, both reached 80% flower opening.

Table 2: Historical records for flower buds, flower clusters and yield

Items	Tree	2004	2005	2006	2007	2008	2009
Flower buds	4/oil			1066	150	1724	160
	5/non			1759	123	1733	317
Flower cluster	4/oil	1374	872	1126	101	2232	128
	5/non	1098	488	1949	97	1596	259
% flower bud open	Oil			>100%	67.3%	>100%	80.0%
	Non			>100%	78.9%	92.1%	81.7%
Yield per tree (kg)	Oil				10.01	61.38	14.55
	Non				8.73	61.46	24.21
Yield per flower cluster (g)	Oil				99	27.5	114
	Non				90	38.5	93
Count	Oil					90.1	85.9
	Non					88.0	83.8
Merchantable yield (kg)	Oil					19.1	4.5
	Non					20.3	8.3
Return ($)	Oil					125.02	29.9
	Non					133.95	57.8
Chill portions		65	57	72	59	68	58

5.2.3 Yield and Nut Quality

In season 2007/08, trees with oil application had more flower buds, flower opening and higher yields than the control trees. In season 2008/09, the tree with oil application had 25% more bud break than the tree without oil application. However, yields harvested did not show clear a difference. The tree without oil application had 1596 flower clusters open and produced 61.46 kg nuts in shell, while the tree with oil application had 2232 flower clusters open and produced 61.38 kg nuts in shell. This seems to show that more than 60 kg per tree is a good crop for trees here. Any flower buds more than 1600 may not produce more nuts. For count size, there is no clear difference between treatments. Table 2 also listed chill portions in different winters. Clearly biennial bearing pattern can be easily found from flower buds, flower cluster opening and yield records. For the whole biennial bearing pattern, Figure 3 provides a clear profile.

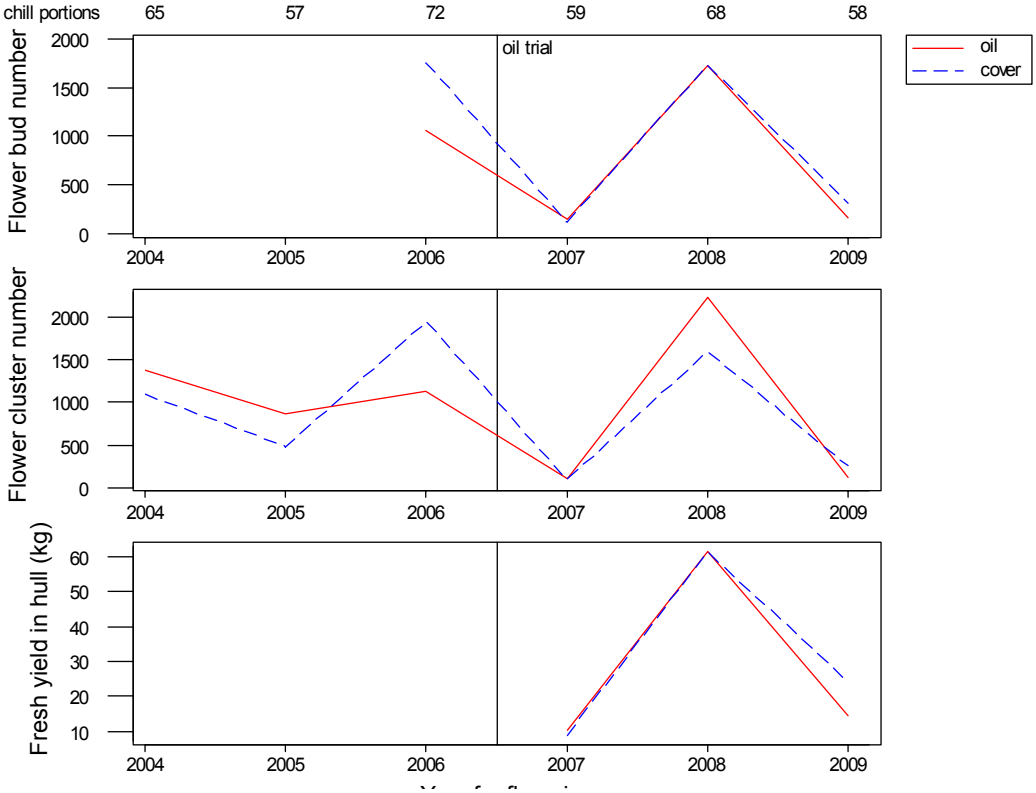


Figure 3: Numbers of flower bud and flower opening and yield between 2004 and 2009

Table 3 and 4 listed quality tests. Without replicates, we can only compare them visually. Generally speaking, the tree without oil treatment had better results for almost all items but it had low crop and return. To compare treatments in 2 years, trees with oil application had higher percentages of blank, pick out, dark stain and other stain.

Table 3: Percentages of nut qualities in physiological aspects in season 2009/10

Season	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
2008-2009	Oil	0.42	53.97	0.00	2.96	4.01	1.47	5.47	8.33	1.01
	Non	0.99	57.11	0.00	5.43	5.60	1.95	7.55	5.77	1.92
2009-2010	Oil	0.33	65.79	0.61	6.08	0.86	0.35	1.21	8.43	4.14
	Non	0.76	76.58	0.00	2.98	0.59	0.17	0.76	5.43	1.93

Table 4: Percentages of nut qualities in stain aspects in season 2009/10

Season	Treat	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2008-2009	Oil	12.09	0.47	1.65	9.47	1.77	N/A	7.67	16.29
	Non	6.95	0.49	0.92	4.38	1.16	N/A	2.94	15.72
2009-2010	Oil	11.39	0.48	0.38	6.86	3.96	0.13	2.80	5.68
	Non	5.20	0.73	0.19	2.99	1.51	0.14	1.22	7.57

5.3 Reflective Mulch Trial 2

5.3.1 Trial Harvest

Table 5: Harvest results

Harvest	Treat	Trunk CSA (cm^2)	Yield /tree	Merch-antable yield	Count	Return ($)	%1st shake	%Blank Shell, FM	%in hull to dry yield
2007	Contral	411	47.4B	18.1B	80.9	117.5B	80.1	6.45	41.3
	Extenday	434	55.3A	22.4A	82.1	148.9A	83.5	6.29	43.2
	P	0.542	0.006	0.000	0.373	0.000	0.135	0.831	0.115
2008	Contral	1.67	3.36b	1.15	85.2	17.5	55.7	10.8	38.2
	Extenday	1.35	7.40a	2.66	85.0	7.4	50.6	10.0	39.9
	P	0.301	0.016	0.130	0.961	0.137	0.421	0.696	0.471

5.3.1.1 Yield

On the 10 May 2007, trunk circumferences at 10 cm above the union of each trial tree were measured. Trunk cross section areas (CSA) were calculated based on the measurement. The second column of Table 5 shows the results. On average, the CSA of trees on the reflective mulch area (R) is a little larger than that of control (N) but this difference was not reached at $p \leq 0.05$ level. In season 2007-2008, numbers under Trunk CSA are increments of trunk CSA instead of trunk CSA itself. Trees above the mulch showed less trunk increase but this difference did not reach a significant level.

In harvest 2007, from the third column of Table 5, trees on the mulch showed significantly higher yield than the control. Among the 12 trees with the mulch, there were only 2 trees that showed lower crop than the control in the same replicates. In harvest 2008, trees on the mulch showed significantly higher yield than the control. Among the 12 trees with the mulch, there were 4 trees that showed lower crop than control in the same replicates.

No significant difference of count size was found between 2 treatments in Table 5 in both harvests.

In harvest 2007, from the sixth column of Table 5, trees on the mulch showed very significantly higher returns than the control. On average, it was $31.40 extra return per tree. On a hectare basis (332 tree/ha and 300 female tree/ha), trees on reflective mulch should have $9420 extra return per hectare. In harvest 2008, trees on the mulch showed very significantly higher return than control. On average, it was $10.10 extra return per tree. Trees on reflective mulch should have $3000 extra return per hectare.

To set up reflective mulch for a hectare, it would cost $8600 but it will last 3 years. This indicates that increased return in the first year should cover the cost for the mulch for 3 years usage. From our experience in trial 1, the real benefit was in the second year.

In both seasons, no significant difference was found between percentages of the first shake, percentages of blank nuts, percentages of in hull to dry yield and percentages of marketable yield. On average, trees in the mulch area showed less blank nuts and with higher percentages of in hull to dry yield and marketable yield.

5.3.1.2 Nut quality

Table 6: Nut quality results

Harvest	Treat	% small	% medium	% Jumbo	% light stain	% narrow split	% pick out	% loose kernel	%Non Split	% floater	% total non split
2007	N	1.00	51.9	0.01	21.1	3.26	12.5	0.06	1.39	2.42	3.94
	R	1.21	49.0	0.36	26.5	3.62	10.4	0.17	0.88	1.62	2.47
	P-value	0.885	0.529	0.423	0.084	0.703	0.265	0.267	0.171	0.100	0.022
2008	N	1.82	51.2	4.07	10.0	4.11	11.2	0.10	1.92	4.75	6.70
	R	2.08	56.6	2.90	9.3	3.06	11.3	0.04	1.73	2.98	4.70
	P-value	0.642	0.260	0.468	0.689	0.169	0.949	0.195	0.940	0.087	0.279

Table 6 shows nut quality results. All items here are the same as payment items. In Table 6, none of the comparison reached a $p \leq 0.05$ level. This indicates that both treatments had very similar nut quality.

Non-split nuts were divided into non-split and floater (non-split with stained shell). Trees on the mulch showed lower percentages of this "total" non-split but floater. In the harvest of 2007, it reached a significant level with a p-value =0.022. This shows physiological benefit reducing non-split nuts through the use of the mulch. In harvest 2008, average percentages of 'total' non-split nuts on trees above the mulch was still lower than the control but did not reach a significant level. This was probably due to less flower buds and the mulch benefiting kernel filling.

In the harvest of 2007, the mulch showed a slightly higher percentage of light stain. Although without significant difference, the mulch showed higher percentages of light stain in all the 3-year trials. However, in the harvest of 2008 the mulch did not show a high percentage of light stain. This could be because the mulch promoted ripening. Harvest time was usually according to the time of the control trees (trees beyond the trial). Trees on the mulch had over mature nuts. In the harvest of 2008, harvest was started a little earlier. Because nuts in CMV Farms were not ready and the trial harvest was started before the end of the first shake for whole orchard. This gave us a chance to test the stain effect from the mulch. Results showed that if harvest is on time, stain should not be a problem for the mulch.

5.3.2 Influence of Reflective Light from the Mulch on Trees in Different Distance

Figure 3 shows measurement results. The unit for the measurement should be $\mu Mol/m^2 \bullet s$. Each sub-graph shows a set of measurements for each particular month, November, December or February. To identify differences, morning measurements are in green, mid-day measurements in red and afternoon measurements in blue. Replicates 1-3 are also in different lines.

In all the 3 sub-graphs, position 2, 3 and 4 showed clear higher values than the rest. This is the major benefit area. Positions 1 and 5 showed lower values than positions 2, 3 and 4 but higher values than the rest. Trees in positions 1 and 5 were used for tying the mulch but they are not fully covered by the mulch. From all the 3 graphs, position 6 still showed higher values than position > 6. Position 6 was just in the south of the mulch. They still showed a little benefit. From position 7 to position 19, they almost showed similar low values. Position 20 showed little higher values. This may be due to some benefit from the mulch in the south. However, this only reflects from replicate 2 but not from replicate 1. This may also be due to the shape of the land.

Reflective in mid-day measurements (red) were not strong comparing with measurements in the morning (green) and in the afternoon (blue) except at the position in replicate 3. Replicate 3 is a young tree with smaller canopy. This indicates that when sun is at side position of the rows, sunlight is easy

to be reflected under canopies. When the sun is in the direction towards the rows, the canopies in the rows shade sunlight. Although absolute sunlight at mid-day is much stronger than morning and afternoon, reflective light is weaker than the other two.

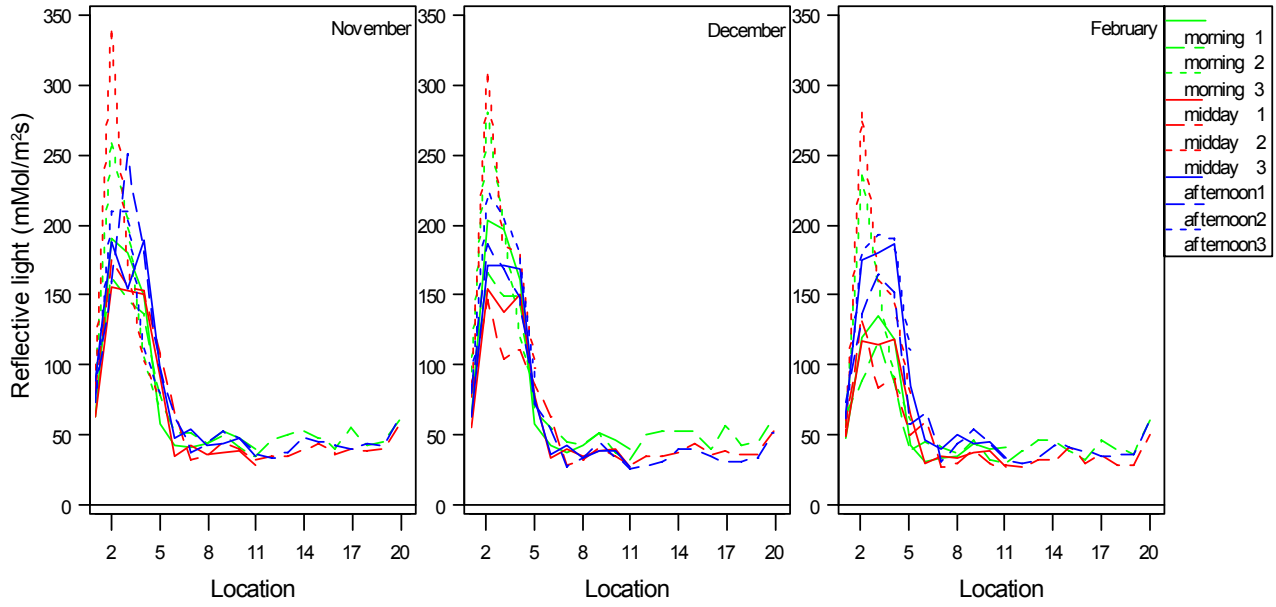


Figure 3: Reflective light intensities in different positions from the mulch as well as in different day time and different months

5.3.3 Comparison of Reflective Capacity between the Mulch under Canopies and Between-Rows

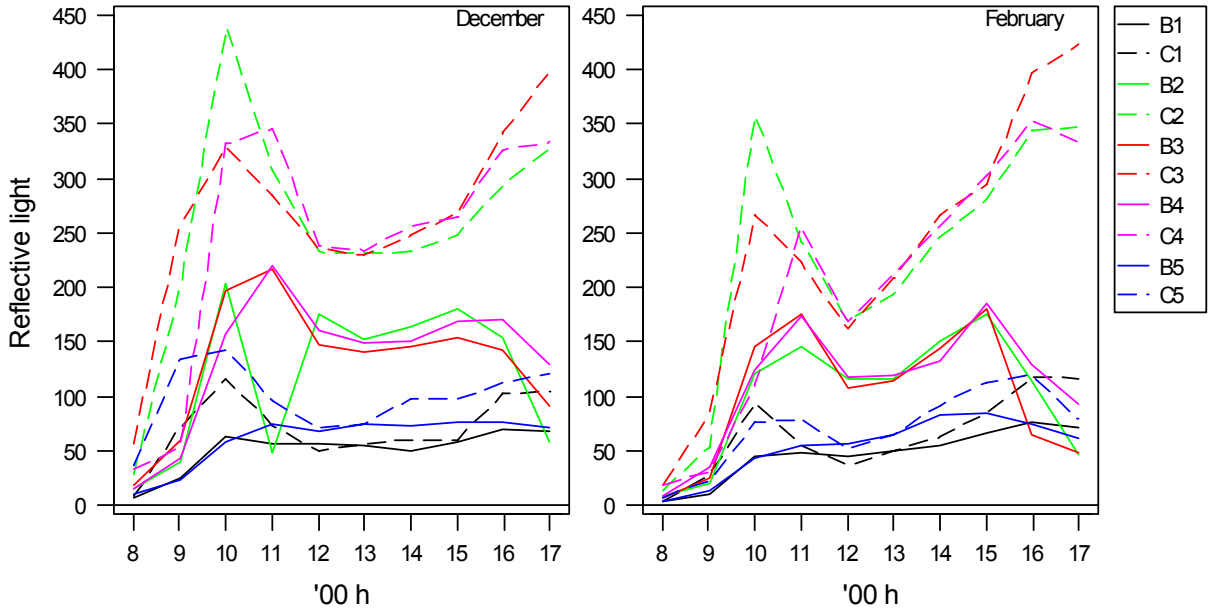


Figure 4: Comparison of reflective light of mulch between under canopy and between-row in different time

Figure 4 shows the measurement results. The left sub-graph shows results in December and the right sub-graph shows results in February. X-axis represents time in 24 hour system. Y-axis represent reflective light in $\mu mol/m^2 \bullet s$. There are 10 lines in the graphs. In legend, B1 to B5 represent trees 1 - 5 for the mulch between rows. Solid lines represent B1 – B5. C1 to C5 represent trees 1 – 5 for the mulch under canopy. Dash lines represent C1 – C5. Both tree 1 are in black colour and tree 5 in blue colour. Trees 2, 3 and 4 are in green, red and pink, respectively.

Trees 2, 3 and 4 of mulch under the canopy show the higher reflective light value than trees 2, 3 and 4 of mulch between rows almost from beginning to the end of the measurements. For tree 4 between 9 am and 10 am, trees above central mulch showed low values which were due to shadings from a big male tree. On average, reflection for trees under canopy is about twice reflection as trees of mulch between rows. Statistical analysis shows a significant different at the p=0.00 level.

Trees 1 and 5 are at the boundary of the mulch. Their exact position is beyond the mulch as shown in Figure 5. In real production with the mulch, boundary trees are just a few trees and not important. Anyway, from this comparison, mulch under canopy (dash lines) is still better than mulch between rows (solid lines).

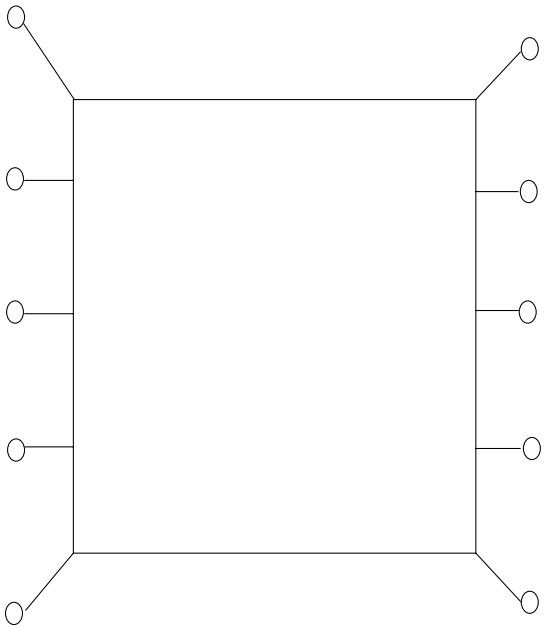


Figure 5: The mulch fixing between rows tying trees in 2 rows. Here circles represent trees

This test also provides a chance for us to view the intensity of the reflective light in different times of the day. Most of the lines showed maximum reflection around 10 am or 11 am. Reason for this difference should be due to orchard variation, especially the high male tree position. Sunlight is very strong between 12 pm and 3 pm in the day light saving period. However, reflection is not strong. This should be because directions of sunlight and row. In this situation, sunlight is shaded by trees in the row. No much reflection was seen in this case. After 3 pm, reflection increased again.

5.3.5 Reflection Capacity Test of Sun-Brite

Table 7: Light intensive measurement (μmol/m^2•s) for treatments in November, December and February

Month	Extenday			Sun-Brite			Fields		
	Time	Sunlight	Reflection	Time	Sunlight	Reflection	Time	Sunlight	Reflection
Nov	12:55	1932	1034	12:59	1960	215	13:16	1989	127
	13:03	1957	996	13:07	1981	278	13:17	1992	136
	13:11	1954	986	13:15	1985	295	13:17	1981	135
Dec	13:27	2040	962	13:22	2055	238	13:23	2063	156
	13:27	2040	937	13:22	2055	244	13:23	2063	153
	13:27	2040	843	13:22	2055	227	13:23	2063	151
Feb	13:25	1908	944	13:31	1921	162	13:32	1916	132
	13:25	1908	926	13:31	1921	162	13:32	1916	135
	13:25	1908	906	13:31	1921	162	13:32	1916	133

Table 7 listed measurement results. For each test, it listed the time of the test, open sunlight and reflective light. Figure 6 summarized the measurements. This is a box-plot graph showing data distribution for each group of tests. X-axis shows reflective light in μmol/m^2•s while Y-axis shows different treatments in different months. From Figure 6, Extenday in all 3 months shows significantly

(p=0.000) higher reflection than the other 2 treatments. The average intensity is about 948 μmol/m^2•s. Sun-Brite shows significant higher values (about 220 μmol/m^2•s) than control (about 140 μmol/m^2•s). However, reflection from Sun-Brite was far lower than that from Extenday mulch and reflective light of Sun-Brite clearly decreases as time increases.

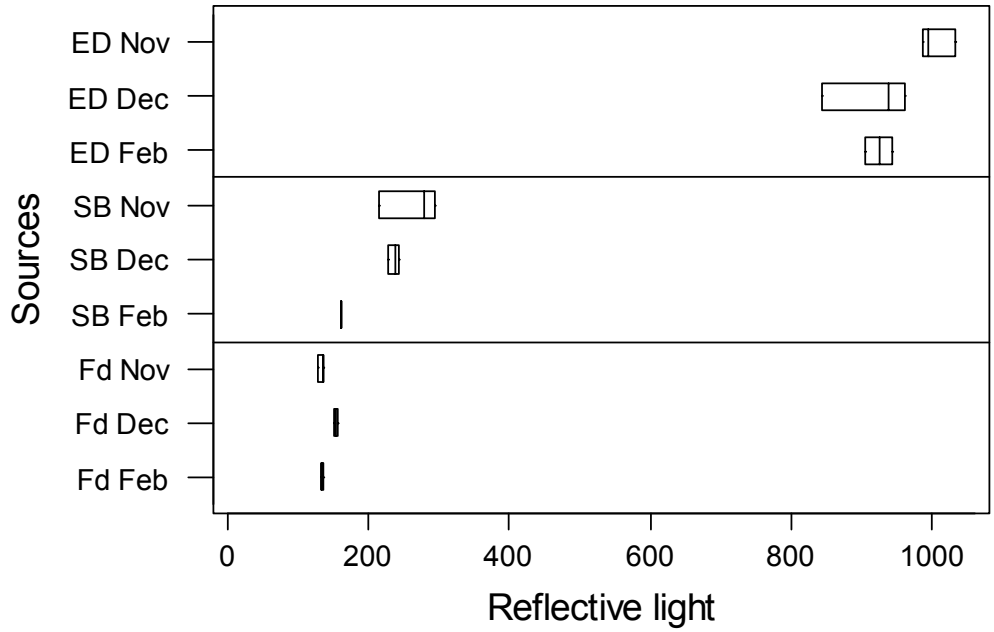


Figure 6 Reflection in area of Sun-Brite, Extenday and common fields in November and December. ED = Extenday, SB = Sun-Brite, Fd= field

Price of Sun-Brite is about ¼ of price of Extenday mulch for a same amount of area. However, it reflection capacity is about ¼ of Extenday mulch. From the trial experience, Extenday mulch should be used at least for 3 growing seasons. Nobody including the suppliers believes Sun-Brite can last 3 growing seasons. If so, there will be no value for further tests in next growing season.

5.4 Reflective Mulch Trial 3

5.4.1 Trial Harvest

5.4.1.1 Yield

Table 12: Yields, count size and returns

Season	Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield (kg)	Return/tree ($)	Accu. Yield in hull/tree
2007/08	Between	3.65	67.0ab	94.8	1.36	9.2	
	Under	6.97	62.7b	89.1	2.69	18.7	
	SunBrite	5.23	73.8a	88.4	1.85	12.7	
	Control	5.85	59.4b	92.9	2.10	14.3	
	P-value	0.317	0.014	0.127	0.296	0.290	
2008/09	Between	66.69a	83.81	88.13b	22.6	151.7	66.69a
	Under	68.22a	82.94	89.13b	22.7	149.3	68.22a
	Plastic	65.46ab	81.60	90.27ab	20.7	137.0	65.46ab
	Control	59.68b	79.87	91.58a	18.9	123.1	59.68b
	p-value	0.035	0.273	0.040	0.213	0.156	0.035
2009/10	Between	8.3ab	86.45	82.3	2.93	19.8	74.9ab
	Under	11.7a	88.00	79.6	4.21	28.3	79.9a
	Plastic	7.9b	84.59	79.9	2.65	17.8	70.9b
	Control	6.0b	83.75	81.1	1.73	11.5	65.7b
	p-value	0.020	0.391	0.426	0.106	0.105	0.007

In season 2007/08, from the third column of Table 12, there is no significant difference of fresh in-hull yield between treatments. Generally speaking, they produced low crop. The previous season was an 'on' year and season 2008 was a big 'off' season. Although we put the mulch there, trees cannot produce more crops without flower buds. In season 2008/09, Extenday both under canopy and between rows had significantly higher yields in hull per tree than the control. For each replicate, control in most cases was the lowest one. In season 2009/10, Extenday under canopy had significantly higher yields in hull per tree than control and white plastic. In both seasons, 2008/09 and 2009/10, merchantable yields and return showed the same pattern but did not reach a significant level. This is purely due to less replicates. In season 2008/09, $27 per tree difference for return made $8100 difference per hectare (300 female tree/ha). In season 2009/10, $16.8 per tree difference for return made $5040 difference per hectare. This difference is enough to cover fitting cost for Extenday.

Accumulated yield is difficult to calculate. In season 2007/08, the treatment included Sun Brite but in seasons 2008/09-2010/2011, this treatment included white plastic. These 2 cannot be mixed together. Thus, accumulated yield calculation started from season 2 as shown in Table 12. All of these show benefit from Extenday use.

From trial 1 and trial 2, and season 2007/08 and season 2009/10 in trial 3, Extenday treatments did not show benefit for count size. However, Extenday treatments showed benefit for count size in season 2008/09 in this trial. Both Extenday treatments showed significant larger nuts than control.

Due to too low crop in season 2007/08, Sun Brite showed a significant higher percentage in shake 1 without clear meaning. In seasons 2008/09 and 2009/10, no significant difference was found between percentages of shake 1.

5.4.1.2 Nut quality

Table 13: Percentages of nut qualities in physiological aspects

Season	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
2007-2008	Between	2.97	57.2	5.9	2.45	0.42	1.18	1.59	7.7	0.14
	Under	2.19	61.6	6.0	2.82	0.39	0.79	1.18	5.5	0.17
	SunBrite	1.07	64.4	6.3	3.15	0.67	1.10	1.77	6.8	0.28
	Control	2.74	56.8	5.5	2.64	0.37	1.09	1.46	9.0	0.29
	P-value	0.068	0.458	0.987	0.594	0.600	0.903	0.842	0.176	0.207
2008-2009	Between	0.33	65.7	1.59a	3.81	3.06	1.99	5.06b	7.0	0.81
	Under	0.37	63.9	0.35b	4.86	3.83	2.52	6.35ab	7.4	0.74
	Plastic	0.80	65.3	0.39b	4.88	2.96	1.98	4.94b	7.3	1.11
	Control	0.62	61.3	0.47b	3.99	4.25	2.62	6.87a	9.0	1.12
	p-value	0.093	0.092	0.010	0.395	0.152	0.701	0.045	0.583	0.479
2009-2010	Between	0.532	61.3	2.93	2.95	1.47	1.33	2.81	7.46	1.93
	Under	0.365	61.4	4.79	2.28	1.12	1.06	2.18	7.33	1.72
	Plastic	0.466	62.0	2.03	2.77	1.22	1.04	2.27	7.69	2.32
	Control	0.407	59.5	4.42	1.93	1.83	2.17	4.01	8.41	1.87
	p-value	0.200	0.684	0.545	0.304	0.517	0.176	0.076	0.803	0.672

From Table 13, there is no clear difference in season 2007/08. In season 2008/09, Extenday between rows showed very significantly higher percentages of Jumbo nuts. In all 3 replicate from quality tests, Extenday between rows had much higher percentage of Jumbo nuts than others. This is another way to show the benefit for nut size from reflective mulches. But this effect did not show in season 2009/10.

'Non split' is non-split nuts without stain while a 'floater' is a non-split nut with stain. From a view of cultivation or physiology, they are both non-split nuts. In season 2008/09, the control had the highest percentages of total non-split nuts and significantly higher percentages than Extenday between rows and white plastic. In season 2009/10, although it did not reach a p-value ≤ 0.05, p-value = 0.076 also shows this benefit. In all 3 seasons, percentages of blank nuts on control tree were always highest on average but did not reach a significant level.

Table 14: Percentages of nut qualities in stain aspects

Season	Treat	% pickout	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2007-2009	Between	9.6	0.164	2.77	6.17	5.86	0.17	0.14	12.5
	Under	8.0	0.151	1.82	5.51	5.00	0.42	0.09	12.5
	SunBrite	7.8	0.141	2.04	5.05	4.48	0.43	0.14	8.6
	Control	10.6	0.183	3.54	6.40	5.99	0.31	0.10	11.1
	P-value	0.713	0.994	0.436	0.847	0.742	0.274	0.872	0.379
2008-2009	Between	7.45	0.152	0.81	5.55	1.59	N/A	3.75b	8.90
	Under	8.64	0.141	1.47	5.95	2.00	N/A	3.73b	7.96
	Plastic	8.54	0.242	2.14	4.99	1.58	N/A	4.72ab	7.62
	Control	10.28	0.107	1.09	7.80	1.71	N/A	5.78a	7.34
	p-value	0.182	0.226	0.525	0.237	0.672		0.037	0.572
2009-2010	Between	9.4	0.62	0.160	7.1	2.72	0.168	3.78	12.0
	Under	10.4	0.65	0.323	8.1	3.89	0.187	3.52	10.6
	Plastic	9.2	0.77	0.282	6.4	2.04	0.053	3.86	12.8
	Control	10.7	0.53	0.227	8.4	3.73	0.009	4.25	10.1
	p-value	0.828	0.821	0.598	0.678	0.321	0.735	0.942	0.475

From Table 14, no significant difference was found in season 2007/08. In season 2008/09, control showed significantly higher percentages of other stain comparing with Extenday treatments. In season 2009/10, Table 14 did not show clear differences for almost all tested qualities.

The mulch showed a little high percentages of light stain. Although without significant difference, the mulch showed higher percentages of light stain in all the 3-year trials. This could be because the mulch promoted ripening. The harvest time was according to the time of the control trees (trees beyond the trial). Trees on the mulch had over mature nuts. If all trees in production are on the mulch at the harvest time the problem will be reduced. In season 2008/09 in trial 2, the mulch did not show a high percentage of light stain. In that harvest, nuts in CMV Farms were not ready and the trial harvest was started before the end of the first shake for whole orchard. This gave us a chance to test the stain effect from the mulch. Results showed that if harvest is on time, stain should not be a problem for the mulch.

5.4.1.3 Trunk circumference increment

Table 15 shows trunk circumferences and circumference increments. From the beginning of the trial, there is no significant difference of trunk circumferences between treatments. In the following 3 seasons, increments did not reach a significant level.

Table 15: Trunk circumference and increment

Treat	Trunk circumference in winter 2007	Trunk circumference increase (cm) between 2007 & 2008	Trunk circumference increase (cm) between 2008 & 2009	Trunk circumference increase (cm) between 2009 & 2010	Trunk circumference increase (cm) between 2010 & 2011
Between	54.8	3.25	0.83	1.10	
Under	54.0	2.62	0.81	1.12	
Plastic[z]	54.6	3.58	0.47	1.59	
Control	54.4	3.29	0.56	1.06	
P-value	0.986	0.287	0.219	0.118	

[z] here in the season 2007/08 is Sun Brite

5.4.2 Nut Number per Cluster

Nut numbers per cluster were counted and recorded on tree basis. Results from analysis of variance based on single cluster listed as follows:

```
Analysis of Variance for N/cluster based on single cluster
Source     DF        SS        MS        F        P
Treat       1     25855     25855    30.22    0.000
Error     399    341340       855
Total     400    367195

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+--
Control   197     43.62     27.04  (-----*-----)
Under     204     59.69     31.23                       (----*-----)
                                  ----+---------+---------+---------+--
Pooled StDev =    29.25              42.0      49.0      56.0      63.0
```

and based on tree

```
Analysis of Variance for N/cluster based on tree
Source     DF        SS        MS        F        P
Tree        5     31748      6350     7.48    0.000
Error     395    335447       849
Total     400    367195

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Tree        N      Mean     StDev  ---------+---------+---------+-------
control1   27     36.89a    27.88   (--------*--------)
control2   68     40.78a    25.68         (-----*-----)
control3  102     47.30ab   27.39                (---*----)
Under1     85     64.01b    33.44                             (----*-----)
Under2     85     56.41b    29.11                       (----*----)
Under3     34     57.06ab   30.25                   (--------*-------)
                                  ---------+---------+---------+-------
Pooled StDev =    29.14                    36        48        60
```

However, to analyze the treatment, we need an analysis of variance on a nested-design. The following table shows results from analysis of variance on a nested-design. Due to relatively bigger variance between trees, especially for control 3 and under 3, treatments did not reach a significant level.

Table 16: Analysis of variance of nested trial design

Source	SS	Df	MS	F	P
Treatment	25855	2	12927.5	1.63	0.3038
Tree	31748	4	7937.0	10.10	0.0000
Error	309592	394	785.8		
Total	367195	400			

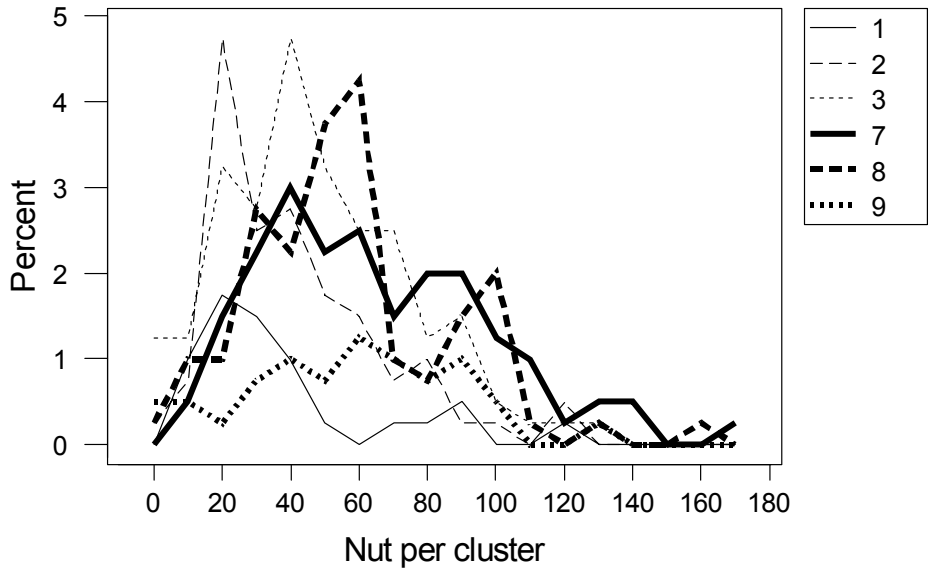


Figure 4: Distribution of nuts per cluster in 6 hand harvested trees

All analysis above seems to show us a clear trend but we need more trees for a significant level. Figure 4 shows exact distribution of nuts per cluster for those 6 trees. Here 1, 2 and 3 are trees of control, while 7, 8 and 9 are trees with mulch under the canopy. This seems to indicate that the trend is clear but more replicates are required to reach a significant level.

These 6 trees only have nut numbers recorded but do not have yield records. Based on the investigation of the nuts remaining on trees in plot 2, yield of these 6 trees were estimated.

5.4.3 Material Tests for Reflective Light

5.4.3.1 Measurement in December 2007

Figure 2 shows results for reflective light measurements. In legend, 6 treatments are listed there. For detail:

Control	is no reflective facility;
Between	is Extenday mulch between rows;
Under	is Extenday mulch under canopy;
Trunk	is white trunk painting;
T+Grape	is white trunk painting plus plastic mulch used for grape under canopy;
Grape	is plastic mulch used for grape under canopy;

Each plot includes 5 trees and only the middle 3 trees are really for the test. This time only the middle 3 trees were tested. This reduced variation within each treatment and standard error bars can be used in graph. Thus, Figure 5 shows 6 colour lines representing 6 treatments. Each point in the graph was averaged from 3 tree measurements with its standard error bar.

From Figure 5, control shows the lowest reflection. Treatment of "white trunk" is the second lowest one. Statistical analysis did not show a significant difference at any hour between these two.

Treatment of “between” showed significantly more reflection than the control and “white trunk” between 10 am and 4 pm. However, another 3 treatments with the mulch under canopy showed significantly more reflection than treatments “between”.

Statistical test shows that both “Grape” and “T+Grape” had significantly more reflection than “under” from 8 am to 4 pm. By way of explanation the mulch used for grapes was 2.5 m wide while the Extenday was 2 m wide. One more metre wide should have more reflection. In addition the mulch for grapes was whiter and smoother than Extenday. This may also increase reflection.

Except at 2 pm, there is no statistical difference of reflection between “Grape” and “T+Grape”. Even at 2 pm, it was the “Grape” that showed significantly more reflection than “T+Grape”. This indicated that “T+Grape” showed high reflection being due to the mulch itself. White trunk did not strengthen reflection. Also, white trunk painting was not statistically better than the control. All these show that white trunk cannot increase reflection.

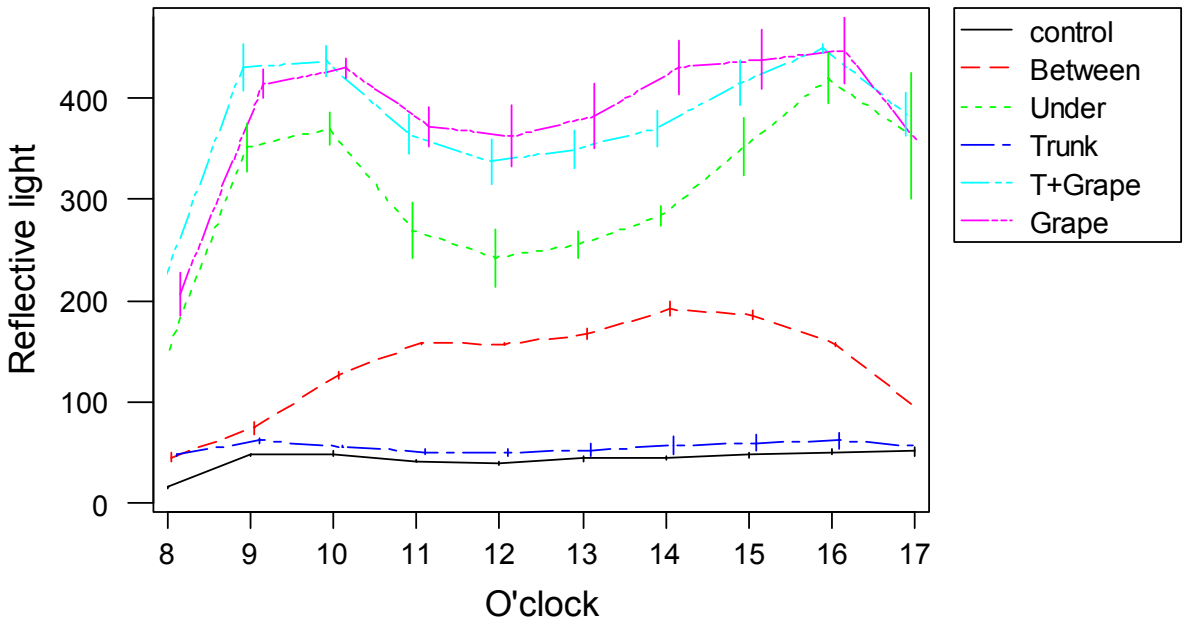


Figure 5: Hourly reflective light measurements under treatments on 17 Dec 2007

5.4.3.2 Measurement in February 2008

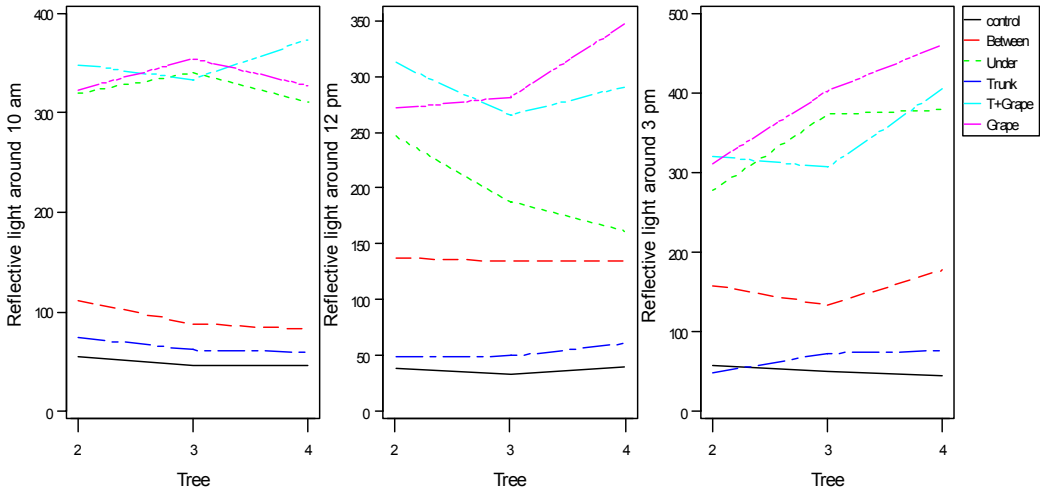


Figure 6: Light measurements in February

Figure 6 shows different structures of graph comparing with Figure 5. Three sub-graphs show 3 measurement times. The conclusion was that they look similar. "Grape" and "T+Grape" showed the best results. This time, "under" showed much close results as "Grape", especially around 10 am and 3 pm. As known, "under" usually does not show good reflection at noon. However, "Grape" had 1 metre wide than "under", reflection at noon was much better than "under". "Between" is still ranked number 4. "Trunk" showed a little better than control but this is really not much.

5.4.4 Comparison of Reflective Capacity in Different Mulch Layout

5.4.4.1 Measurements in October 2007, January and February 2008

Figure 2 shows the measurement results. There are 4 columns showing results in October, November, and January and February. Within each column, there are 3 rows showing reflective light around 10 am, 12 pm and 3 pm, respectively. In each subgraph, X-axes represent tree positions while Y-axes represent reflective light in $\mu mol/m^2 \bullet s$. There are 8 lines in subgraphs. Solid lines show trees in rep 5 (bottom 20 trees in Figure 1) while dash line show trees in rep 6 (top 20 trees). Different colours represent different treatments as shown in legend.

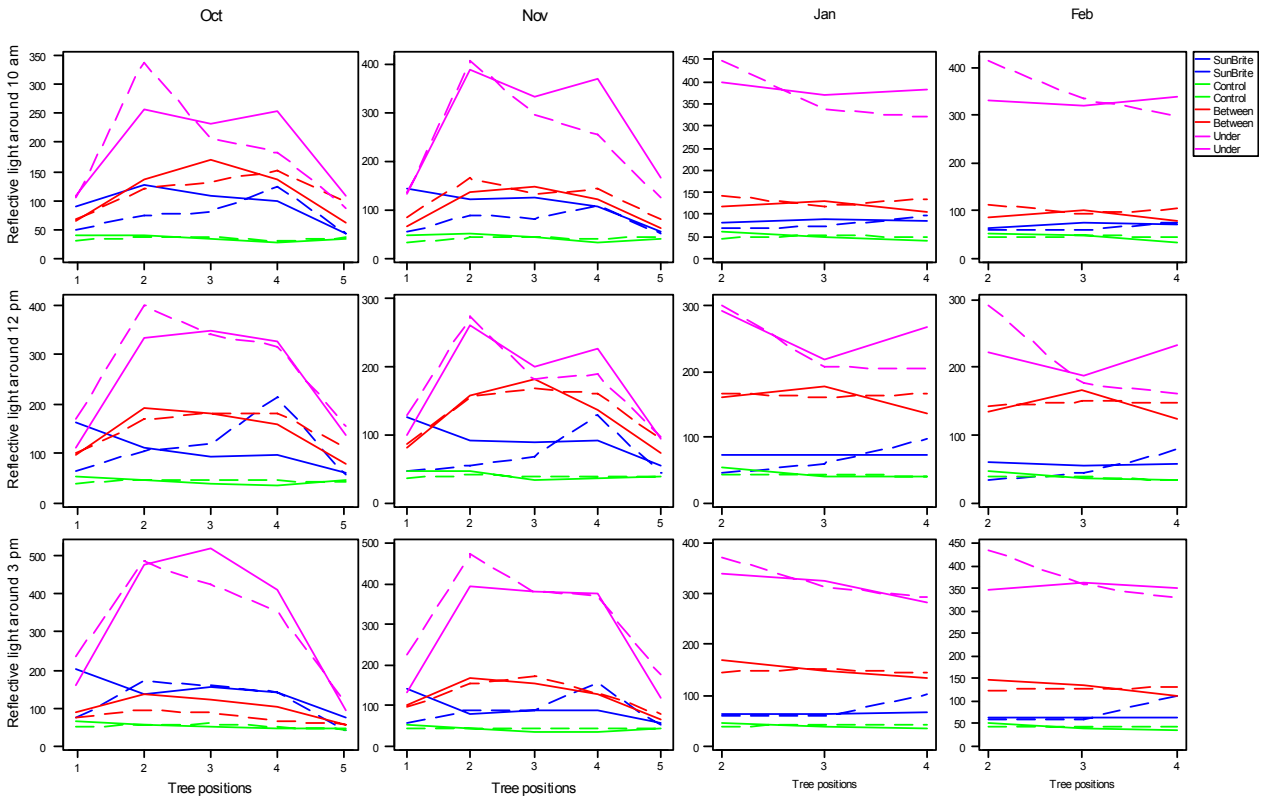


Figure 7: Comparison of reflective light of mulch between treatments and time

In the measurements in October and November, almost all treatments, trees 1 and 5 have lower reflection than trees 2, 3 and 4 (Note: tree 1 in rep 1 of Sun-Brite and tree 4 in rep 2 of Sun-Brite were relatively small trees with higher reflection. These 2 trees are only for light measurement but not for yield record). Trees 1 and 5 are at the boundary of the mulch. Their exact positions are beyond the mulch. In commercial production with long mulch, boundary trees are just a few trees and not important.

The major part is the tree 2, 3 and 4. Trees 2, 3 and 4 of the Extenday under canopy show significantly higher reflective light values than the Extenday between rows. In the October test, around 10 am and 12 pm, the Extenday between rows showed significantly higher reflection than Sun-Brite, however, around 3 pm, Sun-Brite showed significantly stronger reflection than Extenday between rows. But in late tests, Extenday between rows always showed better results than Sun-Brite. This implies that Sun-Brite was a good reflective tool just after it was sprayed. With increase of time, its reflective capacity becomes weaker and weaker. It still looked better than the control.

All the measurements, 4 treats x 4 months x 3 times x 3 tree positions x 2 replicates = 288 measurements, were pooled together for an analysis of variance. Analysis of variance showed that differences between treatments, between months and between measurement times reached a $p \leq 0.001$ level. Differences between tree positions reached a $p \leq 0.05$ level but no significant difference between replicates was found ($p=0.953$).

Table 17: ANOVA results for treatments, months, measurement times and tree positions

Treat	Reflect	Month	Reflect	Time	Reflect	Tree	Reflect
SunBrite	90.13C[z]	Oct	163.32A	10	146.07B	2	158.55a[y]
Control	42.41D	Nov	152.30AB	12	135.28B	3	144.91b
Between	140.00B	Jan	144.78AB	15	164.18A	4	142.06b
Under	321.49A	Feb	133.63B				
p-value	0.000		0.001		0.000		0.020

[z]Different capital letters show difference at $p \leq 0.01$;
[y]Different small letters show difference at $p \leq 0.05$.

From Table 17, in overall, Extenday under canopy showed very significantly ($p \leq 0.01$) higher reflection than Extenday between rows. Extenday between rows showed very significantly higher reflection than SunBrite. SunBrite showed very significantly higher reflection than control.

Besides the major conclusion, monthly comparisons showed that October had the strongest reflection while February had the weakest one. Measurement time comparison showed that 3 pm had the highest averages while 12 pm had the lowest averages. This probably shows that when the Sun position was low, the reflection was more useful.

As know, tree positions 2, 3 and 4 should not have significant difference. However, this test showed a significant different at the level of $p \leq 0.05$. From Figure 7, this is mainly due to tree 2 in rep 2. Its measurements were usually higher than trees 3 and 4 in the same replicate. The tree was a little bit smaller as was the front tree (tree 1) but they could not be defined as young trees.

5.4.4.2 Day-length measurements in November 2007

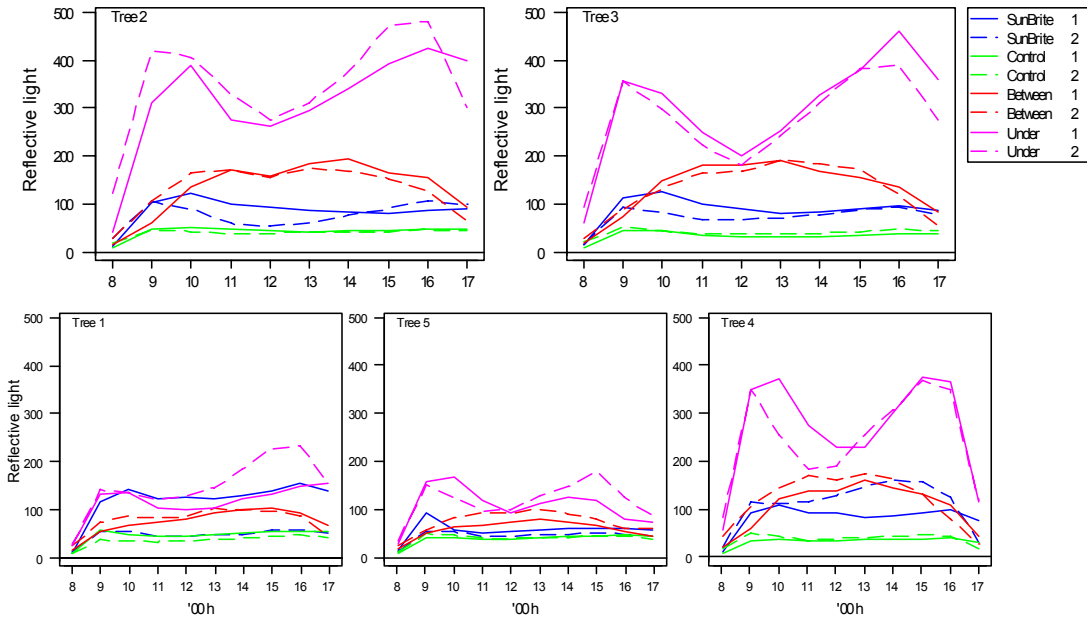


Figure 8: Reflective light in different times in different tree locations

From Figure 8, there was continuous change through time for each individual tree position. Each treatment/replicate has 5 trees. The key comparisons are trees 2, 3 and 4. For the whole graph containing 5 sub-graphs, trees 2 and 3 are put in larger sub-graph positions.

For trees 2, treatment of Extenday under canopy showed significantly higher reflection than the rest from beginning to the end. Treatment of Extenday between rows showed better results than treatment of Sun-Brite between 10 am and 4 pm. And there is no clear difference between treatment of Extenday between rows and treatment of Sun-Brite at 8 am, 9 am and 17 pm. Treatment of Sun-Brite showed clearly more reflection than the control.

For trees 3, it showed the same results as trees 2. Trees 4 showed very similar results as trees 2 and 3 except treatment of Sun-Brite in replicate 2. That was a young tree resulting in higher reflection. Although they were in low values, trees 1 and 5 also have similar results as above except treatment of Sun-Brite in replicate 1. This trial was set up in a 40-tree row and the first tree of replicate 1 of treatment of Sun-Brite is the first tree in that row facing north. In front of that tree there is a big gap. This is the reason for higher reflection.

For normal production with longer mulch what we were mainly concerned with were trees like trees 2, 3 and 4. Results were clear for reflection that the order was Extenday under canopy, Extenday between rows, Sun-Brite and control. Table 18 showed the detailed analysis. Besides significant differences between treatments, tree location and measurement time showed significant differences, especially around 11 am and 12 pm, where the reflection showed lower values.

Table 18: ANOVA results for treatments, measurement times and tree positions

Treat	p=0.000	Testing time (o'clock)	p=0.000
Sun-Brite	78.56 c	Around 8	26.65 c
Control	38.56 d	Around 9	121.27 ab
Between	103.91 b	Around 10	128.26 ab
Under	223.82 a	Around 11	111.49 b
Tree position	p=0.000	Around 12	105.59 b
1	83.92 c	Around 13	118.30 ab
2	146.97 a	Around 14	129.42 ab
3	134.34 ab	Around 15	140.42 a
4	124.51 b	Around 16	136.50 ab
5	66.33 d	Around 17	94.21 b

5.4.4.3 Measurements in December 2008

Figure 9 shows the measurement results on 8 December 2008. There are 3 sub-graphs for measurements around 10 am, 1 pm and 3 pm. Treatment of the Extenday under canopy show significantly higher reflective light values than all the others while control shows significantly lower reflective light values than all the others. Around 10 am and 3 pm Extenday, between the rows showed similar reflection to white plastic. Around 1 pm, all plastic showed low values while Extenday between rows had much sunlight and showed higher reflection. It should be mentioned that tree 4 in replicate 2 of plastic was a young tree. Thus its reading was usually higher than others but this does not represent the treatment level.

