Carmel Growth Disorder

Dr Prue McMichael
Scholefield Robinson Horticultural Services Pty Ltd

Project Number: AL08015

AL08015

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the almond industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the almond industry.

ISBN 0 7341 2527 5

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

Horticulture Australia

Scholefield Robinson
HORTICULTURAL SERVICES

Carmel Growth Disorder
Final Report
AL08015

Prepared for	:	Horticulture Australia Ltd and Almond Board of Australia
By	:	Scholefield Robinson Prue McMichael *et al*
Date	:	November 2010

HAL Project No. AL08015

PROJECT TITLE
Carmel Growth Disorder Final Report
Prepared for: Horticulture Australia Ltd and Almond Board of Australia

Scholefield Robinson Horticultural Services Pty Ltd
ACN 008 199 737
ABN 63 008 199 737

PROJECT LEADER

Dr Prue McMichael
Principal Consultant/Plant Pathologist
Scholefield Robinson Horticultural Services Pty Ltd
PO Box 650
Fullarton SA 5063

PROJECT TEAM

Dr Kate Delaporte
Mr Garth Swinburn*
Dr Ben Thomas
Ms Alison MacGregor*/Yasmin Chalmers
Dr Peter Scholefield
Ms Cheryl Jenkins

PO Box 650, Fullarton SA 5063
Ph: (08) 8373 2488
Fax: (08) 8373 2442
srhs@srhs.com.au
www.srhs.com.au

*PO Box 1446
Mildura VIC 3502

PURPOSE OF REPORT

This report was commenced in response to a widespread growth disorder observed in Carmel almond trees in spring 2008. It aimed to identify the cause of the bud growth failures and to identify practices and conditions that may have contributed.

ACKNOWLEDGMENTS

The Scholefield Robinson team is particularly grateful for the support provided by the Almond Board of Australia, the Commonwealth government and Horticulture Australia Limited (HAL). Mr Ben Brown and the co-operating orchards have provided valuable assistance.

DISCLAIMER

November 2010

TABLE OF CONTENTS

List of Tables

List of Figures

List of Appendices

MEDIA SUMMARY

The Australian almond industry observed in spring 2008 a widespread bud growth disorder in the pollinator, Carmel. Carmel was the only cultivar seriously affected in Australia. The disorder presented as extensive areas of bare wood, tufted terminal growth on some shoots, sparse canopies, poor leaf out and in some cases reduced flowering and low nut set. This project was initiated to determine the potential cause of this disorder and its likelihood of spreading. The outputs include several reports and a Fact sheet and literature review on bud development and factors that affect it.

This 18-month investigation focussed on bud dissections from 'affected' and 'non-affected' trees. The Carmel trees were from orchards across three production districts that had experienced recent heatwaves. Bud dissections from affected trees, revealed that many buds that appeared to be normal externally, had internal necrosis. Damaged buds did not recover and central buds sustained more damage generally than outside buds, at each node.

Damaged buds were visible by early summer, but the incidence and extent of damage increased as the season progressed. Predictions of bud emergence problems in spring could be made from bud dissections in the previous autumn, with some accuracy. Budsticks with high levels of central bud damage had low leaf out. Necrotic outside buds generally had a low percentage and/or delayed transformation to flower buds. Field visits and dissections have provided evidence that once bud failure is seen, an increasing proportion of the tree's canopy becomes unproductive each season.

In general, trees planted in 2005 and later appear to have suffered more bud failure than mature trees in the same districts. There is evidence that during the almond planting boom, which peaked in 2006-07, budwood was sourced from locations outside the industry's Monash budwood repository. Our testing of Monash buds demonstrated their superior health status and size and it is recommended that future Carmel budwood only be sourced from this location. Specifically, the low budsticks closest to the original tree bud are more likely to support buds with low bud failure potential. Similarly, trees from the original buds/budstick introductions of Carmel into Australia, are likely to have low bud failure potential and their locations other than at Monash, and their orchard performances during recent heatwaves, should be determined in further investigations of bud failure.

The contribution of bud genetics to non-infectious bud failure in Carmel in California has been well researched. It has been found that bud genetics determine the bud failure potential, but high temperatures at critical bud development periods, and annual growth rates, influence the onset of bud failure. It appears that Australia's Carmel bud failure is not readily distinguishable from non-infectious bud failure in California.