There were a few reasons for low reflection of white plastic. Firstly, it was narrower than the mulch under the tree. Whole plastic rolls were cut into two and each part was 1.25 m wide. After burying it in the soil, good fixing may remain 90 cm for reflection, bad fixing may remain 70 cm. Secondly, it is difficult to fix the plastic as it was very smooth and plain. If there was a pleat, it will be kept for the whole season. Above the pleat, dirt, sand, even leaves fallen from last season or a lot of rubbish may store there to reduce reflection. This never happened for Extenday.

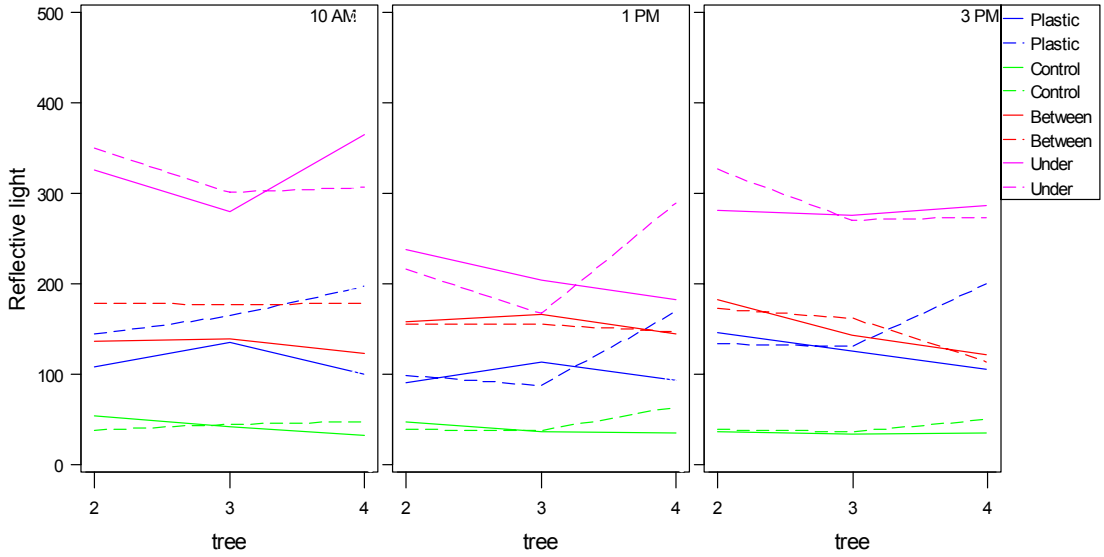


Figure 9: Comparison of reflective light of mulch between treatments and time

5.4.5 Soil Temperature Test

5.4.5.1 Variation in a growing season

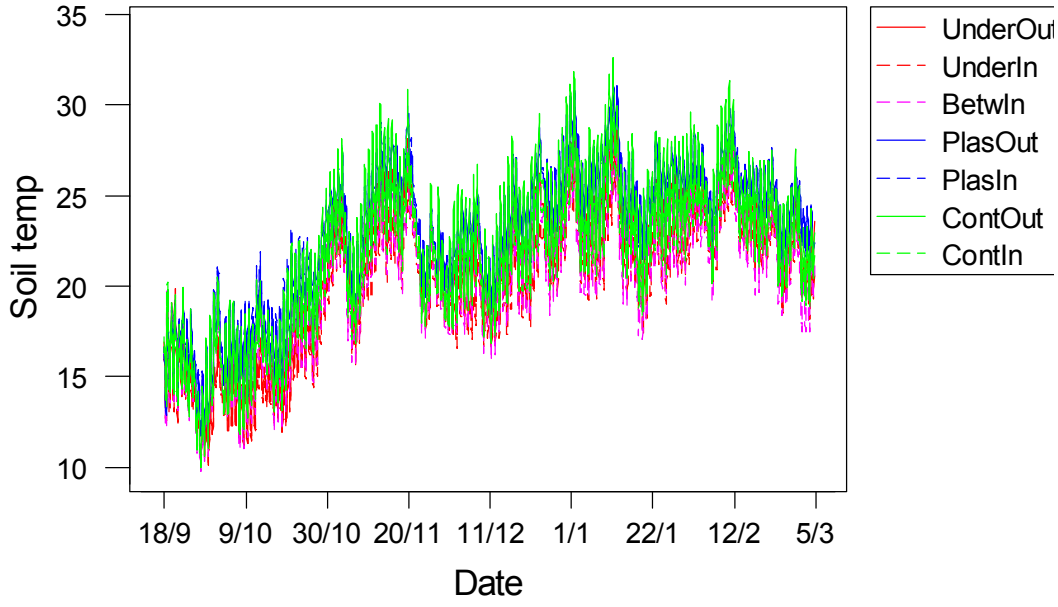


Figure 10: Soil temperatures in different locations

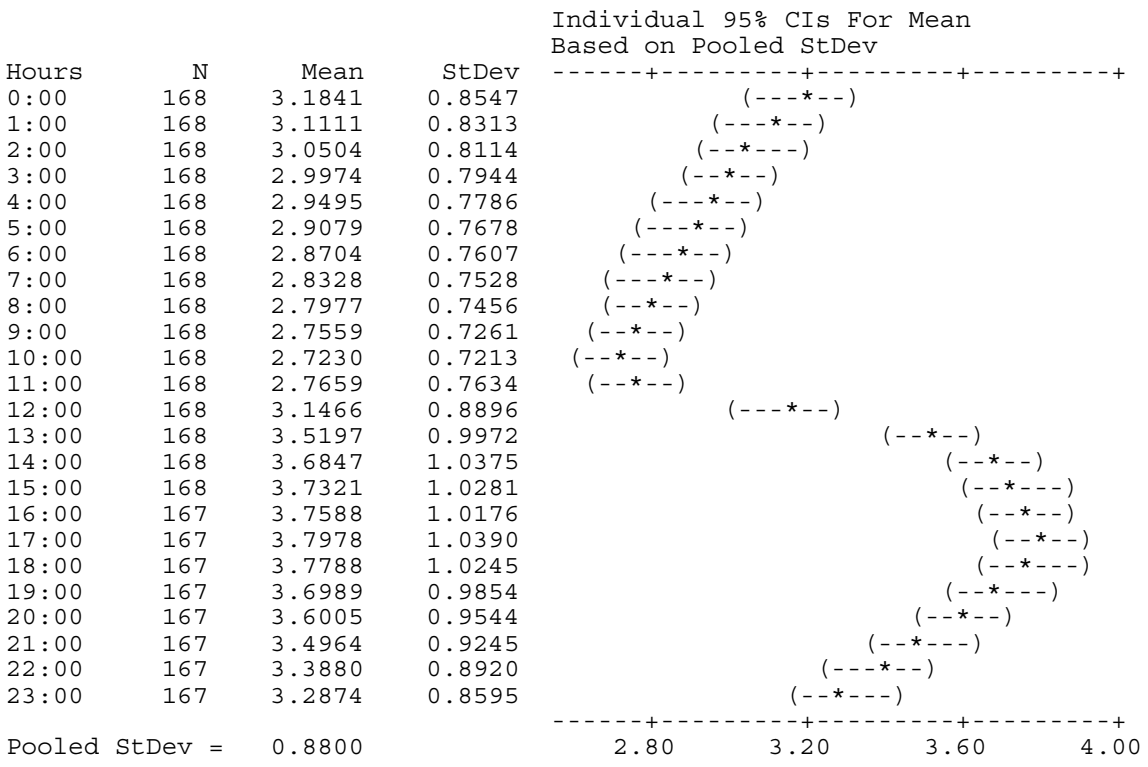
```
Analysis of Variance for Soil temperature
Source     DF        SS        MS        F        P
Source      6   38637.4    6439.6   426.49    0.000
Error   28161  425200.7      15.1
Total   28167  463838.1

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level           N      Mean StDev  -----+---------+---------+---------+-
Under Out    4024    20.793 4.060              (*)
Under In     4024    19.652 3.603  (-*)
Between Out  4024    20.530 3.696           (*)
Plastic Out  4024    22.924 4.007                                    (*)
Plastic In   4024    22.894 3.909                                    (*)
Control Out  4024    22.315 4.249                                (*)
Control In   4024    21.157 3.630                    (-*)
                                  -----+---------+---------+---------+-
Pooled StDev =    3.886             20.0      21.0      22.0      23.0
```

Figure 10 showed hourly temperature changes between 18 September 2009 and 4 March 2010 in different locations. As per the legend red and pink are for Extenday, blue for while plastic and green for control. Figure 10 clearly shows that red and pink were at the bottom while green and blue were at the top. This indicated that the mulch reduces instead of increasing soil temperature.

5.4.5.2 Effects in different hours

Under the mulch, temperature reduction compared with the control was different in different hours. To test this effect, a variable was created being the difference between the control inside and the mulch under canopy inside for each hour. The following analysis of variance showed this pattern. The maximum difference between the mulch and control is usually around 18:00 or 19:00. After 18:00 or 19:00, this difference reduces, until 10:00 in next day; then this difference increases gradually until 18:00. In the data logger recording, no day light saving hours were applied. Thus 18:00 here in summer time actually was 17:00 as we would normally have.

```
Analysis of Variance for difference between control inside and Extenday under
canopy inside in different hours
Source     DF        SS        MS        F        P
C8         23   563.382    24.495    31.63    0.000
Error    4000  3097.636     0.774
Total    4023  3661.018
```



5.5 Stylar End Lesion

5.5.1 Trial in 2007

5.5.1.1 Tree investigation

Field observations were carried on 1, 17, 22, 29 November, 6 and 13 December 2006. No abnormal observations were found on the 1 November. However, serious damages on leaves and nuts were found on 17 November after 4 foliar applications. Both double concentrations created damage problems on nuts and leaves. Half concentrations (0.4%) looked better, especially for $Ca(NO_3)_2$. In conclusion $Ca(NO_3)_2$ showed better results than $CaCl_2$, even with higher concentrations in the first application; both of double concentrations created serious damage problems on leaves and nuts.

5.5.1.2 Leaf analysis for trial SEL in February

For the SEL trial in this 'on' year, we decided to apply 2 Ca chemicals in 3 concentrations plus a control giving a total of 7 treatments. We decided to take 1 leaf sample for each treatment in February. The results are listed as follows:

Table 1: Leaf analysis results

Treatment	N	P	K	S	Na	Ca	Mg	Cl	Cu	Zn	Mn	Fe	NO_3	B
1.6% $CaNO_3$	2.29	0.136	1.590	0.128	0.020	2.348	0.510	0.263	131.62	45.94	29.2	84.3	459	165
0.8% $CaNO_3$	2.19	0.130	0.904	0.130	0.023	2.656	0.644	0.380	198.15	45.72	29.0	78.3	58	220
0.4% $CaNO_3$	2.03	0.124	0.965	0.121	0.022	2.957	0.737	0.414	176.37	41.17	31.4	81.6	100	240
Control	2.13	0.123	1.144	0.121	0.031	2.407	0.576	0.406	195.17	39.90	28.0	85.4	48	194
1.00%$CaCl_2$	2.12	0.121	0.772	0.120	0.024	2.662	0.648	0.740	146.11	39.38	25.6	82.6	39	200
0.50%$CaCl_2$	2.24	0.134	1.145	0.137	0.030	3.356	0.774	1.002	85.23	41.09	38.7	86.6	88	220

0.25%$CaCl_2$	2.21	0.129	1.054	0.133	0.033	3.060	0.748	0.684	137.31	41.43	36.8	90.1	89	280

There were no replicates and these result are for reference information.

Generally speaking, leaves with Ca treatments had higher Ca contents than the control except 1.6% $CaNO_3$. This is probably due to sampling error. However, the pattern was not clear that high concentration treatments must have high Ca content in the leaf analysis.

5.5.2 Trial in 2007/08

5.5.2.1 Trial in Production Scale at Stage 3

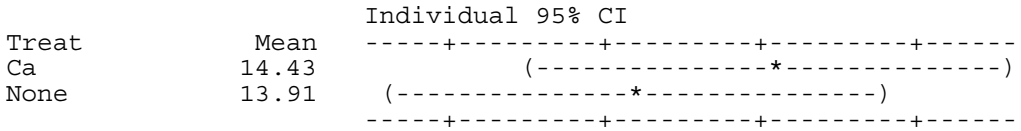
5.5.2.1.1 Yield Harvest

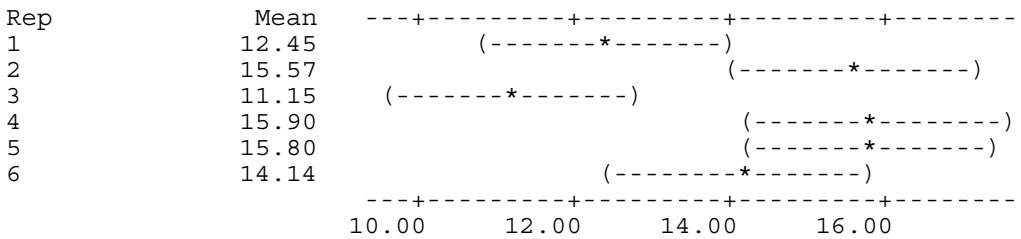
This trial was harvested on 5 March and 13 March 2008. In the first harvest, all bins were transported into APPC for weighing. In the second harvest, nuts were weighed in the fields before transferring into production bins. In both harvests, 10 kg of nuts were collected for each row for nut quality test. The following data shows harvest records.

Row	Treat	Rep	NoTree	Yield	Yield/tree	%1st shake
2	Ca	1	16	197.04	12.32	93
5	None	1	22	276.78	12.58	95
8	None	2	28	430.49	15.37	94
11	Ca	2	29	457.47	15.77	94
13	Ca	3	26	308.16	11.85	90
16	None	3	38	397.29	10.46	91
19	None	4	42	613.81	14.61	91
22	Ca	4	30	515.54	17.18	92
24	Ca	5	25	381.93	15.28	91
27	None	5	46	751.13	16.33	94
30	Ca	6	50	709.64	14.19	93
33	None	6	48	676.47	14.09	92

Analysis of Variance for Total Yield/tree

Source	DF	SS	MS	F	P
Treat	1	0.8267	0.8267	1.00	0.363
Rep	5	39.3923	7.8785	9.55	0.014
Error	5	4.1258	0.8252		
Total	11	44.3448			





Due to big variations between rows, no significant difference was shown in the above analysis. However, due to different percentages of rootstock Pioneer Gold in trial rows, this may be one of the major factor influencing SEL nuts. Figure 4 showed when the percentages of Pioneer Gold rootstocks increased, the percentages of SEL nuts increased. This implies that co-variance analysis will be more efficient for this case.

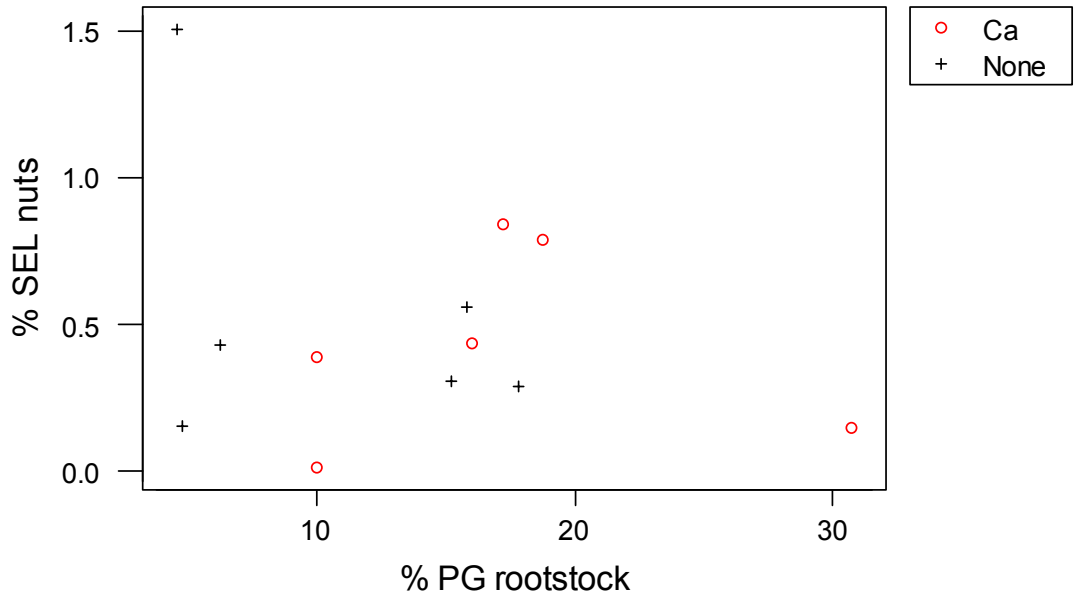


Figure 4: Relationship of percentages between of SEL nuts and Pioneer Gold rootstocks

Table 2: Comparison of SEL nuts percentages between treatments in total, shake 1 and shake 2

Treatment	Total	Shake 1	Shake 2
Ca spray	0.436%	0.444%	0.330%
Control	0.541%	0.530%	0.670%
p-value	0.496	0.506	0.128
Covariance p-value	0.178	0.265	0.763
Covariance p-value without transformation	0.072	0.053	0.840

Table 2 compared the percentage of SEL nuts for rows with and without Ca spray in shake 1, shake 2 and total by 2-way variance of analysis (ANOVA) above the double line and by covariance analysis (CANOVA) below the double line. For data in percentages, data were transferred by formula $\arcsin\sqrt{\frac{percentage}{100}}$ to obtained p-values. ANOVA showed no clear difference between treatments in total SEL nut percentage. CANOVA showed a difference reached at p=0.072 level. Although it did not reach a standard level of 0.05 it was very close. For a big production-scale trial in 4 hectare area, with different rootstocks, young trees and sick trees, a difference at p=0.07 level should be considered seriously.

5.5.2.1.2 Nut Quality

Table 3 shows the comparison for all stain nuts. Ca treatments produced low percentages of chocolate stain, but total dark stain, Golden stain and other dark stain were high. Light stain was a little low.

Table 3: Comparison of stain nuts percentages between treatments in total

Treatment	Dark Stain	Golden Stain	Chocolate stain	Other dark stain	Light stain
Ca spray	4.17%	2.39%	0.44%	1.34%	6.48%
Control	2.83%	1.63%	0.54%	0.66%	7.45%
p-value	0.327	0.568	0.496	0.045	0.290

Table 4 listed quality comparison for other items. Ca treatments seem to increase percentages of the number 1 grade of small nut and of adhering hull.

Table 4: Comparison of nuts qualities between treatments in total

Treatment	% No1 grade small	% No1 grade medium	% No1 grade Jumbo	% narrow split	% non-split	% blank, FM	% adhering full	% pickout
Ca spray	2.48%	69.8%	3.76%	2.41%	1.05%	5.52%	2.10%	6.60%
Control	1.44%	72.1%	4.54%	2.61%	1.41%	5.17%	0.74%	3.80%
p-value	0.078	0.366	0.495	0.336	0.594	0.521	0.096	0.167

Pickout = adhere hull + dark stain + shell damage

5.5.2.2 Trial in concentration and timing test at Stage 1

Table 5 showed investigation results for leaf damage. 0.8% $Ca(NO_3)_2$ is normally used in this work in these years. However, 0.8% $Ca(NO_3)_2$ as a late spray (last 3 sprays) showed more leaf damage than in early applications.

Table 5: Investigation results on leaf damages for different treatments

Date	1% KNO_3	control	Late 0.8% $Ca(NO_3)_2$	0.4% Ca	Early 0.8% $Ca(NO_3)_2$	p-value
23 Nov	0.0B	0.0B	1.3A	0.0B	0.2B	0.000
30 Nov	0.0B	0.0B	1.4A	0.1B	0.1B	0.002

This investigation also examined nuts with black tips, black sides and others and no significant difference was found.

5.5.3 Trial in 2008/09

Observations of the trees indicated there was no unusual damage. This indicated that Ca application during B spray may be useful in the future work.

5.5.4 Observation in 2009/10

Investigations were conducted on SEL nuts at Kaniva Pistachios on 1st December and orchards around Renmark on 2nd December. These orchards showed very light SEL nuts, probably 1 nut found for 2 or 3 trees. Investigations of SEL nuts at Kyalite Pistachios on 9th December produce much lighter SEL nut numbers found, probably 1 nut in 20 trees. Clearly SEL nuts did not result in big losses in this season.

5.6 Other Symptom Related to SEL Nut

5.6.1 Trial 2006/07

The tolerant test in 2006/07 led to black-side nuts. This was another chance for us to understand reasons for black-side nuts. The direct results are in Table 1.

Table 1: Elements in nut samples in treatments from direct results.

Treat	Nut	N	P	K	S	Na	Ca	Mg	Cl	Cu	Zn	Mn	Fe	NO_3	B
$CaNO_3$	Good	2.74	0.261	1.31	0.149	0.013	0.164	0.081	0.063	24.6	33.4	10.0	34.5	352	10.7
$CaCl_2$	Good	2.95	0.299	1.40	0.166	0.013	0.205	0.090	0.150	23.6	42.5	10.2	39.4	278	18.0
Control	Good	2.46	0.286	1.33	0.161	0.013	0.136	0.084	0.088	18.4	36.2	10.5	34.3	241	12.2
$CaNO_3$	Bad	3.38	0.323	1.64	0.179	0.013	0.326	0.096	0.081	43.6	41.5	13.0	36.8	572	18.3
$CaCl_2$	Bad	3.79	0.402	1.69	0.206	0.019	0.523	0.120	0.564	33.4	50.0	13.3	48.4	258	22.6

From Table 1, clear differences can be found showing that bad nuts had much higher values for elements. Table 2 lists the ratios of each element between good nuts and bad nuts (average of ratios from treatments $Ca(NO_3)_2$ and $CaCl_2$. Good nuts in the control were not included in this calculation). Here ratios of Cl and Ca were the highest ones being above 2. All the rest averaged around 1.29.

Table 2: Ratio of elements between bad nuts and good nuts.

Element	N	P	K	S	Na	Ca	Mg	Cl	Cu	Zn	Mn	Fe	NO_3	B
Ratio	1.26	1.29	1.23	1.22	1.23	2.27	1.25	2.52	1.59	1.21	1.30	1.15	1.28	1.48

Bad nuts were damaged nuts with black-sides. It could be imagined that the damage resulted in high respiration with higher use of carbohydrates but not inorganic elements. Results of tissue analysis are based on dry matter. Thus in damaged nuts, elements take bigger amounts than normal nuts. This also happened in results in 2005/06 (Table 3). Black-side and black-tip had relatively higher values for N, P, K, S, Na, Mg, Cu and B. However, Black-side had high Ca while black-tip had low Ca compared with good nuts. This indicates that nut damage and carbohydrate use may raise elements to a certain level. However, if an element is really low, it cannot be changed to high just. Historical analysis on black-tip nuts and SEL nuts, Ca content was still low in the nuts.

Table 3: Element comparison of black-tip nuts and black-side nuts with control and Ca treated nuts from report in season 2005/06

Source	N	P	K	S	Na	Ca	Mg	Cl	Cu	Zn	Mn	Fe	NO_3	B
Black-side	2.83	0.32	2.00	0.15	0.025	0.200	0.099ab	-0.003c	26.6	30.5	7.2b	44.3	2d	25a
Black-tip	3.07	0.33	2.03	0.18	0.021	0.101	0.104a	0.000c	21.3	34.0	10.4a	48.7	0d	23ab
$CaCl_2$	2.32	0.25	1.67	0.12	0.014	0.139	0.081b	0.255a	20.5	31.5	9.7a	34.7	85b	15c
Control	2.36	0.26	1.72	0.13	0.018	0.115	0.078b	0.193b	21.3	37.2	10.1a	34.0	70b	14c
$Ca(NO_3)_2$	2.37	0.28	1.82	0.15	0.011	0.137	0.085b	0.194b	22.0	36.3	10.4a	39.2	126a	17bc
$CaCl_2$ D	2.28	0.26	1.74	0.14	0.020	0.116	0.082b	0.181b	20.0	33.5	9.2a	44.9	36c	18bc
Control D	2.20	0.28	1.66	0.13	0.017	0.085	0.073b	0.204b	20.5	32.4	9.9a	38.6	36c	16c
$Ca(NO_3)_2$ D	2.03	0.29	1.85	0.13	0.013	0.104	0.071b	0.203b	19.1	33.4	9.8a	47.8	30c	20b
P	0.09	2.89	1.12	1.70	0.21	0.09	0.01	0.00	0.73	0.88	0.02	0.08	0.00	0.00

5.6.2 Trial 2007/08

5.6.2.1 Direct Results

Table 4 and 5 show analyzed results directly from the laboratory reports. From these tables the control had significantly lower N and S than all others. The control also had much lower P, K, NO_3, Na, Zn and Fe. Should we accept this result? Control nuts had lower values for most of the elements. Clearly, this cannot be true.

Table 4: The first parts of tissue analysis results of sampled nuts

Symptom	N	P	K	S	Ca	Mg	NO_3
New symptom	2.32ab	0.28ab	1.63b	0.143a	0.109b	0.072b	45.3b
Black-tip	1.95b	0.27b	1.93a	0.143a	0.136a	0.085a	86.0a
Black-side without Ca	2.30ab	0.30a	1.81ab	0.151a	0.141a	0.085a	47.7b
Black-side with Ca	2.51a	0.30a	1.81ab	0.160a	0.140a	0.078ab	57.7b
Control	1.60c	0.26b	1.52b	0.120b	0.138a	0.074ab	42.3b
p-value	0.000	0.024	0.007	0.006	0.096	0.094	0.002

Table 5: The second parts of tissue analysis results of sampled nuts

Symptom	Na	Cl	Cu	Zn	Mn	Fe	B
New symptom	0.014ab	0.148b	13.0b	28.8b	10.4	35.0a	19.3c
Black-tip	0.017a	0.234a	19.9a	37.9a	13.6	31.6ab	28.2a
Black-side without Ca	0.013b	0.150b	13.8b	28.9b	11.9	33.4ab	24.9b
Black-side with Ca	0.013b	0.135b	14.6b	31.8ab	11.3	33.1ab	23.8b
Control	0.011b	0.137b	14.0b	26.4b	10.7	28.5b	20.5c
p-value	0.029	0.000	0.000	0.029	0.569	0.125	0.000

5.6.2.2 Ratio of K/Ca and Ca/Mg

The ratios of K/Ca, Ca/Mg and K/Mg were used in element study over a long period. Although different nuts have different respiration rates, respiration costs organic nutrition. The elements such K, Ca and Mg should keep the same manner in the nuts. In this way, use one of them as the constant to divide the others, this constant may be more reliable. This operation will not be affected by respiration.

Table 6: The ratios of K/Ca, Ca/Mg and K/Mg for different samples

Symptom	K/Ca	Ca/Mg	K/Mg
Black-side	15.0a	1.52b	22.6
Black-tip	14.4a	1.59b	22.9
Black-side without Ca	12.8ab	1.66ab	21.4
Black-side with Ca	13.0ab	1.80ab	23.4
Control	11.0b	1.86a	20.6
p-value	0.064	0.039	0.630

From Table 6, although the one-way analysis of variance did not show a significant level (p=0.064), Fisher test showed that the control had significant lower K/Ca than black-side and black-tip. Ca/Mg show much clearer results than the control which had significant higher Ca/Mg than new symptom and black-tip. Both results mean that the control had higher Ca content than black-side and black-tip nuts. K/Mg did not reach a significant level and did not show any valuable result for this work.

Black-side nuts either on trees with Ca spray or on trees without Ca spray did not show a significant different from the control in this test.

From this analysis, it can be inferred that nuts with either black-side or black-tip have a low Ca level. Ca application may improve this problem. This was proved in investigations in this season. Also, in Mallee Orchard Pistachio, Ca spray also showed to reduced black-side nuts. This season, no SEL nut was found. Ca application should reduce black-side nuts.

Black-side nuts look not to have Calcium deficiency symptoms. Last year's results showed that black-side nuts had too higher Ca than the control. However, test in this season did not show this result. Further studies are necessary for black-side nuts.

5.7 Hand Pruning Trial to Overcome Alternate Bearing

Fresh yield in hull per tree was a most practical parameter, which directly links with production. Table 7 showed the harvest results for yield averages for each treatment. Different letters follow the number showing a significant difference at $p \leq 0.05$ level. The last row each year showed a p-value from the 2-way analysis of variance.

For fresh yield in hull per tree, the Control showed significant higher yield than the other 2 treatments in 2005, significant higher yield than treatment Middle in 2006 and highest yield on average in 2007. Treatment Aggressive had the highest yield on average in 2008. No significant difference of biennial bearing indices was found between treatments although treatment Aggressive had the smallest index. However, for cumulative yields in 4 years the control still showed the highest ones, which was significantly higher than treatment Middle. This is also clear from Figure 1.

Table 7: Harvest results for yield

Year	Treat	Yield /tree (kg)	Mercha ntable yield (kg)	% First Shake	Biennial bearing index	Count size	Total income ($/tree)	Cummul ative yield/tree	Nut /cluster
2005	Aggressive	42.8b	14.2b	80.5	N/A	92.8	87.6b	N/A	
	Middle	42.4b	14.9b	78.1		93.2	93.9b		
	Control	49.2a	17.5a	76.1		94.1	108.7a		
	p-value	0.003	0.002	0.244		0.522	0.004		
2006	Aggressive	35.7a	13.5ab	89.3	9.1	80.4	85.55b	78.5b	32.8ab
	Middle	30.7b	11.8b	89.9	15.8	79.1	74.85b	73.1b	35.4a
	Control	37.7a	15.1a	89.5	14.1	79.7	97.95a	86.9a	30.8b
	p-value	0.020	0.006	0.917	0.429	0.320	0.002	0.002	0.021
2007	Aggressive	40.2	14.5	74.0	8.2	76.4	101.24ab	118.7b	
	Middle	38.1	15.0	75.8	14.4	77.7	97.30b	111.1b	
	Control	43.3	14.7	73.1	12.5	77.6	113.88a	130.2a	
	p-value	0.134	0.905	0.472	0.257	0.180	0.036	0.004	
2008	Aggressive	14.9	5.90	32.5	21.1	89.1	39.2	133.6ab	
	Middle	11.9	4.73	35.1	27.9	91.3	30.9	122.5b	
	Control	13.3	5.25	34.7	28.2	90.1	35.0	143.5a	
	p-value	0.688	0.742	0.907	0.292	0.641	0.716	0.027	

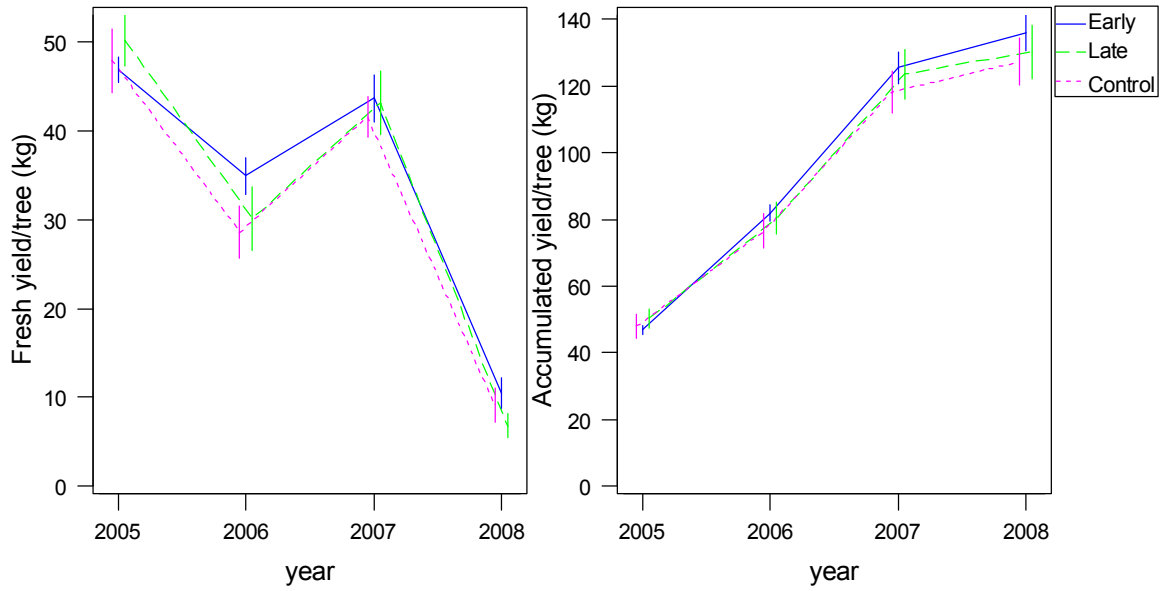


Figure 1: Yield and accumulative yield per tree between 2005 and 2008

Table 7 also shows that merchantable yield/tree had the similar pattern as fresh yield. In the first 3 seasons the Control showed the highest returns than the other 2 treatments due to higher yields. Count size did not show clear differences in both seasons.

Also, before harvest 2006, 20 clusters were harvested from each trial tree. Table 8 shows that the control had the lowest nut numbers per cluster while treatment Middle had the highest ones.

Table 9: Percentages of nut qualities in physiological aspects

Year	Treatment	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damage shell
2005	Aggressive	3.60	41.1	0.00	4.81	4.37	4.59	8.96	6.24	0.75
	Middle	4.75	42.0	0.01	5.40	3.78	3.45	7.23	6.16	0.85
	Control	5.46	40.3	0.04	5.59	4.29	3.62	7.90	5.38	0.73
	p-value	0.121	0.780	0.563	0.506	0.473	0.094	0.104	0.142	0.720
2006	Aggressive	2.37	51.0	0.03	9.17a	6.54	3.61	10.15	3.24b	0.70
	Middle	2.10	47.7	0.04	7.44b	6.40	3.81	10.21	4.78a	0.50
	Control	2.07	54.1	0.03	7.45b	4.50	3.05	7.55	3.15b	0.66
	p-value	0.619	0.121	0.979	0.050	0.145	0.442	0.103	0.000	0.246
2007	Aggressive	0.95	56.6a	0.40	5.92	3.29	2.51	5.80	5.62	0.36
	Middle	0.42	49.8c	0.63	4.39	3.13	2.45	5.41	6.31	0.77
	Control	0.73	53.3b	1.10	4.13	2.71	2.69	5.58	5.38	0.86
	p-value	0.546	0.027	0.621	0.175	0.543	0.831	0.543	0.598	0.093
2008	Aggressive	2.39	53.5	2.80	3.32	1.54	1.34	2.88	5.26a	0.13
	Middle	2.96	52.2	3.03	3.73	1.33	1.09	2.42	5.32a	0.16
	Control	3.36	58.7	3.37	3.03	0.90	0.70	1.60	3.34b	0.21
	p-value	0.548	0.058	0.764	0.877	0.142	0.218	0.103	0.018	0.719

In 'off' seasons 2006 and 2008 the Control had significantly lower percentages of blank nuts. In 2006 the Control also had significantly lower percentages of narrow split nuts than treatment Aggressive.

Table 9: Percentages of nut qualities in stain aspects

Year	Treatment	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	% other stain	% light stain
2005	Aggressive	14.72	0.20	4.59a	9.11	2.26	6.39	20.4
	Middle	12.27	0.15	2.29b	8.74	2.68	6.27	22.0
	Control	13.13	0.18	2.75b	9.15	2.46	6.60	22.0
	p-value	0.091	0.454	0.018	0.919	0.156	0.903	0.708
2006	Aggressive	8.93	0.07	1.10	6.91	0.90	6.01	15.0
	Middle	10.12	0.12	0.95	8.45	0.98	7.47	17.5
	Control	8.27	0.08	0.93	6.46	0.87	5.59	17.3
	p-value	0.322	0.275	0.979	0.137	0.760	0.134	0.462
2007	Aggressive	6.8	0.09	1.68	4.71	1.83	2.88	17.8
	Middle	9.2	0.06	1.97	6.30	1.93	4.37	23.6
	Control	9.7	0.05	2.15	6.39	1.94	4.44	20.2
	p-value	0.222	0.677	0.746	0.563	0.904	0.693	0.119
2008	Aggressive	10.38	0.01c	1.13	8.64	8.15	0.30	19.5
	Middle	13.08	0.13b	1.55	10.90	10.08	0.33	17.1
	Control	10.76	0.24a	1.02	9.24	8.65	0.01	15.6
	p-value	0.064	0.001	0.342	0.212	0.300	0.093	0.326

Table 9 shows returns and percentages of commercial requirements. There were no many significant differences between treatments. 2 significances in Table 9 did not show clear meaning.

In year 2 and year 3 with maintaining the pruning, trees with "severe" and "mid" pruning treatments were open inside and extended outside into higher positions. At the end of year 3, tree heights of those 2 treatments looked higher than the control. However, in slant mechanical pruning in winter 2007 at CMV Farms, those trees were cut more severe than the control and lost some of their advantage in the pruning treatments last 2 years. Due to reasons mentioned above, the importance of keeping this trial is questioned. This trial was stopped after 4 years.

5.8 Hand Thinning Trial to Overcome Alternate Bearing

5.8.1 Yield

Table 6 shows the harvest results for yield averages for treatments. To describe the variations for treatments, standard deviations are attached after a sign "±". The last row in each year shows a p-value from the 2-way analysis of variance.

Table 6: Comparison of yield parameters between treatments in harvest 2005 - 2008

Year	Treat	Yield in hull (kg/tree)	Merchantable yield (kg/tree)	Nut /cluster	Biennial bearing index	Count size	Total income ($/tree)	Cummulative yield/tree
2005	Early	46.9±4.5	15.9			94.1	97.49	46.9
	Late	50.2±9.0	17.6	N/A	N/A	94.3	107.54	50.2
	Control	47.9±11.3	16.7			94.2	103.55	47.9
	p-value	0.711	0.547			0.978	0.579	0.711
2006	Early	35.0±6.7	12.0	32.3±18.3	0.151b	84.6	72.58	81.8
	Late	30.1±11.4	11.0	34.3±19.9	0.264a	82.9	66.71	80.4
	Control	28.6±9.2	10.1	32.8±18.5	0.274a	83.1	61.12	76.5
	p-value	0.227	0.222	0.515	0.037	0.236	0.414	0.385
2007	Early	43.7±2.66	16.8	29.9±0.85	0.105a	81.8	109.5	125.5
	Late	43.2±3.60	17.4	29.8±1.99	0.175b	81.1	114.2	123.6
	Control	41.6±2.32	16.8	28.7±1.93	0.183b	79.8	109.1	118.1
	p-value	0.825	0.970	0.880	0.006	0.263	0.845	0.353
2008	Early	10.4±1.73	4.2		0.295	93.4	27.5	135.9
	Late	6.8±1.35	2.8	N/A	0.404	90.8	17.8	130.3
	Control	9.1±1.94	3.9		0.390	94.6	25.4	127.3
	p-value	0.319	0.327		0.060	0.584	32.6	0.304

Fresh yield in hull per tree is a most practical parameter, which directly links with production. There was no significant difference between treatments in all years. In the harvest of 2005, most of the trees had yielded around 45 kg/tree. On average, the late thinning was the most while the early thinning was the least. Thinning reduced variations of yields between trees, especially the early thinning. In 2006 there was no hand thinning. Treatments of early thinning showed the highest yield while control showed the lowest yield. In 2007 there was hand thinning again. The early thinning showed the highest yield while the control showed the lowest yield although the early thinning were aggressively hand thinned again. But 2-way analysis of variance did not reach a significant level. This was expected. Before an 'on' year, we hoped that thinning did not reduce crop but benefit crop in the following year. In 2008 there was no hand thinning as this was a big 'off' year in the 4-year cycle. Before bud break, flower shoots per tree were counted for each of the trial trees. The trees with early thinning last year showed more flower shoots per tree (74.2 ± 10.2) than the other 2 treatments (late thinning with 47.4 ± 8.4 and control with 49.4 ± 9.7) but the difference did not reach a statistical level. From harvest, the early thinning showed the highest yield but 2-way analysis of variance did not reach a significant level. Figure 2 summarized average yields per tree of each treatment for 4 harvests with standard error bars. The left graph shows average yield per tree while the right graph shows accumulated yield per tree. For early thinning, it was the lowest average in harvest 2005; in harvest 2006, it became the highest average and it kept the highest average in harvest 2007 and 2008. Among the 3 treatments, it showed the least reduction in the off-season. Table 6 shows a lower biennial bearing index (Zhang, 2009) from early thinning than the other 2 at the p-value of 0.06. This implies

that nutrition saving from thinning 2004/05 benefits the crop in 2005/06, and the nutrition saving from thinning 2006/07 benefited the crop in 2007/08.

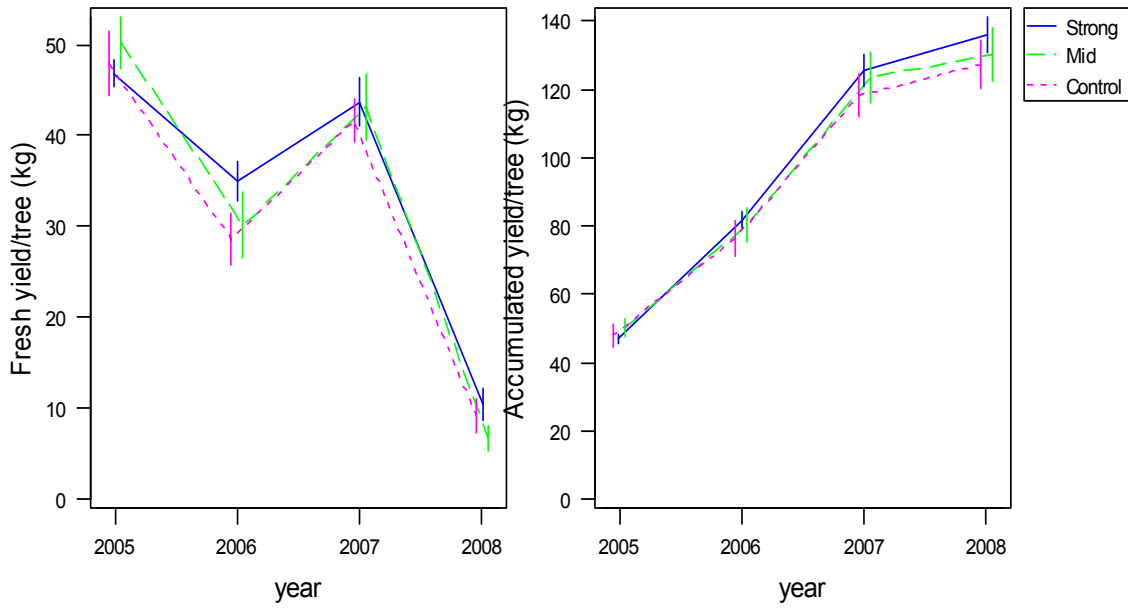


Figure 2: Average yield/tree in harvest 2005 – 2008

In Figure 2 the right graph shows results for accumulative yields in the 4 trial years. Early thinning clearly showed the highest accumulative yields in the 4-year period and the control was the lowest one. Unfortunately this difference did not reach a statistical level.

Table 6 also shows that merchantable yield/tree had the similar pattern as fresh yield. Also, before harvest, 20 clusters were harvested each trial tree in 2006 and 2007. Nut numbers per cluster did not show significant differences between treatments although treatment strong showed the highest average but just 1 nut more than others per cluster.

5.8.2 Nut Quality

Table 7: Percentages of nut qualities in physiological aspects

Year	Treatment	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damage shell
2005	Early	3.48	38.9	0.03	5.08	5.2	5.0	10.11	5.98	0.49
	Late	4.16	38.6	0.02	4.80	4.9	4.7	9.65	5.16	0.53
	Control	5.34	38.1	0.03	5.26	4.6	5.1	9.74	5.24	0.52
	p-value	0.095	0.942	0.894	0.711	0.762	0.807	0.918	0.244	0.930
2006	Early	3.25	57.2	0.151	6.14	3.52	2.81	6.33	4.26	0.723
	Late	2.19	57.6	0.286	5.44	3.31	3.05	6.35	4.69	0.719
	Control	3.00	57.5	0.188	5.47	4.48	2.51	6.99	4.87	0.816
	p-value	0.130	0.982	0.423	0.327	0.085	0.529	0.719	0.544	0.714
2007	Early	0.75	46.3	0.14	5.29	2.96	2.56	5.52	5.46	0.688
	Late	0.59	44.8	0.32	5.59	2.86	2.03	4.89	4.99	0.628
	Control	0.65	43.7	0.36	5.44	3.24	2.02	5.26	5.07	0.439
	p-value	0.882	0.593	0.936	0.976	0.882	0.332	0.671	0.880	0.307
2008	Early	3.13	52.0	2.54	3.62	0.81	1.06b	1.87b	5.31	0.120
	Late	4.25	51.5	2.44	3.69	1.35	1.77a	3.12a	4.59	0.162
	Control	3.63	52.1	3.40	4.36	0.84	1.15b	1.99b	4.82	0.309
	p-value	0.653	0.862	0.207	0.447	0.090	0.024	0.001	0.679	0.435

For percentages of nut qualities in physiological aspects (Table 7), 2 significant differences were found in season 2007/08. They were percentages of floating and total non split nuts. In this finding late thinning showed the worst results. This has no importance to the trial.

No significant difference was found for percentages of nut qualities in stain aspects between treatments in different years (Table 8).

Table 8: Percentages of nut qualities in stain aspects

Year	Treatment	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	% other stain	% light stain
2005	Early	14.9	0.22	3.55	10.44	2.53	7.91	21.4
	Late	15.8	0.09	3.02	11.85	2.26	9.59	21.8
	Control	14.1	0.14	2.67	10.74	2.41	8.01	22.0
	p-value	0.559	0.175	0.443	0.487	0.799	0.270	0.895
2006	Early	13.51	0.114	3.07	9.78	2.08	7.71	9.01
	Late	12.20	0.124	2.33	9.11	1.60	7.48	11.16
	Control	11.94	0.126	1.72	9.38	1.61	7.77	9.95
	p-value	0.420	0.989	0.325	0.782	0.289	0.967	0.238
2007	Early	10.6	0.134	1.89	7.78	2.61	5.14	25.7
	Late	8.8	0.087	1.50	6.31	2.36	3.95	29.8
	Control	10.2	0.052	2.52	6.95	2.35	4.60	29.2
	p-value	0.460	0.087	0.507	0.568	0.795	0.589	0.432
2008	Early	13.5	0.115	2.15	10.6	10.1	0.214	17.9
	Late	13.1	0.131	2.07	10.4	9.9	0.128	17.2
	Control	13.3	0.073	2.36	10.0	9.7	0.040	16.4
	p-value	0.969	0.644	0.854	0.949	0.976	0.141	0.796

5.9 Mechanical Pruning Trial at CMV Farms

5.9.1 Yield

Table 2: Yields, count size and returns

Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Return ($)
No	13.5	95.4	80.5	5.00	34.80
Pruned	12.6	95.1	80.0	4.60	32.00
p-value	0.579	0.333	0.680	0.522	0.507

From Table 2, pruned rows had a little lower yield on average but far from a significant level. Also, pruning did not make count size different.

5.9.2 Quality

Quality tests are listed in Tables 3 and 4. Pruned rows showed significantly lower pick out and damaged shells but significantly higher non-split nuts and total non-split nuts. Pick out was a sum of damaged shell, dark stain and adhere hull. In Table 3 and 4, pruned rows showed all these items with lower values compared with the non-pruned rows, but damaged shells reached a significant level. We cannot explain the reason for low damaged shell from pruning. Further observations are required.

Table 3: Percentages of nut qualities in physiological aspects

Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
No	0.471	62.9	4.26	1.35	1.45	1.20	2.66	6.76	1.69
Pruned	0.327	63.0	4.39	1.67	2.64	1.58	4.21	6.29	1.14
p-value	0.241	0.967	0.988	0.416	0.008	0.111	0.012	0.411	0.027

Table 4: Percentages of nut qualities in stain aspects

Treat	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
No	5.04	0.408	0.164	3.18	1.29	0.134	1.62	16.2
Pruned	4.28	0.408	0.146	2.98	1.04	0.151	1.67	15.4
p-value	0.021	0.746	0.648	0.288	0.245	0.592	0.703	0.851

However, pruning made percentages of non-split nuts significantly higher than non-pruned rows. This also led to a high percentage of total non-split nuts.

It is too early to make any conclusion.

5.9.3 Tree Response

The following tables were investigated in winter 2010.

Rows	2	3	6	7	10	12	14	16
Average	50	50	50	30	30	40	30-50	50
maximum	90	90	70	70	60	60	70	80

Investigation before pruning on the pruning side

Rows	1	4	5	8	9	11	13	15
Average	40	30	30	25	30	25	30	30

5.10 Mechanical Pruning Trial at Kyalite Pistachios

5.10.1 Pruning Checking

After pruning, an investigation was carried out to find pruning intensity levels. Although we want to set up a correct pruning height for a row, it is impossible to set up a unique pruning height to suit every tree, particularly in Kyalite Pistachios, where tree heights have very big variations. In this situation, a pruning height that fits most of the trees will be the best choice.

Every tree in the trial rows were check and marked the maximum-age wood to be pruned for that tree. For example, if a tree was topped with 1-year-old wood, 2-year-old wood and 3-year-old wood at the same time (this is normal phase). This tree was marked as 'maximum-age wood pruned' as 3. Thus maximum-age wood pruned has been marked as 2, 3, 4 or 5. For young trees (defined as not flower buds including sick trees), they are marked as "0". Based on results of this investigation, summarized numbers of trees under different maximum-age pruned for each row.

To evaluate pruning results in each row, a score was calculated as a sum of percentage of each age multiplied by that age. Most of them were between 2 and 3. That indicated that on average the maximum age pruned was between 2 and 3. For treatment averages, double topping was 2.44, single topping was 2.29. This indicated pruning taken correct height and angle.

5.10.2 Yield

Table 2: Yields, count size and returns

Harvest	Treat	Yield in hull/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Return ($)	Accumulated yield in hull/tree (kg)
	On-year 1 side	51.2	78.6	91.6	16.0	102.50	
2009	On-year 2 sides	49.0	78.1	92.7	15.5	98.15	
	p-value	0.132	0.792	0.061	0.246	0.166	
	On-year 1 side	11.4b	65.8b	79.9	3.83	23.28	62.5
2010	On-year 2 sides	14.7a	73.5a	80.5	4.78	29.44	63.7
	Off-year 2 sides	12.5ab	61.1b	78.1	4.21	26.61	
	p-value	0.032	0.001	0.079	0.096	0.086	0.600

In harvest 2009, pruning on 1-side had higher average yield in hull per tree, merchantable yield per tree and return than pruning on 2-sides (Table 2). In 2010, pruning in an 'on' year 2-sides had significantly higher average yield in hull per tree than pruning in an 'on' year 1-side. Merchantable yield per tree

and return for pruning in an 'on' year 2 sides also showed higher values on average than pruning in an 'on' year 1 side. For yield and return, pruning in an 'off' year 2 sides were in the middle. Pruning in an 'on' year 2 sides also showed significantly higher percentages of yield in shake 1 than the other two. Table 2 also listed accumulated yield. Pruning in an 'on' year 2 sides had a low crop in 2009 and this led to no significant difference for accumulated yields between treatments. Pruning 'off' year 2 sides showed the best count size but did not reach a significant level.

5.10.3 Quality

In 2009, for the quality tests in Tables 3 and 4, most of them did not show any clear difference. However, pruning in an 'on' year 1 side showed higher percentages of blank nuts than pruning in an 'on' year 2 sides while pruning in an 'on' year 2 sides showed higher percentages of light stain nuts than pruning in 'on' year 1 side. However, these 2 characters did not show clear differences in the following year.

In 2010, pruning before an 'off' year 2 sides showed significantly higher percentages of medium unstained nuts than the other two. This treatment also showed lower percentages of total non-split nuts. For all other elements they did not reach a significant level.

Table 3: Percentages of nut qualities in physiological aspects

Harvest	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
2009	On-year 1 side	1.85	59.3	0.30	8.25	8.99	2.30	11.3	5.39a	1.32
	On-year 2 sides	1.84	55.9	0.26	8.59	10.16	2.74	12.9	4.23b	1.29
	p-value	0.873	0.149	0.604	0.726	0.346	0.294	0.300	0.038	0.818
2010	On-year 1 side	0.33	46.3b	1.76	3.83	7.98	6.13	14.11a	7.93	1.52
	On-year 2 sides	0.33	47.0b	1.89	4.17	7.32	5.30	12.63ab	7.88	1.32
	Off-year 2 sides	0.27	51.9a	2.76	3.78	6.72	3.99	10.71b	8.00	1.24
	p-value	0.749	0.018	0.207	0.813	0.425	0.069	0.035	0.987	0.524

Table 4: Percentages of nut qualities in stain aspects

harvest	Treat	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2009	On-year 1 side	7.45	0.18	1.15	5.00	1.40		3.72	5.95b
	On-year 2 sides	7.49	0.27	0.84	5.36	1.59		3.64	8.46a
	p-value	0.944	0.239	0.418	0.399	0.366		0.836	0.017
2010	On-year 1 side	15.92	0.43	1.83	12.1	3.22	0.24	7.12	9.37
	On-year 2 sides	13.31	0.55	2.10	11.7	3.31	0.20	6.61	10.20
	Off-year 2 sides	13.16	0.60	1.35	10.3	2.62	0.14	6.01	8.83
	p-value	0.322	0.195	0.293	0.471	0.307	0.444	0.627	0.599

5.11 Research on Chilling Requirement

5.11.1 Greenhouse

5.11.1.1 Greenhouse work

5.11.1.1.1 Determine which bud development stage used in this work

To test 50% flowering within 3 weeks, firstly, we needed to identify which bud development stages should be used from the 6 stages recorded. Because we were looking at the whole population a simple average could not be examined by a statistical procedure. Thus, averages on a tree and shoot basis should also be considered. Results describe the percentages in different levels.

Figure 1 shows results for total averages of percentages of bud break in different stages. Dates of collection times can be found in Table 1. A line at 50% is placed in each sub-graph for the reader to easily identify 50% bud break.

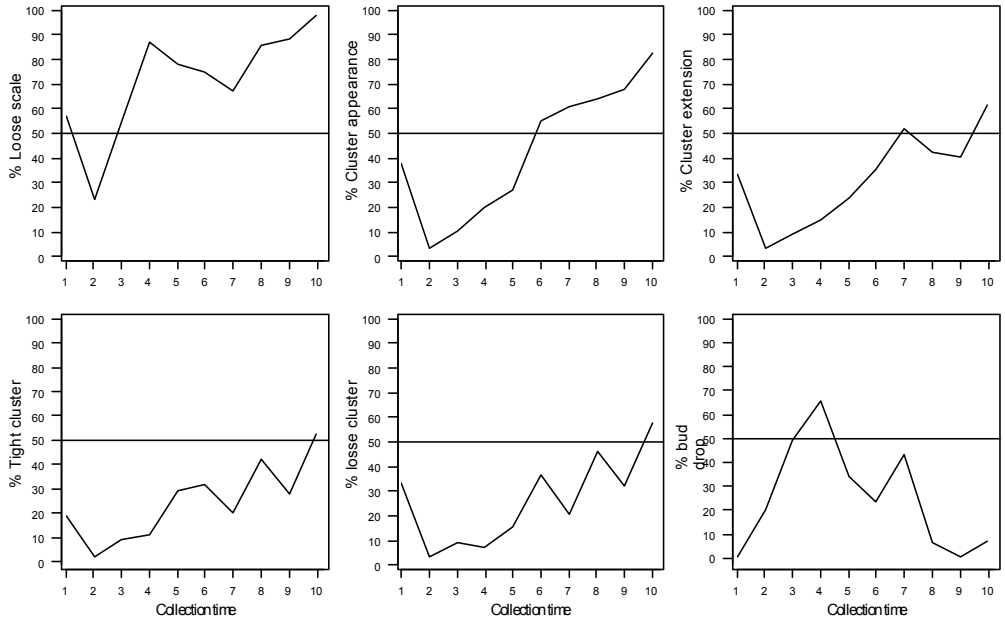


Figure 1: Percentages reaching different stages in collection dates

Figure 2 shows averages for the percentages on a tree basis. Each average was from 2 sources, percentages from an eastern shoot and that from a western shoot.

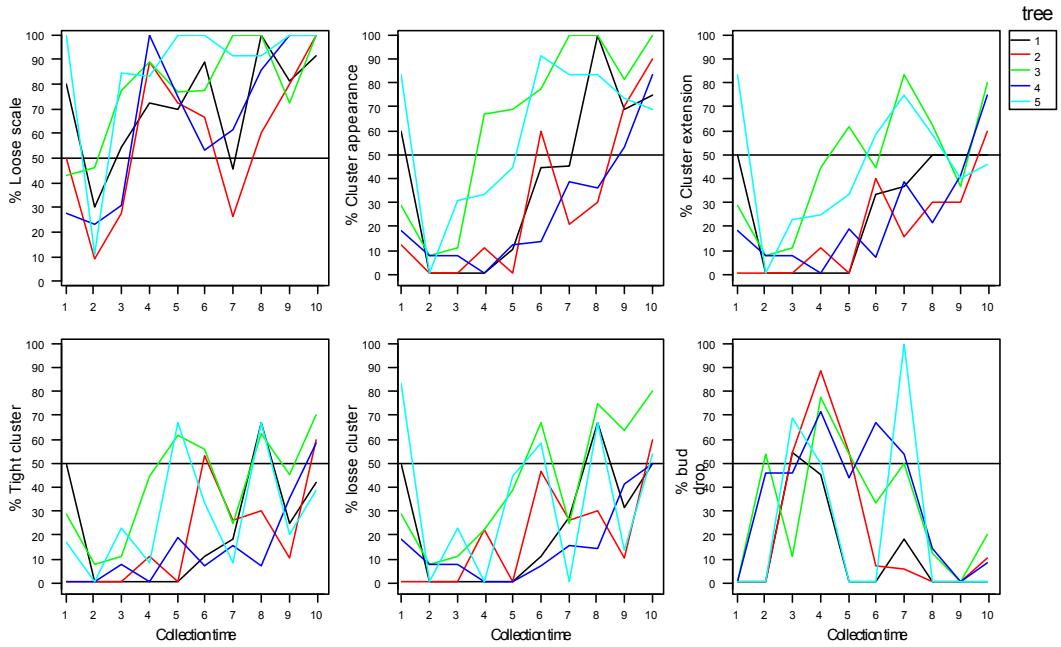


Figure 2: Percentages reaching different stages in collection dates on tree basis

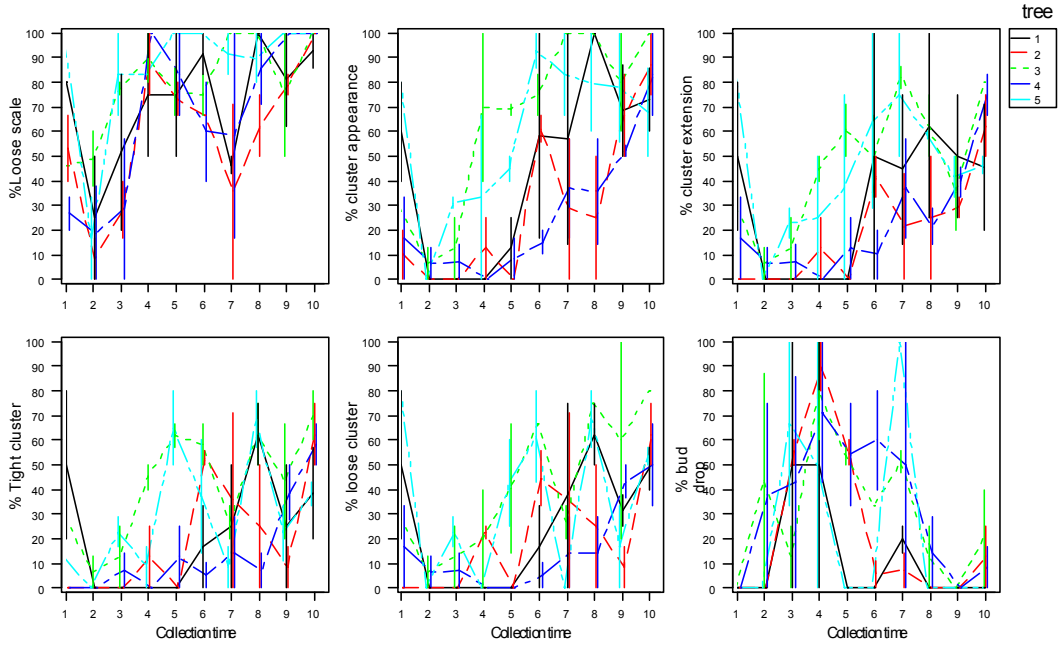


Figure 3: Percentages reaching different stages in collection dates on tree basis with standard error bar

Figure 4 summarises the minimum, 1st quartile, medium, 3rd quartiles and maximum for percentages of each shoot for each collection date. Again, the last 4 stages were not consistently reached by 50% of buds.

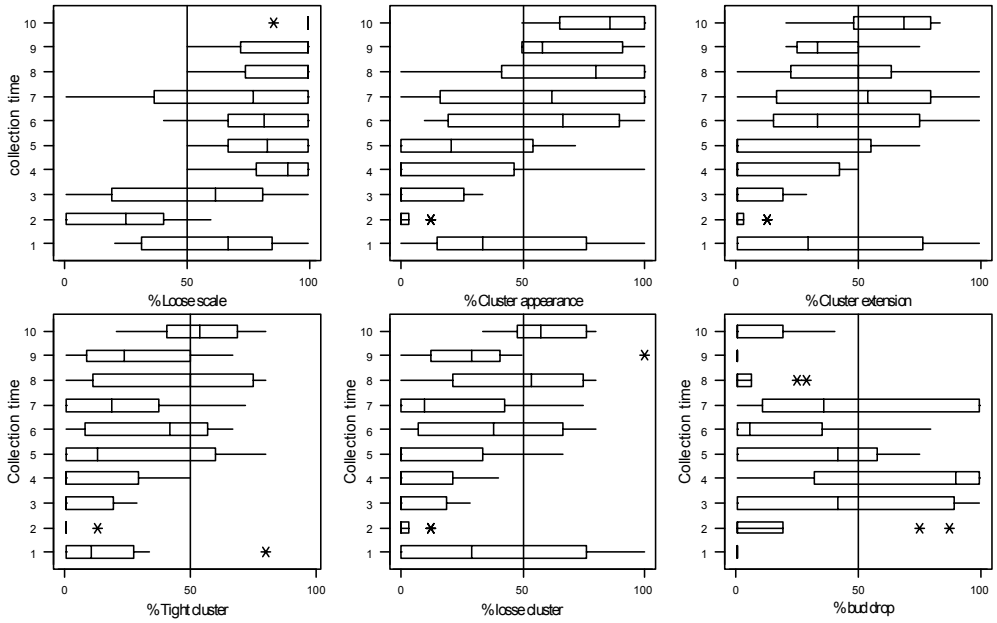


Figure 4: Percentage population description for bud development stages in different collection dates

From Figure 1-4, % cluster extension, % tight cluster, % loose cluster and % bud drop barely reached 50% and therefore they were of little use in this work. On the other hand, loose scale was too early to be used. Felker and Robitaille (1985) divided cherry bud development into 6 stages and they used stage 3 as the standard for chill completion. Ghariani and Stebbins (1994) used the green tip stage for apple and pear. Clearly, for chilling completion and bud break, the importance is bud break instead of full bloom. When using cut shoots, bud break should be easily reached. Full bloom needs more nutrition support. Cut shoots cannot reach full bloom due to nutrition problems not lack of chill. For our work, cluster appearance was the stage we used to describe budbreak.

5.11.1.1.2 Chill requirement fulfillment

50% bud break is not the sole measure of chill completion. Bud break within 3 weeks of cutting is another standard. Figure 5 illustrates frequencies of buds reaching cluster appearance (bud break) for each collection date. Three weeks are shown in the graphs by the vertical bars.

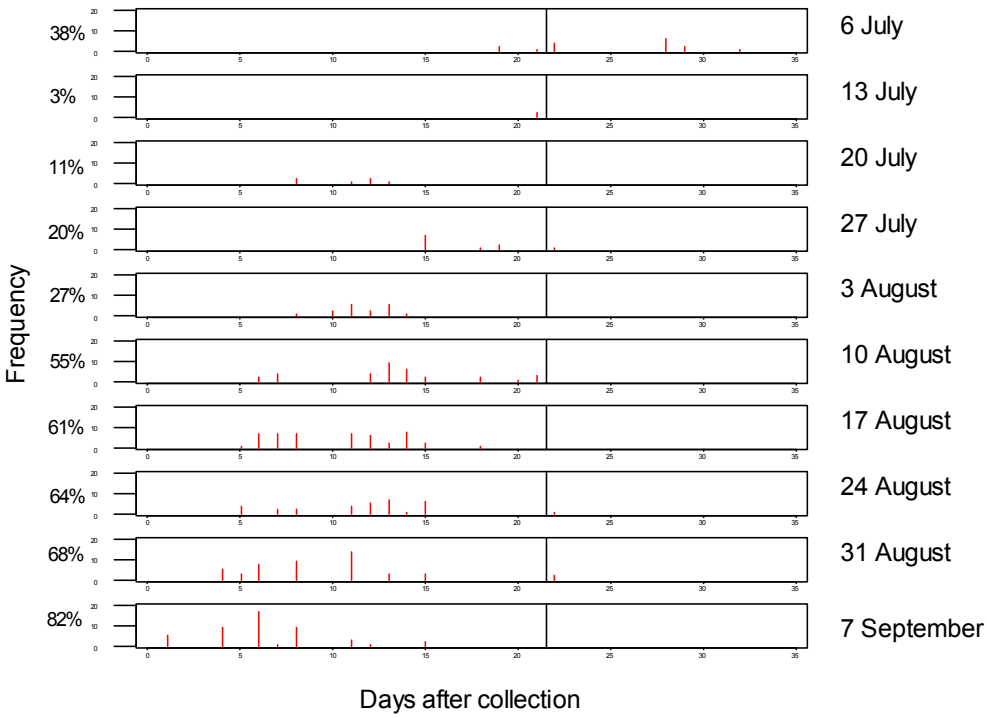


Figure 5: Distribution of days after collection reaching cluster appearance

As shown in Figure 5, 6 July had a relatively higher percentage of buds reaching the cluster appearance stage. However, most of them reached cluster appearance after 21 days. This does not fit the chilling completion standard. Although on 3 August buds reached cluster appearance within 3 weeks less than 50% of the buds reached cluster appearance. This indicated that chilling completion started just before 10 August. Looking at Figure 2, the trees reached 59% cluster appearance between 27 July and 31 August. One tree reached 50% cluster appearance on 10 August and then dropped back below 50% before again reaching 50% on 31 August. This indicated that on average chilling completion was finished before 10 August; for individual trees, it was completed between 27 July and 31 August, actually a month variation.

The average chill completion date was should be 10 August. Analysis also showed no difference between shoots from the east and west side of the tree.

5.11.1.2 Modeling

5.11.1.2.1 Chilling starting

In warmer climates where temperatures may vary for considerable lengths of time above and below the compensation point, determining when to begin accumulating chill unit is problematic (Seeley, 1996). Erez (e-mail 7/04/2006) confirmed that the starting time of chilling accumulation is definitely an unsolved problem. In the California pistachio industry for Chill Hour Model, they normally use total hours with temperatures ≤ 7.2°C from 1 November (Beede et al, 2005), this should be equivalent to 1 May in Australia.

In the original Utah Model, Richardson, Seeley and Walker (1974) suggested that positive chill-units (CU) begin to accumulate just after the day in the fall when the largest negative accumulation is experienced. Seeley (1996) describes the method in detail. Since negative CUs accumulate above 16°C and average late summer temperatures exceed this threshold, plotting CU accumulation in late

summer gives an increasingly negative curve until average temperatures drop below negation levels and positive CU accumulation begins (Figure 6). This maximum negative accumulation can be used as the starting point for physiological CU accumulation. Based on the maximum negative accumulation method, Table 3 lists starting dates for Utah model in different years and different locations.

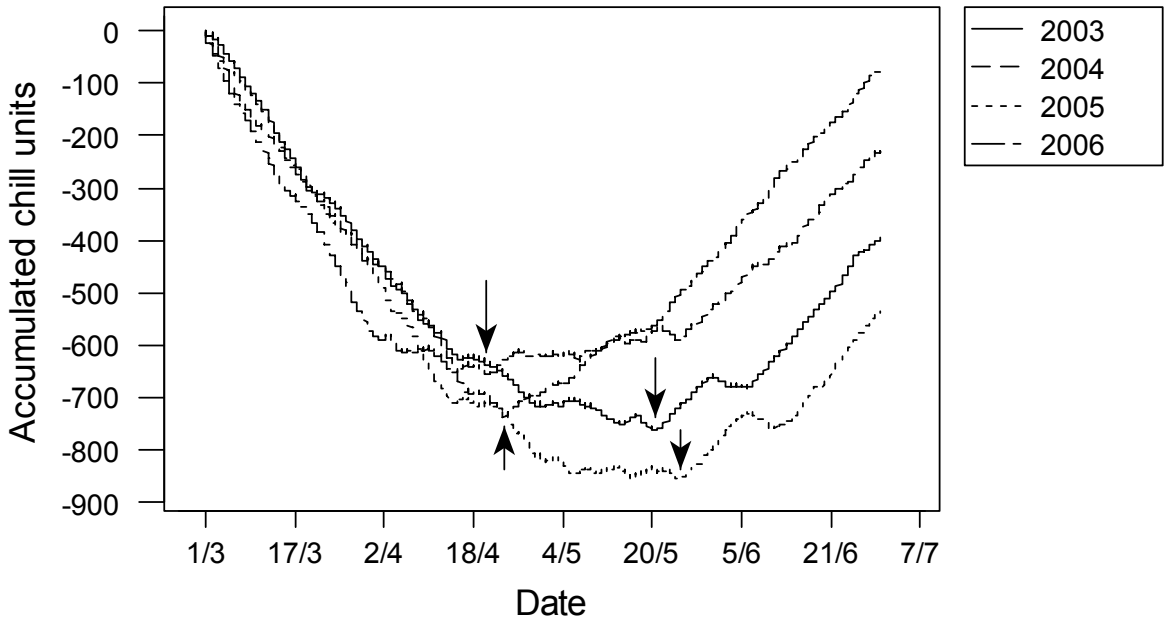


Figure 6 : Starting point for chill unit accumulation in cold climates based on Mildura data

Table 3 Chilling starting dates based on Utah model

Year	Swan Hill	Mildura	Renmark	Wagga	Nhill	Lameroo
1999	14/4	20/4	20/4	N/A	N/A	N/A
2000	30/4	30/4	30/4	6/5	N/A	N/A
2001	19/4	16/5	16/5	N/A	N/A	N/A
2002	9/5	16/5	16/5	1/5	N/A	N/A
2003	29/4	21/5	21/5	20/5	N/A	N/A
2004	23/4	23/4	23/4	18/4	N/A	N/A
2005	6/5	25/5	25/5	11/5	6/5	6/5
2006	14/4	21/4	20/4	6/4	5/4	14/4
2007	18/5	21/5	21/5	19/5	9/5	18/5
2008	26/4	26/4	26/4	21/4	26/4	26/4
2009	25/4	26/4	26/4	26/4	25/4	25/4
2010	4/5	11/5	10/5	26/4	24/4	4/5

When the Dynamic model is used (Fishman et al, 1987a), the model automatically calculates the starting date (1 portion accumulation) as listed in Table 4.

Table 4 Chilling starting dates based on Dynamic model

Year	Swan Hill	Mildura	Renmark	Wagga	Nhill	Lameroo
1999	29/3	22/4	8/4	N/A	N/A	N/A
2000	22/4	22/4	22/4	19/4	N/A	N/A
2001	22/3	13/4	13/4	N/A	N/A	N/A
2002	27/4	27/4	27/4	27/4	N/A	N/A
2003	17/4	17/4	17/4	17/4	N/A	N/A
2004	19/4	25/4	25/4	24/4	N/A	N/A
2005	20/4	16/4	16/4	1/5	20/4	6/3
2006	6/4	9/4	17/4	2/4	2/4	2/4
2007	10/5	10/5	10/5	10/5	25/3	10/5
2008	28/3	27/4	27/4	28/3	27/3	28/3
2009	16/4	26/4	26/4	26/4	6/4	7/4
2010	12/4	6/5	6/5	12/4	12/4	12/4

Seeley (1996) also raised 2 alternative methods but he discarded them in practice. One potential alternative is the use of vegetative maturity, the absence of regrowth after defoliation or decapitation. However, this physiological benchmark is labour and plant material intensive, time consuming, and dependent on climatic factors that occur after the point event. Another alternative is natural defoliation, which has been suggested as a phenological benchmark for beginning CU accumulation. Couvillon (email 21/04/2006) suggested that after 60% of the leaves have fallen in the fall. However, leaves may senesce over varying lengths of time, become inactive due to stress or other stomatal closure conditions while still being attached and/or be inactivated by cold temperatures yet remain on the tree for extended periods. At present, the maximum negative accumulation is best for distinctly seasonal climates (Seeley, 1996).

In our work, 60% leaf fall was not recorded. Thus we cannot use leaf fall for this work at present. We used 1 May to test the models of $\leq 7.2°C$, 0-7.2°C, $\leq 7.5°C$. The Utah model and the Dynamic model automatically use their own calculation for chilling starting.

5.11.1.1.2 Chilling hour requirement of 'Sirora' pistachio

As chill fulfillment was on 10 August, if we calculate the accumulated hours or units or portions from chill starting date to the chill fulfillment dates, we will obtain the hours or units or portions for the models.

Based on maximum negative accumulation in Utah model, the starting date in 2006 was 21 April for Dareton, Mildura and Renmark. Table 5 listed calculation results for all the models. Here 2 chill fulfillment dates are assumed, 10 August. Results for model ≤7.2°C are listed in column 2. Sometimes they use total hours with temperatures between 0° and 7.2°C within the same period (Column 3). Australian scientists Hobman and Bass (1986) suggested using total hours with temperatures ≤ 7.5°C. For 'Sirora' pistachio they suggested an average of 600 – 800 hours (Column 4). Besides numbers of hours, Richardson et al (1974) proposed a Utah model with chilling unit (CU) and its results in column 5. Erez et al (1988) raised a dynamic model for chilling requirements by portion accumulation. Using data from the UC Davis California website, their accumulation starts on 1 March (Column 6).

Table 5 Accumulation of chilling hours from 1 May or units from 21 April or portions from 1 March (Dynamic model)

Completion date	Hours ≤7.2°C	Hours 0-7.2°C	Hour ≤7.5°C	CU	Portion
10 August	659	645	713	990	59

5.11.2 Model Comparison

5.11.2.1 Identification of lack-chill seasons

The reason for the study on chill requirement was that low-chill seasons may reduce the crop of fruit trees significantly. When we study chill effects, we first need to identify low-chill seasons.

Figure 7 shows the biennial bearing pattern of pistachio production between 2000 and 2010 in selected orchards in the Mildura/Swan Hill and Renmark regions. In the Mildura region, the biennial bearing pattern was clear, 2001, 2003, 2005, 2007 and 2009 were 'on'years. In the Renmark region, 2001, 2005, 2007 and 2009 were clearly 'on'year crops but 2003 was not. This resulted in very even yield between 2001 and 2004 but a large increase in 2005. This indicated factors other than biennial bearing influenced yield. Winter chill for Renmark in 2002 was low. This is supported by observations of bud break. This low chill led to low yield in the 'on'year of the biennial bearing cycle.

Another low-chill winter in both the Mildura and Renmark regions was the winter of 2005. Bloom in 2005 was late and in some orchards bloom was delayed by more than 4 weeks and some flowers were still emerging in February 2006, just before harvest. After we started chill study work, 2007/08 and 2009/10 were also recorded as low chill seasons.

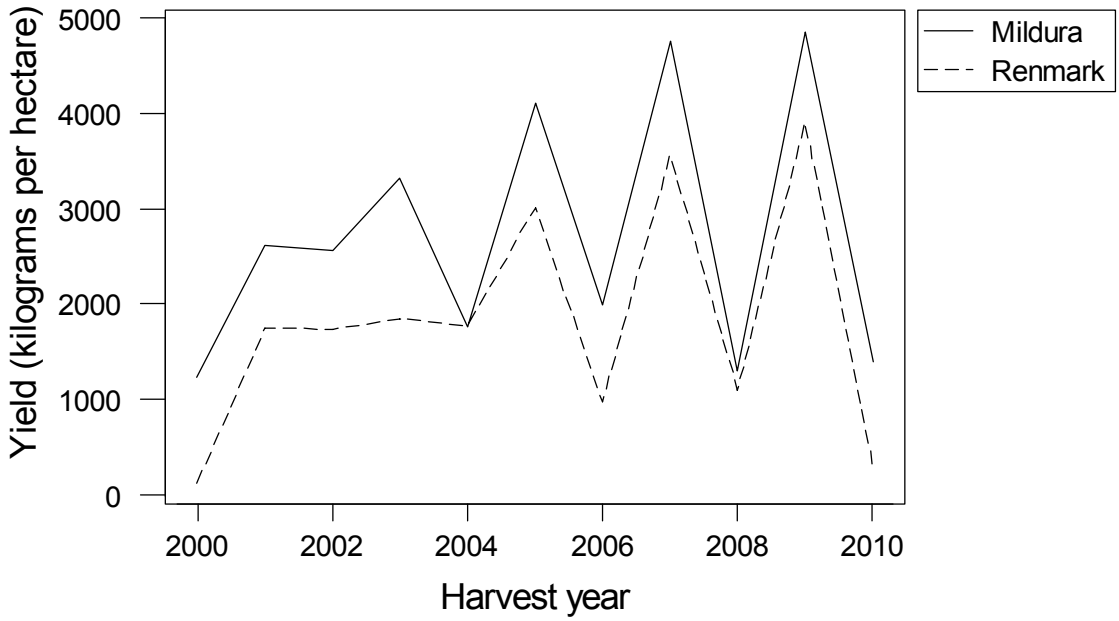


Figure 7: Average yields for 6 orchards in the Mildura region and 4 orchards in the Renmark region 2000 – 2010

5.11.2.2 Model comparison

As mentioned in Chapter 1, there are 5 chill models that may influence our work. They are

- Numbers of hours ≤7.5°C (Hobman and Bass, 1986).
- Numbers of hours ≤7.2°C
- Chill Hours Model: numbers of hours between 0 - 7.2°C
- Utah model (Richardson et al, 1974)
- Dynamic model (Fishman et al, 1987a).

Although all routine work includes 6 meteorological stations, which are around our pistachio orchards, 4 of them never have records with low chill. The following discussion will only focus on 2 major stations, i.e. Mildura and Renmark.

Table 6 lists accumulative hours and units and portions from starting dates to 31 August each year for each station. In Table 6, orange areas show low chill seasons. In Table 6, * Indicates poor match between observed evenness of bud break and that predicted by the models.

Table 6 Historical chilling accumulations up to 31 August each year, chill requirement for hours ≤7.5°C is 713, for hours ≤7.2°C is 659, hours 0 - 7.2°C is 645, for Utah model is 990, for Dynamic model is 59 portions according test in winter 2006.

Region	Model	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
	≤7.5°C	791	819	726	699	684*	798	629	998	711	768	538	782
	≤7.2°C	673*	767	704	676	677*	702	656	896	674	703	486	738
Renmark	0-7.2°C	602*	706	645	591	620*	652	604	727	573	657	445	645
	Utah	813*	1100	958*	767	953*	956*	795	975*	838	1122	678	1030
	Dynamic	59	70	64	56	63	63	55	68	55	65	55	65
	≤7.5°C	736	773	675*	691*	666*	733	564	937	789	729	556	703*
	≤7.2°C	612*	719	653*	655*	640*	633*	581	831	728	667	506	655*
Mildura	0-7.2°C	600*	715	635*	640*	629*	625*	574	773	688	662	488	644*
	Utah	1023	1294	1063	941*	1077	1097	857	1137	1020	1152	823	1198
	Dynamic	66	75	63	59	64	65	57	72	59	68	58	69
	≤7.5°C	949	1040	840	852	776	934	807	961	1105	831	773	941
Swan	≤7.2°C	796	979	779	812	747	827	780	912	1037	775	705	864
Hill	0-7.2°C	773	963	739	770	732	809	768	842	987	765	693	842
	Utah	1308	1551	1263	1161	1293	1481	1162	1352	1363	1423	1257	1494
	Dynamic	72	82	74	68	70	78	67	79	69	76	73	79
	≤7.5°C							844	1036	895	862	721	876
	≤7.2°C							782	985	822	782	656*	817
Lameroo	0-7.2°C							768	956	804	778	653	815
	Utah							1247	1549	1373	1579	1416	1546
	Dynamic							71	85	71	84	79	82
	≤7.5°C							870	1072	884	899	658	962
	≤7.2°C							792	1008	810	817	563*	904
Nhill	0-7.2°C							785	962	797	814	563*	894
	Utah							1358	1661	1468	1732	1542	1733
	Dynamic							70	91	75	93	83	86
	≤7.5°C		1227		1119	922	1115	861	1264	1055	1077	850	1153
	≤7.2°C		1124		1047	837	1026	819	1203	989	999	796	1078
Wagga	0-7.2°C		1118		978	813	989	802	1079	928	976	755	1044
	Utah		1910		1499	1477	1772	1381	1548	1486	1685	1445	1714
	Dynamic		90		76	73	86	70	88	70	84	79	85

Results from the winter 2006 greenhouse work (Table 5) were used as standards for each chill model to compare historical chill and industry yield records. The Dynamic Model correctly indicated the chill in all years when matched to flowering and yield records.

The Utah Model indicated poor chill for Renmark in 1999, 2001, 2003, 2004 and 2006, and for Mildura in 2002, when industry yield records indicated sufficient chill. Winter 2006 was a very good year for chill (Table 6) and warm weather in September led to bud break in spring being 10 days earlier than usual. However, in 2006 Renmark only had 975 Chill Units this was not reflected in the observed bud break and yield was excellent.

Both the Chilling Hour Model and Model with hours ≤ 7.2°C incorrectly indicated that 1999 and 2003 were low chill years for Renmark. In these years yield data indicated sufficient chill. Similar inaccurate predictions were made for Mildura in 1999, 2001- 2004 and 2010.

Model with hours ≤ 7.5°C incorrectly indicated that 2003 was a low chill year for Renmark. In these years yield data indicated sufficient chill. Similar inaccurate predictions were made for Mildura in 2001- 2003 and 2010.

The other 4 regions have no low chill records as well as no low chill accumulation in all the models.

Over the 5 years the Dynamic Model produced more reliable predictions that the other models.

5.11.3 Standards on 15 August

Oil application should start in middle of August. Thus, another evaluation from starting dates to 15 August is necessary for decision making for oil application although this is not the final evaluation for the years.

Before the end of the chill process, we had to make a decision on oil application. In California, it is based on a calculation up to 15 February. From our experience in 2005 CMV Farms obtained the best oil application results between 22 and 24 August. This indicated that we should make the decision based on calculation on 15 August instead of 31 August. This section discusses what may happen up to 15 August for chill process.

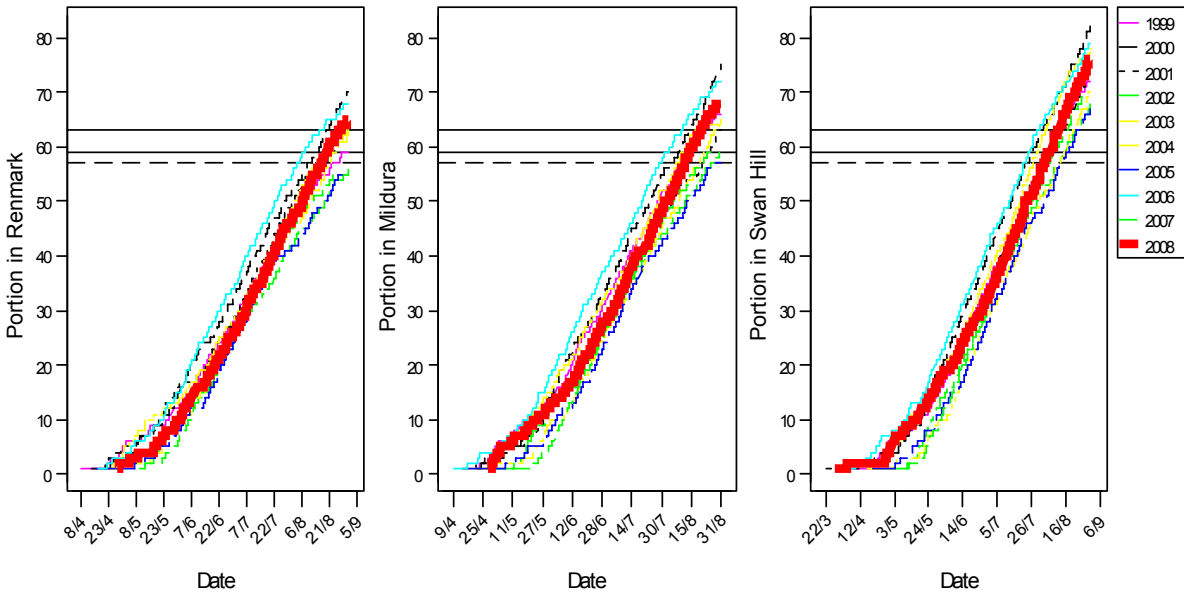
Table 7 only lists results from Dynamic model. Before evaluating if chill is sufficient up to 15 August, it is necessary to know how many portions can be gained from 15 August to 31 August. This may tell us how much portion lag can still be added in the period between 15 and 31 August. Table 7 lists the portion increases between 15 and 31 August in different years in Mildura and Renmark.

Table 7: Portion increase between 15 and 31 August

Year	Mildura			Renmark		
	15 Aug	31 Aug	gain	15 Aug	31 Aug	gain
1999	61	66	5	54	59	5
2000	64	75	11	60	70	10
2001	54	63	9	56	64	8
2002	52	59	7	49	56	7
2003	54	64	10	53	63	10
2004	62	65	3	58	63	5
2005	51	57	6	49	55	6
2006	66	72	6	63	68	5
2007	55	59	4	51	55	4
2008	60	68	8	56	65	9
2009	55	58	3	51	55	4
2010	58	69	11	56	65	9

From Table 7, the average portion increased between the 15th and 31st August was 6.917±2.906 and 6.833±2.290, for Mildura and Renmark, respectively. Although $6.917-2\sigma=1.22$, this is for 95% in the double tail test. In this particular case, we were only interested in tail in 1 side (small side). In this way, 95% security is 2.138 and 3.067. In the other words we can say, there is only less than 5% possibility that Mildura cannot get 2.138 portions from the 15th to 31st August, while Renmark gets 3.067 portions. To make this easily and more confident, we assumed that from the 15th to 31st August, these areas at least can gain 2 portions in 95% of the years. Thus, 57 portions were decided as a boundary for winter oil application.

Providing information from routine work, weekly portion accumulation was provided from early June to end August through a website. Figure 8 is an example. In Figure 8, the historical portion accumulation lines are thin lines while current year line is a thick line. Horizontal lines show portions at 57, 59 and 63.



This was the early stage of our chill work. For production safety, maybe we should apply oil if any location shows problem. However, if all the locations show no problems, like the spring of 2006, our model work should tell growers that oil application is unnecessary. This does not only reduce cost but also reduce infection of *Botrytis* strikes.

5.11.4 Bloom Date Prediction

Growing degree hour (GDH) requirements for bloom, following fulfillment of the chilling requirement were also calculated based on greenhouse work. In the spring of 2006, at Dareton Research Station, GDH from fulfillment of chilling requirement (10 August) to 50% bloom was 9633. Based on GDH of 9633, 50% bloom dates were predicted and listed in Table 8. In three of the four years, the predicted bloom date was within one day of the actual date. However, the prediction in 2007 was very poor, by the end of August Chill Portions were below the chilling requirement with bloom in the field occurring 20 days after the predicted date. The bloom date for the five trees at Dareton was later than other orchards in that area which bloomed only two weeks after the predicted date.

Table 8 Accumulation of Chilling Hours, Chill Units and Chill Portions for chilling requirement each winter with predicted (based on 9633 GDH established in 2006) and actual bloom dates and field GDH (twigs taken from Dareton Research Station, Industry and Investment, New South Wales). Chilling Hours calculated from 1 May to chilling fulfillment date and Chill Units and Chill Portions calculated from 1 Mar. to chilling fulfillment date.

Winter	Chilling fulfillment date	Chilling Hours	Chill Units	Chill Portions	50% bloom date predicted	50% bloom date in the field	GDH
2006	10 August	645	990	59		20 Sept.	9633
2007	13 Sept.	677	919	58	8 Oct.	7 Nov.	17297
2008	3 Sept.	569	1078	62	9 Oct.	10 Oct.	9818
2009	9 Sept.	412	752	60	17 Oct.	16 Oct.	9411
2010	25 August	535	1123	61	9 Oct.	10 Oct.	10063

5.11.5 Orchard Visit

5.11.5.1 2007

Chilling process had become a notable problem for our pistachio production. For research purposes, how to define chilling process each year was a key work for further research. Also, the difference of chilling responses from locations was another key issue for further research. As from early November 2007, the RFO visited most of the orchards in group around Renmark (5th and 6th November), Mildura (8th November) and Swan Hill (12th November).

Generally speaking, flowering process had been completed during the visits. This probably implied that there were no big problems for the chilling process that winter. All the 3 locations showed similar development. But oil application made clear difference for flowering process.

Table 9: Orchard list for oil application or not

6% oil application	No oil application
CMV Farms	Mallee Orchard Pistachio
Kyalite Pistachios	Bob Hodgson
Peter Weir	Lill
Frank Levak	David Peake
Rodney Hand?	Eric Wright
	Murrawee

Investigation showed that orchards with oil application have nut development 2 weeks earlier that the orchards without oil application. During the visit, all the orchards with oil application had big nut shells while the orchards without oil application had nuts in “chicken and hen” stage.

5.11.5.2 2008

Chill requirement was a problem for our pistachio production. To evaluate and record the yearly status for industry orchards is important. Daily marking of flowers during bloom period is important. After the flower marking, it is the correct time to visit orchards. Flower-advanced orchards reach nut size increase, while slow-flower orchards still show some whole clusters with very small nuts. This is the reason that the work is undertaken during this period.

Orchard visits were conducted around Nangiloc on 29th October, and around Blanchetown and Renmark between 5th and 6th November, and Kyalite Pistachios as well as Swan Hill on 7th November.

The orchards that applied winter oil were: CMV Farms, Kyalite Pistachios, Redlynch, Peter Weir, Don and Chris Lill, Colin and Lois Caelli;

The orchards that did not apply winter oil were: Eric Wright, Boojerahla, Frank Levak, Bob and Joan Hodgson, Murrawee.

Generally speaking, oil application in late August still showed flower advantage, but this advantage was not as big as last year. When Redlynch showed all clusters with big nuts, Eric Wright's orchard still had flower just opening; When Weir and Lill showed all clusters with big nuts, Hodgson's orchard had some whole cluster with small nuts. Caelli's orchard showed advanced flowers than Murrawee. In Murrawee, they did not apply our "standard" oil application. Instead, they applied oil in July with some application. Thus, their flowers were not very delayed comparing with Caelli, but there was clearly delay.

The only exception was Frank Levak. He did not apply oil this winter (he usually applies oil). We did not find any small-nut there. But his crop was not very high that season. Clearly the reason was that his orchard was fully shaded. In the orchard, there were no gaps between rows. The status was only light crop on the top of the trees. Most trees did not have a heavy crop. If some trees have a crop there must have been a gap due to a broken branch or a missing tree. From there, sunlight shows its importance.

5.11.5.2.1 Symptom documented

During orchard visits, the RFO noticed a symptom around orchards. Quite a few trees had a relatively large area with nuts but without leaves (Photo 1). This appeared only in heavy cropping orchards such as KP, CMV, Weir and Hodgson. Light cropping orchards did not have this problem that year. KP, CMV, Weir had oil application, but Hodgson did not have oil application. This implied that there was no affect due to oil application. Heavy pruning areas in KP were also checked and, although it was lighter than other areas, there were still a lot of symptoms. Andrew Bowring said this problem was also seen in 2003. He also noticed that *terebinthus* was more serious than Pioneer Gold. This report raised this issue for further consideration.

Photo 1 Nut bearing without leaves

5.11.5.3 2009

Chill requirement was a problem for our pistachio production. To evaluate and record the yearly status for industry orchards is important. Daily marking of flowers during bloom period is important. After the flower marking, it is the correct time to visit orchards. Flower-advanced orchards reach nut size increase, while slow-flower orchards still show some whole clusters with very small nuts. This is the reason that the work is undertaken during this period.

Orchards around Nangiloc were visited on 27th October, Kyalite Pistachios and orchards around Swan Hill on 30th October, and Kaniva Pistachios on 4th November. Visits to Kaniva Pistachios and Mallee Orchard Pistachios occurred on 1st December, and orchards around Renmark on 2nd December.

Oil application in late August showed flower advantage very clearly. It was about 3 weeks or more ahead. This was due to low chill winter. In the November visit, Redlynch showed all clusters with big nuts, Wright's orchard still had flowers just opening. In the December visit, Lill's orchard showed a very few flower clusters being open, Weir's orchard showed a lot of opening flower clusters and some non-break buds. Weir's orchard had good flower buds that year. Late flowers and non-break flower buds showed clear results for yield reduction. Orchards of Hosgson and Levak had no application of winter oil and had the same problem. However due to a light crop in both orchards, yield influence were not as significant as Weir's orchards. Visits to orchards of Ruediger and Permedah highlighted that both orchards applied winter oil and they looked much better than non-oiled orchards.

Low chill this year clearly showed yield reduction if there was no oil application. Also, the Renmark area showed a worse response than the Mildura area. On the visit on 2nd December, all had no oil application, trees at Dareton station showed better performance than trees in Renmark.

In this trip, the problem for low chill was not found in orchards at Kaniva and Pinnaroo. This indicated that data from meteorological stations used were reliable.

5.11.5.4 2010

Visited orchards are listed in Table 3.

Table 3 Visiting schedule

Date	3 Nov	4 Nov	5 Nov	8 Nov	9 Nov	12 Nov
Growers	RedLynch* Wright Robb	Mallee* Orchard Pistachio	Kaniva Pistachio	Hodgson Reudiger*	Lill* Levak Permedah*	Tripodi Caelli KP*

NB: * indicates winter oil application for improving bud break.

During these visits we compare orchards in close locations with or without winter oil application. The observation for oil effect was clear but was less than the 1st week.

For all orchards, 'on-year' crop was clear. Mallee Orchard Pistachio showed extremely heavy crop in all blocks.

5.11.6 Other Varieties

From the winter of 2008, chill process was tested for male PT134 (red in CMV Farms) and PT198 (green in CMV Farms). From the winter of 2009, chill process for 'Kerman' was tested.

Table 10 Chill requirement test for male trees and 'Kerman' at Dareton Station

Source	Spring	Date	Portions	% buds	% shoot	50% bloom
Male	2008	20 Aug	57	78.1	90	3 Oct
PT198	2009	2 Sep	58	57	80	10 Oct
(green)	2010	18 Aug	58	79	90	9 Oct
Male	2008	3 Sep	63	41.4	60	8 Oct
PT134	2009	16 Sep	61	59	80	20 Oct
(red)	2010	25 Aug	61	92	100	12 Oct
Kerman	2009	Not till 16/09	>63			
	2010	Not constant				

From the 3-year tests (Table 10), Male PT198 (green) showed chill requirements with 57 portions and Male PT134 showed 61 portions. This indicated that PT198 had less chill requirement than 'Sirora' while PT198 had a little more chill requirements than 'Sirora'. In low chill years, PT198 may have more influence than 'Sirora'.

Since the spring of 2009, old 'Kerman' trees were useful for chill requirement tests. In spring the of 2009, for 'Kerman' trees, shoots did not reach 50% bud break in the last sampling (16th September) and did not reach 50% full bud break on the 29th October. This indicated that 'Kerman' needs > 63 portions to complete its chill process. In the spring of 2010, 'Kerman' got 60% bud breaks in shoots and buds on 25th August. However, after that, 'Kerman' showed low bud break on 1st, 10th, 15th and

22nd September. Thus, we cannot confirm bud break on 25th August was true or just by chance. Those trees are old and weak. Also, shoots taken from those trees had too many flower buds. All these may affect percentages of bud break. 'Kerman' work needs further improvement.

5.11.7 Decision Making for Winter Oil Application

Table 11 lists the increase in Chill Portions between 15th August and 31st August in Mildura and Renmark. The minimum gain in the last 12 years was three in Mildura and four in Renmark. To allow for variance we assume that two Chill Portions can be obtained in this period. Thus, 57 Chill Portions accumulated by 15th August indicates chilling requirements will be fulfilled. If chill accumulation is below this on 15th August., winter oil should be applied.

Table 11: Number of Dynamic portions accumulated at 15th August and 31st August at Mildura and Renmark and the number of portions accumulated in the interval.

Year	Mildura			Renmark		
	15 August	31 August	Gain	15 August	31 August	gain
1999	61	66	5	54	59	5
2000	64	75	11	60	70	10
2001	54	63	9	56	64	8
2002	52	59	7	49	56	7
2003	54	64	10	53	63	10
2004	62	65	3	58	63	5
2005	51	57	6	49	55	6
2006	66	72	6	63	68	5
2007	55	59	4	51	55	4
2008	60	68	8	56	65	9
2009	55	58	3	51	55	4
2010	58	69	11	56	65	9
Average gain			6.9			6.8

5.12 Winter Oil Application Study

5.12.1 Bloom Observation

In the season 2008/09, besides a few normal observations, in the morning of 6th of October 2008, the responses were independently observed. Tthe same conclusions that in replication 1, 2 and 3, treatment 6% was clearly earlier for budbreak than 3%. However, in replication 4 and 5, there was no difference. Those trees were similar at 3% oil application at replication 1, 2 and 3. In 2009/10, investigations were made on the 15th October. Rows with 6% oil treatment still showed advanced flower opening. In 2010/11, flower opening were checked and confirmed that 6% had earlier flower opening than 3% treatment.

5.12.2 Yield

In these 3 seasons, Chill Portions were 68, 58 and 69 in winter 2008, 2009 and 2010, respectively.

From Table 2, in the season 2008/09, there was no significant difference of yield in hull per tree, merchantable yield per tree, count size and return per tree between 3% and 6% winter oil application. All p-values were > 0.800 indicating their high similarity. A significant difference was found in

percentages of shake 1. Treatment 3% showed earlier ripening than 6%. In stage 3, average yield in hull per tree was 44.7 kg, merchantable yield per tree was 15.2 kg and count size was 88.7.

In season 2009/10, treatments of 6% winter oil application had significantly higher yield in hull per tree, merchantable yield per tree, return per tree and percentage of shake 1 than 3% winter oil application.

Table 2: Yields, count size and returns

Season	Treat	Yield/tree (kg)	% shake 1	Count	Merchantable yield/tree (kg)	Return $/tree
2008-2009	3%	50.7	89.0	87.3	17.28	110.6
	6%	50.5	85.7	87.4	17.40	111.3
	p-value	0.887	0.001	0.893	0.847	0.871
2009-2010	3%	12.0b	86.4B	82.4	4.1B	27.90B
	6%	15.6a	89.2A	81.5	5.6A	38.60A
	p-value	0.012	0.003	0.496	0.009	0.007

5.12.3 Quality

Table 3: Percentages of nut qualities in physiological aspects

Season	Treat	% small	% medium	% jumbo	% narrow split	% non split	% floater	% total non split	% blank	% damaged shell
2008-2009	3%	0.441	57.5	0.488	5.65	6.23	2.93	9.16	7.08	1.13
	6%	0.517	56.5	0.513	6.12	8.30	3.24	11.54	6.83	1.74
	p	0.477	0.672	0.588	0.727	0.075	0.370	0.071	0.650	0.005
2009-2010	3%	0.558	65.2	2.72	2.30	1.60	1.35	2.95	7.73a	2.00
	6%	0.583	65.2	3.37	1.79	2.01	1.43	3.44	6.56b	1.30
	p	0.939	0.990	0.561	0.084	0.328	0.834	0.392	0.021	0.054

In the season 2008/09 there was quite a lot of differences in nut quality tests. In general, 3% oil application showed bad results for stains, among them, gold stain and dark stain reached statistically different level. Other stain, adhering hull and pickout were close to the statistically different level. Treatment 6% showed more physiological defects such as damaged shell, loose kernel and non-split nuts.

In the season of 2009/10 treatment of 6% winter oil application had significantly lower percentage of pick out, blank nuts and FM. Also low percentages of narrow split at p-value = 0.084.

Table 4: Percentages of nut qualities in stain aspects

Season	Treat	% pick out	% loose kernel	% adhere	% dark stain	% gold stain	%SEL nut	% other stain	% light stain
2008-2009	3%	10.74	0.151	1.15	8.13	2.29	N/A	5.62	8.8
	6%	8.83	0.227	0.89	5.87	1.31	N/A	4.31	8.9
	p-value	0.058	0.087	0.143	0.017	0.002		0.164	0.982
2009-2010	3%	6.00a	0.410	0.274	3.68	1.49	0.270	1.82	12.1
	6%	4.69b	0.475	0.182	3.19	1.05	0.135	1.83	13.9
	p-value	0.024	0.359	0.384	0.247	0.100	0.119	0.930	0.433

5.13 Winter Oil Dipping Trial

The first time that differences were visible was 17 September. Shoots with 6% oil dipping on 20 and 27 August clearly showed advanced movement. 24 September showed a similar pattern. Within a tree, people can see that dipped shoots demonstrated earlier bud break than the rest (Photo 1).

Photo 1: Earlier bloom for single shoots with oil application

Each dipped shoot was compared with the most advanced non-dipped shoots. Results are listed in Table 1. From the application dates, 13 August showed least advancement. On 1 October we observed 2 advanced shoots for 6% that had been treated on 13 August. This was again observed on the 10th October. This indicated that 13 August dipping showed less effect. Application on 20 August showed clearly advanced effect, especially with 6% oil application. However, applications on 27 August showed a clear advancement with 3% oil application.

Table 1: Investigation on advanced bud break comparing with advanced non-treated shoot

Application date	Concentration	Shoot advanced than advanced non-treated shoot				Extremely late bud break	
		1/10/2008	6/10/2008	10/10/2008	14/10/2008	10/10/2008	14/10/2008
13/08/2008	3%	2	2	2	2	1	2
13/08/2008	6%	2	6**	6	6	2	2
20/08/2008	3%	3	3	3	2	2	1
20/08/2008	6%	7	7	7	7	0	0
27/08/2008	3%	6/9***	6	6	6	0	0
27/08/2008	6%	4/9	4	4	4	0	2*

NB: ** 4 of these shoots are only ahead by 1 or 2 days for 6% of 13/08
*** 6/9 and 4/9 mean that 6 and 4 shoots with bud break within total 9 shoots.

This seems to show us that early application stage, 6% oil showed more effect; at a late application stage, 3% oil showed reasonable results. This result was similar as results obtained from stage 3 of CMV Farms where 6% oil application showed better results than 3% treatment on the 19th August.

On the 10th October, we noticed some shoots with non-break buds at the late bloom stage. Those shoots were recorded. In late October and mid November, we checked those shoots and found that all the non-break buds dropped. This highlights the reason why we cannot find non-break buds after bloom period on pistachio trees.

5.14 A Study on Climate Factors on 'Sirora' Pistachio Nut Size

5.14.1 Maximum Temperature Exploration on Nut Size

Contour map of r-values is a tool to find key period in agricultural study (Zhang and Thiele, 1992). Table 1 shows contour map for r-values of average nut weights with maximum temperatures. The r-values for each row and column combination show correlation of the count and the average maximum temperature from the month in that row to the month in that column. For example, in the first row, -0.08 is the r-value of the weight with maximum temperature in the previous October, and 0.05 is the r-value of the weight with average of maximum temperatures between previous October and previous November, etc. Digitals are surrounded by lines showing r-value at $p \leq 0.05$ ($|r| \geq 0.205$), bold digitals show r at $p \leq 0.01$ ($|r| \geq 0.267$).

Table 1: Contour map of r-values of nut weight with maximum temperatures from previous October to current March

	Oct-	Nov-	Dec-	Jan-	Feb-	Mar-	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Oct-	-.08	.05	.05	.03	-.03	.01	.01	.00	-.04	-.04	-.02	-.02	.02	.11	.16	.11	.12	.13
Nov-		.15	.11	.06	.00	.04	.04	.03	-.02	-.02	.00	.00	.05	.14	.19	.13	.15	.16
Dec-			.04	.00	-.08	-.01	-.03	-.04	-.11	-.10	-.05	-.05	.01	.12	.17	.11	.13	.14
Jan-				-.03	-.13	-.04	-.07	-.08	-.15	-.13	-.07	-.07	.00	.11	.17	.10	.12	.13
Feb-					-.15	-.02	-.04	-.05	-.13	-.11	-.06	-.06	.01	.12	.17	.11	.12	.13
Mar-						.18	.11	.05	-.04	-.03	.01	.00	.06	.17	.22	.14	.15	.16
Apr							-.03	-.03	-.11	-.09	-.05	-.05	.02	.14	.19	.12	.13	.14
May								-.04	-.16	-.12	-.05	-.05	.03	.17	.22	.14	.15	.16
Jun									-.24	-.15	-.05	-.05	.05	.19	.25	.15	.16	.18
Jul										-.01	.08	.04	.12	.25	**.29**	.19	.20	.22
Aug											.11	.05	.13	**.28**	**.33**	.21	.22	.23
Sep												-.03	.12	**.31**	**.36**	.21	.22	.23
Oct													.20	**.42**	**.43**	.24	.24	.26
Nov														**.43**	**.38**	.19	.19	.21
Dec															**.29**	.02	.05	.09
Jan																-.20	-.09	-.04
Feb																	.09	.15
Mar																		.10

NB: bold digitals are at p=0.01 level; normal digitals surrounded in lines at p=0.05 level

Table 1 shows that maximum temperature between the current October and December was a major factor in influencing on nut size. The significant markers appear between current May to March and are concentrated between July and December, and peaked between October and December (r=0.43). Thus, the key study should be focused on between October and December. Significances between July

and December are due to October, November and December existing in that period. Detailed study was conducted around October and December.

Table 2 further shows that maximum temperature during hull enlargement and shell hardening was a major factor influencing nut size. To focus key periods, Table 2 shows contour map for r-values of maximum temperatures between October and March on 10-day averages. To express date intervals, "10-1" represents the first ten days of October, "10-2" represents the second ten days of October, etc. Due to the use of the key periods, more digitals reached significant levels. To show the key period clearly, cells are surrounded by lines showing r-value ≥ 0.30 while bold digitals show r-value ≥ 0.40. The peak point is between '10-2' and '11-2'. Here the r-value is 0.52, which is much higher than 0.43 on monthly basis. This will be the new focus period.

Table 2: Contour map of r-values of nut weight with maximum temperatures on 10-day averages and their combinations from October to March

	10-1	10-2	10-3	11-1	11-2	11-3	12-1	12-2	12-3	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3
10-1	-0.12	0.22	0.19	0.33	**0.44**	**0.42**	**0.40**	0.39	**0.42**	0.37	0.31	0.24	0.21	0.27	0.24	0.21	0.24	0.26
10-2		**0.48**	0.34	**0.48**	**0.52**	**0.51**	**0.48**	**0.45**	**0.47**	**0.41**	0.35	0.27	0.24	0.29	0.27	0.24	0.27	0.28
10-3			0.09	0.37	**0.43**	**0.43**	**0.40**	0.36	**0.40**	0.34	0.27	0.20	0.17	0.22	0.20	0.17	0.20	0.22
11-1				0.36	**0.42**	**0.43**	0.39	0.34	0.38	0.32	0.26	0.19	0.16	0.21	0.19	0.16	0.19	0.21
11-2					0.35	0.34	0.30	0.28	0.34	0.28	0.21	0.14	0.11	0.17	0.15	0.12	0.15	0.17
11-3						0.12	0.13	0.17	0.26	0.21	0.12	0.05	0.02	0.10	0.07	0.05	0.08	0.10
12-1							0.11	0.17	0.28	0.21	0.10	0.01	-0.01	0.07	0.05	0.02	0.06	0.08
12-2								0.15	0.27	0.21	0.08	-0.01	-0.03	0.06	0.03	0.01	0.05	0.06
12-3									0.28	0.19	0.00	-0.08	-0.09	0.01	-0.02	-0.04	0.00	0.02
1-1										0.03	-0.17	-0.20	-0.18	-0.07	-0.10	-0.11	-0.07	-0.05
1-2											-0.32	-0.26	-0.22	-0.10	-0.12	-0.14	-0.11	-0.08
1-3												-0.17	-0.16	0.02	-0.03	-0.06	0.00	0.03
2-1													-0.06	0.13	0.07	0.03	0.11	0.14
2-2														0.32	0.22	0.10	0.19	0.24
2-3															-0.13	-0.16	-0.02	0.03
3-1																-0.07	0.05	0.10
3-2																	0.15	0.22
3-3																		0.09

NB: bold digitals are at r ≥ 0.40; normal digitals surrounded in lines at r ≥ 0.30

Figure 1 shows results for further calculations on a daily basis between 1 October and 31 December. At the top of the figure, "O" represents 1 October; "N" represents 1 November; "D" represents 1 December. We cannot read further details in the contour map. It was 92 days from 1 October to 31 December. We cannot put 93 rows with clear digitals in an A4 page. This paper can provide a rough image for us to understand effects of temperature on nut size. Different colour areas show the effects. According to our reading in Tables 1 and 2, Figure 1 marked low colour area at r ≥ 0.40. This major area started about the 10th October, and major area was around November and December. Dark colour area at r ≥ 0.50. Except for a few small areas, the major dark area was between 10 October and late November with darker colour for peak values of 0.54 between 11 October and 21 November.

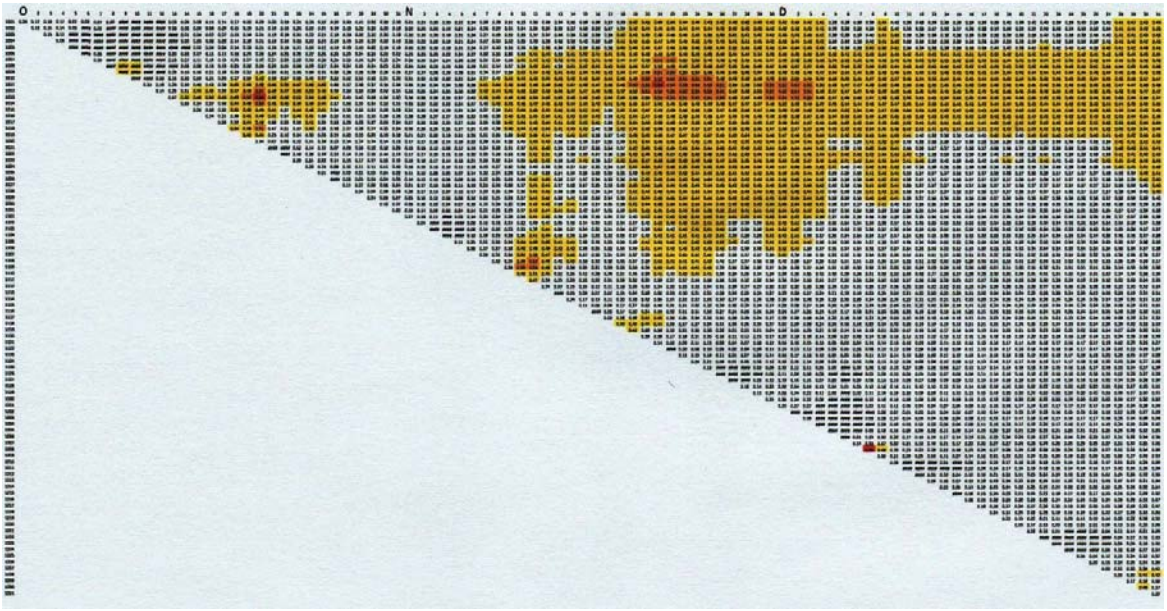


Figure 1: Contour map of r-values of nut weight with maximum temperatures on daily averages and their combinations from 1 October to 31 December

This Figure seems to tell us that higher maximum temperatures from 10 October to late December, even January, benefit nut size. The key period is around 11 October to 21 November. It is not necessarily that it is because of the temperatures between 11 October and 21 November. Actually the temperatures around neighbour dates all have high r-values. It can be imagined that data from different years, based on different bloom starting dates, may lead to the peak value drift around, but it could not be too far from this period.

For significant values, they were all positive. This indicates that when maximum temperatures at that period are high, nut weight was heavy. In Australian pistachio areas, most bloom periods start around 1 October. Here 11 October was a late bloom period or early hull enlargement period. From late bloom to shell hardening, higher maximum temperatures induced larger nut size.

5.14.2 Effects of Winter Chill on Nut Size

Hourly temperatures ≤7.2ºC between 1 May and 31 August showed significant correlation with pistachio nut size with r=0.26*. (In this paper, * represents significant at p=0.050 level, ** represents significant at p=0.010 level, *** represents significant at p=0.001 level.) This is much weaker than the relationship with maximum temperatures during hull enlargement with r=0.54***. This seems to show temperatures in hull development periods play an important role.

5.14.3 Other Cultivation factors

Other cultivation factors were tested but their correlation with nut weight were much weaker than maximum temperatures during hull enlargement. Table 3 also tested influences from yields and major elements in leaf analysis on nut weight. None of them reached a significant level in the test.

Table 3 r-values between nut weight and relevant factors

Variables	Yield/ha	Hours ≤7.2ºC	N	P	K	Ca	Mg	S
r-value	0.18	0.26*	-0.17	0.17	0.08	-0.10	-0.18	-0.05

NB: * significant at p=0.05 level

5.14.4 Multiple Regression Analysis for Nut Size

Multiple regressions provide a further solution. As mentioned in the last 2 sections, temperatures and winter chill may influence nut weight. Multiple regressions not only can put them together in 1 regression formula but also can test the importance between factors.

The formula below shows multiple regression results based on average maximum temperature between 11 October and 21 November ($MaxT_{11\ Oct\text{-}21\ Nov}$) and hours ≤ 7.2°C between 1 May and 31 August. Both variables reached very significant levels in the multiple regressions (Table 4). The regression equation is

$$\text{Weight} = 0.219 + 0.0278\ MaxT_{11\ Oct\text{-}21\ Nov} + 0.000192\ \text{hours} \quad (R = 0.61^{***}) \qquad [1]$$

From Table 4, 2 independent variables have 1 degree of freedom, but the sum of the square of $MaxT_{11\ Oct\text{-}21\ Nov}$ are about 4 fold as hours ≤ 7.2°C between 1 May and 31 August. This indicated the importance of $MaxT_{12\ Oct\text{-}11\ Nov}$ for nut size. From formula [1], every 1 ºC increased, individual nut weight increases 0.03 grams.

Table 4: Coefficients test in multiple regression

Predictor	Coefficients	Sum of square	Standard deviation	T test	P-value
Constant	$2.18x10^{-1}$		$1.20\ x10^{-1}$	1.82	0.071
$MaxT_{11\ Oct\text{-}21\ Nov}$	$2.78x10^{-2}$	0.184	$4.18\ x10^{-3}$	6.65	0.000
$Hours_{\leq 7.2°C}$	$1.92x10^{-4}$	0.048	$5.74\ x10^{-5}$	3.35	0.001

Winter chill showed significant effect on nut weight. Hours ≤ 7.2ºC between 1 May and 31 August reached p=0.001 level in the multiple regression with $MaxT_{11\ Oct\text{-}21\ Nov}$.

Figure 2 shows this relationship. When the average maximum temperature increases, the nut weight increases. Figure 2 actually uses 2-dimensional plane to show a 3-dimensional plot. Here actual hours ≤7.2°C between 1 May and 31 August cannot be expressed in the figure but different hour ranges are expressed in different marker shapes. Under similar temperatures, markers in • (> 800 hours) normally are on the top, then * and, × and + (< 645 hours) usually at the bottom. This indicated that under the same maximum temperature, trees with good winter chill produce larger nuts.

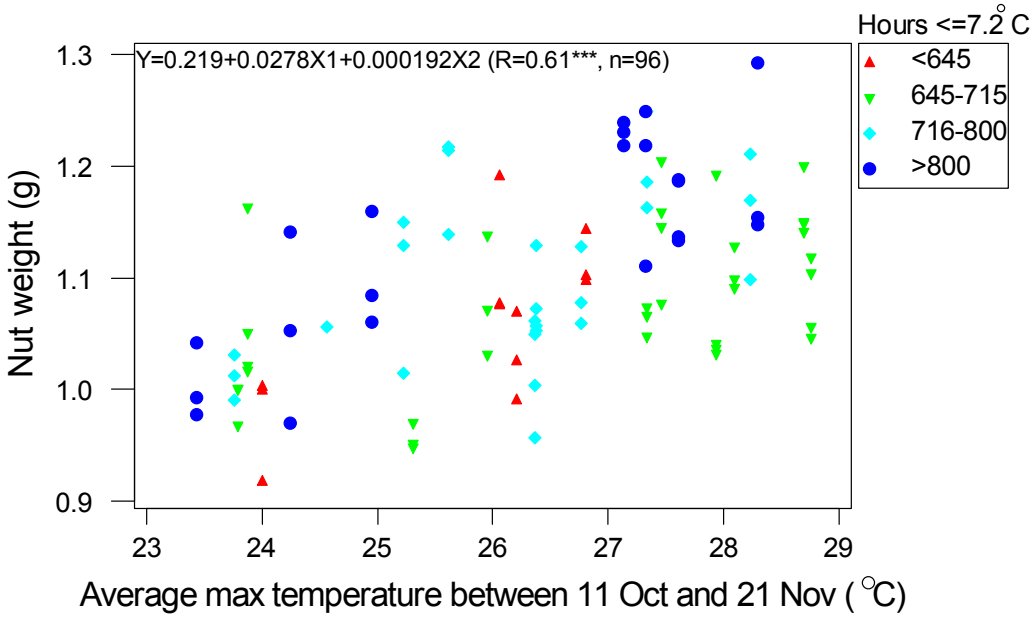


Figure 2: Relationship between nut weight and average maximum temperatures between 11 October and 21 November under different hour's ≤ 7.2°C between 1 May and 31 August

6 DISCUSSION

6.1 Chill Requirement

The Pistachio industry in Australia requires a reliable model to determine the accumulation of chill to assist in orchard management decisions. Provided the role of chilling models as nothing more than proxies for winter chill is recognised, using a chilling model to quantify both a cultivar's chilling requirement and the amount of chill available at a given location becomes possible, if not completely accurate (Luedeling 2009a). The Dynamic Model has been found to be better or at least equal to the Utah Model in studies at various locations (Allan et al 1995, Ruiz et al 2007, Viti et al 2010, Luedeling et al 2009a, Alberquerque et al 2008). In our study, the range of Chill Portions measured was relatively small (58-62). In late August and early September between two and six Chill Portions can accumulate in one week and had we collected samples more frequently the range may have been smaller. After five years observations it appears that the chill requirement for 'Sirora' pistachio is 59 Chill Portions. In those years when Chill Portions reached 59 no reduction in yield due to chill was observed. The only year where greenhouse experiments indicated a chill requirement of <59 Chill Portions was 2007, which was also the only year where poor correlation between predicted and actual bloom was observed at the Dareton Research Station. This discrepancy may be due to inconsistencies in the 10-shoot samples collected. At Renmark, Chill Portions were <59 in 2002, 2005, 2007 and 2009 all years where observations of uneven bud break and poor yield also indicated that these were years with insufficient winter chill. Mildura had Chill Portions of <59 in 2005 and 2009 which correspond with observations of uneven bud break and low crop if no winter oil was applied.

The large variation produced by the other two models makes selecting a chilling requirement value for these difficult. There was poor correspondence between the calculated chill and observed bud break with both the Chilling Hour and Utah Models. The Chilling Hour Model focuses on hourly temperatures between 0 and 7.2°C. However, in the Mildura and Renmark regions, hourly temperatures ≤ 7.2°C are usually infrequent as shown in Figure 1. Even in the cold winter of 2006, there were still many days where the minimum temperature was > 7.2°C. Providing temperatures between 7.2 and 12.9°C are useful for the chill process, it is obvious that the Chilling Hour Model is not suitable for those areas.

If the chilling requirement of 990 Chill Units determined in 2006 is used to interpret historical temperature records in Renmark, all years except 2000 and 2008 and 2010 should have had insufficient winter chill. Even for 2006, a very high chill year, the Chill Units accumulated in Renmark were still below the "standard". Of course, if the 752 Chill Units calculated in 2009 were used as a "standard", almost all years would have been deemed to have had sufficient chill. In the winter of 2006 in Renmark, warm temperatures resulted in 249 hours with -1 unit and 243 hours with -0.5 units. In the winter of 2008 in Renmark, the Utah Model had the highest values in the last 12 years when there were only 194 hours with -1 and 231 hours with -0.5 in Renmark. However, not only did both the Chilling Hour Model and the Dynamic Model have lower values in 2008 than 2006, but bloom in 2006 was earlier and shorter than in 2008. So, even when the Utah Model indicated that chill in 2006 was low, the plant response indicated that chill was high. The frequent warm temperatures experienced in winter resulting in so many negative units make the Utah Model unsuitable at least for the Renmark region.

Average winter temperatures in Renmark and Mildura are very similar. However, Renmark has higher maximum and lower minimum temperatures than those of Mildura. Under this situation, Renmark usually has more Chilling Hours ≤ 7.2°C than Mildura because it has lower minimum temperatures. However, Renmark usually has less Chilling Units than Mildura because it has higher maximum

temperatures. When the Chilling Hour Model (0-7.2°C) is used, the hours <0°C at Renmark do not account for any extra chill and this results in a similar amount of Chilling Hours as Mildura.

Predicted temperature increases due to climate change will reduce the amount of available winter chill (CSIRO and Bureau of Meteorology, 2007). Any reduction in winter chill will present challenges to growers with changes in management practices and perhaps even cultivars needed to accommodate new growing conditions (Luedeling et al 2009c; Luedeling and Brown, 2010). Consequently, it is important to identify the most suitable model for this region and also one that will continue to be useful in the future in a climate with increased temperatures. Luedeling et al (2009a) indicated that the Dynamic Model has the least variation compared with the Chilling Hour and Utah Models and our studies have shown that for the pistachio growing regions of south-east Australia this is also true. The Dynamic Model has also been shown to be the Model that best describes the bud break and yield responses of pistachio to chill in south-east Australia.

Between leaf fall and bud break, there are two phases necessary to break dormancy, chill requirement and heat requirement. It is not known when these two phases start and end. Luedeling et al (2009a) tried to calculate this "intersection point" based on "phenological observations". Harrington et al (2010) believes "that chilling and forcing can occur at the same time (in the temperature range where they overlap)". If that is the case then there will always be some inaccuracies when using existing chill and heat accumulation models to predict bloom dates in regions that experience warm temperatures during the "chilling" season. For this study we assumed that heat requirements start immediately after the chill requirement fulfillment. From our observations, when winter chill is satisfied then the assumption that heat accumulation only starts when the chill requirement is achieved appears to be valid and the prediction of bloom dates is accurate. However when winter chill is low, prediction of bloom dates is less accurate and it therefore appears that chill and heat accumulation is a much more complex process. Based on calculations from the Dynamic Model, predictions of 50% bloom dates were within one day of the actual bloom date for all years except 2007 (Table 2). Predictions were also made for the Mildura and Renmark regions with reasonable results except 2007 (unpublished data). In 2007, temperatures in August, September and October were higher than average. This resulted in the chill requirement fulfillment being achieved much later than usual. Fishman et al (1987a) assumed that dormancy completion could be proportional to an accumulated amount of some changes in plants such as an accumulation of a chemical substance or changes in physical structures and called this the "dormancy breaking factor (DBF)". When the chill requirement is fulfilled within the normal period, it may be assumed that all buds obtain enough DBF. When the chill requirement is not fulfilled within the normal period, the DBF produced only satisfies a proportion of buds. These buds break earlier while some other buds still require DBF and break late. This may explain why we observed differences in bud break in the greenhouse and in the field. In winter 2007 the shoots collected and placed in the greenhouse had accumulated enough DBF for 50% bud break to occur while those left on the tree did not.

A relationship between chill accumulation, 50% bud break in the greenhouse, bloom in the field and yield has yet to be established. Many authors (Erez and Lavee, 1971; Richardson et al, 1974; Shaltout and Unrath, 1983; Linsley-Noakes and Allan, 1994) have tested chill fulfillment but they mainly focus on bud break. However, growers are more interested in the influence of chill accumulation on yield and how the ability to predict chill accumulation can influence orchard management practices to maximize yield. It is important to include consideration of the yield when comparing models.

In the Pistachio industry in Australia, winter oil has been commonly used in most orchards for some years. Oil application in mid-late August may advance bud break by 0-4 weeks or more compared with orchards without winter oil application. From our observation in the field, if by 31 August, Chill

Portions were ≥ 68, oil application had no influence on bloom date or yield. When Chill Portions were between 61 and 67, oil application advanced bloom by about one week but our observations indicated that it had no influence on yield. When Chill Portions were between 59 and 60 we observed no clear reduction in yield, however, oil application advanced bloom by about two weeks. When Chill Portions were ≤ 58, without application of oil yield was reduced and oil application advanced bloom by three or more weeks.

Winter oil application plays a major role in pistachio production during low-chill years. All data shown above was based on chill accumulations to 31 August each year. However, the decision to apply oil to alleviate low chill needs to be made before 31 August. Mid-late August is the best time for winter oil application in Australia. Thus, by mid-August growers need a prediction of chill accumulation to decide whether or not to apply oil. Based on our data, 57 Chill Portions accumulated by 15 August provides a reasonable level of confidence that chilling requirements will be fulfilled. If chill accumulation is below this on 15 August, winter oil should be applied. Otherwise, the possibility of reduction in yield is high. Five years of practical application shows this estimate (57 Chill Portions) works well. Even though occasionally this model will underestimate the final Chill Portions, such as in winter 2010 when 56 Chill Portions had accumulated by 15 August and nine Chill Portions accumulated between 15 August and 31 August, growers have still accepted it as a management tool that provides security in years where sufficient chill may not accumulate.

We also need to discuss relationships between orchards and meteorological stations. Kyalite Pistachios should be in Swan Hill meteorological station zone. Portions in Swan Hill were never below 67. However, Kyalite still showed clear symptoms of low chill in the spring of 2005. In latitude, Kyalite is 47 km north to Swan Hill, while Swan Hill meteorological station (Aerodrome) is 5 km south of Swan Hill. Thus, Kyalite Pistachios is 52 km north of the Swan Hill meteorological station. Actually orchards around Swan Hill seem not to have good chill response as Swan Hill meteorological station shows. Another reason for this may be the station is too close to the lake area. This made weather records different from dry areas. It seems that portions from Mildura meteorological station are more reliable for orchards around Mildura, Robinvale, Kyalite, even Swan Hill. This is worth keeping the comparison in the future. In our industry, some big orchards have their own meteorological measurements. Unfortunately this data cannot be used for chill calculation. Those may provide more reliable predictions in the future.

6.2 Winter Oil Application

Winter 2008 had 68 portions based on Mildura meteorological station and oil application trials did not show any significant differences. However, the winter of 2009 had 58 portions and yield differences were clearly shown in the trial. Trees with winter oil application at 6% had 1.5 kg more merchantable yield per tree or $10.50 more return than trees with 3% winter oil application. Especially in ‘off’ years, this is of significant importance.

Nut maturity shows an interesting result. In season 2008/9, percentages of shake 1 of treatment 3% were significantly higher than 6%. Or we say, treatment at 6% made flower open earlier but made nut ripen later. But in season 2009/10, winter oil at a 6% application also led to early flower open and early maturity, i.e. higher percentages of nut harvest in the first shake.

There was no significant difference of blank shells and FM in season 2008/09 between treatments. However, in season 2009/10, winter oil at a 6% application also led to significantly lower percentage of blank shells and FM. This implies that early bud break benefited nut development.

In season 2008/9, treatment at 3% showed higher percentages for stains such as golden stain and dark stain. We cannot imagine this is due to chemical application: chemical at 3% gave worse results than at 6%! The other explanation is the late harvest comparing with treatment at 6%. Thus treatment 3% had a little higher stain.
In season 2009/10, on average, dark stain, adhere hull and damaged shells were lower in treatment of 6% than 3%.

Also in season 1, treatment of 3% had significantly lower percentages of damaged shells but this time it is in contrast.

In season 2008/9, treatment of 6% showed significantly higher percentages in damaged shells ($p=0.005$), but in season 2009/10 treatment of 6% showed low percentages on those at p-value of 0.054.

Treatment of 3% showed higher percentage of pick out in season 2008/09 ($p=0.058$) and in season 2009/10 ($p=0.024$). Treatment of 3% also showed high percentages of dark stain and golden stain in both seasons with significant difference in the first season. Further observation is needed to disclose the reason why high concentrations of winter oil lead to low percentages of stain nuts.

Costs of winter oil application were calculated. If the oil price is $2/L, an extra 3% per ha will cost $108 per ha. For a return in this year, based on the price in 2004, it is $10.5 per tree, or $3150 per ha. It is much more than the costs at $108 per ha.

Our conclusion here is that in low chill years, 6% winter oil application showed clear benefit. For common season, what should we do? In satisfied with the chill year, does 6% winter oil damage nuts?

This attempt showed some advances for scientific methods in our future work. Field trials for oil application in the dormancy period is difficult. It normally needs 4 rows for protection rows. This needs a big area for a small trial. After we have confirmed the best oil concentration, oil application date may still be a problem. California papers proved that mid August was better than either mid July or mid September. For our application, we still need to identify the better application dates within the 31 days of August. The general feeling is that the best time is around 18 – 28 August. But we need further evidence. We also need to know if those dates are fixed every year or they are drifting year by year based on chill progress. This kind of work is difficult to deal with in the field but is easy to work by oil dipping methods.

This is the first time for us to set up this kind of observation. We got some results. However, this work clearly showed shortages. Firstly, we did not set up control shoots. Thus we used advanced non-oiled shoot as the control. In practice, set up control shoots before may be easier for this work. Secondly, 6 trees show their own bud break speeds. Each tree has a particular treatment of concentration x date. Advanced bud break may be due to the treatment, or may be due to each single tree. Although human judgment may correct this, different treatments on the same tree will be fair for this comparison. If we intend to use this method, further work is required.

6.3 Nut Size

Pistachio nut size is different from fruit size. For example, temperatures a month before harvest still have strongly influences on fruit size on 'Royal Gala' apples (Zhang and Thiele, 1992). Apple size depends on flesh. Late influences are still useful. But pistachio nut size is equivalent to the seed in the apple. No reports record the influence of climatic factors on apple seeds. Nut size of pistachio is also

different from almonds. Most commercial grades of almonds do not have shells, whilst pistachios do. For the market, pistachio nut grading depends on maximum length of shells to pass grading holes. Shell size is the key factor for pistachio nut size. Average nut weight in this paper was transferred from nut count size. It is subjective to shell size. Although late kernel development may improve a little nut size, it is very limited after the shell has hardened. Undeveloped kernels may lead to high percentages of closed shells but do not affect the size very much. This indicates that nut size study on pistachio is quite different from other fruit or nut crops. During the hull enlargement period (around 11 October to 21 November in the southern hemisphere), maximum temperatures stimulate large shells. Crane and Al-Shalan (1974) reported that the quick diameter increase period of pistachio nuts is around mid-April to mid-May in the northern hemisphere also supports this finding.

At the same time, good previous winter chill should benefit flower buds before flowering. Well developed flower buds have potential to bear larger nuts. Commonly winter chill is considered as a natural change, or a "yes/no" change. If plants undergo enough winter chills, plant will have no problem with production. However, this paper shows that winter chill also has a qualitative influence. It affects nut size. Due to weak influence, it is not easy to be found.

Table 3 actually shows a non-significant positive correlation between nut weight and yields. That means that when yield increases, nut size increases. We cannot imagine that high yields produce large nuts. Our database has 10-years of records. In 5 'on' years, the average maximum temperature between 11 October and 21 November was 26.8°C while that for 5 'off' years was 25.9°C. T-test showed difference at p=0.003 level. In this circumstance, heavy crops in the 'on' years, under better temperature during hull enlargement period, did not produce small nuts as could be expected. This result leads us to find the relationship difficult between nut weight and crop load in this database.

It should be emphasized that in some cases, temperatures showed much stronger influence than crop load. For example in 'Royal Gala' apple predictions in New Zealand, when temperature increases 1°C between December and January, every apple may increase 9.7 grams. It is more useful than reducing half of the whole crop (Zhang and Thiele, 1992). We cannot evaluate the effect of crop load on nut size of the pistachio at this time. It is hoped to add this item into the model in future work.

Computer modeling was developed in the late 20th century. Modeling techniques provide benefits in a range of areas such as synthesizing knowledge, generation and testing of hypotheses, developing technologies for the fruit production system and decision support system. In knowledge development, visualization is important to help humans further understand the natural process (Atkins, 1999). Contour mapping is a tool for us to view climatic effect in our production.

To understand the key period of nut size is important. This not only can provide methods for people to increase shell size or nut size, but also can control nut size to reduce percentage of close nuts for big nut variety.

6.4 Tree Management

Although Aggressive pruning obtained the highest average yield during the last season, from an accumulative yield point, the Control is still the best results. This is probably due to tree re-structure in pruning treatment. Control keeps wide tree shape while both treatments made the tree thin and higher. In this process, much bearing area is lost in re-structure but any new bearing area needs time to re-build. This may be the reason for yield reduction. However, this shape is the better shape for production, especially reducing branch broken. How to evaluate this will be difficult.

From trial result, hand thinning did not significantly reduce crop in year 1 but increased crop in year 2. Year 3 was expected to be the same as year 1 but it showed better performance than year 1. Year 4 kept the trend for yield increase comparing with other treatments. This may be due to nutrition savings in the early bloom stage. However, none of them reached a significant level. This created an obstacle for conclusion making. From a biennial bearing point, harvest 2008 was a big off-year. Although early hand thinning obtained the highest yields among the treatments, it did not stop the big crop drop in the big 'off' year. This, at least, shows that hand thinning only has a limited benefit for crop adjustment. Due to the limited effect on biennial bearing and the lack of measure for implementation the hand thinning trial had to be stopped here. However, if equipment for thinning is available and cheaper, it is still a potentially useful measure in controlling biennial bearing in pistachio production.

6.5 Reflective Mulch

In the 1st trial, we put the mulch together and obtained higher yields for trees above the mulch in 2-years time. In the 2nd trial, we arranged the mulch as randomized plots and we still obtained higher yields comparing with the control although in year 4 both treatments had low yields due to a big 'off' year. The 3rd trial started last season. Due to a big 'off' year in harvest 2008, all treatments had low yields in season 1. Reflective mulch did not show an advantage in a big 'off' year. However, from seasons 2 and 3, the mulch showed significant effect on yield as well as in nut size. In season 2, the whole of CMV Farms had a high yield. However, the mulch area showed much higher yields than average. Control with 60 kg nut in hull per tree should be reasonably high yields based on an 18 tree average. Trees above Extenday produced 67 or 68 kg nut in hull per tree. This number is higher than 51.7 kg/tree in the highest average stage (stage 1) and the 39.0 kg/tree in stage 5. This is the highest records historically for a group of trees in the pistachio industry in Australia. Furthermore, Tree 5 at Kyalite Pistachio was a famous high-yielding tree (Zhang, 2005). In its 16-year record, there were 2 records with yield > 80 kg, one was 88.5 kg in 2003 and the other was 83.8 kg in 2007. In row 65 in Kyalite Pistachio, another tree with a record > 80 kg was tree 21 in 2007. However, in the reflective-mulch trial this season, there were 5 trees with yields in hull > 80 kg, 4 of them above Extenday under canopy and 1 of them above Extenday between rows. Two trees from these 5 trees have the yields above 85 kg. They came from both Extenday treatments. We should recognize the influence from the mulch.

The study of PAR (photosynthetically active radiation) reflected from the orchard floor back onto the trees showed that, under bright conditions, Extenday reflected some 15% of the incident radiation into the lower canopy compared to 2% for the control trees, which compares well with other reports (Grout et al, 2004). The spectral distribution of the reflected light was similar to that of incident sunlight but there were significant differences in the red:far-red ratio between reflective material. The red:far-red ratio for incident radiation is 1.27. Extenday reflects light that is very close to incident light in ratio of red:far-red (1.06) whereas the red component is much reduced when reflected from grass (0.13) (Grout et al, 2004). As well known in apple thinning studies, light is an important factor for fruit set. Shading trials always prove low fruit set. In contrast, extra light may benefit fruit set or nut set. Furthermore, when mulberry flowers were exposed to eight different colours of light, seed set was higher in red and orange light and lower in black compared with other light colours (Chowdhuri et al, 2003). All these seem to suggest that the improved red:far red ratio of the extra light reflected into the lower canopy had an improvement on nut set. Pistachio have panicles flower clusters and a single cluster may have 200 - 250 flowers (Shuraki and Sedgley, 1996). In our hand thinning trial and pruning trial, we investigated 1800 clusters and found maximum nut numbers per cluster were 119 with averages around 30. This indicates room to increase nut numbers per cluster if light can do this. We believe this could be the reason for reflective mulch increasing yields immediately. In addition, Runkle and Heins (2001) working on flowers and vegetables found that environmental shortening of red light can suppress

flower initiation or development. This may explain yield increase in 'off' years by the mulch but we need further evidence for this point.

Extra high yield may increase biennial bearing. This was not the result according to yields in year 2 in our 1st reflective mulch trial and Year 3 in our 3rd trial. There is a hypothesis for this although it may be difficult to prove. Yield can be divided into nut numbers per cluster and cluster numbers per tree. As known, nut or fruit clusters strongly reduce flower bud initiation by gibberellins in the twig close to the clusters. This influence is local and does not affect the whole trees. New shoots without nut stimulation by mechanical pruning with good flower initiation prove this. In this way, pistachio growers rather want more nuts per cluster than more clusters per tree in a same yield although the former still has its shortcoming. This will enhance the practicality of the mulch.

Mulch location is another research topic for us. After we proved yield increases from the mulch, mulch location was raised. Reflective light test from the bottom of the canopy strongly suggests advantages of under canopy mulch. But trial harvest this season did not support this very much. Although treatment of under canopy produces 1 kg more nuts than treatment of between rows without a statistical difference, the latter produced a significantly higher percentage of Jumbo nuts than the former with higher return than the former on average. The mulch under canopy does reflect more light into the bottom of the canopy. This may increase nut setting but they were not large nuts. The mulch between rows reflects less light into the bottom, it strengthens light in the middle area of the canopy. This area is the place to bearing big nuts as known in apple production. Thus we obtained this result. Our original purpose for mulch was to increase nut size and we obtained this result n season 2. We also established a model for nut size and we recognized the key period for nut size. Further application may be developed in this area. In the other view, mulch under trees may reduce damage from vehicle passing between rows.

Plastic mulch used for grapes showed better results for light reflection than Extenday at the same position – under canopy. It's cheaper and whiter and showed possibility for further application. However, it has shortcomings which are discussed. Maybe white plastic across the whole area is better than Extenday, which is a knit. However, after rainfall, water cannot pass through white plastic and can remain on white plastic for the whole day. This may be a kind of loss in agriculture production. We compared our application with vegetable growers. When they use large plastic sheets for vegetables or watermelons, there are so many holes for the plants, this may allow the water into the ground. For our application, 2.5 m wide, maybe 100 m, 200 m long without any holes, water will have no chance to move into the ground. Another problem of white plastic is root damage when white plastic is buried under the canopy. However, harvest showed that it ranked between Extenday and the control but close to Extenday. This further explains the importance of extra light. Under condition of root damage, extra light still produced high yields than the control. However, problems of root damage and the fixing process makes this practice difficult.

It is easy when staples are used to fix Extenday onto ground. The material is tough. After fixing, there was no any problem for the whole season. When the same method to fix plastic mulch was used within just 10 days the mulch was broken under the staples. This mulch is much more fragile. Some areas were refixed with a piece of plastic knit under each staple. Although it showed better results the plastic breaking still happened quite often. Generally speaking, this mulch does not suit fixing by staple or hook. For this kind of mulch the best fixing method is to bury it into the soil using a machine.

The mulch may provide better **soil moisture**. It may provide a function like buried drip line. However, we need data to prove this. Portable EnviScan is the best choice. But old methods, metal box burns soil should be cheap enough for the practice.

Last discussion is the price. Currently Extenday is \$1.40 per m^2. Plastic mulch used for grapes or for vegetable is about \$ 0.20 – 0.30 per m^2. However, we clearly know that Extenday can be used at least for 3 growing seasons. As for irrigation requirement we had to redraw the mulch before harvest each year. Can we re-use the plastic mulch? If we cannot, the price will be very similar.

In our trial history, we tried pruning but it did not clearly show yield increase. We tried hand thinning but it did not show yield increase clearly or immediately. We tried different nitrogen applications but it did not show any yield difference in the first application season. However, the mulch does. Except a very 'off' year, the mulch always shows yield increases in the application season. We need to pay more attention to this.

6.6 SEL Nuts

SEL nuts are specially produced in 'off' years. We underwent SEL nut damage in harvests in 2000, 2004 and 2006. However, almost no SEL nuts were found in orchards in season 2007/08 and 2009/10. We should understand the reason for our future work. In 6 years of work, we almost believe that SEL nut is due to Calcium deficiency. Most of the Calcium activity is related to its capacity for coordination to provide stability for life. Calcium is predominantly in the cell walls and at the plasma membrane. Its function is the regulation of membrane permeability and related processes and the strengthening of the cell walls. Pectates are kinds of glue between the cell walls. The degradation of pectates is mediated by polygalacturonase, which is drastically inhibited by high Ca^{2+} concentrations. In agreement with this, 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 (fruit or nut) increases the risk that the tissue content of Calcium falls below the critical level required for the maintenance of membrane integrity, leading to typical so-called Calcium-deficiency-related disorder (Marschner, 1986). Our SEL nut developed under these conditions. Tissue analysis supports this explanation. Calcium spray seemed useful in the trial area. Cold spring usually prevents Calcium absorption. An increase in the concentration of Calcium in the external solution leads to an increase in the Calciun level in the leaves but not necessarily in low-transpiring organs such as fleshy fruits supplied predominantly via the phloem. High transpiration rates of the whole shoot often decrease rather than increase the Calcium influx into 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). Root pressure is dependent on root respiration and low temperature is one of the factors influence on root respiration.

In Figure 5, spring 1999, 2003 and 2005 showed cold temperatures and resulted in SEL development. However, spring 2007 and 2009 showed warm weather.

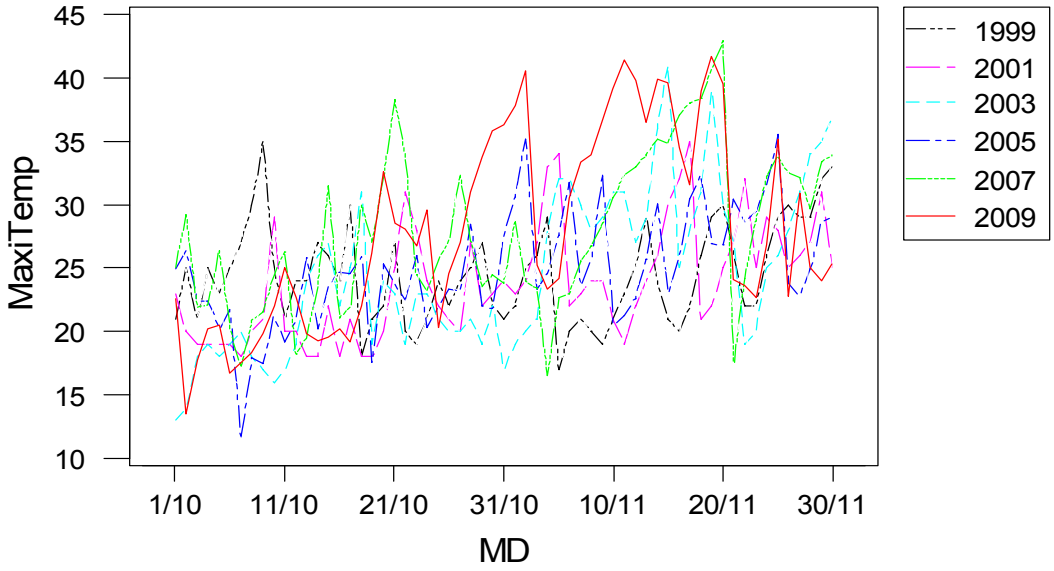


Figure 5: Daily maximum temperature in Oct and Nov between 1999 and 2009

Figure 6 further explains this phenomenon. Figure 2 (within the results section) used accumulative maximum temperatures with a base temperature of 25°C. From Figure 2, spring 2007 and 2009 showed outstanding results.

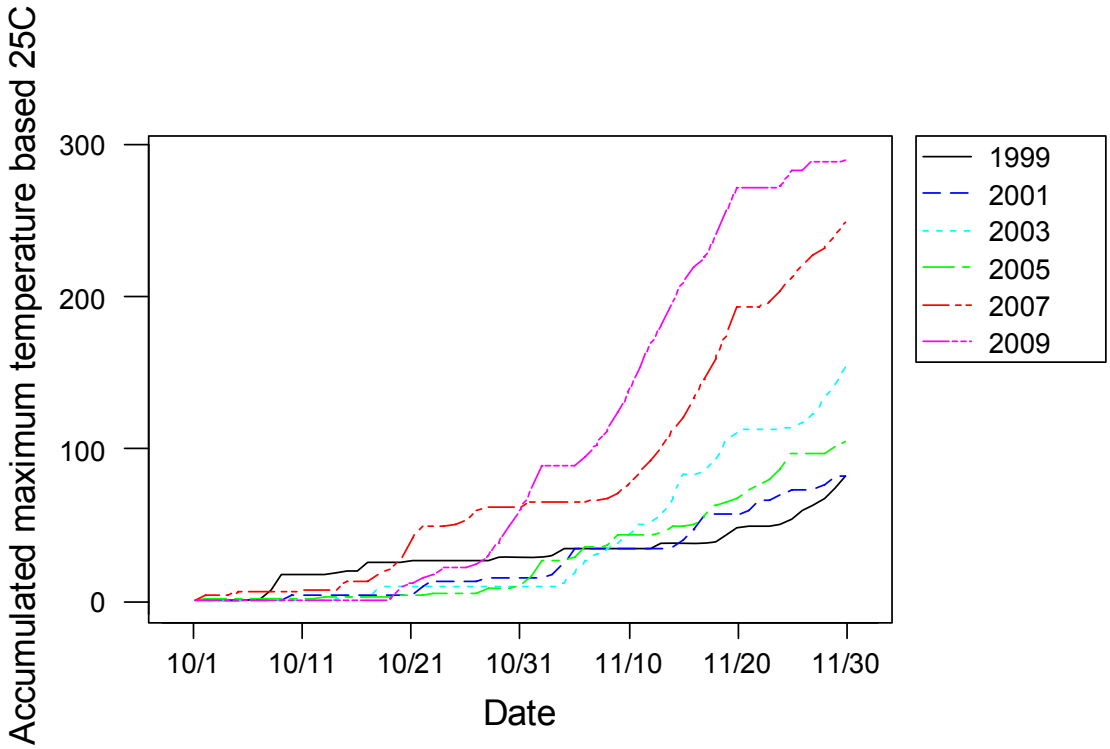


Figure 6 Accumulative maximum temperatures with base temperatures of 25°C in Oct and Nov

The 2002 harvest showed an unusual result an it is explained in this paper. From all 3 graphs, spring 2001 did not show any better spring than 1999, 2003 and 2005. In checking with Andrew Bowring, he thought that year was not a severe SEL nut year, but it was not as clean as 2007/08. Andrew also thought that harvest 2002 was a relatively high-cropping off-year. All of these may reduce SEL appearing. Anyway, further clarifying harvest 2002 is necessary.

7 TECHNOLOGY TRANSFER

7. 1 Conferences

- Zhang, J. and Joyce C. 2009. A study on climate factors on 'sirora' pistachio nut size. Oral presentation. V International Symposium on Pistachios and Almonds. Sanliurfa, Turkey, 6th - 10th October, 2009
- Zhang J. 2009. A study on application of reflective mulch in pistachio production. V International Symposium on Pistachios and Almonds. Sanliurfa, Turkey, 6th -10th October, 2009

7. 2 Field day

A research information day was held by the Pistachio Growers Association Incorporated for growers at CMV Farms, Robinvale, Victoria, on 21st July 2006, 8th August 2007, 25th June 2009, 7th May 2010 and 14th September 2011. Presentations will be given on the Research Field Officer's trials on nutrition, pruning, nut size and quality, and harvest date prediction. There will be opportunity for grower questions and discussion, and in the orchard demonstrations.

7. 3 Orchard visit

This is one of the tasks for this position. Besides research on particular topics, contact with pistachio growers and improvement on their production capacity were carried out in last 3-year work.

Based on voluntary principle, an 18-grower group has been organized. Pruning advice in orchard has been provided last winter and will provide every winter during pruning period. This work also analysed historical data for the 18 orchards. Comparison of yields based on tree or hectare, and nut size etc also provided for the growers. Fertilizer analysis is under the way. From fertilizer analysis, maximum application in our growers of N was about 10 times of minimum application. This trend is definitely needed to be improved. This work will help most growers to find production problem and improve their production.

7. 4 Chill hours.

Using the results of the Chill Hour modelling PGAI has prepared annual chill hour assessments and recommendations. These have been distributed to all growers on the 15th August each year to assist the growers understand what chill hours their orchard may have received and what action needs to be taken.

The following is a copy of the 2011 Chill hour report.

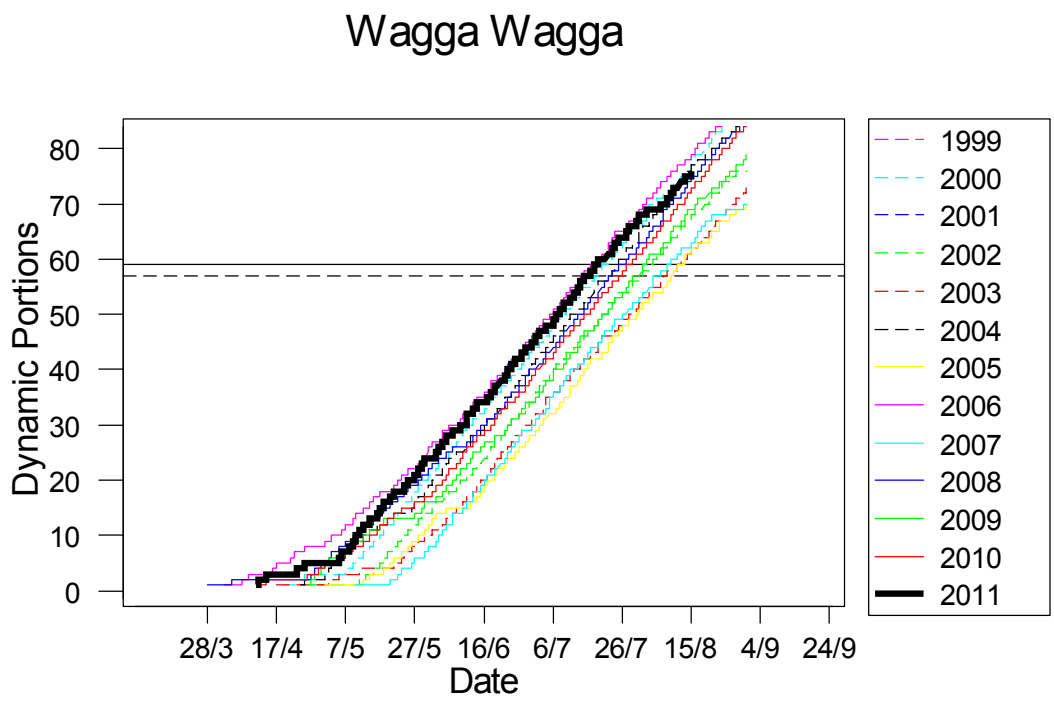
PGA Chill Newsletter Number 3 - 2011-12 Season

16th August 2011

Winter Chill 2011

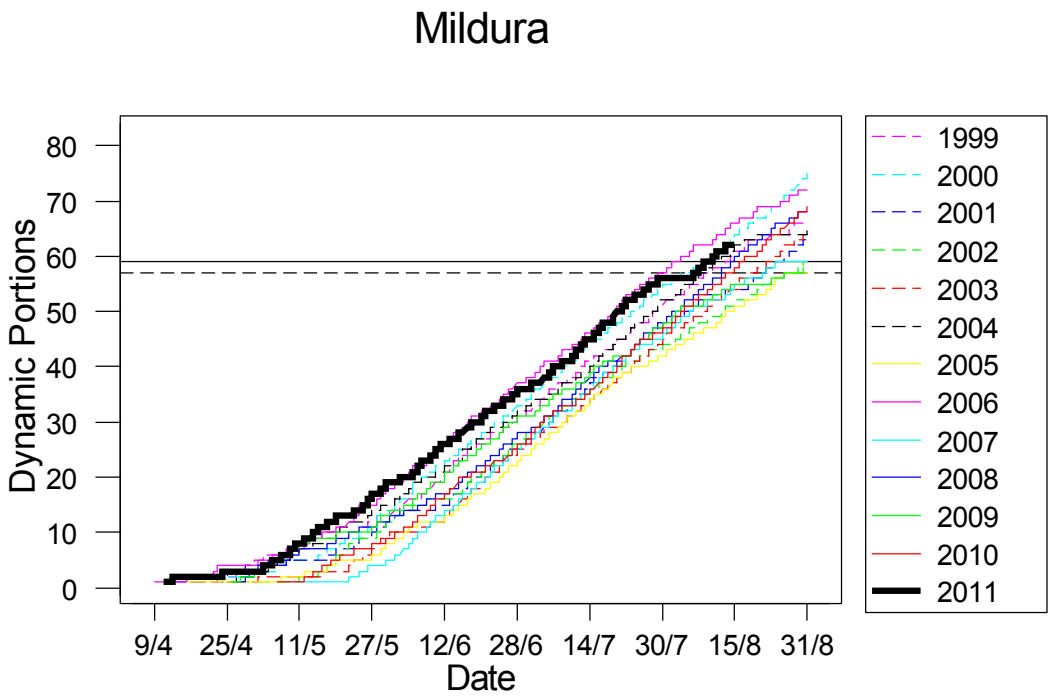
Whilst there are prospects for the accumulation of additional chill over the next weeks, the PGA concludes its reporting of the Dynamic Model chill portions on 15th August each season. This cutoff date is to allow growers to take remedial action in the event of insufficient chill.

The PGA research, lead by Dr Jianlu Zhang, shows that 59 Dynamic Portions to 31st August provide pistachios with sufficient chill. The research and annual data collection is funded by the financial contribution of most pistachio growers with additional support from the Australian government through Horticulture Australia Ltd.
The data for 2011 shows that the Riverland had insufficient chill; the Swan Hill- Mildura region just had sufficient chill; the other sites covered by the PGA all showed sufficient chill. The details for each site are shown below.
All of the charts show a flat portion of zero chill accumulation in the first week of August. It really is not known what this week of record high temperatures will do. But, it is unlikely to be a positive impact on chill accumulation.



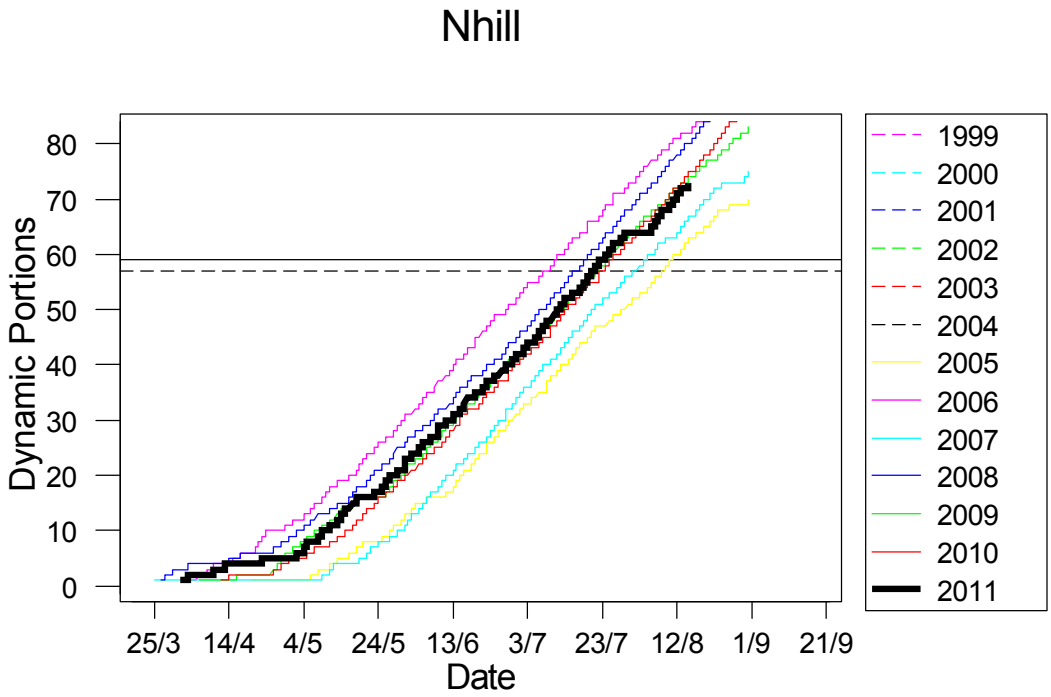
PGA research shows that 59 Dynamic Portions between 1st March and 31th August is sufficient chill. 57 Dynamic Portions to 15th August will in 95% of years produce 59 Portions by 31st August.

Wagga Wagga - It is 76 Portions. This is well above the required 59 Portions.



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.

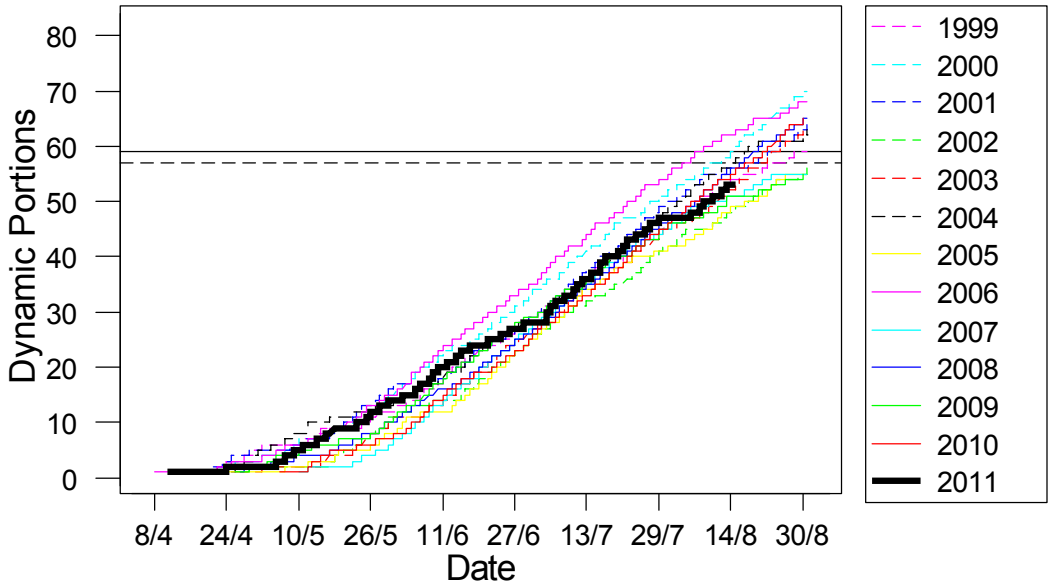
Swan Hill/Mildura – It is 62 Portions. This is just above the required 59 Portions.



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 – It is 73 Portions. This is well above the required 59 Portions.

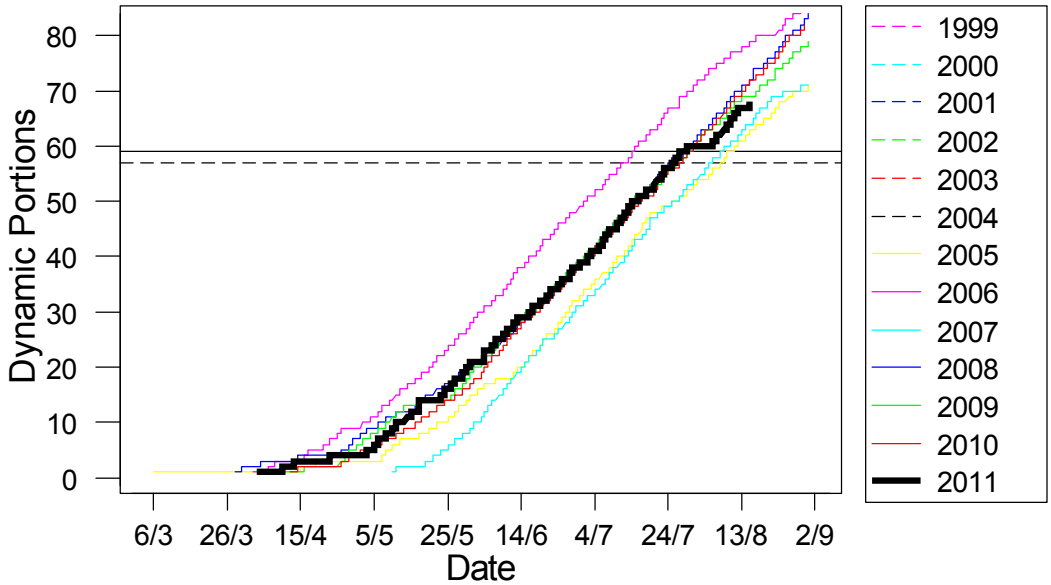
Renmark



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 – It is 53 Portions. This is below the required 57 Portions required by the 15th August. Riverland growers should be seriously considering remedial action as out lined in below.

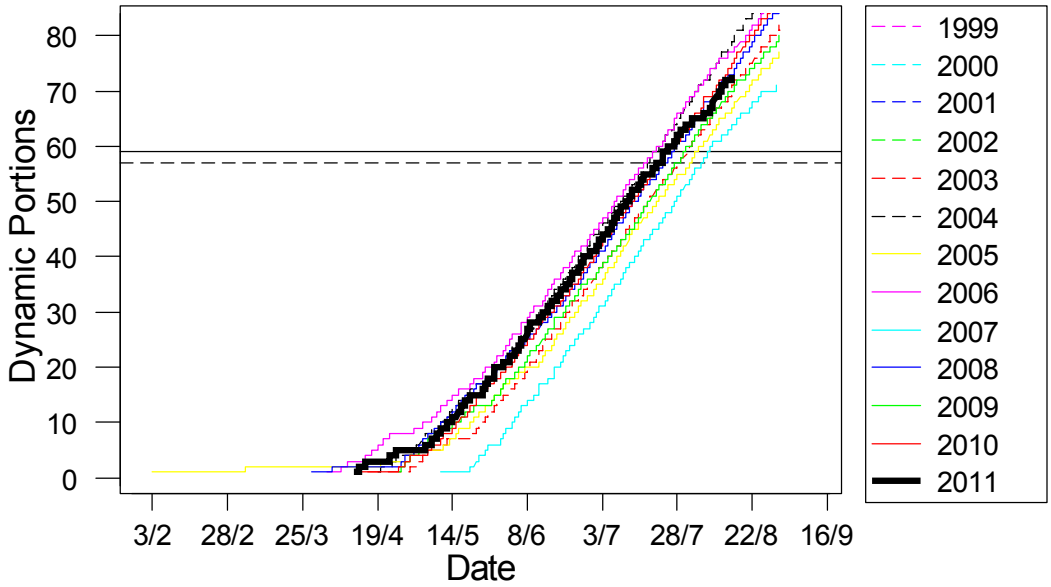
Lameroo



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.

Lameroo – It is 68 Portions. This is above the required 59 Portions.

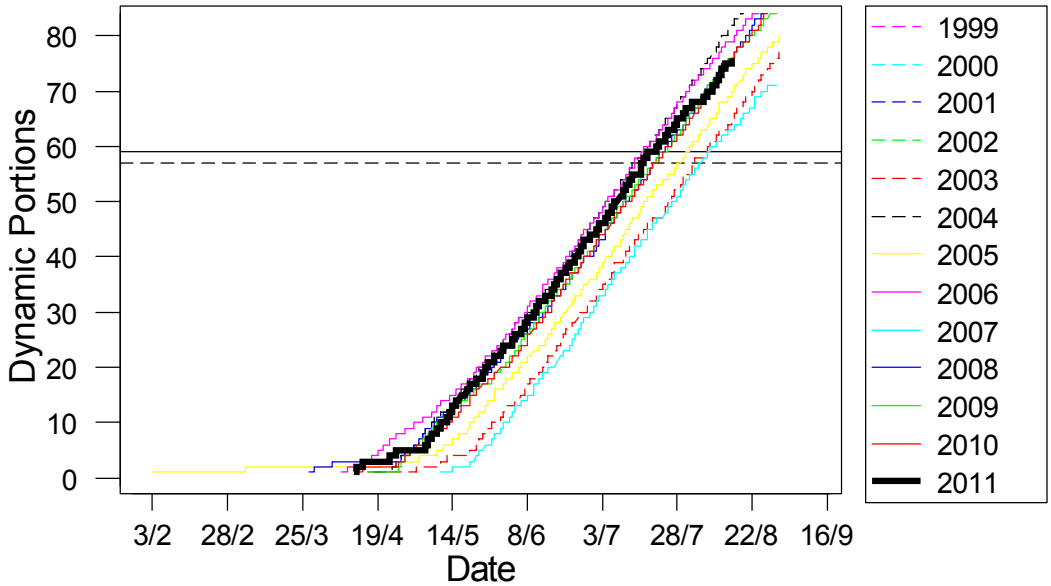
Yarrawonga



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 – It is 73 Portions. This is well above the required 59 Portions.

Albury



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 – It is 76 Portions. This is well above the required 59 Portions.

Chill Background Information

Pistachios are extremely sensitive to lack of winter chill.
Lack of chill will result in very uneven opening of flowering buds; some will not open until November or December, many buds will not open at all.
PGA research, supported by the Australian government through Horticulture Australia has shown the Dynamic Chill model to be the most appropriate method to measure the chill requirements of pistachios. The research has shown that *Sirora* pistachios require 59 Dynamic Chill Portions between 1st March and 31st August. The research also shows that 57 Dynamic Chill Portions to the 15th August will in 95% of years accumulate to 59 Portions by 31st August.

Mitigating the effect of low winter chill

Insufficient winter chill causes uneven bud break and perhaps some buds not to open at all. Sometimes buds will delay opening until November or December resulting in poor or non-pollination. Late opening buds will have late maturing nuts; some buds will mature so late that they cannot be commercially harvested.

PGA research by Dr Jianlu Zhang has shown that the Dynamic Model provides the best guide to measuring the amount of chill received each season. His work shows that less than 59 Dynamic Portions of chill between 1st March and 31st August are insufficient 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 have shown limited adverse affects from oil application. In the one 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

Application rate: Spray volume is dependent on tree size, but must be applied to the point of runoff. It is critical that bud scales are thoroughly wetted. On average size trees, the application rate is up to 1,800 litres/ha.

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

7. 5 Reports/Publications

- Zhang, J. 2006. An update on pistachio rootstock trials - Comparing the performance of Pioneer Gold 1, UCB#1 and *Pistacia terbinthus* when grafted to Sirora. *Australian Nutgrower*. 20(3):22-24.
- Zhang, J.; Cox, G. 2007. Chemical control of budbreak: a review. *Australian Nutgrower*. 21(2):29-36.
- Zhang, J. 2009. A study on biennial bearing of pistachio trees. *Australian Nutgrower*. 23(3):32-34.
- Zhang, J. and Joyce C. 2009. A study on climate factors on 'sirora' pistachio nut size. *Acta Horticulturae* (submitted)
- Zhang J. 2009. A study on application of reflective mulch in pistachio production. *Acta Horticulturae* (submitted)
- Zhang, J. 2010. A nitrogen trial for pistachio production. *Australian Nutgrower*. 24(4):22-23.
- Zhang, J. and Taylor, C. 2011. The dynamic model provides the best description of the chill process on 'Sirora' pistachio trees in Australia. *HortScience* (Accepted)

8 RECOMMENDATIONS

Further work on N application trial is necessary. This may allow Australian growers to more efficiently and economically use this major fertilizer. Based on nutrition studies, Australian leaf analysis standards for pistachio should be established.

The chill requirement study of 'Sirora' pistachio trees over the 5 years, from 2006 to 2011, obtained successful results. A scientific paper has been accepted by HortScience in USA. Further work is required for other varieties.

Winter oil application has shown to reduce crop reduction. Further studies on what situations require winter oil and any possible side effects of winter oil are required.

A model for nut size prediction has been developed. The results have been proposed and accepted by Acta Horticulturae. Predictions over 2 years have obtained reasonable results but further validation is required for this work.
More climate models should be created for bloom date predictions, heat requirements and maturity predictions including new methods.

For the research of controlling stylar end lesion in nuts the first step is reviewing historical data. The second step is to find which element(s) are useful for controlling stylar end lesion nuts and the third step is to prove the findings. The fourth step is to find the correct timing and concentration for this work and the fifth step is to find an efficient way to apply Calcium.
Current results may allow industry to report that the second step is completed and Calcium is probably the main factor for stylar end lesion nuts. We have almost completed all the five steps and reached a practical process for controlling stylar end lesion in nuts.

Mechanical pruning is an important project. It seems to improve tree structure and reduce alternate bearing as well as reducing costs. This study may also assist in understanding the process of biennial bearing of pistachio trees. When should this technique be used - prior an 'off' year or an 'on' year? All these need further work to answer some of the outstanding issues.

Reflective mulch trials showed good results. The trials proved that reflective mulch can increase crop with the mulch under the canopy showing good results. Further work is required including timing of the mulch application, whole growing season or a key period in a growing season and/or what kinds of mulch colour are most efficient.

9 ACKNOWLEDGMENTS

The authors would like to acknowledge the

- Financial support by Horticultural Australia Ltd and The Pistachio Growers' Association Inc,
- Field and laboratory work support from APPC, CMV Farms and Kyalite Pistachios,
- Sharp critics and valuable suggestions by, Dr Ben Robinson, Peter Weir, Andrew Bowring, James Meyer and Chris Joyce and the research committee members, and
- Laboratory work by, Ms Cathy Taylor (Victorian DPI).

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SECTION 2: Management procedures for Fungal Problems facing the Australian Pistachio Industry

1. INTRODUCTION:

Fungal problems have existed in Australian Pistachio orchards for some time. With the exception of one orchard, the damage to date has been at a low commercial cost. During the extreme season of 2010/11, fungal diseases have exploded causing substantial commercial loss. The current estimate is that 20-40% of the expected on-crop of 2,000 tonnes has been lost. The market value to growers of this loss is $3.2 million to $6.4 million for the 2011 season alone. The final loss cannot be determined until after harvest in April 2011.

The principal pathogen identified, so far in 2010-11, is Anthracnose (*Colletotrichum acutatum*) with some detections of *Botryosphaeria sp.*

Anthracnose is known in other Australian crops such as avocados, olives and almonds. It has been reported in Pistachio crops overseas, but not extensively. *Botryosphaeria* is very well known in Californian Pistachios and California has established protocols for its management.
The PGAI is seeking proposals for the development of a grower information pack including a management procedures manual to manage these fungal diseases in Australian Pistachio orchards. It is envisaged that the manual will include recommendations on the likely weather triggers for an outbreak; cultural practices that will minimise an outbreak, and spray programs that will minimise the impact of an outbreak.

It is envisaged that to produce such a manual, the service provider will need to obtain significant information on Anthracnose to be able to recommend a protocol. For *Botryosphaeria* it is likely that review, and modification as appropriate, of the Californian protocols will be sufficient. However, should the study show that a different approach is required for Australian conditions, then such alternatives should be included in the final manuals.

2. METHODOLOGY.

The initial work in this section of the project include:-

1. Initiation of a work plan and the undertaking of an early field visit to view the problem within the orchard and collect samples.
2. Collation of information and data from local, Australian and International researchers and growers including a thorough literature review.
3. Review initial information and where necessary expand the consultation and prepare initial documentation.
4. Conduct and review with SARDI in vitro tests on fungal growth parameters and fungal sensitivity to a range of fungicide chemistries.

Dr Prue McMichael from Scholefield Robinson Horticultural Services was brought onto the Pistachio Growers' Association Inc team to assist with the program of ascertaining the problems incurred by the Australian Pistachio Growers during the 2010/11 harvest.
The role was to implement a program to collect and collate information for the identification of the problem through

- Field visits for newly observed fungal problems within the orchard, and

- Literature review for newly observed fungal problems within the orchard, and
- Input from local, Australian and International pathologists for newly observed fungal problems within the orchard.
- Coordinate the laboratory work with SARDI

3. RESULTS:

The following outputs have been achieved as part of this milestone:

a. Grower Survey.

A grower survey form was prepared and distributed to all Australian Pistachio Growers to source relevant information on the fungal problem over the 2010/11 season.
A copy of this form is attached as Appendix A to this report.
The information supplied was collated by the PGAI Research Field Officer Jianlu Zhang and then forwarded to Prue McMichael for utilisation within her research component of this project.

b. Field Visit

To commence work on the serious fungal diseases encountered this season Garth Swinburn and Prue McMichael carried out orchard inspections and information gathering during the week 21st to 25th March. The information that was collected related to the

- time of disease onset this season,
- description of the earliest symptoms,
- conditions prior to disease onset,
- variation in symptom development and severity,
- spray programs that have been trialed,
- block' variability – e.g. by tree age, rootstock,
- hard/hedge pruning or edge effects (and therefore canopy humidity and air movement),
- orchard sanitation effects (pruning's/leaf litter versus cleared under canopy).

Weather details that had already been collated by the PGAI Research Field Officer were also made available.

From the field visits the following activities occurred:-

1) The collected samples - leaves, petioles, rachises and nuts – were incubated to find almost all have *Colletotrichum* present, even in the absence of significant symptoms before surface sterilising. The leaves with concentric rings had some *Alternaria* but also *Colletotrichum* and it was difficult to identify which came first, as *Alternaria* was both a pathogen and a secondary organism. It was assumed that the midrib leaf lesions and blade spots include both fungi and this differs from the USA literature to some extent as it suggests there are no/few leaf symptoms associated with anthracnose. The marginal "burn" observed on leaves was still to be looked at more closely.

2) Prue McMichael met with Barbara Hall and discussed the *in vitro* laboratory chemical efficacy tests that should be conducted. The limitations of such tests

were considered (i.e. gives indication of efficacy on mycelial growth but not other life cycle stages) but PGAI agreed that industry needed these tests to get an early indication of efficacy. From these results PGAI will be able to reduce the number of products to be tested in more detail later (in greenhouse trees or field trees).

3) The next step to be considered was greenhouse testing of products as 'protectants' or 'eradicants.' Such work will ideally be carried out over winter so that spring field trials could be quite focused.
The greenhouse stage required getting some potted trees soon, keeping them in the greenhouse (to keep leaves on) and at different stages inoculating leaves and spraying them with test products. Inoculation of leaves with the fungus, followed by spraying will test a chemical's eradicant potential; whereas spraying first followed by inoculation will test the protectant capacity

4) What remains unknown before next season was the survival potential and location of *Colletotrichum* spores. The *Botryosphaeri*a is known to survive in wood and on mummied nuts, and in leaf material. In other hosts, *Colletotrichum* spp. survives well in infected twigs, mummies and infected leaf matter but from the field visits it did not look like this fungus had moved into wood from the rachises but this needs to be confirmed through further laboratory testing.
It was assumed that
   - any nuts or rachises that remain on the tree, will harbour spores over the winter, and
   - these spores will be capable of infecting green shoots and nuts in the spring/summer, and
   - spores will survive in infected plant material on the ground and that rain splash will elevate them to low shoots and fruit in the spring.

   What is not known is what survival there might be in surrounding vegetation or weeds at this stage.

5) The *Botryosphaeri*a and *Colletotrichum* spp. isolates from pistachios were sent to California and the *Colletotrichum* isolate were also sent to Western Australia for identification confirmation.

c. Consultation with other researchers and people of experience

A number of parties have assisted in supplying relevant scientific and technical information and these include:-.

- Californian researchers
  Themis Michailides: THEMIS@uckac.edu
  Bob Beede: Bob.Beede@co.kings.ca.us

- Qld DPI
  Liz Dann: elizabeth.dann@deedi.qld.gov.au

- Rod Edmonds on 07 3896 9414.

- BOUNDARY BEND OLIVES,
  Simon Robb, M: 0437 985 778 E: s.robb@boundarybend.com

- University of Western Sydney, Centre for Plants and the Environment
  Associate Professor Robert Spooner-Hart Phone +61 245701429 Mobile 0414 953 129 Email r.spooner-hart@uws.edu.au

- Pathologists who have done work for some growers
  SARDI : Barbara Hall Barbara.Hall@sa.gov.au
  SRHS : Prue McMichael prue@srhs.com.au

d. Technical Information Sheets

Following the literature review, consultation with other parties and consideration of the available data on the incidence in Australian orchards, the project service provider has made some initial recommendations for the management of the disease in Australian orchards.

Through the inputs of Prue McMichael, Chris Joyce (Chair of the Pistachio R&D Committee), Trevor Ranford (Project Manager/Executive Officer, PGAI), Andre Bowring (Pistachio grower) and Barbara Hall (SARDI) a range of technical information sheets have been prepared which includes some of the management recommendations.

The following Technical Information Sheets have been prepared and are attachments (PDF) to this report:-

(1) Technical Information Sheet No 1 – *Anthracnose of Pistachios during 2010 – 11*
(2) Technical Information Sheet No 2 – *Report on the Project work on Anthracnose*
(3) Technical Information Sheet No 4 – *Panicle and Shoot Blight ("Bot') of Pistachios.*

e. Literature review

There was belief that there was considerable literature available on both Anthracnose and *Botryosphaeria sp*.

A full bibliography of literature needed to be prepared and made available to PGAI for placement on the website as part of the final disease management manual.

Prue McMichael undertook an initial literature review in relation to the fungal problems for Pistachio crops.

A copy of the Literature Review titled *"Fungal diseases of Pistachios Literature Review"* is attached to this report (PDF).

f. Laboratory work by SARDI

The South Australian Research & Development Institute (SARDI) were contracted through Scholefield Robinson Horticultural Services to undertake laboratory tests on the sample collected from the orchard visits with the aim of identifying the relevant diseases.

Further work was then conducted in the following areas:-

(1) Temperature versus growth evaluation
(2) Fungicide Evaluation
(3) Fungicide Evaluation on Plants
(4) Timing of infection by *Colletotrichum sp*

The work by SARDI has continued from March and the interim results have been forwarded to PGAI on a regular basis. Additional work has been undertaken during the period and the most current results are attached to this report (PDF).

4. TECHNOLOGY TRANSFER

The information collected, collated and prepared has been distributed to ALL Australian Pistachio growers through:-

a. e-mail, or

b. hard copy (postage) where appropriate, and

c) placed on the members section of the Pistachio Growers' Association Inc web site.

In addition articles have been prepared and printed in recent editions of the Australian Nutgrower.

5. CONCLUSIONS:

The work on Anthracnose (*Colletotrichum acutatum*) and *Botryosphaeria sp* is continuing as part of a new project PS11001 – Development of Fungal Management program for Australian Pistachio production.

6. ACKNOWLEDGMENTS

The authors would like to acknowledge the

- Financial support by Horticultural Australia Ltd and The Pistachio Growers' Association Inc,
- Field work support from CMV Farms and Kyalite Pistachios,
- Input from Andrew Bowring, James Meyer and Chris Joyce and the research committee members, and
- Laboratory work by Barbara Hall and others at SARDI.

APPENDIX A: Grower letter and survey.

Pistachio Growers Association Incorporated

27 Ludgate Hill Road
ALDGATE, SOUTH AUSTRALIA, 5154

Executive Officer: Trevor Ranford
Mobile: 0417 809 172

18th February 2011.
Dear Grower,

CONFIDENTIAL GROWER SURVEY

Urgent Fungal Diseases Survey

There has been wide spread fungal damage to many Pistachio crops this season. The main pathogen has been *Anthracnose* with some examples of *Botryosphaeria*. This is the first occasion that these fungal diseases have caused such wide spread damage and extensive damage in Australian Pistachios. Losses of 20% to 40% in some orchards have been reported. The Pistachio Growers' Association Inc (PGAI) is commencing a major project to develop a management manual for growers together with gaining approval for a wider range of chemicals than are currently available.
Anthracnose is known in a number of Australian crops, e.g. olives, avocados, almonds. There are limited reports of its activity in Californian Pistachio crops, but only limited examples. Considerably work will be required to develop protocols for managing this disease in Australian Pistachios. It is essential that the PGAI researchers have access to as much data as possible on the behaviour and incidence of the fungal diseases this season, from as many growers as possible**.**
All information provided will be confidential and only available to the PGAI researchers. No information on individual orchards will be disclosed.
Even if your orchard has not been affected, your weather and spray program may provide the researchers with insights to management techniques.
If you do have damage to your crop it is critical that the pathogen is correctly identified. It is possible that there are other causal agents besides *Anthracnose* and *Botryosphaeria*.
Barbara Hall at SARDI has experience with this identification. She can advise how to collect and send the samples. Phone 08 8303 9562 email: barbara.hall@sa.gov.au . The cost is about $240.
Attached is a questionnaire with an outline of the information required. Please add all information you have and any additional information and comments that you think may be of assistance.
Now that the spores of these pathogens are widely spread in Australian orchards, it is likely that it will require only moderate conditions to set off another serious attack in future seasons. It probably will not require the extreme wet conditions of 2010/11 to cause the same damage.
Your urgent assistance is required and appreciated.

If you have any concerns and/or issues please contact me on 0417 809 172.

Yours faithfully,
Trevor Ranford.
Executive Officer
Pistachio Growers' Association Inc.

Confidential
Pistachio Industry Fungal Disease Survey

Orchard: .....

Location: ……

Contact name ..... Phone.....

Email .....

Fungal Diseases 2010/11
Have you noticed fungal damage this season? YES NO
If yes, please estimate the extent of the damage:
…….....

Has the pathogen been identified? YES NO
If yes, please attach copy of pathology report.

Please comment on when you first noticed the damage and any features of its spread:

.....

Is the disease still active? YES NO

Describe:.....

Did you use the BUDMON program to check fungal development in fruit buds? YES NO
If yes, please attach the BUDMON results for as many seasons as you have available.

Do you have any photographs of the damage? YES NO
If yes, please forward these ensuring that the date of when the photograph was taken is noted.

Weather Data
Do you have an on farm weather station? YES NO
If yes, can you provide the readings from September 2010 to the present?

If no, please advise the most appropriate BOM weather station for your orchard. ……

Spray Data
Please attach details of your 2010/11 spray program. Include any winter fungal sprays.
The full details of the chemicals, spray rates and dates of application are vitally important.

Please forward your response:
By e-mail to: Dr Jianlu Zhang pgai@iinet.net.au
By mail to: PGAI, 27 Ludgate Hill Road, ALDGATE. SA. 5154.

Pistachio Growers Association Incorporated

ANTHRACNOSE of PISTACHIOS

during season 2010-11

Prue McMichael
Scholefield Robinson Horticultural Services Pty Ltd

Technical Information Sheet No 1

The 2010/11 growing season was unique and the pistachio industry like many others, faced challenges never before experienced. The abnormal conditions were those of unprecedented spring, summer and autumn rainfall, and of mild summer temperatures – both contributing to the extensive development of 'anthracnose'. This fungal disease was first reported on Australian pistachios in 2001, but there is little specific information about it on pistachios. We can however learn about the fungal life cycle, and conditions that favour its spread and infection, from literature on other host plants and a literature review will present this information soon.

A healthy, late season cluster (Source: Pistachio Growers Assoc.)

How did this fungus cause an epidemic in 2010/11?

Colletotrichum spp. spores, frequent rain events and mild temperatures, trigger anthracnose epidemics on many hosts. The longer the presence of free water (and mild temperatures), the greater is the chance the fungus will infect its host, but the plant tissue and its age influence the timing and type of symptoms. Rain will splash spores from active lesions to new sites of infection, thereby increasing the severity and incidence of the disease in an orchard.

The 2010/11 growing season lacked the usual periods of high-extreme heat (days with maximum above 40°C). Summer heat may have inhibited *C. acutatum* growth, because early information from on-going laboratory experiments suggests our pistachio isolates will grow rapidly in high humidity at 20°-25°C, and at a negligible rate, above 35°C and below 5°C.

By the end of the 2010/11 season, leaves, rachises and nuts on both our scion varieties, were severely infected. How much of it was initiated in spring, rather than autumn, is unknown. It appears that all soft pistachio tissue, including flowers, leaves, rachises, hulls and green shoots, are susceptible to infection throughout the season. From early spring, close inspections of all plant parts, is needed. Infection of immature fruit occurs but may not result in extensive symptoms until the fruit starts maturing. As harvest approaches, lesions on infected clusters will rapidly expand and coalesce, hulls blacken, and the pink-orange spores of the fungus will become visible *en masse*.

What causes pistachio anthracnose?

In Australia, we believe *Colletotrichum acutatum* (rather than *C. gloeosporioides*) is the cause. This fungus has a wide host range that includes almonds.

Range of nut symptoms from discreet, sunken lesions to blackened hulls

What should I be doing now if my orchard suffered from anthracnose in 2010/11?

Infected rachis (with spores) supporting fruit not showing visible symptoms of anthracnose

C. acutatum survives over winter in pistachio buds, and in lesions on infected fruit, rachises, leaves and twigs that remain on the tree, or on the orchard floor.

The following are some early recommendations.

- **Sanitation.** This is very important, albeit expensive.
  - **Re-shake to remove all infected nuts and rachises.**
  - **Remove, mulch and/or incorporate under-tree debris** (so fungus is not splashed from under canopy to lower limbs and leaves in spring).
- **Don't prune during rain.**

It is not yet clear if a forced leaf drop (as with urea or zinc) and fungicide application after, would deliver economic benefits in affected orchards.

- **Understand the underlying threat for next season.**
  - Monitor the fungi in dormant buds. (BUDMON tests can detect *Botryosphaeria* and *Colletotrichum* infection in buds

With knowledge of bud infection levels, and the relative susceptibility of pistachio tissue, we could utilise free moisture, humidity, temperature data, to predict disease outbreaks. This would assist growers in optimising the timing and placement of fungicide applications.

Several contact and systemic fungicides are effective against *Colletotrichum* spp. on other hosts. Some also have reported efficacy against other pistachio fungal pathogens, including *Botryosphaeria* sp. (panicle and shoot blight) and *Alternaria* sp. (*Alternaria* late blight). A series of fungicides are currently being screened in SARDI laboratory trials and we hope that data to support permit applications, will be available before spring 2011.

Infected cluster and leaf symptoms (March, 2011)

Midrib and blade lesions. (Source: Pederick & Hall, SARDI)

More seasonal information and chemical usage information will be supplied in further technical leaflets and at a grower workshop planned for September 2011.

For further information, please contact Prue McMichael at Scholefield Robinson (08) 8373 2488 or visit www.pgai.com.au

Pistachio Growers Association

Scholefield Robinson HORTICULTURAL SERVICES

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HAL Horticulture Australia

Pistachio Growers' Association Incorporated

PANICLE AND SHOOT BLIGHT ("Bot") of PISTACHIOS

Technical Information Sheet No 4

Prue McMichael
Scholefield Robinson Horticultural Services Pty Ltd

The unprecedented volumes of spring, summer and autumn rainfall and the mild summer temperatures contributed to the extensive development of fungal pistachio diseases in 2010/11. A disease caused by a form of *Botrytosphaeria* sp. was present again in 2010/11 but was not as destructive as anthracnose (see Technical Information Sheet No. 1). Similar pistachio diseases caused by fungi in family Botryosphaeriaceae, have been reported in Italy, California and South Africa. In California, '***panicle and shoot blight***' is the official name of the disease on pistachios, but the colloquial name there and in Australia, is simply **"Bot"**.

Late spring shoot blight ("Bot strike")

"Bot" strikes on male tree

What causes "Bot"?

In the US, *Botryosphaeria* spp. are pathogens of 35+ plant genera, and nut hosts include almond and walnut, as well as pistachios. In Australia, a primary cause is *Neofusicoccum parvum*. In California, more than one fungus is involved, and the most frequently isolated is *N. mediterraneum*.

"Bot strikes" amongst healthy foliage (summer)

How do I identify "Bot" in my pistachio orchard?

The epidemiology of the disease in Australia has not been researched and the full range of symptoms on plant parts is unknown. We can however be guided by the description of symptoms in California.

During dormancy, look for **hard-stuck, black rachises**. In California these are consistently infected by *Botryosphaeria* sp. and are a source of spores (that ooze from fruiting bodies called pycnidia) the next season, unless removed.

Black lesions may form at the **base of shoots and rachises** arising from **infected buds**. During the late spring, look for **weak, wilted shoots** supporting off-colour leaves. These **"Bot strikes"** are easily seen amongst healthy foliage. Elongated, **black lesions on leaflet midribs**, petioles and leaf stems are damaging. Midrib lesions expand, killing the leaf blades, and the fungus moves into petioles, leaf stems and shoots, often girdling them. Depending on where the lesions form, leaves fall prematurely, with or without their leaf stems or petioles.

Infection at the base of a rachis below the branching points, results in **rachis death** and the subsequent **dehydration and starvation (but not infection)** of nuts on the panicle beyond the point of rachis collapse. These nuts turn brown-tan and have no lesions.

Midrib infection in process of killing leaf

Nut infection is the most serious form of the disease. Nuts become infected through natural openings or wounds. Infection in immature nuts remains latent for some time, but small quiescent, black lesions also develop. In warm wet conditions the fungus grows from the infected nuts, into peduncles and towards the rachis and shoot. **Infected nuts have black hulls** that later turn silver-grey when pycnidia form on the dead tissue.

In California, if the fungus reaches current season wood before dormancy, sunken, black cankers form. Pycnidia in cankers release viable spores for as long as 6 years.

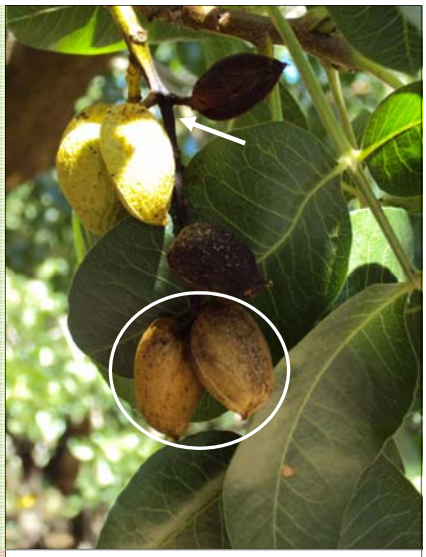
Cankers have not yet been observed in infected Australian pistachios.

How does "Bot" infect pistachios?

Summer and autumn rains are particularly damaging because they splash the spores from the mouth of pycnidia, onto susceptible tissue. Spring rains disperse spores to opening flowers and fresh, soft, susceptible tissue. Autumn rains and warm temperatures are conducive to secondary infection, and therefore inoculum build-up before dormancy.

This is a warm weather disease. Low temperatures slow the infection processes and disease development. Most early spring vegetative 'infections' are seen as "strikes" only after temperatures and humidity increase in late spring. Latent fruit infections become active and visible later in summer. Spore germination in California, is favoured at 24-36°C.

Wetness periods of 9-12 hours (and >12°C) allow germ tubes to infect host tissue. Disease development is most rapid at 27-33°C, and pycnidia form at temperatures around 30°C. The optimal growth temperature for *N. parvum* from Australian pistachios is 30°C.



Infected nuts (black) causing rachis collapse (↑) and starvation of other nuts beyond point of rachis collapse (brown).

What should I be doing NOW if my orchard has "Bot"?

Nut dehydration, starvation. Rachis infected, but not nuts.

'Bot' once threatened the entire California pistachio industry, but today the disease is well- managed by integrated approaches that include excellent orchard **sanitation, fungicides, insecticides**, and cultural practices centred on strategic **pruning** and irrigation. Management differs in young and old orchards.

- **Minimise the threat for next season**
  - **Clean up** in dormant season—remove stuck rachises, pruning debris, vegetation harbouring *Botryosphaeria* spp.
  - **Monitor** fungi in dormant buds (BUDMON tests)
  - Plant only healthy nursery stock
- **Minimise disease development within the growing season**
  - Know the s**ymptoms** of 'Bot', anthracnose and Botrytis infection
  - Use **ONFIT** to evaluate latent infection of immature fruit
  - **Irrigation**—Don't wet trees; avoid long periods of high humidity. Short sets in daylight on consecutive days are preferable to long sets
  - **Apply protectant fungicides** from flowering to shell hardening.* Apply at approx 3.2 km/hr
  - **Pruning**—In young orchards, prune out strikes and cankers (5-7cm below infection). In older orchards, improve airflow and reduce humidity

*The fungicides registered for use on pistachios in California, and ranked *highest* for efficacy against "Bot" are: *pyraclostrobin + boscalid* (Pristine®) and *fluopyram+trifloxystrobin* (Luna Sensation®). Also ranked highly: azoxystrobin (Amistar®), pyraclostrobin (Cabrio®) and 6 others (Adaskaveg et al., 2011).

Bot References
http://www.apsnet.org/publications/apsnetfeatures/Pages/Pistachio.aspx
http://ucce.ucdavis.edu/files/repositoryfiles/ca4403p6-69466.pdf
http://ucanr.org/repository/cao/landingpage.cfm?article=ca.v046n06p28&fulltext=yes

Chemical evaluations – University of California (Adaskaveg et al, 2011)
http://www.uckac.edu/files/106962.pdf

More seasonal information and chemical usage information will be supplied in further technical leaflets and at a grower workshop planned for September 2011.

For further information, please contact Prue McMichael at Scholefield Robinson (08) 8373 2488 or visit www.pgai.com.au

Pistachio Growers' Association

Scholefield Robinson HORTICULTURAL SERVICES

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Scholefield Robinson
HORTICULTURAL SERVICES

Fungal diseases of Pistachios
Literature Review

Prepared for : Pistachio Growers' Association Inc (PGAI)

By : Prue McMichael

Date : August 2011





Pistachio Fungal Diseases – Literature Review

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PURPOSE OF REPORT

This literature review is a summary of information on two important fungal diseases present in Australian pistachio orchards, and on two other diseases to which we believe Australian pistachios, are vulnerable. It is intended to be a resource for the industry and should be considered a dynamic document.

ACKNOWLEDGMENTS

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DISCLAIMER

AUGUST 2011

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1 INTRODUCTION

Pistachios (*Pistacia vera* L.) are perennial, dioecious tree, native to western Asia. The extensive root systems, tougher leaves, and windborne pollen of pistachios, assist their natural survival in hot, dry locations, including some deserts. It is not surprising therefore that spring, summer or autumn rains, high humidity, and poorly-drained soils in commercial production areas, have contributed to pistachio diseases for which there is no apparent genetic resistance.

Fungi have no capacity to photosynthesise and therefore their existence relies either on colonisation of dead material (saprophytes) or parasitism of living material (pathogens). As pathogens, they may cause disease in plants, animals and/or humans. Fungal plant pathogens are diverse in their capabilities for host interaction, infection, distribution, reproduction and survival. Australian pistachio orchards have at times suffered serious losses attributed to fungal and bacterial pathogens.

This literature review is a collation of information on three above-ground fungal diseases of pistachios in Australia, and a fungal disease believed not to be present in Australia. Understanding the processes in pathogenesis is important if disease management is to be optimised. This literature review presents information that will allow us to better understand the epidemiology of the diseases and the environmental influences on their development, on their hosts, and on the pathogens themselves. It is hoped that this knowledge will increase our capability to monitor and manage the diseases.

The review has included literature on the following fungal diseases of pistachios: anthracnose, panicle and shoot blight, Alternaria late blight, and Septoria leaf spot.

2 VISUAL GLOSSARY

A glossary and visual description of some phytopathological terms included in Appendix 1.

Healthy cluster

3 ANTHRACNOSE

3.1 What causes anthracnose?

The name 'anthracnose' describes diseases of a variety of plants, caused by fungi that produce spores in fruiting bodies called 'acervuli', and characteristic sunken lesion symptoms. Anthracnose is caused by a number of fungi within the class Ascomycetes, but most are in the genus *Colletotrichum*. Leaves, petioles, peduncles, flowers, fruit and shoots are primarily infected and fruit spots and rots, defoliation, flower blights are typical symptoms. The host range of *Colletotrichum* spp. includes woody and herbaceous ornamentals, conifers, flowering perennials, and annuals, including grasses.

Anthracnose development regardless of the host plant, is influenced by free water (usually rain, but also overhead irrigation) and warm temperatures (high humidity). All *Colletotrichum* spp. have waterborne and splash-dispersed spores. The spores usually germinate and infect hosts, but the degree of colonisation of the host at the time of early infection, is influenced by the host tissue and environmental conditions, and may result in latent and/or quiescent infections, or extensive disease. A *Colletotrichum* sp. may exhibit pathogenesis variations on different hosts, and on different tissues of the same host (Peres et al., 2005; Sreenivasaprasad and Talhinhas, 2005). There appears to be some specialization about tissues attacked on each host, at given times (Table 1), making anthracnose an early season and pre-harvest problem on many hosts and a post-harvest and storage problem on others (Pruskey, 1996; Prusky and Plumbley, 1992).

Table 1 : Plant tissues affected by *Colletrotrichum acutatum* on selected hosts

	Apple/peach	Blueberry	Strawberry	Almond
Young leaves/twigs	-[a]	(+)	+	+
Flowers	-	(+)	+	+
Fruit preharvest	-	+	+	+
Fruit postharvest	+	+	(+)	(+)
Roots/crowns	-	-	+	-

–[a] = No symptoms; + = symptoms; (+) = symptoms occur but not common (Source: adapted from Peres et al, 2005)

3.1.1 *Colletotrichum* spp.

The taxonomy within the genus has long been unclear and 66 species names appear in common usage (Hyde et al., 2009; Cai, 2009). Older literature often attributes anthracnose of flowering perennials to *C. gloeosporioides.* *C. gloeosporioides* and *C. acutatum* however have similar morphological characteristics, extensive cultural variability and overlapping host ranges that include commercially-important hosts.

Despite morphological features being no longer considered reliable for distinguishing species, there are several similarities and differences worth documenting. The general morphological features that *C. acutatum* and *C. gloeosporioides* colonies may share on synthetic media, include their white, young mycelial growth, that later becomes grey, cream, orange or pink. Comparisons of colonies on benomyl-amended synthetic media (at 1.0 µl/ml) provide more useful distinctions. *C. acutatum* grows, albeit at a reduced rate on such media, while the growth of other species including *C. gloeosporioides* is completely inhibited (Peres et al., 2005). McKay and colleagues (2009), working on *C. actuatum* of almonds in Australia, reported the majority of colonies from infected almonds ranged from bright pink to carmine, but only *C. acutatum* excretes red-pink pigments into the media. Both species produce pink-orange spores, but the end shapes of the ellipsoid spores vary marginally with the conidia of *C. acutatum* usually rounded at one end only (Bernstein et al., 1995).

On plants, the acervuli of each species also have some distinctive features. *C. acutatum* generally has no, or very few, black spikes (setae), while acervuli of *C. gloeosporioides* reliably have several to many.

Despite similarities, molecular methods and vegetative compatibility groups have confirmed *C. acutatum* to be distinct from *C. gloeosporioides. C. acutatum* is reported to be a genetically-distinct collection of closely-related isolates, with little host specificity (Peres et al., 2005). *C. acutatum* has distinct genetic groups (Damm et al., 2010). In Australia, the *C. acutatum* isolates recovered from a range of hosts were placed primarily into Clades 1 and IV (that correspond to groups A3 and A5 of the world 8-group classification) (McKay et al., 2009).

C. acutatum is correctly named at present and in full, as *C. acutatum* J.H.Simmonds ex J.H. Symonds. This name reflects the asexual stage of the fungus. Its sexual stage is *Glomerella acutata* Guerber & J.C. Correll. This stage has not been found in nature and therefore ascospores are unlikely to have a significant role in pathogenicity or distribution but they presumably explain the genetic variability found within the species (Wharton and Dieguez-Uribeondo, 2004).

G. gloeosporioides (Penz.) Penz. & Sacc. and its sexual stage *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk, are not universally accepted as being correctly named (Phoulivong, et al., 2010; Cai et al., 2009; Hyde et al., 2009).

3.2 Anthracnose on other hosts

Anthracnose is a well-documented disease on mangoes, strawberries, blueberries, almonds, olives and avocados, but the behaviour of the fungi is not the same on all hosts. The impact and location of fruit infection on some hosts, varies during the season and the symptom descriptions on mangoes and avocados for example, often reflect the extent of damage. 'Anthracnose' implies extensive lesions, but 'stem rot' is confined to the area around the attachment points. 'Pepper spot' of avocados and tear stain of mangoes appear to describe the multiple, small lesions that form early in a season but fail to become extensive until the fruit nears maturity (Giblin et al., 2010; Fitzell and Peak, 1984). The literature suggests infection of mango fruit occurs around wounds, while avocado fruit exposed to sun appear to be the first affected (Giblin et al., 2010; Peres et al., 2005). Both hosts suffer many postharvest losses due to anthracnose.

Of importance in all susceptible hosts, are the sources of inoculum (spores) that are present at the end of winter, ready for dispersal in spring rains, and the potential for spore production during the growing season that allows a repeating cycle of infection. The literature suggests infected leaves in the canopies of avocados and mangoes are the main source of spores which splash during rain events onto immature fruit (Fitzell, 1979; Fitzell and Peak, 1984).

In almonds, infected fruit mummies and peduncles from the previous crop harbour the fungus over winter and contribute most inoculum in spring (Wharton and Dieguez-Uribeondo, 2004) and in blueberries the mycelium that survives winter in twigs and dormant flower buds, is important. The fungus in infected buds, grows as dormancy is broken if temperatures and moisture are conducive. It then colonises surrounding tissue and may produce spores in the lesions once the infected tissue is dead. The periods of peak spore production in several hosts including blueberries, is at bloom and again late in the season when fruit matures.

In contrast to other hosts, *Colletotrichum* spp. on olives appear not to penetrate the cuticle of olive leaves, but rather leaves are the site of secondary conidia formation. Epiphytic survival on olive leaves is more important than survival of spores elsewhere on the plant.

3.3 Infection by *Colletotrichum* spp.

Infection of host plants by fungi, generally involves three stages – pre-entry, entry and colonisation. There is little specific information on the steps in the infection process of *C. acutatum* on pistachios, but much may be extrapolated from published research on other hosts (Diequez-Uribeondo et al., 2005; Wharton and Diequez-Uribeondo, 2004; Duthie, 1997; O'Connell, 2000; Peres et al., 2005).

Amongst the research are demonstrated host-pathogen relationships where *Colletotrichum* spp. behave as necrotrophs, biotrophs or hemibiotrophs (Peres, et al., 2005; Sreenivasaprasad and Talhinhas, 2005). *Necrotrophs* kill their host cells as they advance so their nutrient source is dead cells. *Biotrophs* survive and live entirely off living cells. On some hosts the pathogen combines these behaviours to initially grow as a biotroph and later as a necrotroph. These *hemibiotrophic* pathogens acquire nutrients from living cells and dead cells during their life cycle (Table 2). *C. acutatum* on almonds and avocados appears to behave as a hemibiotroph, while the fungus on strawberries is necrotrophic (Wharton and Diequez-Uribeondo, 2004; Diequez et al., 2005). The mechanisms that alter the processes to trigger active, destructive parasitic fungal growth (steps 4 and 5 below) are not well known. Several mechanisms and explanations have been offered.

Despite variation in the pathogenesis processes and timeframes, the general infection steps toward pathogenesis by *Colletotrichum* spp. are similar and may be summarised (and presented as steps below) as: spore lands on plant in a splashed water droplet. If the site and conditions are suitable, the spore will adhere to the host, germinate and at the end of hyphae either an appressorium or a secondary conidium, will form. A penetration 'peg' pressures and breaks through the host wall. The fungal colonisation of the host thereafter, the development of symptoms, and the host response, are affected by many conditions discussed later in this review.

Pre-entry

1. Spore lands on surface of susceptible tissue and germinates
   - 1a. Hyphae spread and fuse over surface
   - 1b. Germ tube differentiates to form secondary spore, or
   - 1c. Appressorium

Entry/penetration

2. Fungus enters plant tissue
   - 2a. Germ tube may enter natural openings: stomates, lenticels, hydathodes, wounds, but more likely
   - 2b. Penetration pores/pegs from appressoria apply pressure to cell walls of cuticle
3. Fungal advancement
   - 3a. Fungal growth arrested in sub-cuticular cells, or
   - 3b. Fungus grows intracellularly, and
   - 3c. Symptoms are absent (*latent*) or appear as non-expanding, small, black lesions (*quiescent*)

Colonisation

4. Fungus colonises plant cells
   - 4a. Fungus grows intercellularly ⇨ colonisation (*disease* becomes apparent)
   - 4b. Fungal growth in 3a infections, recommences (*disease* becomes apparent)
5. Extensive cell damage, death, and lesions expand
6. Acervuli form
7. Spores are produced and cycle re-starts late in season or after over-wintering

As for most pathogens, the nature of host tissue, pathogen biology and the environment, influence the infection processes and disease development. For example, petal tissues appear to allow direct infection in a range of conditions without the formation of appressoria. However, germ tube differentiation is strongly influenced by the conditions and therefore may take 3-48 hours; spore production and dispersal are affected by moisture and temperature; and visible symptom development is affected by the maturity of the fruit (Wilson et al., 1990; Sangeetha and Rawal, 2009; Thomas et al., 2008).

The time and location of fungal infections cannot always be determined visually. Latent infections are those in which the fungus has entered the host, but does not colonise it for a period (latent period). The pathogen and host co-exist for a time without displaying symptoms, reduced function or viability, eg. appressoria form but the fungus becomes 'dormant' in the sub-cuticular, epidermal cells (step 3a) (Table 2).

Table 2 : *Colletotrichum* spp. behaviour on different tissues and hosts

Type and location of fungal action	Host and affected plant parts			
	Blueberry	Sweet orange	Strawberry	Almond
Biotrophy: Tissue	Immature fruit	Mature leaves	Leaf, petioles	Leaves and fruit tissues
Structures	Appressoria	Appressoria, 2°conidia[a]	Appressoria, 2° conidia	Appressoria, 2° conidia
Duration	Weeks	Months	Days	Hours
Necrotrophy: Tissue[b]	Ripe fruit	Flowers	All tissues	All tissues
Overwintering: Tissue	Bud scales, dead twigs	Mature leaves	Leaves, petioles	Mummies, dead twigs
Structures	Appressoria, conidia, mycelium	Appressoria	Appressoria	Conidia, mycelium

(Source: Peres et al, 2005) [a] 2° secondary conidia; [b] tissues on which acervuli and conidia are produced

Quiescent infections appear inactive and non-expanding for a period. *Colletotrichum* spp. on many hosts appear early as quiescent, small, black, sunken lesions (Peres et al., 2005). "Pepper spots" on avocados are symptoms of quiescent infection. The variation in symptoms on infected fruit reflects the stage of host development rather than the pathogenic capacity of the fungus, eg. the fungal cause of pepper spots on immature avocado fruit is equally capable of causing extensive anthracnose on mature fruit. The host response that contains the early infections is temporary, and apparently over-turned by fruit maturity.

Although latent and quiescent infection biology is not well researched, the impact of such infections has been demonstrated in several host-pathogen relationships. Later in the season, latent infections become actively parasitic and the disease incidence on mature fruit reflects the earlier incidence of latent infection (Talhinhas et al., 2010). The strong correlation of latent infection with disease incidence, is the premise on which the overnight freezing incubation technique (ONFIT) testing is based and it provides useful predictive information on the existing end-of-season threat, by mid-season when fruit is still immature.

The *Colletotrichum* spp. and fungi in the Sclerotiniaceae are common pathogens that form quiescent and latent infections. In blueberries, the abundant acervuli on mature fruit arise from activated latent infections by spores splashed onto immature fruit. In olives, spores of *Colletotrichum* spp. germinate and grow over the surface of immature fruit, penetrate the fruit but no symptoms appear for 30+ days.

Amongst the fungi in the Sclerotiniaceae, the grape-*Botrytis cinerea* relationship is similar. *B. cinerea* infects grape flowers early but young berries do not become colonised (and therefore 'diseased') until after veraison. If a single diseased berry exists within a cluster 10 days after veraison (due to activation of the earlier latent infection), there is potential for the entire bunch to

be diseased by harvest. Activated primary infections (from bloom) and rapid secondary spread from them later in the season, are responsible.

3.3.1 Activation of quiescent and latent infections

The plant stages susceptible to latent and quiescent infection are important to recognise. If they are known, and conducive environmental conditions have presented, the crop is at risk. If it were possible to control latent infections, the end of season losses would be minimised. Treatments to extend latency, curb the activation of the fungus from quiescent infections, or extend immature fruit host responses into maturing fruit, would also have potential.

The "resistance" of immature fruit to infection by fungi including *Colletotrichum* spp., has several potential explanations. The basis of them relate to physiological and physical changes in the host cell wall, and in response to metabolic changes and/or stress.

- Anti-fungal compounds (toxic) within immature fruit inhibit pathogen growth.

These have been shown to exist in avocado peel (Prusky, 1996; Prusky and Plumbley, 1992). As avocados mature, the concentration of these substances in the peel decreases, while enzyme activity increases, allowing colonisation.

- Nutrients from unripe fruit do not sustain the fungal growth.

Ripening involves biochemical changes that include the conversion of stored carbohydrates to soluble sugars. In blueberries infected by *C. acutatum,* disease development is correlated with acidity and phenolic levels (Sangeetha and Rawal, 2009).

- Enzyme releases by the fungus do not allow colonisation.

Unlikely, since enzymes from *Colletotrichum* spp. have been shown as capable of destroying cell structure, killing cells, degrading carbohydrates, and hydrolysing cuticles.

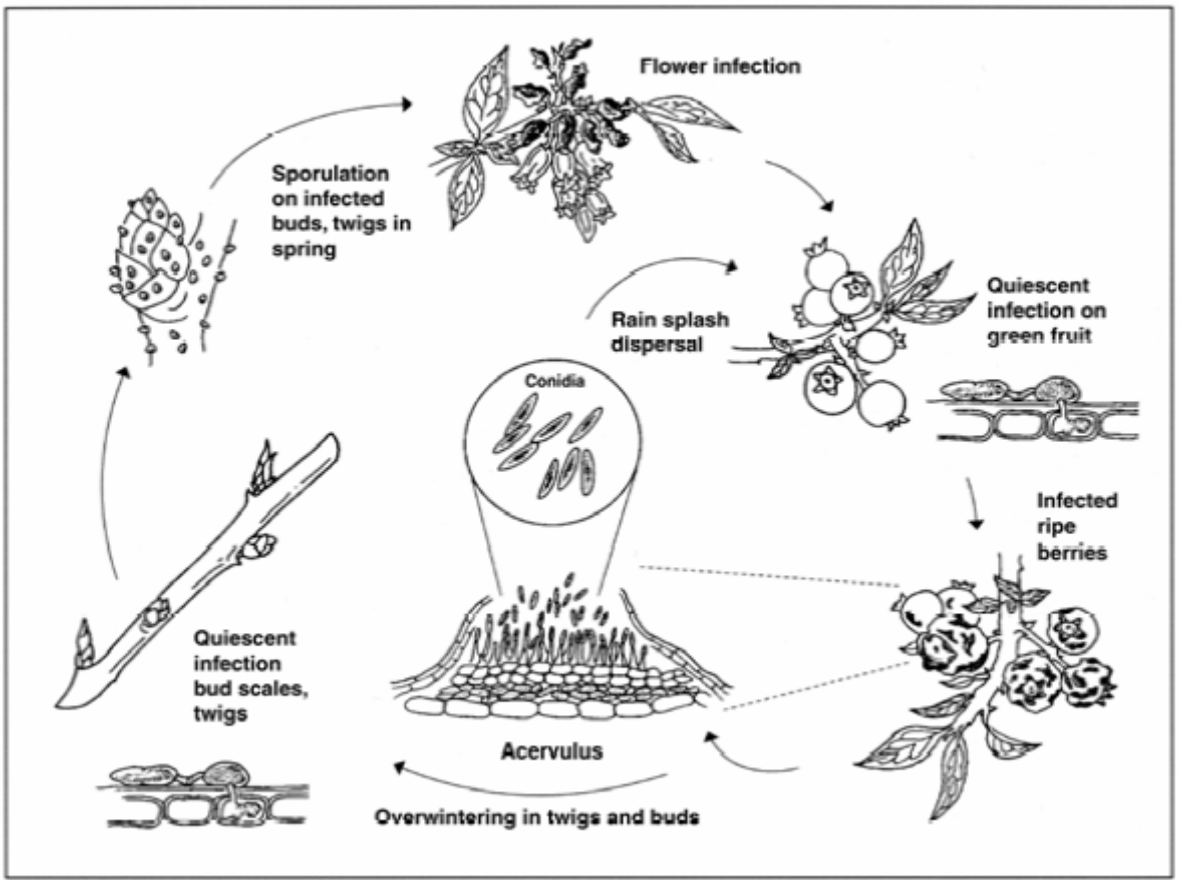
- The unripe fruit responds with phytoalexin production.

This role of phytoalexins has not been shown in mangoes and avocados, but capsidiol is thought to limit infection of capsicums (Wharton and Diequez-Uribeondo, 2004).

The epidemiology of *C. acutatum* is complex as both pathogenic and non-pathogenic stages seem to occur within its life cycle (Peres et al., 2005). Although each stage has not been studied in Australia on pistachios, our observations suggest that the *life* and *disease* cycles of *C. acutatum* on pistachios may be more similar to that described for blueberries, than for almonds. Figure 1 visually describes the life and disease cycle of blueberry ripe fruit rot (Peres et al., 2005). Blueberries, mangoes, strawberries and avocados for example develop extensive symptoms on the harvested, commercial product (ripe fruit), but almonds and pistachios are not marketed as harvested. However, extensive pistachio hull infection by *C. acutatum*, is indicative of extensive shell staining stained and low quality kernels.

The field and laboratory observations made on pistachios during the 2010/11 season that indicate similarity with the well-researched blueberry disease cycle are: survival of the fungus in buds, potential for bud and flower infection in spring resulting in quiescent and latent infection of immature clusters; the quiescent infection of leaves; the primary necrotrophic stage on ripe tissue (eg. hulls), rather than leaves; the potential for a secondary cycle within the season, as evidenced by presence of spores on infected, mature hulls. In addition, there is evidence of survival of *C. acutatum* in mummied nuts and infected rachises also. It is however unknown if *C. acutatum* survives in pistachio wood or lignified twigs.

Figure 1 : Disease cycle of blueberry ripe rot caused by *Colletotrichum acutatum*



Source: Peres et al., 2005

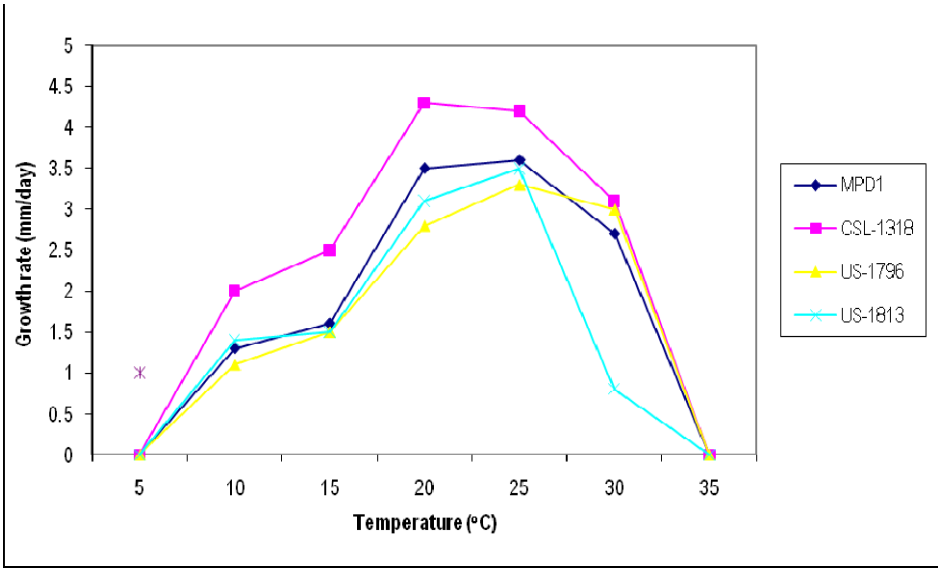
3.4 Anthracnose – contributing factors

Environmental conditions, especially mild temperatures and free moisture, influence infection by *Colletotrichum* spp. **Temperature** and **moisture** affect fungal growth and sporulation, infection processes and symptom development.

3.4.1 Conducive temperatures

The growth of *Colletotrichum* spp. is affected by temperature. The effect of temperature on the rate of mycelial growth of *C. acutatum* isolates is shown in Figure 2. The optimum growth temperatures for isolates of *Colletotrichum* spp. from different hosts, do not vary greatly. Papaya isolates grow most rapidly at 30˚C, while the growth optimum for almond, peach, lupin and strawberry isolates is 25˚C (Thomas et al., 2008). In SARDI experiments two isolates of *C. acutatum* from Australian pistachios, had a growth range of 10-35˚C, with one isolate's peak growth being at 25˚C and the other with an optimal growth range of 15-30˚C, without a clear peak (Hall et al., 2011).

Figure 2 : Mean mycelial growth rate (mm/day) of almond isolates of *C. acutatum



(Source: adapted from McKay et al., 2009)

* Almond isolates: MPD1 and CSL-1690 (from Australian almonds); US-1796 and US-1813 (from Californian almonds). Isolates incubated in dark for 7 days at 5 to 35°C.

In California, research suggests the temperature range over which most *Colletotrichum* isolates (from a range of hosts) grow in culture is 6-32°C (Adaskaveg and Hartin, 1997, Thomas et al., 2008). Optimal temperatures for the infection process are less clear, but it is known that infection of mature and immature strawberry fruit is highest at 25-30°C.

In vitro, several researchers have found that as temperatures increase from 12-26°C, the incubation period decreases, and time taken for spores to develop, decreases. As the incubation temperatures increased, so too did lesion severity. On lupins for example, the mean time for lesions to appear at 12°C was 11.5 days, and at 26°C, only 4.3 days. At 12°C, spores formed on average by day15, but it took only 6.2 days, at 26°C. When inoculated lupins were held at 5°C or 35°C for seven days, there was no apparent growth of the fungus, and it was unclear if colonisation would have occurred. However, once these plants were returned to 20°C, the fungus grew as expected. We may conclude from this that high or low temperatures may curb disease development on some hosts, but do not kill the fungus (Thomas et al., 2008).

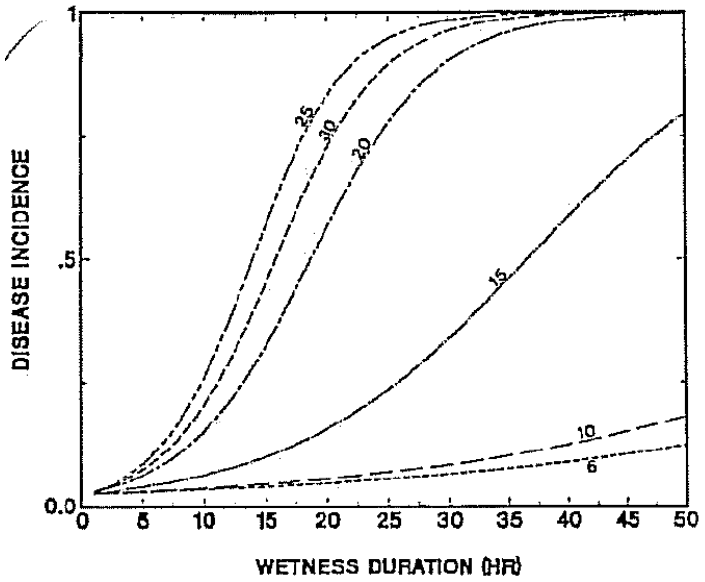
Winter chill temperatures do not appear to reduce the survival of the fungus significantly. There is evidence of *Colletotrichum* sp. survival on many forms of organic matter, eg. on weed hosts (Peres et al., 2005), as an endophyte on non-hosts, in soil that is not saturated, as a saprophyte in lesions formed by other pathogens, and in infected buds and plant parts that remain in canopies or on orchard floors, over winter.

3.4.2 Conducive moisture conditions

In areas that routinely suffer anthracnose, rain in spring, summer and/or autumn is common. Anthracnose is an irregular problem in locations that have hot, dry summers, and only occasional autumn rain. In practice, rain in warm springs ensure suitable conditions for infection, as well as splash distribution of spores to flowers, emerging new shoots, and developing, immature fruit. Rain in summer and autumn spreads spores from both older and new infection sites. The environmental conditions (including dew) near harvest influence the repeating, secondary infection cycle, incidence of new infections, lesion severity and their rate of development. This coincides with re-activated fungal growth from latent infections and hence the rapid loss of yield and quality of matured fruit either at harvest or soon after harvest. The interaction of wetness and temperature on strawberry infection is shown in Figure 3.

Fungicide trials have demonstrated however that autumn rain can trigger significant losses, even in orchards that have not suffered spring or summer rain. Fungicides applied only in response to autumn rain were insufficient to control the disease, and it was therefore presumed that latent infections had contributed earlier in the season, to the final disease incidence and severity (Talhinhas et al., 2010). Similarly, in the unusually wet and warm spring (attributed to El Niño) of 1998 in California, almond anthracnose was severe even in orchards with no history of the disease. Although the early rains fell when temperatures were cool, anthracnose appeared by mid-spring as the temperatures increased (Aust Nutgrower, 1999).

Figure 3 : Effect of wetness duration and temperature on the predicted infection of immature strawberry fruit by *C. acutatum*.



(Source: Wilson et al, 1990)

Rain promotes anthracnose, especially when temperatures are mild-warm. Rain not only disperses spores by splashing them from the surface of lesions to exposed plant parts, but also provides the extended periods of free moisture necessary for the spores to infect the host. The distance spores may be splashed is influenced by wind; the surface characteristics under the canopy and within the orchard (ground cover, debris); the rain intensity and the density or sparseness of vegetation near the inoculum source/s (Yang et al., 1990).

The minimum wetness periods that ensure spore release, germination and infection of all susceptible hosts and plant parts, are not known. Wilson and colleagues (1990) working on strawberries, demonstrated that infection of strawberry fruit by *Colletotrichum* sp. increased as the wet period increased, at all temperatures between 6°C and 30°C (Figure 4). They also confirmed that mature fruit are more susceptible than immature fruit, at the same moisture levels and temperature. To demonstrate this, immature and mature strawberries were inoculated with spore suspensions. High humidity and free moisture at the inoculation sites were maintained for designated time intervals between 0.5 to 51 hours. At the pre-determined time, the inoculated fruit were dried and moved to the test temperatures for eight days.

3.4.2.1 Contributing factors in orchards

The factors that influence anthracnose in orchards are the same as those that influence the disease on annual hosts – temperature, free moisture, and humidity. In commercial orchards of susceptible hosts (eg. pistachios, apples, olives, mangoes and avocados), fruit is often the worst affected plant part and therefore fruit maturity is also an important contributing factor. Farming

practices are capable of manipulating orchard conditions, thereby making them important in both anthracnose minimisation and management.

Plant spacing contributes to the potential for anthracnose epidemics in wam-wet seasons. In young orchards, direct sunlight and air flow through canopies is high and even susceptible hosts may show little evidence of the disease because leaf wetness periods are minimised. As tree canopies expand, air flow and direct sunlight are impeded. Pruning and hedging manipulate these factors and reduce the drying time of leaves and fruit within canopies, after rain.

Although uncommon in Australia, overhead irrigation is still practised in some orchards of susceptible hosts, other than pistachios. Such irrigation promotes anthracnose particularly if its timing and duration, create long wetness periods and slow drying conditions. Mini-sprinklers may indirectly affect anthracnose through the creation of long periods of high humidity, or directly by wetting low hung leaves and branches. The duration of high humidity can be manipulated by preventing under tree irrigation during evenings, removing cover crops and under-canopy debris, ensuring drainage is effective and that water does not pool under trees.

There is little opportunity to manipulate the latent periods or activation of quiescent infections, although high and low temperatures, and fungicides, may influence the rate of activated fungal growth. Fruit maturity has a greater influence on the rate of activated fungal growth and it is difficult to manipulate this.

Neighbouring orchards of susceptible hosts may have some significance if they mature earlier than the orchard in question, during a wet autumn. This has some relevance in Queensland where mangoes and avocados are commercially grown in the same regions and in other areas where almonds and pistachios are in close proximity (Giblin et al., 2010). However, infected olive orchards near almonds and pistachios are not likely to be a significant source of inoculum because olives mature in winter. Inoculum sources within the orchard (i.e. infected debris and plant tissue within canopies) are more important than external inoculum sources. Orchard sanitation and fungicide applications reduce inoculum, but their effectiveness is largely dictated by environmental conditions and the density of viable inoculum sources that remain (eg. in buds and bud scales; or as an epiphyte on other tissue).

Isolates of *Colletotrichum* spp. differ in their relative pathogenic capability on non-wounded and wounded tissue. Almond and peach isolates are capable of infecting both, and our initial observations suggest that the same is true of pistachio isolates (Adaskaveg and Hartin, 1997). It is recommended however that farming practices that potentially wound developing fruit, be avoided or timed not to coincide with rain events.

3.5 Anthracnose management

The range of literature and advice on the management of anthracnose is evidence of its complexity. Successful management of this disease, regardless of its host, requires understanding of the epidemiology of the fungus, the disease cycle and relative susceptibility of host tissues, inoculum sources, conducive weather events and their timing relative to host development stages.

The approaches to management have largely focussed on *avoidance* (eg. the provision of clean planting material), pathogen reduction (eg. sanitation, removal of overwintering inoculum), and host protection (eg. the application of protectant fungicides). No single method may be relied upon.

If more specific information were known about the various periods of tissue susceptibility, the duration of latent periods under given conditions, the mechanisms that trigger active, pathogenic growth after latency and the mechanisms that determine latency and quiescence, the success of current approaches would be increased. Current approaches to anthracnose management in orchards are discussed below.

3.5.1 Cultural

Cultural practices, in the form of manipulated irrigation, pruning and phytosanitation, are discussed above and are relevant generally to pathogen reduction.

3.5.2 Chemical – Fungicides

Much of the literature on anthracnose includes discussion of chemical management programs. In all, the emphasis is on complete and continuous coverage of susceptible tissue, starting at early flowering in perennials, and ahead of rain events. Early treatment appears to be critical to later season control of the disease.

The literature on chemical management of anthracnose, emphasises several key points:

- Fungicides alone are insufficient. Cultural practices that increase air movement, and sanitation are also needed
- Multiple applications during conducive conditions are necessary, from early flowering
- Preventive (*protectant*) programs rely on early recognition and understanding of conducive (wet, warm) conditions, and complete coverage of emerging susceptible tissue
- No current formulations of active constituents are reliably *curative*, i.e. control/eliminate established 'anthracnose'
- Some products with *eradicant* activity, may be successful if applied within 2-3 days of a conducive rain event
- Rotations of products with different modes-of-action are necessary to reduce the threat of resistance development and loss of efficacy.

The correct choice of fungicides involves consideration of their efficacy, mode-of-action (and therefore potential resistance, and cross-resistance), rainfastness, and withholding periods. The timing and placement of applications are important because the periods of tissue susceptibility are long, and all new expanding tissues, including buds, leaves, flowers, and fruit need to be protected during wet periods. Protecting immature fruit is essential.

Both contact and systemic (translaminar) fungicides have been trialled for efficacy against anthracnose, and are discussed in the literature. Laboratory screening of fungicides suggests that *Colletotrichum* isolates from different hosts do not always have the same sensitivity to fungicides (Adaskaveg and Hartin, 1997). *In vitro*, the literature suggests all tested isolates appeared sensitive to captan, while the growth inhibition by benomyl (a benzimidazole fungicide), is variable across the isolates. In general, a range of literature suggests *C. acutatum* is less sensitive to benzimidazoles, than *C. gloeosporidoies*.

The effective contact, broad spectrum active constituents (and their chemical group classification) mentioned in literature include:

- *chlorothalonil* (chloronitrile group)
- *copper* (inorganic)
- *mancozeb, ziram* (dithiocarbamates), and
- *captan* (phthalamide)

In vitro fungicide screening against two *C. acutatum* isolates from Australian pistachios, confirmed the efficacy of chlorothalonil (Hall et al, 2011).

The systemic and translaminar active constituents identified as being efficacious against *Colletotrichum* spp. on some hosts (peach, almond, strawberry), in a range of literature, include:

- *myclobutanil, metaconazole, propiconazole*, and *tebuconazole* (demethylation inhibitors in the triazole group – DMI-triazoles);
- *imazalil* and ***prochloraz*** (DMI- imidazole group)
- *azoxystrobin,* ***pyraclostrobin***, and ***trifloxystrobin*** (Quinone outside inhibitors -QoIs)
- *boscalid* (pyridine-carboxamide)
- *thiophanate-methyl* (methyl benzimidazole carbamate –MBC)
- *fenhexamid* (hydroxyanilide SBI Class III)
- ***fludioxonil*** (phenylpyrrole)
- *fluazinam* (pyridine, QiI)

and several pre-mixes of the above (tebuconazole+trifloxystrobin; boscalid+pyraclostrobin). Within pre-mixes one component may have systemic activity while the other may be more resistant at the surface and act as a contact fungicide (eg fludioxonil).

In vitro fungicide screening trials conducted by SARDI on two *C. acutatum* isolates recovered from Australian pistachios in 2011, confirmed the efficacy of cyprodinil and carbendazim, in addition to the above active constituents shown in bold. They were tested at label rates, half rates and one-tenth rates (Hall et al, 2011). The same experimentation however did not indicate consistent efficacy at the label rates, on both isolates, of: boscalid, tebuconazole, fluopyram, fenarimol, pyrimethanil, myclobutanil or fenhexamid.

There is little data on eradicant activity, but several reports name prochloraz and the strobilurins as having eradicant activity (2-3 days kick back). They are however still recommended for application as protectants.

3.5.3 Biological

Antagonistic organisms that limit the growth of *C. acutatum* have been reported on mangoes and avocadoes. They have not been proven on a commercial orchard scale but some clearly out-compete *C. acutatum* for infection sites.

The likelihood of latent infections and the established correlation of them with disease incidence suggests that biological control systems are unlikely to prevent infection or cure existing disease. They may however reduce the number of available infection sites or delay the onset of symptoms. Several products (eg. Aspire™, BioSave™, AQ10™ and Trichodex™) have been developed to protect fruit post-harvest (Wharton and Diequez-Uribeondo, 2004). Their use and relative efficacy have not been widely reported, although grower testimonials exist. One related to anthracnose on pecans and is included in the Australian Nutgrower (June 2011).

3.5.4 Genetic host resistance

There are few examples of resistant cultivars amongst susceptible host populations, but the pathogenicity of fungal isolates appears to vary on particular hosts. Because of the array of susceptible plant parts on most hosts, and the long duration of susceptibility, breeding or selection for resistant perennial hosts has little likelihood of success. However on annual hosts, and on hosts on which infection is limited to one plant part (eg. apple, blueberries, olives), this approach offers some hope.

'Natural avoidance', rather than genetics may explain the reduced impact of the disease on some cultivars. It appears to be linked to flowering or maturity times, rather than physical and genetic barriers to infection, or to induce host responses. In almonds, despite all *C. acutatum* isolates

being pathogenic, smaller lesions develop on infected, detached Non-pareil fruit, than on other cultivars like Carmel (Diequez-Uribeondo et al., 2005). This observation reflects that observed in the orchard also. Non-pariel does not suffer severe anthracnose, and this may be due to its earlier flowering, when temperatures may be too cold for fungal growth and infection. However, during weather that draws out the flowering period, or delays flowering (eg. in low chill years), Non-pareil may become infected, albeit less rapidly than other cultivars.

3.6 Predicting and monitoring for anthracnose

3.6.1 Monitoring for anthracnose

When specific details are known about the epidemiology of a disease, prediction models may be useful in preparing for its onset and minimising its impact. Disease prediction models generally rely on accumulated microclimatic data and disease incidence data. In their development, extensive reviews of weather events and disease history are required and interpreted (Fitzell et al., 1984; Dodd et al., 1991; Duthie, 1997; Dieguez-Uribeondo et al., 2002; Wharton and Diequez-Uribeondo, 2004). The monitoring of weather is the basis of advisory services. Useful prediction models exist for apples (apple scab) and grapes (downy mildew). See Section 8.

Predicting anthracnose is difficult because its development is determined by environmental conditions and the host stage of development. Each is constantly changing. The critical roles of temperature and moisture in the development of anthracnose make weather monitoring necessary in anthracnose prediction and management. The specifics of rainfall volume, duration and intensity, required to release *C. acutatum* spores, allow germination and infection are not known on all hosts, but in general, growers of susceptible perennials should assume *Colletotrichum* spp. spores to be present and epidemic potential to exist, when persistent rain at temperatures above 10°C, is forecast during the growing season.

The presence of infected buds may be confirmed during dormancy through BUDMON testing. An explanation of this system is given in Section 4.8, and the July 2010 results from a representative NSW orchard are tabulated below (Table 3).

Table 3 : BUDMON – *Colletotrichum* sp. detections in buds, July 2010

Buds	Block 1	Block 2	Block 3	Block 4
No infection	61[x]	72	79	68

[x] Buds from NSW orchard with no detected infection - expressed as a percentage of total number of buds examined.

Monitoring symptoms on immature fruit is important also as there is a good correlation between early season latent (and quiescent) infections, and disease incidence at harvest. Latent infection may be determined through ONFIT testing of immature fruit (see Section 4.8). Quiescent infections may be seen by November in Australian pistachios, as small, black, sunken lesions.

3.7 Anthracnose in Australian pistachios

3.7.1 What we know about anthracnose on pistachios in Australia

Anthracnose on pistachios was first reported in Australia in 2001 (Ash and Lanoiselet, 2001a, b). Other than in a sub-tropical orchard around Tamworth, NSW (Neal Albert, pers comm.) where it has caused on-going losses since 2001, the disease was not considered significant until 2010/11. The casual organism has been confirmed as *C. acutatum*.

Infected leaves, buds, rachises and immature and mature fruit on both scions planted in Australia (Kerman and Sirora), have been observed. Although not yet observed, it is likely that both the male and female flowers are susceptible to infection. Spores have been observed in lesions on rachis tissue, leaves and fruit and each of these may be the source of secondary infection during a growing season. Lesions around lenticels on fruit have been observed, but there is no evidence that natural openings or wounds are needed for *C. acutatum* to infect pistachios.

Dormant buds harbour *C. acutatum*, as do leaves and rachises gathered from trees and the orchard floor after harvest and two frosts, in June. It is possible that as buds break dormancy the fungus grows and infects surrounding tissue in wet spring conditions. Small, black lesions on immature fruit and some leaves were reportedly observed by November 2010. It is presumed these fruit lesions were quiescent infections by *C. acutatum*.

There is no quantitative evidence suggesting the rootstocks *Pistachia terebinthus* or *P. integerrima* (Pioneer Gold selection) influence the susceptibility of scion tissue, to anthracnose. However some growers commented in 2011 that the trees on *P. terebinthus* had an extended flowering period, and anthracnose in them appeared more extensive by the end of the season.

At Dareton, NSW where a trial was established in the 1990s, the severity and incidence of leaf symptoms was visibly greater on some trees. Given that the tree and row spacing in the planting is not uniform and the trees have not been pruned in the last five years, it is possible these observed differences reflect canopy density (and humidity), rather than genetically-based tolerance or susceptibility.

Short term permits for use of benomyl, iprodione, and azoxystrobin on pistachios were granted between 2002 and 2004. Since then azoxystrobin, captan and iprodione have been registered for use. The usual fungicide program in pistachios has not previously targeted anthracnose, and it was inadequate for this disease in 2010/11.

Phytosanitation has the potential to reduce the inoculum sources and it is recommended that infected rachises, fruit, leaves etc be removed post-harvest from the trees and under-tree area.

3.7.2 What does anthracnose look like on pistachios?

On flowers

This is yet to be observed and described. Survival of the fungus in buds has been demonstrated but it remains unclear if many infected reproductive buds die before they emerge, or if they remain symptomless until spring. Blackened, flower buds were observed 2010. It is likely that vegetative buds and male flowers of pistachio are also susceptible.

On leaves

Infected leaves develop symptoms similar to those caused by other fungal diseases of pistachios. Symptoms include small elongated lesions on the midrib, from which larger lesions expand into the blade. Elsewhere on the blade the lesions are larger, and have irregularly bleached areas and margins.

The timing of the leaf symptoms, in relation to the first quiescent infections on developing fruit, is not known. *C. acutatum* was recovered from Australian pistachio leaves with symptoms, in November 2010. Defoliation of severely-infected trees was not reported. In California, pistachio anthracnose has occurred only once and at the time leaf symptoms were not described.

Infected cluster with visible lesions.

Infected cluster with range of lesion sizes.

Lesions on infected hulls.

Sporulation on infected hulls.

Rachis lesions—sunken, black, with sporulation in centre.

Stuck, dead rachises. Good sanitation requires their removal.

Leaf lesions extending from midrib lesion into leaf stem.

Leaf lesions extending from midrib lesion.

On fruit

From the 2010/11 season, we can assume latent infections were numerous. Quiescent infection of immature fruit was observed as tiny black, sunken lesions in spring and summer. The lesions are composed of dead cells but the fungus within them remains viable. These lesions did not expand rapidly until near harvest. As the fruit matured within three weeks of harvest, some growers saw the first evidence of extensive fruit infection. The symptoms presumably resulted from reactivated fungal growth in previously latent and quiescent lesions, and from new infections triggered by the weather conditions at the time. The lesions rapidly coalesced and blackened the hull of most nuts. Most fruit also has dark-stained shells and/or kernels.

On rachises

Lesions formed at branch junctions of rachises. The lesions were black, sunken and as they aged, orange-pink spores formed in their centre. Our limited experience with this disease in the field suggests that rachis lesions were visible before extensive fruit lesions.

3.7.3 The pistachio anthracnose epidemic during Australian season 2010/11

Weather played a significant role in the anthracnose epidemic on pistachios, in all production regions in 2010/11.

The key features of this season were:

- *Record rain*

During the 2010/11 growing season, each of the production districts suffered unprecedented spring, summer and autumn rainfall. The volume of rain received far exceeded any previous growing season rain records, and ranged from a total for October-March, of 352mm in Lameroo, SA to 775mm in Mildura, Victoria. Most pistachio orchards reported over 550mm in the growing period, rather than the usual 80-100mm.

The rain events in 2010/11 were prolonged, frequent, and some were of high intensity. Most regions had at least six rain events in each month of the growing season. Areas around Lameroo, SA and Ouyen, Victoria had their wettest month in December (up to 168mm), while the wettest month in Mildura, Victoria and Wagga, NSW was February (up to 188mm). All other districts had their heaviest falls (up to 228mm) in January, 2011.

The wet spring ensured the spread of spores and latent and quiescent infections. There is a lack of specific information on when and where spores are most likely to be produced in such a season, and on the duration of latency when rain is persistent. There is reasoned speculation from other hosts that the majority of latent infections occur in the wettest part of a growing season, and therefore it appears the pistachios were susceptible throughout the season, when suitable temperatures prevailed.

The wet summer ensured further spread of spores, and new infections that presented later as high disease incidence and severity. The wet autumn ensured fruit destruction and quality problems related to blackened hulls, stained shells, and high inoculum loads over winter in buds, leaves, rachises, mummied fruit, and debris.

- *Mild temperatures*

The 2010/11 season was also abnormal in another sense. The rain events during the season were associated with high humidity and mild temperatures. The summer did not include the usual duration of high temperatures, nor the extreme heat (days with maximum above 40˚C), that may have inhibited fungal growth and spore development. In Robinvale, Victoria for example, the average daily maxima for December 2010, January 2011 and February 2011 were 27.8˚C, 31.5˚C and 29.8˚C respectively, several degrees lower than the long-term averages for the region.

- *Late yield and quality losses*

Most pistachio growers, horticulturists and plant scientists in Australia were unfamiliar with the disease anthracnose of pistachios, prior to harvest in March 2011. They were generally unaware of the potential for latent infection on this host, until 3-4 weeks before harvest when the symptoms and fruit decline increased at an exponential rate. At this time, the industry's shortage of registered, effective products for late-season use became apparent. The on-going rain changed situations even during the harvest period, with early harvest appearing 'reasonable' and late harvests, abandoned.

3.7.4 What is currently unknown about Australian pistachio anthracnose

Several growth parameters of *C. acutatum* isolates from Australian pistachios, and their fungicide sensitivity, have been investigated (Hall et al., 2011). However, the temperatures conducive to spore production and germination, and the duration of wetness/high humidity periods required to support these processes on immature fruit and leaves, are unknown.

The volume, frequency and timing of rain events, critical to the initiation of the Australian anthracnose epidemic, are currently unknown. The relative importance of spring, as opposed to autumn rain, on the observed anthracnose severity and incidence in pistachios, is also unclear. Had the wet, 2010 spring been followed by a dry, hot summer and autumn, pistachio anthracnose may have been limited, but given the potential for the fungus to survive dry periods and overwinter in a range of host tissue, inoculum levels would have remained high. For this reason, sanitation, even in a low disease year, is important in disease management.

The sensitivity to fungicides of *C. acutatum* within infected pistachio tissue, is unknown, but through *in vitro* fungicide screening, several effective systemic active constituents and chlorothalonil as a contact, have been identified. The capacity of these fungicides a) to curb latent infections and reactivation of the fungi in quiescent lesions, b) to provide kick-back eradicative responses, and c) to control other pistachio pathogens (eg. *Botrytis, Botryosphaeria,* and *Alternaria* spp.), is yet to be determined for Australian isolates.

4 PANICLE AND SHOOT BLIGHT AND OTHER DISEASES CAUSED BY *BOTRYOSPHAERIA* SPECIES

4.1 Diseases caused by *Botryosphaeria* spp. on other hosts

Several imperfect and perfect stages of *Botryosphaeria* species infect a wide range of perennial hosts in temperate climates (Appendix 2). Economically-important hosts include apples, almonds, pistachios, walnuts, stone fruit, grapes, ornamentals, olive, prune, avocado and blackberries. Landscape trees and *Eucalyptus* spp. are also affected and may be important sources of inoculum in some locations. On some hosts, extensive cankers form, while on others, fruit rot is the most damaging effect.

In Australia's Hunter Valley, nine different *Botryosphaeria* spp. have been isolated from wine grapes (Qui et al., 2011). In California, all nuts appear to be susceptible to particular fungi classified within the Botryosphaeriaceae family (Table 4), with almonds and pistachio appearing more sensitive than walnuts and pecans. Major limbs and trunks of almonds and walnuts may be attacked by the same fungi, resulting in tree death, lower limb dieback or band canker (Michailides and Morgan, 2004). Fungal 'gummosis' of peaches in the eastern USA is caused by several species (*B. dothidea, B. obtusa* and *B. rhodina*) but *B. obtusa* appears not to be pathogenic on all hosts from which it has been recovered (Copes and Hendrix, 2003). The disease does not occur in Californian peaches, despite the presence of the fungi.

Table 4 : Fungi within Botryosphaeriaceae that cause nut diseases in California

Fungal species	Almond	Pistachio	Walnut
Neofusicoccum nonquaesitum	✓	--	--
N. parvum	✓	✓[a]	✓[b]
*N. mediterraneum**	✓	✓	✓
Macrophomina phaseolina	✓	✓	--
Botryosphaeria dothidea	✓	✓[a]	✓
Diplodia seriata	✓	✓[c]	✓
Dothiorella sarmentorum	✓	--	--
Lasiodiplodia theobromae	✓	✓	✓

(Source: Michailides and Morgan, 2010)

* Reportedly the most common species recovered from pistachios in California
a These species have been reported on pistachio in Greece
b Reported from walnuts in Spain and Greece
c Also reported on pistachio in South Africa.

4.2 What causes Panicle and shoot blight on pistachios?

This disease has been reported on pistachios in Greece, Italy, South Africa, Australia and California. Extensive research on it has been conducted by Dr. Themis Michailides and his team in California. They recognise *Neofusicoccum mediterraneum* as the main cause of pistachio panicle and shoot blight in California (Michailides and Morgan, 2010). While this imperfect (pycnidial) stage of *B. dothidea* has been recovered from pistachios in California, the perfect stage (perithecia of *B. dothidea*) has not.(Michailides 2008, 2009). Perithecia however have been found on other hosts (walnuts, almonds, olives, Eucalypts, blackberry and some native

redwoods) near pistachio orchards (Michailides and Morgan, 2004; 1993)[1]. It is possible that ascospores in these locations account for longer distance spread of disease.

Neofusicoccum parvum is currently considered the primary cause of the disease found in Australian pistachios, that is similar to panicle and shoot blight. In-depth investigation of the disease and the potential of other related fungal species to contribute to it however is yet to be undertaken.

Pistachios are not usually killed by this disease, because older wood, trunks and limbs are not attacked. Panicle and shoot blight (or "Bot") however has caused economic yield losses up to 40% (Michailides, 1990; Michailides and Morgan, 2004). The economic impact of the disease is primarily related to panicle infections. Infected buds and flowers reduce the number and size of nuts in clusters, while the loss of leaves through petiole infection and defoliation, also contribute to yield reduction.

4.3 What does panicle and shoot blight look like?

On buds

Infected buds may die before spring in which case they appear as black and shrivelled (Britton and Hendrix, 1989). Many infected buds however have no external symptoms of infection. Internally they may have dark discolouration concentrated at the base.

In California, visible cankers may form around infected leaf and bud scars, but cankers have not been observed in Australian orchards. The cankers do not kill the infected wood, but pycnidia formed within them, add to inoculum loads within an orchard. Infection of male flowers may move further into wood, forming larger cankers.

Weak shoots and clusters that arise directly from partially-infected buds, soon wilt and die.

On leaves

In California, leaves on both male and female trees are susceptible to infection by *B. dothidea*. Small, black elongated lesions on the midribs and petioles are often the first symptoms seen. These lesions may expand rapidly and are important in the disease development. By late spring, leaves on infected shoots lose colour and turn brown.

Leaf infection at the base of the petiole is usually the result of bud infection. In this case no midrib lesions form, but the whole blade wilts, discolours and dies and the petiole and the leaf fall prematurely. Late infections do not lead to defoliation, and the petiole will often remain attached. The fungus does not usually reach the wood when infection is late in the season.

Lesions on leaf blades form later and are generally quiescent secondary infections. Initially they are small, angular black lesions and most do not expand. If some expand, it is usually on older leaves that become 'blotchy'. These lesions have diffuse margins with chlorotic halos.

On shoots

Infected shoots form on both male and female trees, when wet weather has occurred at bloom. Lesions may form at the base of infected shoots. Leaves on these shoots die as a result of girdling (causing dehydration), rather than as a direct result of fungal infection. If the petiole is

[1] Since we are largely referencing Californian data in this review, it is appropriate to adhere to their practice of referring to *B. dothidea* as the cause, and to acknowledge that most Californian growers simply call the pistachio disease "Bot".

girdled, leaves will fall prematurely. Shoots may also become infected via leaf infection. The lesions on the leaf midrib may extend toward the petiole, causing leaves to lose colour and wilt before the petiole.

By late spring-early summer, infected shoots and leaves become brown and die ("Bot strikes"). They are easily detected amongst the contrasting healthy, green leaves, shoots and clusters. There is usually no fungal sporulation on shoots killed by *Botryosphaeria.* This observation helps distinguish shoots killed by *Botryosphaeria* sp. from those killed (usually earlier in spring) by *Botrytis cinerea*. The symptoms of *B.cinerea* infection are crook-shaped flagged shoots in early spring, with brown-grey sporulation at the base. They develop under cooler conditions than *Botryosphaeria* (Michailides, 1990).

Recent Botryosphaeria strike (summer)

Botryosphaeria strikes on male tree

On rachises and fruit clusters

Dead, black rachises, firmly stuck during winter, are a clear indication that the disease is present in the orchard. In California, there are two important sources of rachis and cluster infection – infected fruit and infected buds. Clusters that develop from infected buds, are weak and often die before they develop fully. Infection is often first seen at the base of the cluster. Depending on the extent of infection at the base, and girdling, the cluster may dehydrate and nuts will turn a uniform beige-tan and no fruit lesions visible.

Botryosphaeria strikes readily detected amongst healthy growth in early summer

A cluster may also become infected via fruit infection. Infections on the surface of fruit are latent for a period, or visible as pin-head sized, black lesions around lenticels. The maturity of the hull has little effect on latency but temperatures and wetness duration are important. From individual, infected fruit, the fungus grows into the peduncles, rachis, and the shoot supporting them. If the sunken, elliptical lesions expand to girdle the rachis, even non-infected nuts die (due to starvation and dehydration).

In mid-summer, black hull lesions enlarge, often at the stem and blossom ends. The nuts killed due to fungal infection are blackened and in autumn, pycnidia form below the epidermis of the hull giving them a silvery-grey and 'peppered' look. The lesions may have a pinkish tinge in their centre. After rain, secondary infections by newly-formed spores may occur. The hull

pycnidia and those that form within bark cankers, add to the inoculum load for the following seasons.

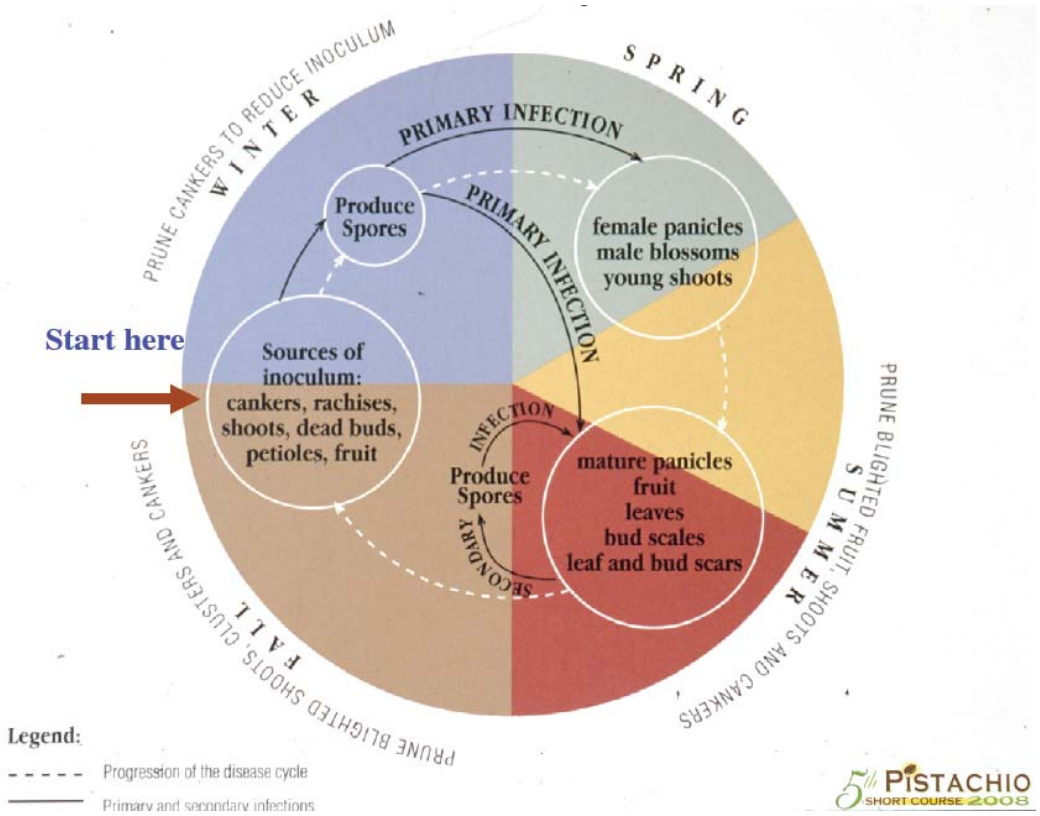
Infected rachises characteristically remain tightly stuck on trees after harvest, and have been found there for up to four years (Michailides and Teviotdale, 2009). The fungus may continue to grow from them into wood, thereby developing sunken cankers near rachis junction points. Rachises that are easily removed are, in California, considered *not* to be infected.

4.4 The disease cycle

The disease cycle for panicle and shoot blight has been described well for Californian orchards by Michailides and Morgan (2004) (Figure 4). The disease cycle for this fungus is influenced by the tree phenology (stage of development) and environmental conditions.

The key features of the disease cycle are: the full range of susceptible pistachio tissue which results in many infection sites within a canopy and locations of fungal survival over winter. Rain during mild-warm temperature periods allows repeated cycles of pycnidiospore release, spore germination, infection, disease development and the formation of pycnidia, within a season.

Figure 4 : California disease cycle of panicle and shoot blight pistachio caused by *B. dothidea*.



(Source: Michailides, 2008; Michailides and Morgan, 2004)

Insidient leaf lesions—not important unless on midrib

Midrib infection in process of killing leaf

Midrib infection in process of killing leaf

Stem infection resulting in death of leaf

Infected nuts (black) causing rachis collapse (↑) and starvation of other nuts beyond point of rachis death (brown).

Typical cluster death due to dehydration, starvation. Nuts not infected, but rachis is.

Cankers are a characteristic symptom in infected pistachios in California

Cankers developed from fungal infection from shoot or rachis moving into wood

Black discolouration in canker is diagnostic

4.5 Infection of pistachios by *B. dothidea*

The majority of information on the infection processes and disease of Californian pistachios has been sourced from Michailides' publications. No epidemiological research has been carried out in Australia on *N. parvum* on pistachios, but the disease cycle is likely to be similar, with rain playing an important role.

Conidia (pycnidiospores) of *N. mediterraneum* cause primary infections in spring and early summer in California, and are the main form of spread of the disease in orchards. Pycnidiospores attack current season tissue – buds, leaves, shoots, rachises and fruit. Disease severity is high when warm, wet weather allows both primary and repeating secondary infection periods, and trees are not well protected by registered fungicides.

Kerman is particularly susceptible to panicle and shoot blight. Observations in Australia since 2009/10 suggest Sirora is also susceptible.

4.5.1 How does infection occur?

Rain plays a major role in the spread of spores and also in infection. Infection requires spores to germinate, free moisture and temperatures above 12°C (or average during rain above 15°C). In wet weather the mucilaginous mass of spores within pycnidia, swells and oozes from the 'mouth' of the pycnida. The spores are then splashed to plant tissue by rain. Insects and irrigation water may also spread spores (Michailides, 1990).

Vegetative and floral pistachio buds (for the following season) become infected in autumn, soon after they form. Non-infected buds and leaf axils may carry spores as contaminants (externally), but once wet or splashed onto other sites, these spores have pathogenic potential. Spores in leaf axils may become active and infect buds, even in the absence of rain. The fungus survives over winter in dormant buds, rachises, mummified nuts, and leaves that were infected during the season but remain on the tree or in debris under the canopy.

In spring, the fungus grows within infected buds and may kill buds or restrict their development. Male flowers are often infected before they dehisce pollen, and the cankers that form around them, usually discolour the surrounding bark and cambium. All opening buds up to the period of fruit initiation are highly susceptible to existing and new infections.

The yield losses attributable to floral bud infection are from lost buds (dead) but more importantly, from the infected, weak clusters (and cankers) that arise from infected buds. Partially-infected reproductive buds produce blighted flowers and infected clusters that often die after a short period of spring growth.

The spores germinate and their germ tubes enter soft, fresh tissue via stomata on leaves and shoots, lenticels on developing fruit and older shoots, and growth cracks. There are few lenticels on one-year-old wood and therefore few infections are initiated there, unless the wood is wounded. Wounds, especially those caused by hemipteran insects and birds, increase the chance of infection, with pycnidia readily forming around wounded areas. The pathogen can also colonise dead wood.

In immature fruit, young leaves and shoots, the infections may remain latent until temperatures increase in the early summer. Cankers, lesions and pycnidia form on infected tissue during the hotter summer weather. The fungus prefers high (optimum 27-30°C) temperatures for growth, so the disease is considered a hot weather disease, despite current season infection often being triggered in the late spring.

It may take 2-3 weeks for an infected cluster to die, although the location of the initial infection site, influences this. If infection occurs at the base of a cluster, the entire cluster may be killed within a week.

Later in summer, rain may trigger secondary infection. Hulls are very susceptible to secondary infection with sources of spores being shoots, rachises, fruit and leaves infected earlier in the current season.

4.6 Panicle and shoot blight – contributing factors

4.6.1 Conducive moisture conditions

Californian researchers have demonstrated that in years with warm, wet springs, disease incidence and severity are high (Michailides and Morgan, 2004). Wet weather promotes the disease by dispersing spores and aiding infection.

Buds collected in the wet season consistently have higher infection levels. Research in California found that the highest percentage of infected buds were detected in the wettest months being, February-March in northern hemisphere (Ntahimpera et al., 2002).

Michailides and Morgan (2004) and Morgan and colleagues (2009) reported that a 4-6mm rainfall was sufficient to disperse spores. Ahimera and team (2004) reported 16 mm rain was needed to release spores, and demonstrated that a water drop from 1m above infected pistachio nuts, resulted in an average spore displacement of 20 mm, and (on average), 23 conidia/drop. In an infected orchard in northern California, an area where summer rain is more prevalent than in southern California, rainwater was tested and shown to carry up to 23,000 spores/ml (Ahimera et al., 2004). The field research also demonstrated collections of up to 67,000 conidia/ml in rainwater falling through infected tree canopies.

Pycnidiospores may be released within 2-3 hours of rain (or irrigation) and the pycnidia may not be exhausted of spores until 10-12 hours later (Michailides and Morgan, 1993). To allow the spores to infect, a period of free moisture is also needed. Michailides and Morgan (2004) found that a wetness period of 9-12 hours was sufficient to allow infection, and promote symptom and disease development. Subsequently it has been recognised that 'wetness period' is of more significance as a predictor or infection periods and disease development, than 'rain duration' (Morgan et al, 2009).

Water stress influences spore germination, germ tube elongation and mycelial growth of *B. dothidea* (Ma et al., 2001). As water potential decreases to below -20 MPa, these growth parameters appear to increase. Dry summers however do not ensure panicle and shoot blight will not develop, as latent or quiescent infection may result from rain at other times.

4.6.2 Conducive temperatures

Temperatures influence most steps in the disease cycle of *Botryosphaeria* spp. on all hosts. On pistachios, pycnidia rarely develop in cool weather. Pycnidia formation is maximised at 27-30°C. Spores however may form at temperatures as low as 10°C, and may be released from pycnidia in old cankers for up to six years and in prunings for 1.5 years (Ahimera et al., 2004). Inoculum loads therefore increase cumulatively over a wide temperature range, until their source (eg. cankers and infected tissues) is removed. On other hosts, *B. dothidea* and *B. obtusa* were found capable of spore production on buds at temperatures above 6°C, but the optimum range for spore production on these hosts, was 24-30°C.

Spore *germination* is favoured by temperatures between 24-36°C. Infection by germinated spores however may occur at lower temperatures (above 12°C) in presence of sufficient moisture, (Michailides and Morgan, 2004). It is therefore presumed that most buds become infected during autumn. *Botryosphaeria* sp. isolates from Californian pistachios grow well at high temperatures, with the optimal range for mycelial growth (as determined in *in vitro* experiments), being 27-30°C. Latent infections may become active in summer when temperatures are in the range of 20-36°C. Disease development is maximised at 27-33°C in California, but humidity may also influence the rate of development (Michailides and Teviotdale, 2009; Michailides and Morgan, 2004).

Two Australian isolates of *N. parvum* recovered from pistachios, had peak mycelial growth at 30°C. They each grew over the temperature range 10-35°C, with the optimal range being 20-30°C. Growth did not cease at 35°C, so it is likely the fungi will continue to grow at higher temperatures, but at a slower rate (Hall et al, 2011).

4.6.3 Contributing factors in the orchard

Irrigation practices may contribute to disease spread in orchards. Although rain plays the major role in the distribution of spores, irrigation water that contacts leaves in the canopy and raises

humidity, will contribute to disease development, incidence and severity. Insect and pollen transmission, and movement via equipment account for very little spread, but are involved at times.

Drought and water stress in orchard trees may also influence the severity of the disease on some hosts. Because infection may occur through growth cracks, wounds and natural openings, avoidance of stress that results in weakened trees, is recommended.

Budding has the potential to spread the disease since the fungus survives well in infected buds. However infected buds rarely take, thus limiting potential transmission of the disease by nurseries, and through this process. Canopy density influences drying times and humidity and therefore pruning practices also influence disease development.

Other nearby hosts may contribute to the threat within an orchard. Olives, almonds, walnuts and some other *Prunus* species are susceptible to *B. dothidea.* Disease onset within some Californian orchards has shown a gradient suggestive of entry from infected riparian vegetation and *Eucalytpus* spp. In one almond orchard, it appears certain that neighbouring, infected walnuts were the inoculum reservoir (Michailides pers. comm.).

4.7 Panicle and shoot blight management

It is important that growers recognise this disease and can distinguish it from others that have similar symptoms at certain times of the season (eg. *B. cinerea*, and citrus flat mite in California) (Michailides, 1990). See Section 7.

Eradication of panicle and shoot blight cannot be relied upon once it is established in an orchard. The disease losses can however be minimised by integrated practices that incorporate orchard sanitation, irrigation and humidity management, pruning and chemical applications. In California, the disease on pistachios is now well managed by growers with an understanding of the epidemiology of this fungus, the optimal timing for implemented practices, and the contributing factors over which they have some control.

Effective management of this disease in young orchards differs from that usually applied in older orchards. Young orchards are more open, but trees are rarely treated with the array of fungicides applied in older orchards.

4.7.1 Cultural

In both young and old orchards, it is important to minimise the number of pycnidia (and therefore, spores). In young orchards where the disease if present, is likely to be sporadic, infected shoots should be removed in summer when they first appear. They should be cut back to 5cm below the affected area (Ahimera et al., 2004).

Pruning that increases airflow and reduces humidity, is worthwhile. Orchard pruning should not be undertaken during wet weather, despite pruning equipment being unlikely transmitters of the fungus. The open wounds provide a surface for natural infection by splashed spores, and pycnidia have been observed at the rim of older pruning cuts in almonds.

In older orchards, sanitation is particularly important. Any prunings should be removed from the orchard floor as viable spores survive in cankers for a long period. Other debris should be removed from under the canopy – to reduce inoculum, and also humidity. Weed-free and cover crop-free orchards are less humid and insect numbers are likely to be lower. Balanced water and fertiliser regimes also assist with canopy and humidity management, and tree stress minimisation.

Other important cultural management practices relate to irrigation. Keeping water out of the canopy and minimising wetness periods are effective means of reducing the spread of spores and new infections. While rain cannot be managed, irrigation water can be manipulated to ensure its throw and trajectory does not wet any part of the canopy. The use of drip irrigation is recommended, and by adjusting the time and duration of irrigation, the periods of high humidity may be minimised. Short irrigations during daylight at lower pressure, are recommended, eg.12-hour sets on consecutive days are preferable to one 24-hour set.

4.7.2 Chemical – Fungicides

The optimal timing of fungicide applications (starting with a bloom spray and followed in summer), in addition to good sanitation and tree monitoring for symptoms, has allowed Californian growers to manage this disease, which at one time appeared could cripple the industry.

California, unlike Australia, has access to a large number of fungicides registered for use on pistachios, making rotations between chemical classes practical. To-date however resistance development has not been a problem in the management of *Botryosphaeria*.

In California, dormant and pre-bloom fungicide applications are not necessary and are considered economically unjustified. This is despite the demonstrated survival of the fungus in pistachios, over winter (Britton and Hendrix, 1989).

Early season (spring) sprays are very effective in California (and presumably in Australia) because history has shown that most rain events occur in spring and decline quickly thereafter, in normal seasons. Several growers (pers. comm.), and Michailides and Morgan (2004) recommend a bloom spray, regardless of the weather conditions, to minimise flower infections. "Bloom" is described in California as the period when terminal buds are 3.5-6.5 cm in length, and full bloom is when the male is dehiscing pollen. However "bloom sprays" are important and are best applied when *shoots* are 3.5-6.5 cm because there is a tendency for vegetative terminals to push out ahead of flowers, in off-years. Californian growers have a wider choice of fungicides registered for use at bloom, for *Botryosphaeria* and *Botrytis* management. They include amongst them, thiophanate-methyl, pyrimethanil and fenhexamid.

Early infections result in more blighted fruit/cluster and more pycnidia development on fruit and leaves. These increase the potential severity of secondary infections and inoculum loads for the following season. Bloom sprays need to be followed by summer applications that protect susceptible panicle tissue up to shell hardening. Extrapolation from Californian data suggests that the critical months for tree protection in Australia are at bloom and from November-January but the timing of rain events will influence this each season.

ONFIT results and the weather guide mid-season fungicide applications. ONFIT results in June (northern hemisphere) are highly correlated with harvest disease incidence, which suggests few latent infections are initiated in summer. Mid-season fungicide applications are usually in response to forecast weather events. Six-eight sprays may be needed in some orchards when disease pressure is high, but with the use of risk-based predictive models and the courage and means to apply fungicides (with kick-back) two days *after* a significant rain event, Michailides believes the disease in many orchards can be managed by as few as two well-timed summer applications of fungicides (or pre-mixes) that include a strobilurin (Michailides pers. comm.).

In California, when BUDMON indicates no infected buds, there is little spring rain, *and* ONFIT indicates no latent infection of immature fruit by June (northern hemisphere), it may be possible to avoid sprays after bloom. Even in such cases, orchard sanitation must be maintained at a high level.

The fungicides registered for use on pistachios, and effective on *Botryospheria* in California, are tabled below. Michailides and Morgan (2004) concluded that strobilurins were the most effective fungicides on this disease in California. Strobilurins as prt of pre-mixes have also performed well. The pre-mix of pyraclostrobin + boscalid (Pristine®) has been ranked the most effective (Table 5). Several growers have reported that propiconazole and azoxystrobin as a tank mix is also very effective. Generally, demethylation inhibitor fungicides have not been as effective on *Botryosphaeria* spp. Chlorothalonil, although ranked as moderately effective, has been linked in some of its generic formulations, to russetting of the fruit hull and shell staining.

Table 5 : Efficacy against *Botryosphaeria* sp. from pistachios, in California

Active constituent	Efficacy
azoxystrobin	+++
chlorothalonil	++
pyraclostrobin	+++
trifloxystrobin	+++
fluopyram+trifloxystrobin	++++
pyraclostrobin + boscalid	++++
pyrimethanil	+++ [y]
cyprodinil + fludioxonil	++
fenhexamid	ND
polyoxin-D	+++
difenoconazole + azoxystrobin	+++
thiophanate-methyl	+ (at bloom)
lime sulphur	+/-

[y] – under low and moderate disease pressure. (Source. Adaskaveg et al., 2011; Michailides, 2008)
+ least effective ++++ - most effective; ND= no data

4.7.3 Host resistance

In California, two *Pistachia vera* cultivars (Sfax and Lassen) have shown resistance to several isolates of *B. dothidea*, but the most commonly-planted cultivar, Kerman, is very susceptible. There is some evidence that Red Aleppo is very susceptible. No other resistant germplasm has been documented, but suckers of several pistachio rootstock species, *P. atlantica*, *P. integerrima*, and the interspecific crosses Pioneer Gold II (PG2) and UCB1, were thought to show some promise in 2004 (Michailides and Morgan, 2004). Sirora is also susceptible as has been shown in Australia where this is the dominant female scion.

4.8 Monitoring and predicting panicle and shoot blight

The relationship between bud infection and panicle and shoot blight at harvest has been described as linear, i.e. given conducive conditions, with 50 % buds infected, one might expect approximately 30% diseased clusters at harvest. Knowledge of bud infection prior to the spring, allows orchardists to prepare for the potential impact of a warm, wet spring. BUDMON is a useful tool for demonstrating *Botryosphaeria* sp. infection of dormant buds. If BUDMON infection levels exceed 7%, or there is a history of panicle and shoot blight, there is a high risk of serious losses, should conducive environmental conditions, present.

Monitoring weather conditions is an essential part of good management, for this disease. Forecast weather conditions allow growers to evaluate the potential disease threat, and prepare for protective treatments.

In California two models to predict infection events have been developed and evaluated. Both models accept the premises that: 1. The spores are only splash dispersed, 2. There is a positive correlation between latent infections (determined at end of spring) and disease severity at harvest, and 3. That the pathogen grows best in warm temperatures, and 4. That moisture and temperature influence disease development.

The *Botryosphaeria* infection model (BIM) determined that infection would be favoured when temperatures were >11°C and accompanying rain included falls of >1mm/h, for >4 hours (Morgan et al., 2009). The data were sourced from weather stations near orchards. The limitations of the model were: no account was taken of the variation in length of infection periods (as determined by temperature), and that 'rain duration' underestimates 'leaf wetness duration'. The relevance of the BIM thresholds in Australian orchards is untested.

The leaf wetness model (LWM) takes into account wetness duration for rain events >4mm, and temperatures at the time of such rain events. It also accommodated prior knowledge that wetness periods separated by <12 hours allowed spores to start, stop and re-commence germination. Such wetness periods were therefore combined in calculations of wetness duration, in this model (Morgan et al., 2009).

Growth chamber experiments with inoculated potted trees confirmed the non-linear relationship between wetness duration (hours) and temperature. Using actual field data collected over a long period, the wetness duration-temperature relationship has been developed into a predictive model where the risk of infection may be predicted as low, medium or high, with consideration also given to the trigger event (>4mm) and number of rain events thereafter (Morgan et al., 2009).

After wet springs, latent infections in developing kernels can also be estimated through ONFIT. It relies on freezing treatment to artificially advance hull maturity. Infected kernels treated this way, once incubated, develop symptoms and the fungus will grow out. At levels of 1-4% on fruit collected in June (northern hemisphere) latent infection is considered 'low', and low disease severity at harvest could be expected. However, results of >10% are a warning that disease incidence and severity at harvest could be very high.

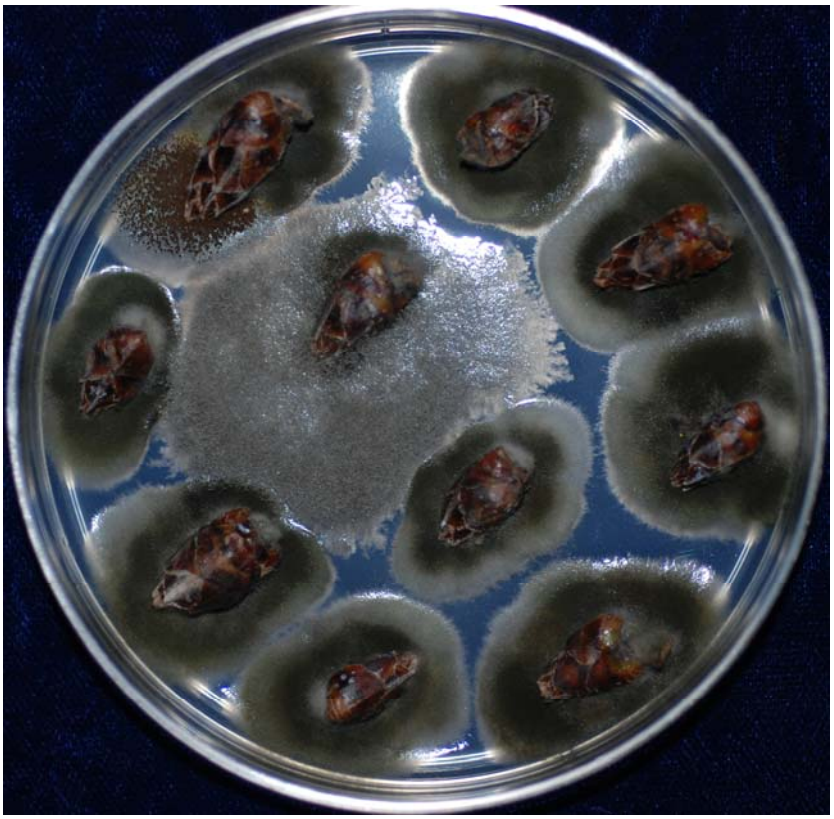
Continuous monitoring in young orchards during the growing season allows early detection of symptoms and removal of blighted shoots and clusters. This summer pruning should be done for at least two consecutive years, before inoculum reduction could be assumed.

4.8.1 Laboratory monitoring services

BUDMON (and ONFIT) testing is commercially available. BUDMON allows the presence of *Botryosphaeria* sp. in dormant buds to be detected prior to the break of dormancy, while ONFIT detects latent infection in immature fruit. For BUDMON, laboratories require 100 buds selected at random during winter months. The samples should not include dead or shrivelled buds. The buds are surface sterilised, and placed on acidified synthetic media suitable to support the growth of *Botryosphaeria* sp. (Figure 5). The same test may also be used to detect *Colletotrichum* sp. overwintering in buds. Infection levels are given as a percentage of infected buds. In California, BUDMON results of 7% or higher, are of concern. BUDMON data[2] from a representative NSW orchard are shown in Table 6.

[2] A review of the technique used in Australia where buds have traditionally been cut longitudinally before placement of synthetic media, is being undertaken and may render these results less meaningful.

Figure 5 : Budmon test plate



(Source: Michailides, 2011)

Botryosphaeria emerging from one infected bud and *Alternaria* spp. from others

Table 6 : BUDMON - *Botryosphaeria* detection[x] in buds, July 2010

Orchard	Block 1	Block 2	Block 3	Block 4
No infection	43[x]	14	38	18

[x] Buds from NSW without infection expressed as a percentage of total number of buds examined.

5 ALTERNARIA LATE BLIGHT

5.1 *Alternaria* spp. as pathogens

Fungi in this genus are ubiquitous and several species have worldwide distribution and are important pathogens of annual and perennial crops. They cause leaf spots, stem blights, fruit spots and rots on a wide range of hosts. A host-specific toxin is part of pathogenesis on some hosts. The same species may be a weak pathogen on some hosts and aggressive on others. Stressed plants and injured plant parts are often more susceptible to attack.

The fungus sporulates on the surface of infected plant tissue producing masses of infective spores. These spores are easily 'rubbed off' and spread to susceptible plant tissue by wind and rain. The fungi may also survive on dead tissue and debris, making them both saprophytes and pathogens.

5.2 What causes Alternaria late blight on pistachios?

The fungi *Alternaria alternata, A. tenuissima* and *A. arborescens* are reported as the causes of this serious foliar and fruit disease in Californian pistachios. The disease appears in late summer, and has been reported, but not investigated in Australian pistachio orchards (Ash and Lanoiselet, 2001c). The disease appears first on male trees late in the season (usually from August in California), and high in the trees.

Spores of *Alternaria* spp. are distinctive and visible often with a hand lens. Their populations increase from mid-summer and in irrigated orchards, the critical period for disease development coincides with these population increases, increased canopy humidity and dew formation (Michailides et al., 1995).

Alternaria spp. infect many crop plants and weeds, fallen leaves and other plant debris. Because all forms of crop residue serve as overwintering sources of the fungus, *Alternaria* spp. capable of causing pistachio late blight, must be presumed to be present in pistachio orchards every season.

5.2.1 What does Alternaria late blight look like on pistachios?

This is a disease of pistachio leaves and fruit. It may result in defoliation, thereby negatively affecting yield in the current season and also in the following season due to the reduced carbohydrate levels. Shell staining and mould contamination have been reported as serious quality issues associated with infected pistachios (Michailides et al., 1995).

Symptoms are generally more severe in "on" years. The first symptoms are usually visible high in the trees, in mid-late summer (from August in California). Leaves on fruit-bearing shoots often show symptoms first, but in some cases male trees have appeared more susceptible than female trees (Holtz, 2002).

On leaves

Leaves develop the first symptoms. The dark brown-black, round or angular lesions are 3-7 mm in diameter. The lesions develop on the blades and older leaves are more susceptible than young leaves. Lesions usually start at the leaf margins and progress inwards toward the mid vein. Petiole lesions also form and are small and black. The lack of early midrib lesions distinguish this disease from panicle and shoot blight, and anthracnose.

As the leaf lesions age, the Alternaria lesions enlarge and merge together to form large areas of blighted tissue. Late in the season, the blighted areas turn black with a reddish-purple halo (Figure 6). Black spores form on the lesions. These may be rubbed off turning the finger black and this distinguishes the lesions from those formed by *Botryosphaeria* sp. These symptoms however may mask existing Botryosphaeria infection. Blighted leaves may fall prematurely.

Figure 6 : Alternaria late blight on pistachios

(Source: New Mexico State University, 2005)

Figure 7 : Premature defoliation of a pistachio tree infected with both Septoria Leaf Spot and Alternaria Late Blight

(Source : New Mexico State University, 2005)

On fruit

Fruit lesions follow leaf lesions, and on immature fruit they appear as small (approx 1mm), black spots initiated in and around hull splits and lenticels. Red halos around lenticels often indicate Alternaria infection. Like leaves, fruit are more susceptible as they mature.

Under high disease pressure, infected mature hulls have black lesions with a purple margin. The lesions are larger (up to 5 mm) in diameter, and in severe cases, cover the entire hull, and stain the shells. Infections are more severe in early-spilt and cracked fruit. Mould in kernels can render them unsightly and off-flavour.

5.3 What promotes infection?

5.3.1 Conducive conditions - humidity

As for anthracnose and other pistachio diseases, Alternaria late blight is favoured by long humid periods, especially in the mid-late part of the season when a fruit load is present. Irrigation, rain and dew, create humidity. In California field trials, trees irrigated with subsurface irrigation had significantly reduced Alternaria late blight, in terms of both incidence and severity, than flood irrigated trees (Michailides et al., 1995). Drip-irrigated orchards also have fewer problems with this disease.

5.3.2 Conducive conditions - temperatures

Alternaria late blight is favoured by high temperatures higher than those conducive to the development of anthracnose. In culture, the fungi grow rapidly at 27-30°C.

5.4 Alternaria late blight management

It is not possible to eliminate this pathogen from the growing environment. Management relies on an integrated approach of humidity manipulation, and fungicides.

5.4.1 Cultural

Good sanitation must be maintained in order to reduce the inoculum sources in orchards.

Minimisation of the duration of high humidity periods in canopies may be achieved through changed irrigation practices. The timing and method of irrigation, infiltration and drainage efficiency, cover crops, and air movement, influence humidity. Winter pruning to open canopies, and reduced irrigation frequency and duration in late summer when trees are most susceptible, are recommended.

Harvest should not be delayed, in orchards with a history of this disease.

5.4.2 Chemical - Fungicides

There are several fungicides that have shown efficacy in California against *A. alternata* on pistachios and other hosts (New Mexico State University, 2005). Michailides (2008) ranked the efficacy of some as shown in Table 7, but some of these rankings may no longer be accurate.

In the United States, Alternaria resistance is widespread. Resistance of this fungus to some strobilurins and pre-mixes containing storbilurins has been reported on pistachios (Michailides and Morgan, 2004). The pre-mix of cyprodinil and fludioxonil; metconazole, and polyoxin-D have recently been reported as largely replacing the strobilurin use for this disease. Polyoxin-D specifically claims to ‘control strobilurin-resistant Alternaria’. These new products and many of the active constituents tabled are not registered for use on pistachios in Australia.

Bloom sprays are not required for this disease but in California mid-season (up to end of July) protection is important. Late sprays are not effective.

Table 7 : Efficacy of active constituents against *Alternaria* spp. in California

Active constituent	Efficacy on pistachios
azoxystrobin	+++*
chlorothalonil	++
pyraclostrobin	+++*
fenhexamid	ND
pyrimethanil+trifloxystrobin	++
trifloxystrobin	+++*
fluopyram + trifloxystrobin	++++*
pyraclostrobin + boscalid	++++*
polyoxin-D	+++
pyrimethanil	++
cyprodinil + fludioxonil	+++
copper hydroxide	+

(Source: Michailides, 2008; Adaskaveg et al., 2011)

*Good control in areas where no resistance has developed.

+ least effective ++++ - most effective; ND=no data

6 SEPTORIA LEAF SPOT – (EXOTIC)

6.1 Septoria leaf spot

Septoria leaf spot on pistachios has not been found in Australia. It is considered therefore 'exotic' to this country. REPORT IT IF YOU SEE IT! The disease was first detected in the USA (in Texas) in 1964. It was found in Arizona in 1986 and by 1988 it was widespread in that state. New Mexico also reports this disease as present. The disease is an on-going threat in the southeast of Arizona, which reflects that region's regular summer rains. August (southern equivalent, February) on average is the region's wettest month, with a monthly rainfall average of 100mm registered from 1992-1996 (Call and Matheron, 1998).

6.2 What causes Septoria leaf spot on pistachios?

Septoria pistaciarum was identified as the cause of the disease in Arizona and New Mexico. This fungus only infects pistachios (New Mexico State University, 2005). Two other *Septoria* spp. are also known to cause leaf spot and premature defoliation. *S. pistaciae* has been found in the US, while *P. pistacina* has been reported in Turkey and Greece (Michailides, 2009). The perfect stages of the fungi are in the genus *Mycosphaerella* sp.

6.2.1 Infection of pistachios by *Septoria* spp.

It is believed that the fungus overwinters as pycnidia on fallen leaves. The fruiting bodies of the sexual stage of the fungus form and readily discharge ascospores during rain in mid-spring. Ascospores are believed to be the primary inoculum. Conidia spread by rain later in the season, are the secondary inoculum.

Septoria leaf spot is initially a cool weather disease and at 10°C, the time taken for symptoms to become visible on leaves is around 10 days. (Matheron and Call, 1998; Michailides, 2009).

6.3 What does Septoria leaf spot look like on pistachios?

On leaves

The first symptoms are usually round to irregular, dark brown, necrotic spots. They form between minor veins on both sides of the leaf, and are not more than 2mm in diameter. The discreet spots form across the blades of infected leaves. Pycnidia form on both sides of the leaf.

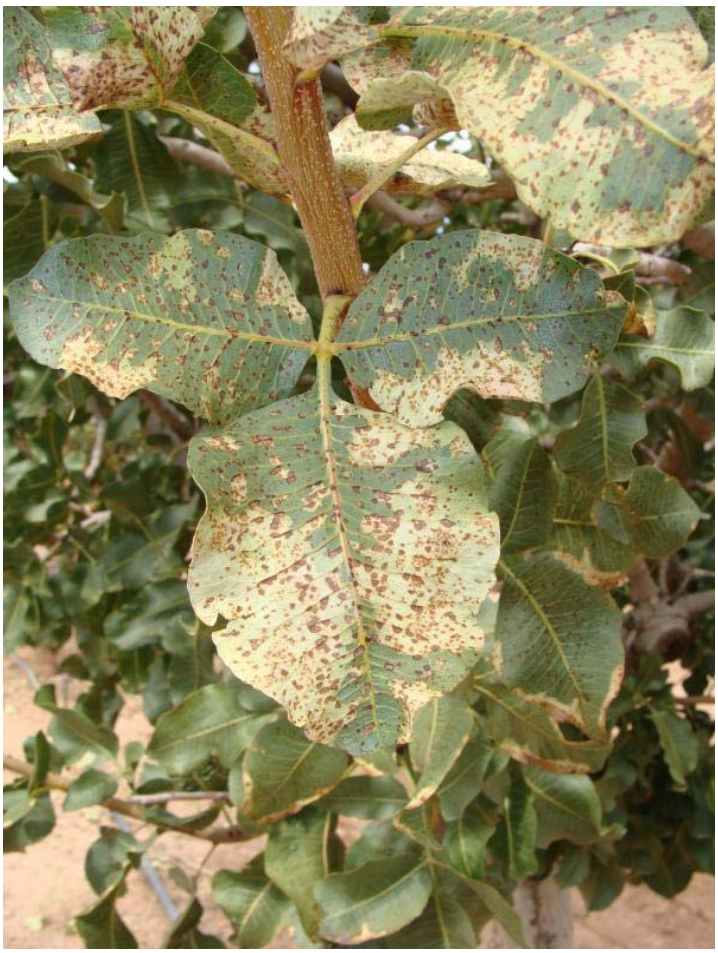
In wet springs, hundreds of spots may be visible by early April in the northern hemisphere (southern equivalent - October). Each spot does not increase greatly. The spots do not coalesce, they remain isolated. Over time however, the infected sections of the leaf may become light tan in colour (Figure 8). Defoliation may occur when infections are severe (see Figure 7).

6.4 Management of Septoria leaf spot

Where hot, dry summers are normal, this disease does not require regular management. However in seasons with high summer rainfall, this fungus like several others on pistachios, has the potential to proliferate. Call and Matheron (1998) carried out field trials and found two applications of chlorothalonil in July and August (southern equivalent January, February) successfully inhibited the development of Septoria leaf spot. Michailides (2009) reported that zineb and mancozeb are also effective. In New Mexico, azoxystrobin, trifloxystrobin, copper hydroxide and chlorothalonil have been registered for use against *Septoria* spp. Copper and chlorothalonil, while effective, should not be used until fruit is greater than 1cm in length.

There appears to be some genetic resistance to this fungus. Kerman is more severely affected than pistachio species *P. chinensis, P. atlantica* and *P. terebinthus* (Call and Matheron, 1998).

Figure 8 : Advanced symptoms of Septoria Leaf Spot.



7 MANAGEMENT OF FUNGAL DISEASES OF PISTACHIOS

7.1 Summary – Chemical management

Table 8 : Fungicide efficacy summary for four pistachio diseases

Active constituent	Efficacy against *Botryosphaeria* spp.	Efficacy against *Alternaria* sp.	Efficacy against *Botrytis* sp.[x]	Efficacy against *C. acutatum*[w]
azoxystrobin	+++	+++*	---	Variable
chlorothalonil	++	++	---	✓
pyraclostrobin	+++	+++*	---	✓
trifloxystrobin	+++	+++*	---	✓
pyraclostrobin + boscalid	++++	++++*	++++	✓, ✗
pyrimethanil	+++ [y]	++	++	✗
cyprodinil + fludioxonil	++	+++	+++	✓, ✓
fluopyram +trifloxystrobin	++++	++++	+++	✗, ✓
fenhexamid	ND	ND	++++	✗
propiconazole + azoxystrobin	+++	+++	---	ND; variable
metconazole	+++	++	+++	ND
thiophanate-methyl	++	---	++	ND

(Source: adapted from Adaskaveg et al, 2011: Michailides, 2008; Hall et al, 2011)

[x] Results for Botrytis, Botryosphaeria and Alternaria relate to Californian conditions and testing
[w] Australian pistachio isolates and *in vitro* testing (Hall et al, 2011) of actives independently, at label rates and lower
[y] – under low and moderate disease pressure. ✗ - not effective, ✓= effective
--- = no effect; + least effective ++++ - most effective; ND = no data
* Resistance reported

Extrapolation from Californian research (Michailides, 2008) has also allowed identification of the critical treatment times likely in Australian pistachio orchards (Table 9).

Table 9 : Proposed critical treatment times in Australian orchards

Fungus/Disease	Critical monitoring periods	Critical treatment months[x]
Botryosphaeria sp. Panicle and shoot blight	Dormant; Spring-summer	Bloom and midsummer **Oct + late Nov-Jan-Feb**
Alternaria spp. Alternaria late blight	Mid-summer - Dec	Mid-summer **Dec-Jan**
Botrytis sp. Blossom and shoot (and some fruit) blight	From bloom while cool, wet	**Bloom – October**
Colletotrichum spp. Anthracnose	Dormant; Spring-summer	? Flowering – end of rain?
Septoria spp. Septoria leaf spot	Spring - summer	*Exotic to Australia*

[x]Proposed most effective application timing **in bold**

7.2 Summary – Symptoms and distinguishing features

Several diseases of pistachio appear similar at certain stages of the disease cycles. To effectively manage diseases, the cause and its life cycle should be known. Table 10 includes some key symptoms on different plant parts and recommended monitoring and treatment times, monitoring and treatment information.

Table 10 : Distinguishing features of several foliar diseases of pistachio

Symptom and location on pistachio	*Neofusicoccum* sp. (*Botryosphaeria* sp.)	Anthracnose *C. acutatum*	Blossom and shoot blight *Botrytis cinerea*	Alernaria leaf spot	*Septoria leaf spot*
Buds, flowers	Symptomless⇨dead At emergence: blighted flowers; cankers near male flowers look like *B. cinerea*	Symptomless⇨black	Blighted flowers – male and female susceptible. Cankers near male flowers	--- No flower symptoms	--- No flower symptoms
Young shoots	Blighted mid-summer; wilted 'strikes'. Black, elongated lesions on shoot base or midribs; dead*	Leaf lesions quiescent Shoot symptoms?	Blighted mid-spring; Brown sporulation at base, wilt in crook-shape, die*	---	?
Petioles	Elongated sunken lesions. Infected via buds or midrib. Defoliation	Black sunken lesions	---	?	?
Leaves	Midrib black lesions⇨blades, petioles. Blades lose colour also if petiole girdled – but no lesions.	Midrib lesions extend into blade	Wilt, die on infected shoots	First sign of disease - marginal burn on older leaves high in tree; late defoliation	Isolated lesions across blade, between minor veins. Overall bleached , spotty
Sporulation	Not on infected shoots. On hulls -grey look	Orange-pink; on petiole, rachis, hull lesions	Beige, buff-coloured; Blighted flowers; base of shoots; male cankers	Black, velvety – can be rubbed off; older leaves; hulls	Primary – conidia? ascospores?
Spores	Mucilaginous, waterborne, splashed	Waterborne; splashed	Powdery - airborne	Powdery - airborne conidia	Waterborne-splashed
Fruiting bodies	Pycnidia inside plant tissue	Acervuli	None usually; sclerotia occasionally	---	Pycnidia
Rachises	Black lesions; collapse	Black lesions	Girdled, collapse	?	?
Fruit - infected	Small black lesions or latent infection of on green fruit Black hull lesions – turning grey by autumn	Quiescent small, black lesions ⇨ black hulls with pink spores, near harvest	---	Lesions with pink-red halo around lenticels; stained shells	---
Fruit - girdled	Uniformly brown-tan if rachis girdled	---	---	---	---
Conducive conditions	Warm wet spring and hot summer	Warm, wet growing season	Cool, wet spring	Hot, late season high humidity.	Cool – hot, wet spring-summer
Notes	Wide range of effective fungicides in California; few in Australia	Anthracnose fungicides largely untested on pistachios	Bloom fungicides – limited range	Widespread chemical resistance	Exotic to Australia

* difficult to distinguish *B. cinerea*- and *B. dothidea*-infected shoots in spring if no sporulation present.

8 PREDICTING FOLIAR DISEASES AND PESTS

See also Section 4.8 on the pistachio leaf wetness model.

In Australia a number of commercial pest and disease prediction services have been developed for a limited number of horticultural crops. In specific disease cases, the output is a 'warning' that weather conditions suitable for infection are forecast or have presented, in a particular region. Individual growers who know the relevant specifics of their orchards relative to the monitoring service sites (eg. elevation, rainfall, disease history), can respond to regional monitoring service warnings most effectively, i.e. adjust their responses (eg. fungicide applications) to reflect their own orchard situation.

Disease predictions allow more informed decisions on disease management, eg. timing, choice of product etc. In crops that have amongst their cultivars, a range of susceptibility to the monitored disease, a selective spray program may be appropriate. Disease prediction models allow planning for applications to tolerant (least susceptible) and non-bearing trees to be minimised or delayed, and for susceptible trees to receive higher priority when opportunities to spray are truncated.

The response to a predicted infection period may be supplementation of an on-going minimal program, rather than the commencement of a treatment program. Protectant fungicides need to be applied before conducive conditions, but when conditions prevent access to the orchard, or remove the recently-applied fungicides, eradicants can be applied if the response time is within the 'curative' period for the products.

8.1 The basis of disease forecasts

To predict diseases, environmental events that influence the development of the fungus and its infection capability, are incorporated into the design of the data collection and interpretation models. Data on rainfall, leaf wetness periods, and temperatures are common components of foliar disease prediction models (Dodd et al., 1991; Duthie, 1997; Fitzell et al., 1984; Dieguez-Uribeondo et al., 2002; Wharton and Diequez-Uribeondo, 2004).

8.1.1 Apple scab on apples

To predict the fungal disease 'apple scab' (*Venturia inaequalis*) on apples, information on temperature and leaf and fruit wetness periods, is needed. The temperature data required are maximum and minimum temperatures, as these influence spore release. The wetness periods are timed as they influence spore germination and infection. Continuous and intermittent rain can induce infection, and dry periods are considered those in which a shaken tree releases no water droplets. The apple scab warning, if given, reflects further interpretation of the conducive events as they relate to the continuity or disruption of wetness periods, spore type (ascospore or conidia) and the occurrence of rain during daylight or darkness. (Thwaite et al, 2002)

The distinction between primary and secondary infection is important for this disease since both conidia and ascospores are formed and ascospores need light as well as moisture to be released (MacHardy and Gadoury, 1989). The two spore types may exist at the same time during the season. Even with rain periods over night, ascospores are not released until around 7 am so the counting of hours of wetness can begin at sunrise. If the rain starts during daylight, counting should also start. However when scab lesions are visible and conidia have formed (generally 9-14 days after primary infection), secondary infection occurs in response to rain, regardless of daylight.

Infection by ascospores of *V. inaequalis* occurs most rapidly at temperatures between 17° and 24°C. Infection outside this range occurs but requires longer wetness periods, and the warning

services in various regions have adapted the data originally presented by Mills and Laplante (1951) and adapted by Stensvand et al. (1997), to suit different regions.

A service based in Orange NSW monitors in the area, the relevant temperatures (from 1°C-26°C) and the corresponding minimum wetness hours needed for an apple scab infection period. An extract from their table is shown below in Table 11. The *average* temperatures during a wet period are used, and therefore max/min thermometers are needed by growers interpreting their own orchard's data. Infection does not occur after 8 dry hours, unless high humidity or cloud cover slow the drying times, in which case it can be 10-11 hours before the threat of infection is removed.

Table 11 : Temperature and time for leaf infection by *V. inaequalis*

Temperature (°C)	Minimum wetness period for infection (hours)		Days until scab lesions visible
	Primary infection (ascospores)	Secondary infection (conidia)	
1	41	37	--
7	15	18	--
9	12	13	17
11	9	10	15
14	7	9	13
17	6	9	9
23	6	8	9
26	11	14	10

(Source: adapted from Thwaite et al, 2002)

8.1.2 Downy mildew of grapes

CropWatch provides grapegrowers in South Australia with timely information on the potential risk of downy mildew, powdery mildew, black spot and light brown apple moth (LBAM). It is a service with 13 automatic weather stations at strategic locations in vineyards around SA's Riverland, hills and southern vales areas.

Downy mildew is caused by the fungus *Plasmopara viticola*. Management of this disease requires well-timed fungicide applications to protect vines and maintain disease control. Grapegrowers have become reliant on the South Australian-developed downy mildew predictive model.

The "10:10:24 rule" is used to predict a primary infection event. The conditions are 10mm of rain at a temperature above 10°C, for at least 24 hours. These conditions are conducive to infection of leaves and the production of sporangia by *P. viticola.*

Once primary infection has occurred the threat of secondary infections increases. The conditions that promote these are '98:13:4', i.e. humidity above 98%, temperatures above 13°C and at least 4 hours of darkness.

8.2 The potential for pistachio disease prediction models

Although there has be no validation of international data published on panicle and shoot blight, or anthracnose of pistachios, under Australian conditions, we have some useful data and techniques for predicting these diseases in our orchards. The most useful at present is BUDMON monitoring which can detect the presence of *C. acutatum* and *Neofusicoccum* sp. in dormant pistachio buds.

8.3 The basis of pest forecasts

To effectively prepare for pest invasions, growers generally must know the history of the pest in their orchard, its life cycle and the influence primarily of temperature on its life cycle.

8.3.1 Codling moth on apples

Predictions of codling moth emergence dates, for example are useful to apple growers who wish to commence insecticide applications at the most effective time, each season. The warning service relies on sex pheromone traps to catch male moths, and electronic sensors to record air temperatures at pre-set intervals from midnight (Thwaite et al, 2002). A software program is used to calculate on a daily basis, the degrees of temperatures above 10°C and below 31°C for the 24 hour period ("degree days"). Below 10°C and above 31°C, the development of codling moth ceases or declines rapidly.

The degree days are accumulated from the 'biofix' (date at which regular emergence of the moths occurs as determined from trapping). Since the accumulated degree days required for egg laying and egg hatch are known, the best timing of the first insecticide spray for the caterpillar stage which damages apples, may be predicted. Different insecticides are effective on different life stages (eg. eggs, newly hatched larvae) and therefore the predictive model assists also with the choice of insecticide.

SCHOLEFIELD ROBINSON
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APPENDIX 1

Glossary and Visual Description of Phytopathological Terms

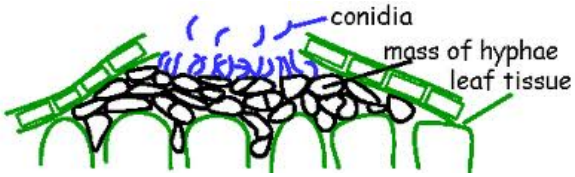
Pistachios

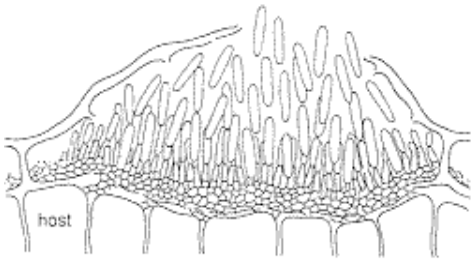
- Flowers

Male (left) and female (right) inflorescences of pistachio (photos courtesy of Dr. Louise Ferguson, University of California, Davis)

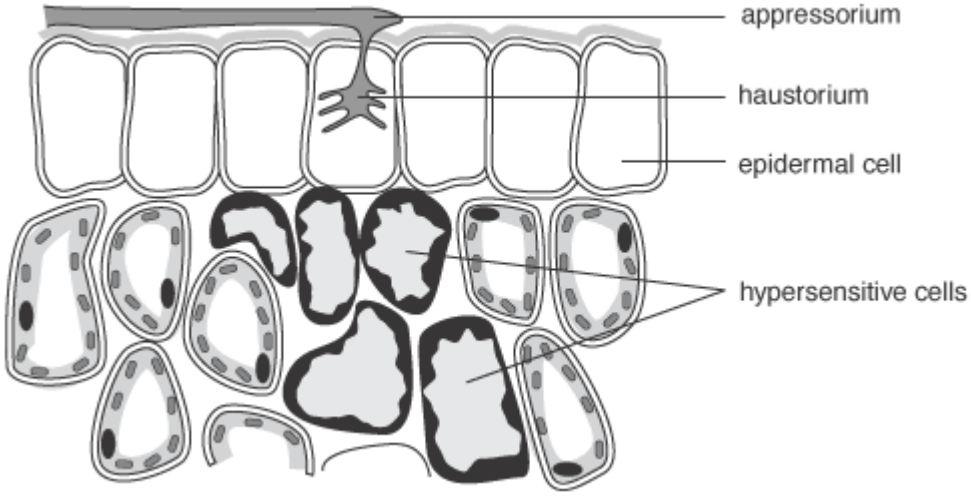
Fungal terms

- *Acervulus* (pl. acervuli) - (saucer-shaped fruiting body of some imperfect fungi, that forms spores that break through epidermis, eg. *Colletotrichum* spp.)



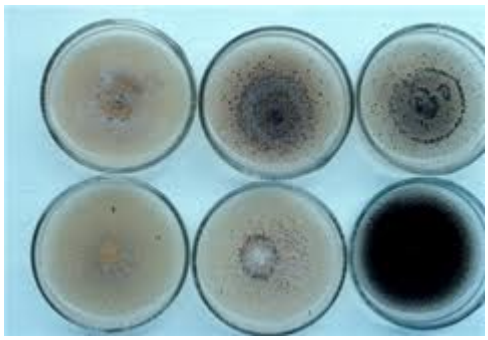


Appressorium (pl. appressoria) – enlarged fungal part that adheres to host surface before penetration



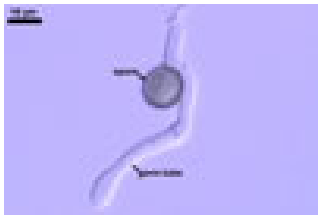
(Image source: http://bugs.bio.usyd.edu.au/learning/resources/PlantPathology/glossary.html)

- *Canker* – necrotic, diseased area that is shallow or extends into soft or woody tissue
- *Culture/isolate* – fungi isolated from host and growing independently from the host, usually on synthetic media



Cultures of *Colletotrichum* spp.

- *Endophyte* – a microorganism, especially a fungus, that lives inside a plant, in a parasitic or mutualistic relationship
- *Fruiting body* – Fungal structure in which either asexual or sexual spores are produced. See pycnidium, perithecium, acervulus
- *Germ tube* – hyphal strand that emerges on 'germination' of a spore



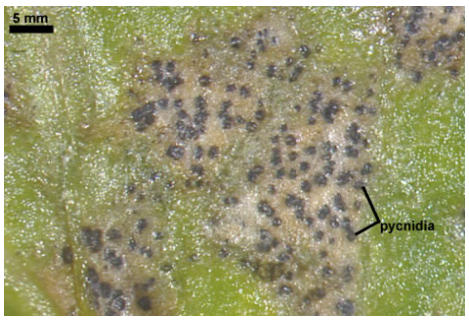
- *Haustorium* – specialised hypha that extracts nutrients from inside host cell (see diagram: appressorium)

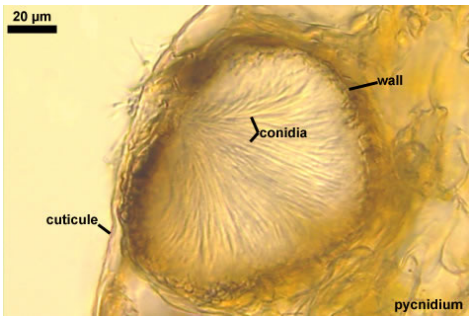
- *Inoculum* – the pathogen parts that can cause infection and create disease. It takes different forms (eg. spores, sclerotia, hyphae), survives in different host parts, and is distributed in different forms by a variety of means
- *Latent infection* – infection of the host, but without associated symptoms
- *Lesion* – a localised and defined diseased area, often sunken (spot, canker)

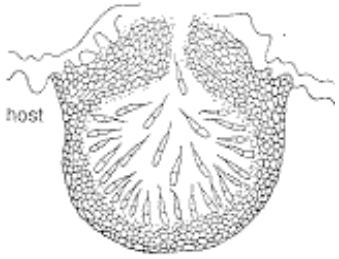
Anthracnose of mango

Anthracnose of beans

- *Mycelium* – the mass of thread-like fungal strands (hyphae)
- *Necrosis* – death of host cells
- *Pathogenesis* – sequence of processes in the host – pathogen relationship that results in disease
- *Primary infection/inoculum* – is responsible for creating early threat and the initial infections in the host, in a given season (usually spring). The source is usually inoculum that has survived in association with the host, or nearby (eg. in seed, on mummies, in residue)
- *Propagules* – fungal bodies capable of independent growth and contributions to pathogenesis (spores, mycelium, fruiting bodies, sclerotia etc.)
- *Pycnidium* (pl. pycnidia) – flash-shaped asexual fruiting body that produces conidia, eg. *Neofusicoccum* spp.







- *Secondary inoculum/infection* – infectious propagules produced and causing infection in the same growing season; often responsible for spread and increase in disease during the season (and for inoculum loads threatening the next season's crop).

- *Setae* – fungal spikes within acervulus.

- *Spores (conidia)* – specialised reproductive form of fungus; conidia are asexual spores.

APPENDIX 2

Hosts from which *Botryosphaeria dothidea* has been isolated in California

Common name	Scientific name	Family
Almond	*Prunus dulcis*	Rosaceae
Apple	*Malus domestica*	Rosaceae
Avocado*	*Persea americana*	Lauraceae
Blackberry*	*Rubus ursinus*	Rosaceae
Black walnut	*Junglans hinsii*	Juglandaceae
Carob seed tree	*Ceratonia siliqua*	Fabaceae
Incense cedar	*Cedrus libani*	Pinaceae
Deodar cedar	*Cedrus deodara*	Pinaceae
Chinese hackberry	*Celtis sinensis*	Ulmaceae
California redwood*	*Sequoia sempervirens*	Taxodiaceae
Cotoneaster	*Cotoneaster frigidus*	Rosaceae
Cottonwood	*Populus deltoides*	Populaceae
English walnut	*Junglans regia*	Juglandaceae
Eucalyptus	*Eucalyptus coccifera*	Myrtaceae
Euonymus	*Euonymus fortunei*	Celestraceae
Silver dollar eucalyptus	*Eucalyptus orbifolia*	Myrtaceae
Feijoa	*Feijoa sellowiana*	Myrtaceae
Fig	*Ficus carica*	Fagaceae
Giant sequoia*	*Sequoiadendron giganteum*	Taxodiaceae
Juniper	*Juniperus occidentalis*	Cypressaceae
Jasmine	*Jasminum officinale*	Jasminaceae
Lemon	*Citrus* × *limon*	Citraceae
Sweet gum	*Liquidambar styraciflua*	Mamamelidaceae
Maple	*Acer* sp.	Aceraceae
Oak	*Quercus* sp.	Fagaceae
Olive*	*Olea europea*	Olivaceae
Orange	*Citrus* × *auranteum*	Citraceae
Pistachio	*Pistacia vera*	Anacardiaceae
Pear	*Pyrus communis*	Rosaceae
Pecan	*Carya illinoensis*	Junglandaceae
Persimmon	*Diospyros kaki*	Ebenaceae
Pine	*Pinus radiate*	Pinaceae
Prune	*Prunus domestica*	Rosaceae
Firethorn*	*Pyracantha coccinea*	Rosaceae
Raymond ash	*Fraxinus augustifolia augustifolia* subsp. *oxycarpa*	Oleaceae
Sycamore maple	*Acer pseudoplatanus*	Aceraceae
Wax leaf privet	*Ligustrum japonicum*	Oleaceae
Western redbud	*Cercis occidentalis*	Fabaceae
Wild rose	*Rosa* sp.	Rosaceae
White willow	*Salix alba*	Salicaceae
Arroyo willow	*Salix lasiolepis*	Salicaceae
Weeping willow	*Salix babylonica*	Salicaceae

(Source: Michailides and Morgan, 2004)

* Hosts on which the sexual stage of the pathogen has been found.

Pistachio disease investigations.

Third interim report September 2011

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Contents

1. TEMPERATURE / GROWTH EVALUATIONS.

1.1. Aim

To determine the optimal temperature for *in vitro* growth of isolates of *Colletotrichum acutatum* and *Botryosphaeria* sp. from Australian pistachios.

1.2. Isolates

C. acutatum isolate 34/11 was recovered from infected pistachio fruit (hulls) from Kyalite, NSW. *C. acutatum* isolate 70/11 was recovered from infected leaves in the same orchard.

Two fungi initially identified[1] as *Botryosphaeria* spp. were recovered from diseased pistachios growing in the Pinnaroo region of South Australia (isolate 116/11) and in Telopea Downs, Victoria (isolate 46/11).

For the purpose of comparison, isolates of two *Botryosphaeria* spp. from grapevines were obtained from the Australian Fungal Collection (NSW DPI, Orange): *B. parva*[2] (DAR79000) NSW 2007 and *B. dothidea*[3] (DAR78224) NSW 2006.

1.3. Methods

Four 9cm diameter petri plates of potato dextrose agar (PDA) were inoculated, for each test isolate. A 6 mm agar plug removed from the margin of a 7-14 day-old culture was placed in the centre of each plate.

Plates were incubated at temperatures ranging from 5-35^{o}C or 10-35^{o}C, for 3 or 6 days. The specific incubation time for each isolate was determined by the colony growth at optimal temperatures. Final measurements were taken before the fastest growing colony reached the plate edge.

Mycelial growth was recorded on two dissects at 90^{o} to each other, and did not include the width of the inoculation plug (6 mm).

Experiment 1: Pistachio pathogens: *C. acutatum* isolates 70/11 and 34/11, and *Botryosphaeria* isolates 116/11 and 46/11 from pistachios were incubated at six temperatures (10, 15, 20, 25, 30 and 35^{o}C) for 6 days before radial mycelial growth was measured.

Experiment 2: Pistachio pathogens and related fungi. *C. acutatum* isolates 70/11 and 34/11, *Botryosphaeria* isolates 116/11 and 46/11, *B. parva* DAR 79000 and *B. dothidea* DAR78224 were incubated at seven temperatures (5, 10, 15, 20, 25, 30 and 35^{o}C) for 3 days before radial mycelial growth was measured. The 25^{o}C incubator failed at the start of the test and was registering temperatures over 35C for the first three days. Radial growth was measured after 9-days incubation.

Experiment 3: Experiment 2 was repeated at temperatures 20, 25, 30 and 35^{o}C.

[1] The isolates have not been identified to species and their purity is now questioned. It is possible the cultures have been contaminated with *Alternaria*. This is still to be confirmed. Isolate 46/11 was confirmed as *Botryosphaeria* sp. by T. Michailides (USA).

[2] Original isolates of *Botryosphaeria* sp. from pistachios (Kyalite, NSW) were confirmed by sequencing to be *Neofusicoccum parvum*, formerly recognised as *Botryosphaeria parva*.

[3] *Botryosphaeria dothidea* is the perfect stage of the recognised cause of 'panicle and shoot blight' of pistachios in California.

1.4. Results

In Experiment 2, the measurements were taken on day 3. By day 9 many of the cultures in the optimum temperature range (20–30°C) had grown to the edge of the plates. The radial growth measurements from 25°C were not included due to the incubator failure.

1.4.1. Colletotrichum acutatum

Both *C. acutatum* isolates from pistachios (70/11 and 34/11), had maximum radial growth at 25°C after 3 and 6 days incubation (Figures 1, 2). At incubation temperatures above 20°C, isolate 34/11 from pistachio hulls grew more slowly than isolate 70/11 from pistachio leaves, after 3 and 6 days of incubation (Figures 3, 4).

In Experiment 1, the growth of isolate 34/11 resulted in two peaks (at 15°C and 25-30°C). This growth pattern was not repeated in Experiment 2. There was no growth of isolate 70/11 at 5°C, but isolate 34/11 grew slowly at that temperature (Figures 1, 2). Neither grew at 35°C in Experiments 2 and 3. Raw data from Experiments 1-4 are included in Appendices 1-3.

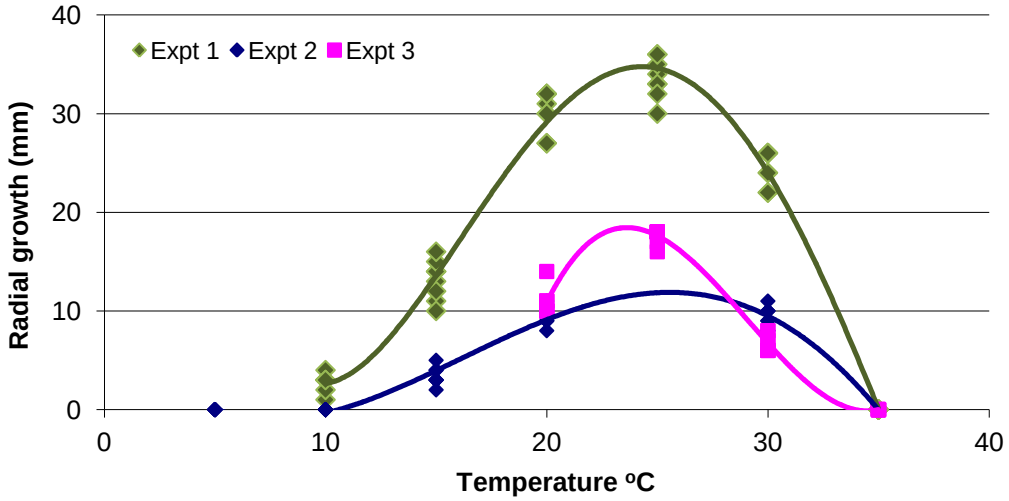

Figure 1. Radial growth of *Colletotrichum acutatum* isolate 70/11 after 6 days (Expt. 1) or 3 days (Expt. 2 and 3). Polynomial regression order 4: Expt. 1 R^2 = 0.9834, Expt. 2 R^2 = 0.9717 and Expt. 3 R^2 = 0.9833

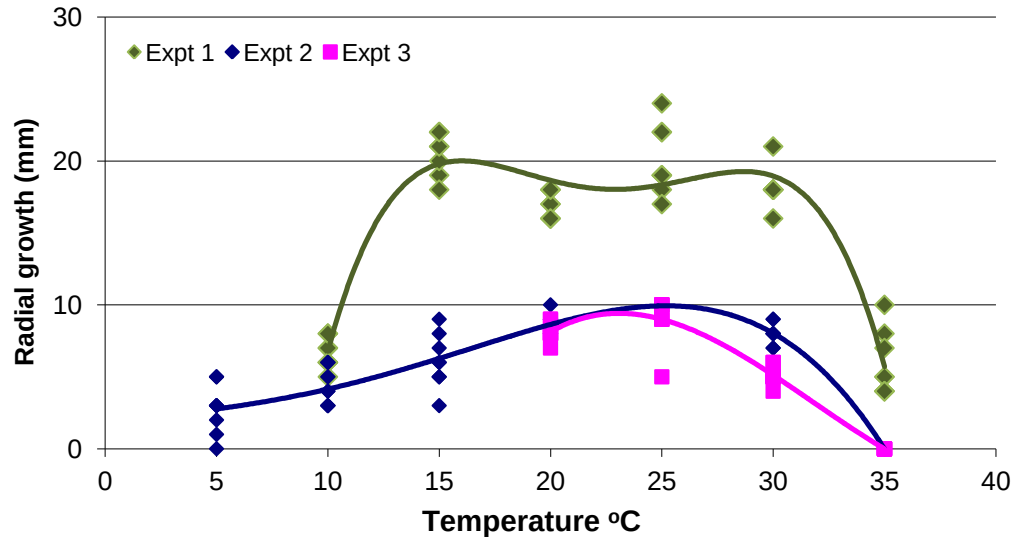

Figure 2. Radial growth of *Colletotrichum acutatum* isolate 34/11 after 6 days (Expt. 1) or 3 days (Expt. 2 and 3). Polynomial regression order 4: Expt. 1 R^2 = 0.9092, Expt. 2 R^2 = 0.8698 and Expt. 3 R^2 = 0.939

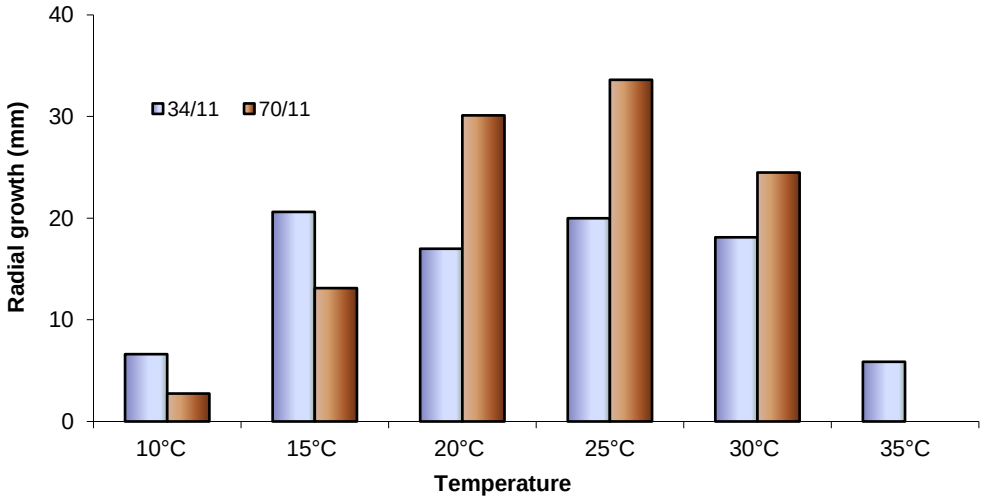

Figure 3. Mean radial mycelial growth of two pistachio isolates of *Colletotrichum acutatum* (70/11 and 34/11) after incubation for 6 days (Expt. 1).

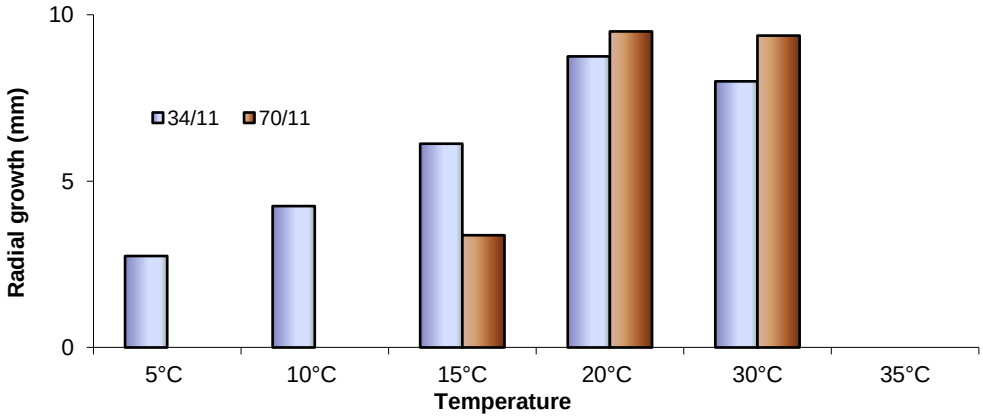

Figure 4. Mean, radial, mycelial growth of two pistachio isolates of *Colletotrichum acutatum* (70/11 and 34/11) after 3-day incubation at 5-35^{o}C (Expt. 2).

1.4.2. *Botryosphaeria/Alternaria* isolates from pistachio

In Experiment 1, the radial growth of pistachio isolates of *Botryosphaeria* sp. peaked after incubation at 30°C for 6 days (Figures 5-7). The growth achieved at each temperature between 20 and 35°C was similar. Growth at 35°C was more extensive than at 15°C.

In Experiments 2 and 3, when incubation was for three days, the optimal growth temperature was nearer 25°C and the optimal growth range was narrower, being 25-30°C (Figures 6, 7).

Each isolate grew at temperatures from 5-35°C. The growth thresholds below 5°C and above 35°C remain undefined.

While the optimal range for mycelial growth of the pistachio *Botryosphaeria* sp. was similar to that of the pistachio *C. acutatum* isolates, the *Botryosphaeria* isolates grew more rapidly at all temperatures (Figure 8).

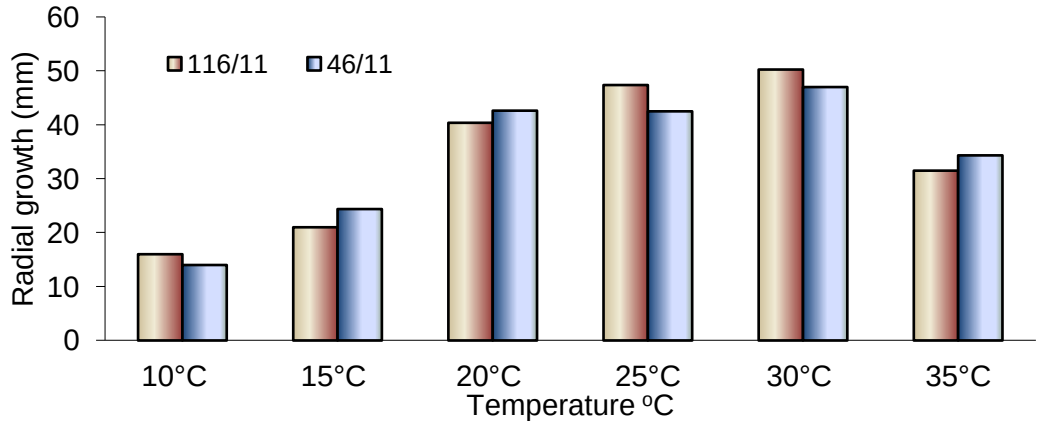

Figure 5. Mean radial growth of two pistachio isolates of *Botryosphaeria/Alternaria* after 6-day incubation.

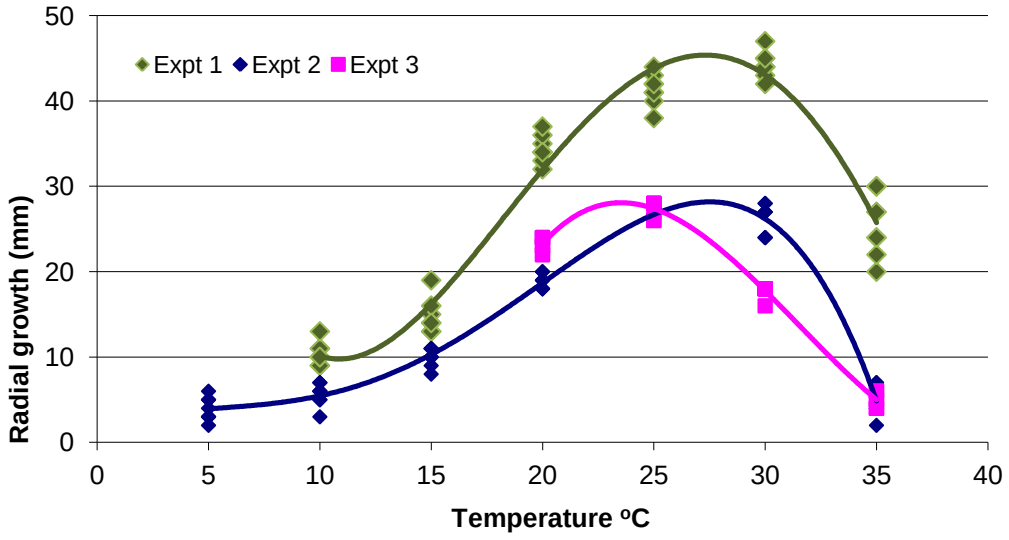

Figure 6. Radial growth of pistachio isolate 116/11 after 6-day incubation (Expt. 1) or 3-day incubation (Expts. 2 and 3). Polynomial regression order 4: Expt.1 R^2 = 0.9612, Expt.2 R^2 = 0.9752 and Expt.3 R^2 = 0.9909

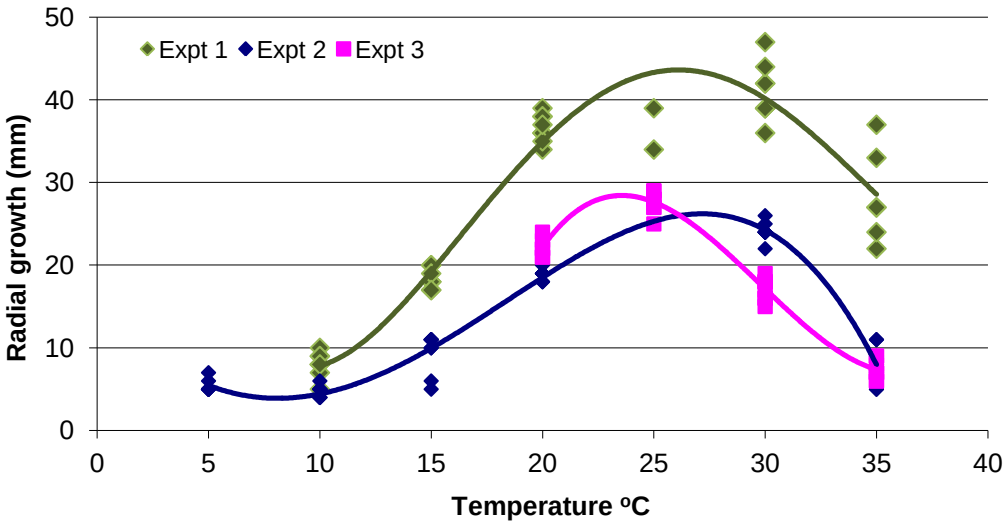

Figure 7. Radial growth of pistachio isolate 46/11 after 6-day incubation (Expt. 1) or 3-day incubation (Expts. 2, 3). Polynomial regression order 4: Expt. 1 R^2 = 0.9316, Expt. 2 R^2 = 0.9602 and Expt. 3 R^2 = 0.9769

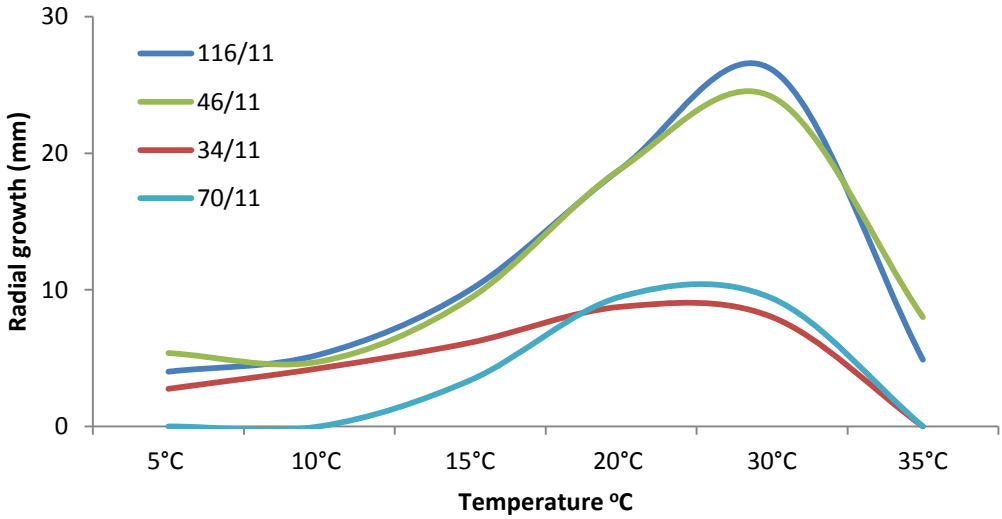

Figure 8. Mean radial growth of pistachio pathogens: *Botryosphaeria* sp. isolates 116/11 and 46/11 and *C. acutatum* isolates 34/11 and 70/11 incubated at temperatures 5-35ºC for 3 days.

1.4.3. *Botryosphaeria* spp. from grapevines

The mycelial growth patterns of two *Botryosphaeria* spp. from grapevines were compared. *B. parva* and *B dothidea* from Australian grapevines grew similarly at all temperatures, with their optimum range being 25-30°C (Figure 9). *B. parva* had a growth peak at 30°C in Experiment 2 and 25°C in Experiment 3 (Figure 10). The growth of the grapevine isolates in the range of 20-30°C was more than twice that of the *Botryosphaeria* sp. (isolate 46/11) recovered from pistachios (Figure 9).

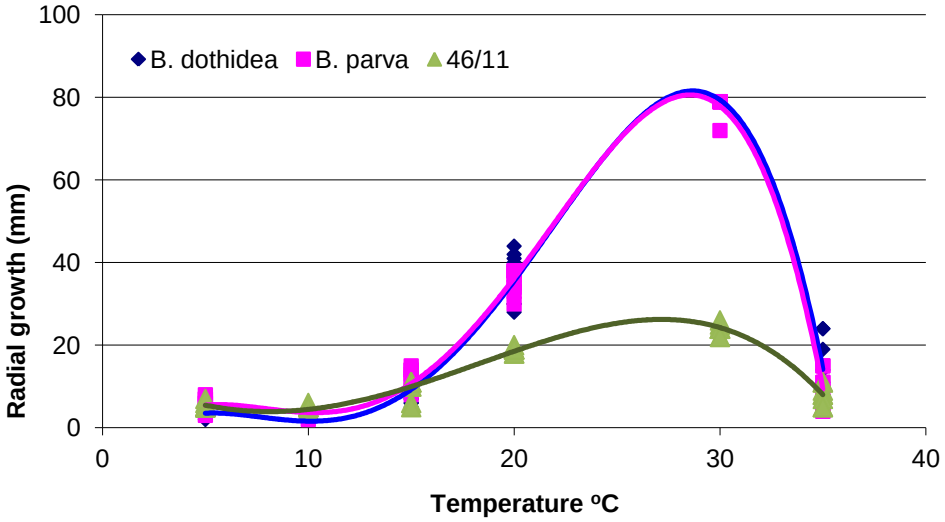

Figure 9. Mean radial growth of pistachio isolate 46/11, and *B. dothidea* and *B. parva* from grapevines, after 3-day incubation at 5-35°C.

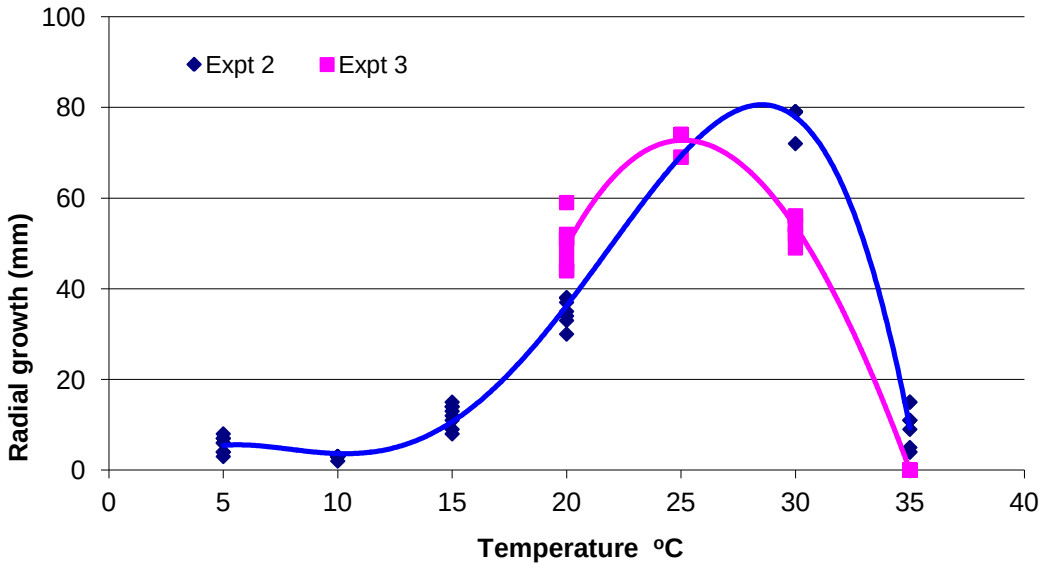

Figure 10. Radial growth of *B. parva* from grapevine after 3-day incubation (Expt. 2, 3). Polynomial regression order 4: Expt. 2 R^2 = 0.99 and Expt. 3 R^2 = 0.9891

1.4.4. Re-growth of fungi after high temperature exposure

In Experiment 2 the 25°C incubator overheated and isolates were exposed to temperatures over 35°C for three days. During incubation at these high temperatures, *B. parva* and *B. dothidea* from grapevines grew slowly. No growth of *C. acutatum* or *Botryosphaeria* sp. from pistachios was observed over the same period. When the cultures were returned to 25°C for 6 days, all isolates resumed growth (Table 1). The growth after 9 days (total) is shown in Table 1.

Table 1. Mean radial growth of fungi over 9 days at various temperatures

Isolates*	Temperature °C[x]			
	20	**>35/25**	**30**	**35**
Pistachio isolates				
Botryosphaeria sp. - 116/11	61.8	67.8	72.5	16.3
Botryosphaeria sp. - 46/11	59.0	72.3	72.5	22.5
C. acutatum - 34/11	27.0	26.5	25.0	0.0
C. acutatum - 70/11	45.4	23.9	28.6	0.0
Grapevine isolates				
B. dothidea	79.0	79.0	79.0	44.9
B. parva	79.0	79.0	79.0	18.4

* 34/11 and 70/11 – *C. acutatum* from pistachios; 116/11 and 46/11 – *Botryosphaeria* spp. from pistachios; *B. dothidea* and *B. parva* – from grapevines.

[x] 9-day incubation at 20, 30 and 35°C - or 3-day incubation at >35°C, followed by 6-days at 25°C.

1.5. Conclusion

The optimal growth range of the *Colletotrichum* and *Botryosphaeria* isolates from pistachios was between 20 and 30°C. The *Botryosphaeria* sp. from pistachios grew faster, especially at temperatures above 10°C, than *Colletotrichum* sp. The *Colletotrichum* isolates did not grow at 35°C, but the *Botryosphaeria* isolates were still active at this temperature. Exposure to >35°C for three days inhibited the growth of the pistachio pathogens, but did not kill them. They resumed growth at 25°C. It appears that summer heat may temporarily halt or slow the growth of the pistachio pathogens, but the arrival of autumn conditions would allow their re-activation. The two *Botryosphaeria* sp. isolates and *C. acutatum* 34/11 from pistachios grew slowly at 5°C. Isolate 70/11 however did not grow at 5°C. This suggests cold spring weather, even if accompanied by wet conditions, may not result in significant disease.

The *Botryosphaeria* spp. from grapevines grew slowly at >35°C, and resumed faster growth when returned to 25°C. The *Botryosphaeria* isolates from grapevines grew significantly faster than those from pistachio at the optimal temperatures. They also grew more rapidly at the 'extremes' of the range. The growth differences amongst the isolates are somewhat unexpected, and they raise further questions about the species of the *Botryosphaeria* pathogens on pistachios.

It is unclear if the environmental conditions affect spore germination, mycelial growth and the infection processes of *Colletotrichum* and *Botryosphaeria* spp., and progress within plant tissue once infection has occurred. Fungal growth and symptom development (and therefore, latency) in infected tissue, are influenced by the nature and maturity of the host tissue also.

2. FUNGICIDE EVALUATIONS

2.1. Aim

To screen *in vitro*, the efficacy of various fungicides for the control of *Colletotrichum acutatum* and *Botryosphaeria* sp.

2.2. Isolates

C. acutatum isolate 214/10 was recovered from infected pistachio fruit (hulls) from Kyalite, NSW. *C. acutatum* isolate 70/11 was recovered from infected leaves in the same orchard in the following season.

As the two isolates from pistachio have not been confirmed as *Botryosphaeria*, the two *Botryosphaeria* spp. isolates from grapevines were tested: *B. parva*[4] (DAR79000) NSW 2007 and *B. dothidea*[5] (DAR78224) NSW 2006.

2.3. Methods

This method was adapted from that described in Adaskaveg, J. E., and Hartin, R. J. 1997. Phytopathology 87:979-987.

The fungicides tested were those that have been reported in published literature as effective on *Botryosphaeria*, *Colletotrichum acutatum*, or more generally on 'anthracnose' of fruit (Table 2). These fungicides were also evaluated for efficacy on *Botryosphaeria* sp.. The fungicides represent a number of different chemical groups, are registered for use in the US and/or Australia, and are either effective individually or as components of efficacious pre-mixes[6].

PDA plates were inoculated with a conidial suspension of a *C. acutatum* isolate (214/10 or 34/11), *B. parva* or *B. dothidea*. The suspension was sufficient to form a thin film across the entire agar surface. 'Growth' of the fungi was therefore the result of spore germination *and* mycelial growth, whereas 'inhibition' reflected either inhibition of spore germination or inhibited mycelial growth.

Each test fungicide was screened at the label rate (high=H), half-label rate (medium=M), or one-tenth label rate (low=L) rate of the formulated product (Table 2). Filter paper disks (12mm in diameter) were saturated with 100µl of distilled water (control) or the test fungicide, air-dried and placed on the surface of inoculated PDA plates.

The plates were incubated for three days at 25°C. The efficacy of the fungicide was determined by the dimensions of the inhibition zone (maximum 1cm) –i.e. zone in which *C. acutatum* did not grow. Inhibition zones were rated from 0 to 3, where 0= no inhibition, 1= < 50%, 2= >50% and 3= 100% inhibition.

[4] Original isolates of *Botryosphaeria* sp. from pistachios (Kyalite, NSW) were confirmed by sequencing to be *Neofusicoccum parvum*, formerly recognised as *Botryosphaeria parva*.

[5] *Botryosphaeria dothidea* is the perfect stage of the recognised cause of 'panicle and shoot blight' of pistachios in California.

[6] Azoxystrobin has permitted use on pistachios in Australia. Chlorothalonil is registered for use on almonds. Pyraclostrobin has a full MRL in Australia for tree nuts (0.01 mg/kg), and international MRLs in pistachio (US – 0.7 mg/kg), Codex and EU – (1.0 mg/kg). Pristine and Switch are registered in the US and Australia on a range of crops, but not for anthracnose. Cyprodinil and fludioxonil have no Aust MRLs in pistachio or tree nuts, but international MRLs exist for pistachios.

2.4. Results

Around several control (water only) disks, there was some fungal inhibition. This occurred in plates of active constituents that are volatile. It is also possible some active constituents diffused further into the agar from infused disks than others.

Colletotrichum acutatum

Trifloxystrobin, carbendazim, prochloraz, fludioxonil and chlorothalonil inhibited the spore germination and mycelial growth of each pistachio isolate of *C. acutatum* (Table 2, Figure 11, Appendix 4) at each of the fungicide rates.

Pyraclostrobin, mancozeb and flutriafol inhibited the fungi at the label rate and half-label rates only.

Azoxystrobin results were inconclusive, with isolate 34/11 being sensitive to the active constituent at each rate, but isolate 70/11 having only partial inhibition at each rate. This highlights the different sensitivity of isolates of this pathogen, in terms of spore germination or mycelial growth.

Boscalid, fluopyram, myclobutanil, fenarimol, pyrimethanil and fenhexamid were either not effective on either of the *C. acutatum* isolates, or had limited effectiveness that was variable between the two isolates.

Botryosphaeria

Mancozeb, prochloraz, fludioxonil and chlorothalonil inhibited the spore germination and mycelial growth of each grapevine isolate of *Botryosphaeria* (Table 2, Appendix 4) at each of the fungicide rates.

Cyprododonil and captan inhibited the fungi at the label rate and half-label rates only.

Carbendazim was variable, inhibiting *B. parva* but not *B. dothidea*.

None of the QoI fungicides (azoxystrobin, pyraclostrobin or trifloxystrobin) inhibited growth completely, although azoxystrobin and pyraclostrobin did provide partial control.

Boscalid, fluopyram, myclobutanil, fenarimol, tebuconazole, pyrimethanil and were either not effective on either of the *Botryosphaeria* isolates, or had limited effectiveness that was variable between the two isolates.

Figure 11. (L) Fenhexamid infused disks with no inhibition of *C. acutatum*. (C) Azoxystrobin infused discs with partial inhibition. (R) Fludioxonil infused discs with complete inhibition of *C. acutatum*.

Table 2. Fungicides, product rates and efficacy.

Fungicide product name	Active constituent	Label rate (per 100L) (High)	*C. acutatum*			*Botryosphaeria* (grape)		
			Inhibition* at label rate (H), ½ rate (M) or 1/10 rate (L)					
			H	M	L	H	M	L
Water	-	-	-	-	-	-	-	-
Miscellaneous								
Captan	800g/Kg captan	125g				+	+/-	-
Dithane rainshield	750 g/Kg mancozeb	200g	+	+	-	+	+	+
Filan	500 g/Kg boscalid	120g	-	-	-	-	-	-
Howzat	500 g/L carbendazim	100ml	+	+	+	+/-	+/-	+/-
Luna	400 g/L fluopyram	100ml	-	-	-			
Scholar	230 g/L fludioxonil	260ml	+	+	+	+	+	+
Teldor	500 g/L fenhexamid	100ml	-	-	-	-	-	-
Unite ultrastick	720 g/L chlorothalonil	210ml	+	+	+	+	+	+/-
DMIs								
Impact endure	500 g/L flutriafol	250ml	+	+	-	+/-	-	-
Folicur	430 g/L tebuconazole	30ml	+	-	-	-	-	-
Mycloss extra	200 g/L myclobutanil	16ml	-	-	-	-	-	-
Octave	462 g/Kg prochloraz	300g	+	+	+	+	+	+
Rubigan	120 g/L fenarimol	30ml	-	-	-	-	-	-

* Inhibition of both isolates - +, inhibition of only one isolate +/-, No inhibition = -. Blank = not yet tested.

Fungicide product name	Active constituent	Label rate (per 100L) (High)	*C. acutatum*			*Botryosphaeria* (grape)		
			Inhibition* at label rate (H), ½ rate (M) or 1/10 rate (L)					
			H	M	L	H	M	L
QoIs								
Amistar	500 g/Kg azoxystrobin	40g	+/-	+/-	+/-	-	-	-
Cabrio	200 g/Kg pyraclostrobin	50g	+	+	+/-	-	-	-
Flint	500 g/Kg trifloxystrobin	15g	+	+	+	-	-	-
Anilinopyrimidines								
Chorus	500 g/Kg cyprodinil	40g	+	+	+	+	+	-
Scala	400 g/L pyrimethanil	200ml	-	-	-	-	-	-

* Inhibition of both isolates - +, inhibition of only one isolate +/-, No inhibition = -

2.5. Conclusion

Several fungicides inhibited spore germination (and subsequent mycelial growth) of *Colletotrichum acutatum* from pistachios and *Botryosphaeria in vitro*. The active constituents are therefore likely to be effective crop protection products in orchards. The QoI group were generally only effective on *Colletotrichum*; however results for azoxystrobin were variable. This product has been used on pistachios previously, and the potential for resistance exists. Apart from prochloraz (Octave), the DMIs were ineffective. Prochloraz is in the imidazole subgroup whereas the others tested are in the triazole or pyrimidine subgroups.

The fungicides mancozeb, carbendazim, fludioxonil, cyprodinil and chlorothalonil were consistently effective on both fungi and should be further field tested.

Some of the fungicides used overseas for control of *Botryosphaeria* (eg. Pristine, a mix of pyraclostrobin + boscalid, Table 3) were less effective in this test. However there may be benefit in including them in any field evaluations, as the efficacy *in planta* may be improved.

Table 3: Fungicide efficacy summary for four pistachio diseases

Active constituent	Efficacy against *Botryosphaeria* spp.	Efficacy against *Alternaria* sp.	Efficacy against *Botrytis* sp.[x]	Efficacy *in vitro* against *C. acutatum*[w]	Efficacy *in vitro* against grape *Botryosphaeria* spp[w].
azoxystrobin	+++	+++*	---	Variable	Variable
chlorothalonil	++	++	---	✓	✓
pyraclostrobin	+++	+++*	---	✓	partial
trifloxystrobin	+++	+++*	---	✓	×
pyraclostrobin + boscalid	++++	++++*	++++	✓, ×	partial
pyrimethanil	+++[y]	++	++	×	×
cyprodinil + fludioxonil	++	+++	+++	✓, ✓	✓, ✓
fluopyram +trifloxystrobin	++++	++++	+++	×, ✓	--, ×
fenhexamid	ND	ND	++++	×	×
propiconazole + azoxystrobin	+++	+++	---	ND; variable	ND; variable
metconazole	+++	++	+++	ND	ND
thiophanate-methyl	++	---	++	ND	ND

(Source: adapted from Adaskaveg et al, 2011: Michailides, 2008; Hall et al, 2011)

[x] Results for *Botrytis*, *Botryosphaeria* and *Alternaria* relate to Californian conditions and testing

[w] Australian pistachio isolates and *in vitro* testing (Hall et al, 2011) of actives independently, at label rates and lower

[y] – under low and moderate disease pressure. ✖ - not effective, ✓= effective

--- = no effect; + least effective ++++ - most effective; ND = no data

* Resistance reported

3. FUNGICIDE EVALUATIONS ON PLANTS

3.1. Aim.

Evaluate the capability of fungicides to control pistachio leaf or nut infection by *Colletotrichum acutatum*.

3.2. Isolates

C. acutatum isolate 214/10 was recovered from infected pistachio fruit (hulls) from Kyalite, NSW.

As the two isolates from pistachio have not been confirmed as *Botryosphaeria, B. parva*[7] (DAR79000) NSW 2007 was used in the initial test.

3.3. Plant material

As leaf material from commercial pistachio trees was not available, leaves were sourced from *Pistacia atlantica* (Mt Atlas Mastic Tree) from the Mediterranean, Canary Islands, located in the Waite Arboretum. Older leaves from last season were still present on the tree.

Visually unaffected nuts were collected in March 2011 by Scholefield Robinson from (NPA 286) and had been stored frozen.

3.4. Fungicides evaluated

Five fungicides (Switch, Pristine, Bravo, Octave and Flint) were selected for the initial testing. These were either used overseas for management of *Botryosphaeria* panicle blight, or had shown efficacy against the two fungi in *in vitro* tests.

Switch was unavailable at the time of this test, and the remaining four fungicides were evaluated at label rates (Table 4).

Table 4. Fungicides evaluated and rate applied

Product name	Active Ingredients (ai)	Rate (per 100L)
Pristine®	252g/kg boscalid 128g/kg pyraclostrobin	40g
Bravo®	720g/L chlorothalonil	160ml
Octave®	462 g/kg prochloraz present as manganese chloride complex	300g
Flint® 500SC	500g/L trifloxystrobin	15g

[7] Original isolates of *Botryosphaeria* sp. from pistachios (Kyalite, NSW) were confirmed by sequencing to be *Neofusicoccum parvum*, formerly recognised as *Botryosphaeria parva*.

3.5. Method

Detached leaves were placed into humidity trays (plastic trays lined with wet "chux" and paper towel inside a plastic bag), two replicate leaves per tray (Fig 12). Frozen nuts were defrosted overnight and placed into small welled trays, eight nuts per treatment (Fig 12).

The leaves and nuts were sprayed with the fungicide solutions until run-off using a small atomiser and allowed to dry in the laminar flow. Sterile water was used as a control treatment.

Each leaf was wounded with a sterile needle on five sites and each nut once. A 6mm diameter plug of mycelium taken from an actively growing culture was placed upside down on the wound (Fig. 12). PDA with no fungi was used as a control. After 24 hours the agar was removed.

Figure 12. Leaves of *Pistacia atlantica* (Mt Atlas Mastic Tree) marked and wounded for inoculation (L) and in humidity trays (Centre). Defrosted nuts in trays with inoculated plug on wounded site.

The extent of lesion development around the wound site was assessed at five and 12 days after inoculation and rated as 0= no lesion development, 1 = minor necrosis around wound site, 2 = some lesion development (~50% of control) and 3 = lesion development equivalent to the untreated control.

3.6. Results and Discussion

By five days, lesions had developed on the leaves treated with water and inoculated (Fig 13). By twelve days, the leaves treated with water and inoculated with *Colletotrichum* were producing orange sporodochia (Fig 13). Lesions were more difficult to assess on the nuts, as many blackened with defrosting (Fig 14).

Octave (prochloraz) prevented any lesion development from *Colletotrichum* on both nuts and leaves at 5 days, but there was some lesion growth on leaves by 12 days after inoculation (Table 5). Flint and Pristine reduced the lesion development of both *Colletotrichum* and *Botryosphaeria* on leaves (Fig 16, Table 5) 5 days after inoculation. By 12 days the lesion size for *Colletotrichum* inoculated leaves had all increased, indicating the fungicides had reduced the effect of the fungus but had not completely prevented infection. Conversely with *Botryosphaeria* inoculated leaves, the fungicides Bravo and Flint effectively maintained the reduced lesion expansion up to 12 days.

All four fungicides reduced the lesion development of *Botryosphaeria*, however only Octave and Pristine provided some extended control of *Colletotrichum*.

Figure 13. Leaves of *Pistacia atlantica* (Mt Atlas Mastic Tree) treated with water and inoculated with *Colletotrichum acutatum*: 5 days after inoculation (L) and 12 days after inoculation (R).

Figure 14. (L) Frozen pistachio nuts 5 days after defrosting were blackened and soft. (R)Leaves of *Pistacia atlantica* (Mt Atlas Mastic Tree) 5 days after treatment with prochloraz and inoculation with *Colletotrichum acutatum*.

Figure 16. Lesions on leaves of *Pistacia atlantica* (Mt Atlas Mastic Tree) 5 days after being inoculated with *Botryosphaeria parva* following treatment with water (L) or Pristine (R).

Table 5. Lesion rating* at 5 and 12 days after *Pistacia atlantica* (Mt Atlas Mastic Tree) leaves were treated with fungicide and inoculated with *Colletotrichum acutatum* or *Botryosphaeria parva*.

Fungicide	Active Ingredient	*Colletotrichum*		*Botryosphaeria*	
		5 days	12 days	5 days	12 days
Pristine	252g/kg boscalid 128g/kg pyraclostrobin	1	2	0	1
Bravo	720g/L chlorothalonil	2	3	1	1
Octave	462 g/kg prochloraz	0	1	0	2
Flint	500g/L trifloxystrobin	1	3	1	1
Untreated	Water	3	3	3	3

*0= no lesion development, 1 = minor necrosis around wound site, 2 = some lesion development (~50% of control) and 3 = lesion development equivalent to the untreated control.

3.7. Further work planned

Repeat on Australian Pistachio leaves and fresh nuts when available, and include Switch

4. TIMING OF INFECTION BY *COLLETOTRICHUM* SP.

4.1. Aim.

To determine the susceptibility of pistachio tissue to infection by *Colletotrichum acutatum.*

4.2. Work so far

Five pistachio plants (one male and four female) have been maintained in the shadehouse at ambient temperature.

They have defoliated and are currently at budswell

4.3. Work planned

One potted tree will be inoculated with a spore suspension of *C. acutatum* at one of the following growth stages.

- Budswell
- Budburst
- Flowering
- Nut formation

Detached leaves and fruit will be exposed to *C. acutatum* spores for various times to determine infection periods.

5. APPENDICES - DATA

Temperature studies. Experiment 1. *Radial growth (mm) of isolates after 6 days incubation at various temperatures. (1) and (2) are the two measurement of the one replicate plate at 90 degrees. * plate contaminated.*

Pistachio isolates	Rep	**Temperature (6 days)**											
		10°C (1)	10°C (2)	15°C (1)	15°C (2)	20°C (1)	20°C (2)	25°C (1)	25°C (2)	30°C (1)	30°C (2)	35°C (1)	35°C (2)
Botryosphaeria Isolate 116/11	1	11	10	13	14	35	32	42	40	43	44	20	22
	2	11	13	19	16	36	34	41	41	44	42	27	27
	3	9	10	16	13	34	33	43	38	47	44	30	30
	4	9	10	15	14	37	34	44	42	45	45	24	24
	Mean	**10**		**9**		**28.4**		**41.4**		**44.3**		**25.5**	
Botryosphaeria Isolate 46/11	1	10	9	20	18	39	34	*	*	42	42	22	*
	2	8	9	17	18	38	35	*	*	39	36	24	*
	3	5	7	20	19	36	38	*	*	39	39	27	27
	4	8	8	18	17	36	37	39	34	47	44	33	37
	Mean	**8**		**18.4**		**36.6**		**36.5**		**41**		**28.3**	
C. acutatum Isolate 34/11	1	6	6	19	18	18	18	24	19	18	18	10	4
	2	5	7	21	22	16	17	22	19	18	18	4	8
	3	7	6	20	21	16	16	19	22	18	21	5	4
	4	8	8	22	22	17	18	18	17	18	16	5	7
	Mean	**6.6**		**20.6**		**17**		**20**		**18.1**		**5.9**	
C. acutatum Isolate 70/11	1	3	4	13	15	32	30	36	35	26	26	0	0
	2	1	3	14	12	31	32	30	33	24	24	0	0
	3	3	2	11	10	30	32	33	36	22	22	0	0
	4	3	3	14	16	27	27	34	32	26	26	0	0
	Mean	**2.8**		**13.1**		**27.6**		**33.6**		**24.5**		**0**	

Appendix 2. Experiment 2 *Radial growth (mm) of isolates after 3 days incubation at various temperatures. (1) and (2) are the two measurement of the one replicate plate at 90 degrees.*

	Temperature (3 days)											
Isolates	Rep	5°C (1)	5°C (2)	10°C (1)	10°C (2)	15°C (1)	15°C (2)	20°C (1)	20°C (2)	30°C (1)	30°C (2)	35°C (1)	35°C (2)
C. acutatum Isolate 34/11 (pistachios)	1	2	3	3	4	3	6	9	10	7	8	0	0
	2	5	3	3	4	6	9	9	9	9	9	0	0
	3	5	3	6	6	8	7	8	8	8	7	0	0
	4	0	1	3	5	5	5	8	9	8	8	0	0
	Mean	**2.75**		**4.25**		**6.13**		**8.75**		**8**		**0.00**	
C. acutatum Isolate 70/11 (pistachios)	1	0	0	0	0	4	3	9	9	10	10	0	0
	2	0	0	0	0	3	3	9	8	11	10	0	0
	3	0	0	0	0	5	3	10	10	9	8	0	0
	4	0	0	0	0	4	2	11	10	9	8	0	0
	Mean	**0**		**0**		**3.38**		**9.5**		**8.38**		**0**	
Botryosphaeria Isolate 46/11 (pistachios)	1	5	5	5	4	6	5	20	19	25	24	11	9
	2	5	5	5	4	10	10	19	18	24	24	8	8
	3	5	6	5	4	11	11	18	19	24	24	5	7
	4	5	7	6	5	11	11	19	19	26	22	11	5
	Mean	**5.38**		**4.75**		**9.38**		**18.88**		**24.13**		**8**	
Botryosphaeria Isolate 116/11 (pistachios)	1	3	3	3	5	10	8	19	19	27	28	7	7
	2	5	3	5	6	10	11	18	18	24	24	6	5
	3	2	4	6	7	9	11	18	20	27	24	4	4
	4	6	5	7	6	10	11	19	20	27	28	4	2
	Mean	**4**		**5.25**		**10**		**18.88**		**25.13**		**4.88**	
B. parva (grapevines**)**	1	3	4	2	3	15	14	37	38	79	79	15	15
	2	6	4	3	3	13	14	38	38	72	79	11	11
	3	8	6	3	3	8	9	35	34	79	79	9	4
	4	7	7	3	3	11	12	33	30	79	79	5	4
	Mean	**5.63**		**2.88**		**12**		**35.38**		**78.13**		**9.25**	
B. dothidea (grapevines**)**	1	3	2	3	3	8	13	42	44	79	79	24	14
	2	3	3	3	3	6	8	39	30	79	79	19	24
	3	4	4	2	2	6	7	40	41	79	79	9	11
	4	3	4	3	2	5	7	30	28	79	79	5	8
	Mean	**3.25**		**2.63**		**7.5**		**36.75**		**79**		**14.25**	

Appendix 3. Experiment 3 *Radial growth (mm) of isolates after 3 days incubation at various temperatures. (1) and (2) are*

measurements of the one replicate plate at 90 degrees.

Pathogen source	**Temperature (3-day incubation)**								
Pistachio	Rep	20°C (1)	20°C (2)	25°C (1)	25°C (2)	30°C (1)	30°C (2)	35°C (1)	35°C (2)
C. acutatum Isolate 34/11	1	9	8	9	9	5	5	0	0
	2	8	8	10	10	5	5	0	0
	3	7	8	10	10	4	5	0	0
	4	9	8	5	9	6	6	0	0
	Mean	**8.1**		**7.0**		**5.1**		**0.0**	
C. acutatum Isolate 70/11	1	10	10	18	18	6	7	0	0
	2	11	11	18	18	6	6	0	0
	3	10	11	17	16	8	8	0	0
	4	10	14	18	18	6	7	0	0
	Mean	**10.9**		**17.6**		**6.8**		**0.0**	
Botryosphaeria Isolate 46/11	1	21	24	29	28	15	16	6	7
	2	*	*	27	25	17	19	8	9
	3	21	22	28	29	16	18	9	7
	4	22	23	27	28	18	18	6	7
	Mean	**22.2**		**27.6**		**17.1**		**7.4**	
Botryosphaeria Isolate 116/11	1	24	24	28	28	18	18	6	5
	2	22	23	27	26	18	18	5	4
	3	24	23	26	28	18	16	6	6
	4	24	22	28	28	18	18	4	4
	Mean	**23.3**		**27.4**		**17.8**		**5.0**	
Grapevines									
B. parva	1	44	44	74	69	56	56	0	0
	2	59	51	74	69	54	54	0	0
	3	51	46	74	74	52	49	0	0
	4	49	52	74	74	56	52	0	0
	Mean	**49.5**		**72.8**		**53.6**		**0.0**	
B. dothidea	1	46	39	79	79	59	56	11	16
	2	49	50	79	79	62	55	11	9
	3	45	48	72	74	52	54	4	5
	4	61	55	74	78	64	64	6	5
	Mean	**43.1**		**76.8**		**58.2**		**8.4**	

**plate contaminated*

Appendix 4. Fungicide screening. *In vitro* efficacy against *C. acutatum and Botryosphaeria*. *Inhibition of spore germination or mycelial growth in presence of* ***fungicide-soaked*** *discs or* ***water-soaked*** *disk (Control = C).* 3 *= 100% inhibition, 2 = >50% inhibition, 1= <50% inhibition and* 0 *= no inhibition. Three rates of fungicide were evaluated, full label rate (H), ½ label rate (M) and 1/10 label rate (L)*

ISOLATE	Rep.	AMISTAR				CABRIO				CAPTAN				CHORUS				DITHANE RAINSHIELD				FILAN			
		C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
		Azoxystrobin 40g				Pyraclastrobin 50g				Captan 125g				Cyprodinil 40g				Mancozeb 200g				Boscalid 120g			
Colletotrichum acutatum 70/11	1	0	2	2	2	0	3	3	3					1	3	3	3	0	1	3	3	0	0	0	0
	2	0	2	2	2	0	1	3	3					0	3	3	3	0	3	3	3	0	0	0	0
	3	0	1	1	2	0	1	3	3					0	3	3	3	0	0	3	3	0	0	0	0
Colletotrichum acutatum 214/10	1	0	3	3	3	0	3	3	3					2	3	3	3	0	1	3	3	0	0	0	0
	2	0	3	3	3	1	3	3	3					1	3	3	3	0	1	3	3	0	0	0	0
	3	0	3	3	3	0	3	3	3					1	3	3	3	0	1	3	3	0	0	0	0
Botryosphaeria dothidea DAR78224	1	0	1	1	1	1	2	2	2	0	1	3	3	1	2	3	3	0	3	3	3	0	2	2	2
	2	0	1	2	2	1	2	2	2	0	1	3	3	1	2	2	3	0	3	3	3	0	2	2	2
	3	0	2	1	1	1	2	2	2	0	2	3	3	0	2	3	3	0	3	3	3	0	2	2	2
Botryosphaeria parva DAR7900	1	0	2	2	2	0	1	2	2	0	0	2	3	0	1	3	3	0	3	3	3	0	1	2	2
	2	0	1	2	2	0	2	2	2	0	1	2	3	0	1	3	3	0	3	3	3	0	2	2	2
	3	0	2	3	3	0	2	2	2	0	1	2	3	0	1	3	3	0	3	3	3	0	2	2	2

ISOLATE	Rep.	FLINT				FOLICUR				HOWZAT				IMPACT ENDURE				LUNA				MYCLOSS XTRA			
		C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
		Trifloxystrobin 15g				Tebuconazole 30ml				Carbendazim 100ml				Flutriafol 250ml				Fluopyram 100ml				Myclobutanil 16ml			
Colletotrichum acutatum 70/11	1	1	3	3	3	0	0	2	2	1	3	3	3	1	1	2	3	0	1	1	1	0	0	1	1
	2	0	3	3	3	0	0	1	2	1	3	3	3	0	1	3	3	0	2	2	1	0	0	2	3
	3	0	3	3	3	0	0	1	3	1	3	3	3	1	0	3	3	0	1	1	0	0	1	1	2
Colletotrichum acutatum 214/10	1	1	3	3	3	0	1	2	3	1	3	3	3	0	2	3	3	0	1	0	0	0	0	1	0
	2	1	3	3	3	0	0	2	2	1	3	3	3	1	2	3	3	0	1	1	1	0	1	1	1
	3	1	3	3	3	0	1	2	3	1	3	3	3	0	1	3	3	1	1	1	1	0	0	1	1
Botryosphaeria dothidea DAR78224	1	0	1	1	1	0	1	1	2	0	2	2	2	0	0	1	1					0	0	0	0
	2	0	1	1	1	0	1	1	2	0	2	2	1	0	0	1	1					0	0	0	0
	3	0	1	2	2	0	1	1	1	0	1	1	2	0	1	2	2					0	0	0	0
Botryosphaeria parva DAR7900	1	0	1	1	2					0	3	3	3	0	1	2	3					0	0	1	2
	2	0	1	1	2					1	3	3	3	0	1	3	3					1	1	1	2
	3	0	2	1	2					0	3	3	3	0	1	2	2					0	1	0	2

ISOLATE	Rep.	OCTAVE				RUBIGAN				SCALA 120SC				SCHOLAR				TELDOR				UNITE ULTRASTICK			
		C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H	C	L	M	H
		Prochloraz 300g				Fenarimol 30ml				Pyremethanil 200ml				Fludioxonil 260ml				Fenhexamid 100ml				Chlorothalonil 210ml			
Colletotrichum acutatum 70/11	1	1	3	3	3	1	0	1	2	0	0	0	0	0	3	3	3	0	0	0	0	0	3	3	3
	2	1	3	3	3	0	1	0	1	0	0	0	2	0	3	3	3	0	0	0	0	0	3	3	3
	3	1	3	3	3	0	1	1	1	0	0	1	1	1	3	3	3	0	0	0	0	0	3	3	3
														1	3	3	3								
Colletotrichum acutatum 214/10	1	1	3	3	3	0	0	0	1	1	1	1	1	0	3	3	3	1	1	1	1	1	3	3	3
	2	2	3	3	3	1	1	0	0	1	2	2	0	0	3	3	3	1	1	1	1	0	3	3	3
	3	1	3	3	3	0	0	0	1	1	1	0	0					1	1	1	1	0	3	3	3
														0	3	3	3								
Botryosphaeria dothidea DAR78224	1	2	3	3	3	0	0	0	1	1	1	2	2	0	3	3	3	0	1	0	1	0	3	2	3
	2	1	3	3	3	0	0	1	1	0	0	1	2	0	3	3	3	0	1	0	1	0	2	3	3
	3	1	3	3	3	0	1	2	2	0	1	0	1					0	1	1	1	0	2	3	3
														1	3	3	3								
Botryosphaeria parva DAR7900	1	0	3	3	3	1	0	1	2	0	0	1	1	0	3	3	3					0	3	3	3
	2	0	3	3	3	0	0	2	2	0	0	0	1	0	3	3	3					0	3	3	3
	3	0	3	3	3	0	1	2	2	0	1	1	1									0	3	3	3

Pistachio Growers Association Incorporated

REPORT ON THE PROJECT WORK ON ANTHRACNOSE

Technical Information Sheet No 2

The Pistachio Growers' Association Inc has been utilising voluntary research contribution funds matched by funds from the Australian Government , through Horticulture Australia Limited to undertake research and extension work on the recent problem of Anthracnose.

Scholefield Robinson Horticultural Services were contracted to undertake the relevant work.

You would have some time ago received a Technical Bulletin on Anthracnose.

Growers are encouraged to revisit that bulletin and implement the actions suggested.

Since that time the following has occurred:-

a) a significant literature review has been undertaken and the final work on that report is being completed and will be available for distribution to growers shortly,

b) Prue McMichael (SRHS) and Andrew Bowring visited the USA and discussed pest and disease issues with Californian researchers and industry. The report on their visit will also be released to growers shortly.

c) The South Australian Research and Development Institute (SARDI) has
d) been undertaken some disease identification and also some initial trials on possible chemicals for the management/control of Anthracnose and *Botryosphaeria* sp. As a result of this initial work PGAI is seeking permits for a number of new chemicals to add to the growers 'toolkit'.

d) A Technical Bulletin on *Botryosphaeria* sp is being prepared (similar to the one on Anthracnose). It will be distributed shortly.

e) A workshop is being organised for the 14th September. More information including registration forms will be distributed shortly.

The issue with Anthracnose in 2010/11 was in part due to the extreme environmental conditions during the flowering, growing and harvest period. But from a lay persons point of view one would believe that the anthracnose inoculums was in the orchard and was just waiting on that extreme weather to express itself.

Will this happen this year? We really do not know but one would normally assume that the level of inoculums is still in the orchard and given another of set of appropriate weather conditions it could express itself again. Growers need to be prepared to deal with this through the right orchard management and practices. The work being undertaken and that will be reported at the workshop is part of giving growers the management tools.

Two key messages came from the Californian study tour:

1. Orchard sanitation is critical to disease and insect control. All good Californian growers reshake the trees in the winter to remove mummies and as many racemes as possible.

2. Slow spray speed is essential for the effective application of fungicides. A maximum speed of 2mph (3.2km/hr) is generally used, and one major grower reported spraying at 3 mph. Australian olive growers have achieved some control of anthracnose by slowing the spray speed. It is important growers recheck operating speeds in their own orchards, and review equipment to ensure that they have sufficient capacity.

Trevor M Ranford
Executive Officer

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ORCHARD SANITATION IS AN ESSENTIAL PART OF REDUCING PEST AND DISEASE LEVELS.

The following was the information from the recent Anthracnose technical leaflet.

What should I be doing now if my orchard suffered from anthracnose in 2010/11?

C. acutatum survives over winter in pistachio buds, and in lesions on infected fruit, rachises, leaves and twigs that remain on the tree, or on the orchard floor.

The following are some early recommendations.

- **Sanitation.** This is very important, albeit expensive.
  Re-shake to remove all infected nuts and rachises.
  Remove, mulch and/or incorporate under-tree debris
  (so fungus is not splashed from under canopy to lower limbs and leaves in spring).

- **Don't prune during rain.**
  It is not yet clear if a forced leaf drop (as with urea or zinc) and fungicideapplication after, would deliver economic benefits in affected orchards.

- **Understand the underlying threat for next season.**
  Monitor the fungi in dormant buds. (BUDMON tests can detect *Botryosphaeria* and *Colletotrichum* infection in buds
  With knowledge of bud infection levels, and the relative susceptibility of pistachio tissue, we could utilise free moisture, humidity, temperature data, to predict disease outbreaks. This would assist growers in optimising the timing and placement of fungicide applications.
  Several contact and systemic fungicides are effective against *Colletotrichum* spp. on other hosts. Some also have reported efficacy against other pistachio fungal pathogens, including *Botryosphaeria* sp. (panicle and shoot blight) and *Alternaria* sp. (*Alternaria* late blight).

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Current Pistachio Permits

Permit ID	Description	Expiry Date
PER9254	Petroleum oil / Pistachio nuts / Black scale and Soft brown scale	30-Sep-11
PER10512	Copper salts / Pistachio nuts / Suppression of Botryosphaeria, Alternaria alternata and Bacterial dieback.	31-Mar-13
PER11980	Azoxystrobin / Pistachio nuts / Alternaria late blight (Alternaria alternata), Botryosphaeria dothidea and anthracnose.	30-Jun-13
PER12332	Chlorpyrifos & Maldison / Tree nuts / Australian plague locust	31-Aug-11

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Pistachio Maintenance Programme

Growers should consider the following for the end of Winter period and early Spring. Leaf analysis and soil test results from the previous season will be a guide to fertiliser requirements for the coming season.

Winter

Superphosphate. Spread along rows at a rate of about 300 kg/ha every 3-5 years or longer. This is said to be more effective than a light application each year.

Potassium. At this stage there is no clear evidence Potassium will benefit pistachios in Australian soils, although the Almond industry generally accepts it does. Also spread along rows at a rate yet to be advised for Australian conditions but in the order of about 100 kg/ha of muriate or sulphate of potash. (Californian work suggests rates much higher, up to 220 kg actual K per ha, in which case use sulphate of potash because of lower salt effect). Potassium is most likely to be required in sandy soils.

Pruning. If pruning for hand harvesting there is no need to prune to the formal vase shape. If the trees are in cropping make two kinds of cuts. Thinning cuts that remove whole branches are used to make room for cultural activities. Heading cuts are made where there are lateral buds which will produce a new shoot next season, rather than in the zone of fruiting buds which will lead to the production of a blind shoot. Remove prunings before spring flush of cover crop growth.

Boron. Apply boron during the period just prior to bud swell through to 20% bud break. If leaf analysis shows boron <120-250 ppm (mg/kg) use Solubor as a spray at a rate of 4 kg/1000 l water (2-5 kg Solubor/ha) and wet trees thoroughly. Symptoms are short internodes, bushy tree appearance, chlorotic (yellow) leaves and misshapen terminal dieback. Californian research has shown Boron to be critical to effective pollination.

Scale. For control of scale and to encourage early and even bud burst (particularly in off year) use white (dormant) oil prior to bud burst (about late August) at a rate of 30-60 l/1000 l of water.

Ants. Chlorphos can be used around trees to reduce the number and activity of ants farming scale insects.

Early Spring

Zinc. Apply a foliar spray of zinc sulphate to young expanding shoots before the spring growth flush is complete and before the leaves have hardened up. Use zinc sulphate at 2-3 kg/1000 l water. Deficiency symptoms are delayed opening of the vegetative and flower buds all over the tree, or on isolated shoots, and terminal leaves may be small. Mildly affected shoots may show mottling between the veins or wavy margins. Symptoms are most obvious early in the season. May consider adding manganese to this spray if there are symptoms of chlorosis of older leaves.

Copper. If leaf analysis shows copper at concentrations <6-10 ppm (mg/kg), use a spray of copper EDTA (chelate) at 0.25-0.75 kg/1000 l (0.5 kg copper EDTA/ha). Spray at the start of the summer growing flush (in December). Symptoms are leaf scorch progressing to a collapse of rapid growth shoot tips, particularly during the summer growth flush. Where copper fungicides are being used, it is unlikely deficiency symptoms will occur, and may be the best way to combat deficiency.

Nitrogen. Applications can be made from mid October to early to mid- February and should be spread over that period. There may be a benefit in weighting application during the nut filling period. Californian results suggest post harvest application is not useful. Recommended application rates are for young trees, 25 kg of actual N/ha applied as little dressings often through the growing season to encourage rapid filling of space. For older trees rates of 150 - 350 kg of actual N/ha may be required. Leaf analysis results from the previous season are a good indicator of requirements, aiming for 2.3%-2.5% leaf N. Older trees require enough N to grow replacement fruiting wood and cope with the stress of maturing a crop. Symptoms of deficiency are delayed bud break, short, thin shoots with red bark and small, pale green leaves with reddened veins.

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BUDMON Testing

"If you are considering BUDMON testing this season for *Colletotrichum* spp and/ or *Botryosphaeria* spp. presence, PLEASE contact Barbara Hall or Sue Pederick at SARDI before bud sampling or sending samples. Their contact details are: Barbara.Hall@sa.gov.au and Sue.Pederick@sa.gov.au.

Please put "BUDMON request" in the subject area of emails.

The SARDI lab will find staggered submission of samples helpful due to their time and space limitations. If testing is requested for *Colletotrichum* sp only, growers can expect a test time of 7-9 days; but *Botryosphaeria* growth is slower and the detection test may take 3 weeks."
If you have not yet done BUDMON testing this winter, you need to do immediately. If you do not, you will have little idea of the fungal problems confronting you this coming season.

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NOTES FROM CALIFORNIA STUDY TOUR JULY 2011

General comments/summary

- Our main problem from 2010/11 harvest was Anthracnose, *Colletotrichum acutatum,* which has only been reported in Californian pistachios on one occasion. The particular orchard in the northern valley has not had a repeat of the disease since this outbreak.

- Although Anthracnose was the major pathogen during Australian 2010/11 season, it was confirmed Botryosphaeria was also present. In addition, reports from BUDMON tests carried out at Kyalite during July 2011 show an increase in latent infections of both Botryosphaeria and Anthracnose. Because Anthracnose was so virulent last year, it is possible it competed strongly with Botryosphaeria, resulting in a low incidence of classic BOT symptoms? Therefore it may also be reasonable to assume that given a more "normal" growing season, Botryosphaeria could become dominant? *If this were the case understanding and controlling Botryosphaeria Panicle and Shoot blight will be just as important as finding the control method for Anthracnose.*

- Growers in California are generally very confident with control mechanisms for Botryosphaeria Panicle and Shoot blight, mainly due to the range of chemicals available to the industry. New chemicals continue to be introduced, and Botryosphaeria has the genetic stability to limit chemical resistance. The cultural practices of removing BOT cankers and winter sanitation are understood, and seem to be well implemented. The combination of chemicals, sanitation, and reducing humidity from irrigation make Botryosphaeria manageable.

- Botrytis Blossom and Shoot Blight is mainly only an issue in the more northern regions or in other areas if rain occurs around bloom period. Basically as you move further south, the rainfall decreases, and the incidence of all disease decreases. This disease needs to be considered significant for Australian conditions given our usual rainfall pattern. Californian growers have reported up to 20% crop loss, from Botrytis infection in the prior season.

- Alternaria Late Blight is now considered by many as the major disease threat to the Californian industry. It has the capacity to cause defoliation (hence affect carbohydrate reserves), current crop loss, and cause shell staining. Alternaria quickly develops resistance to chemicals, and because it occurs in late summer, options for chemical control become less as harvest date gets closer. Three species cause this disease in California. At east one of those species is widespread in Australia and has a broad host range. It has been found on pistachios. The degree of infection however is unclear. Last season it was particularly difficult to assess Alternaria because Anthracnose was so virulent.

- Anthracnose is one of the significant diseases of Almonds in California, again mainly in the northern counties. The disease expresses itself differently in Almonds, even though it is thought to be the same pathogen *Colletotrichum acutatum*. However it is possible guidance may still be taken from Anthracnose control in Almonds, particularly in regard to choice of chemicals, and the effect of leaving new tissue unprotected.

- Whilst growers in central valley or south often achieve season long disease control with as few as 3-4 fungicide applications, in northern counties 5-8 is more the norm. Australian average conditions are considerably wetter than even the northern counties, so it would be reasonable to expect us to require a minimum of 5-8 applications for BOT control.

- General comment: Application speeds. Most CA growers do not exceed 2 mph.

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Horticulture Australia