A review of temperatures during recent growing seasons in the Riverland (Renmark, SA), Sunraysia (Mildura, Vic) and the MIA/Riverina (Griffith, NSW) revealed that the heatwaves of March 2008, January 2009 and November 2009 have likely contributed to the widespread expression of bud failure, especially in young trees. Young trees have extensive annual growth, and limited reproductive growth. The November 2009 heatwave appears correlated with the widely-reported poor leaf out in Carmel this spring (September 2010). Bud failure in previously non-affected trees has been seen this spring, as was predicted through the autumn bud dissections. Some affected trees show evidence of three years of abnormal growth and 90% canopy bareness, yet none have died.

Bud failure, once triggered cannot be eliminated or controlled. Although our cursory attempt at top-working was not successful, top-working is a recognised management option for non-infectious bud failure in California. It relies however on the capability to identify good buds (low bud failure potential). Orchard management requires consideration of the economic viability of affected trees. Our recommendation is to remove young (fourth leaf or younger), affected Carmel trees if more than 30% of the canopy is bare.

TECHNICAL SUMMARY

The 'bud failure' disorder was widely reported in spring 2008. This disorder affects tree growth and the economic viability of young, affected Carmel trees. It presents as extended areas of bare wood that reflect primarily failed vegetative bud development. Dark lesions around failed buds and rough bark are also associated with the Carmel bud failure in Australia. The failure to 'leaf out' has been more apparent than the failure to flower, however severely-affected trees with poor nut loads, have been observed. Vegetative growth in affected trees is generally sparse and often in tufts at the terminal end of shoots. Basal and terminal buds are formed during the spring and autumn (respectively) of the previous season, when temperatures are cooler and this likely explains their on-going development. The worst affected Carmel trees, as indicated by our investigations and surveys, have been planted since 2004.

The practical investigations in this project focussed on bud development and the health of buds at various stages, along budsticks from the Riverland and western Sunraysia, the Riverina, and from Monash, the site of the industry's budwood repository. The dissection of buds on lateral shoots that arose from basal buds of either *affected* or *non-affected* wood, was undertaken at regular intervals. A review of the literature on bud initiation and development has been undertaken and a Fact sheet prepared for the almond industry. Temperature effects have also been reviewed.

Internal bud necrosis was visible as early as December of the previous season (at the time current season nuts were maturing). March bud dissections however provided the clearest indication of potential leaf emergence and/or flowering problems, in the forth-coming spring. The greater the proportion of damaged buds (from dissections), the poorer was the observed leaf out and the sparser the spring canopy. The percentage of damaged buds on laterals from affected wood was far higher than that of buds from non-affected wood, regardless of the location of the orchard. A high percentage of Monash buds showed no damage throughout the sampling periods, and no Monash trees developed sparse spring canopies.

Field visits to the MIA, Riverland and Sunraysia in spring 2009 revealed that young trees identified as *affected* in spring 2008 remained that way. There were no signs of recovery and the bare wood percentage of canopies had increased. Similarly, non-affected trees (as identified in spring 2008) also had maintained that status and had full canopies in spring 2009. Mature trees appeared not to suffer bud failure at a visible level in that season, but later onsets have since been reported.

Continued sampling of budwood for a second season in one Riverland orchard revealed that minor bud damage was visible by December 2009 in both *affected* and *non-affected* trees. Damage increased in February and March, 2010. With the March observations of damaged buds in previously non-affected (and affected) wood, widespread leaf out problems, and new onsets of bud failure were predicted for spring 2010. By September 2010, Carmel trees displaying extensive stretches of bare wood and sparse canopies were reported from all districts. Up to 20% of canopies of previously non-affected trees were bare and up to 90% of affected canopies were bare, and some mature trees also displayed some significant bud failure. Once triggered, bud failure renders an increasing proportion of the canopy unproductive, each season. Young affected trees, given their extensive annual vegetative growth, suffer greater economic impact of bud failure, than mature trees with late onset bud failure.

The reviews of the temperature triggers for non-infectious bud failure (NBF) in California, and the temperature extremes of the past five seasons in Australian almond regions, revealed abnormal heat waves to be a feature of the period, in Australia. In California, NBF researchers have found spring temperatures and particularly those over 27°C in May/June (northern hemisphere) to correlate closely with vegetative bud failure onset in the following spring. Given this, the Australian heatwave in November 2009, has likely contributed to the widespread bud failure, and new onsets of bud failure, reported in spring 2010. Similar temperature extremes preceded the 2008 widespread onset of bud failure and their timing influenced flower development, as well as vegetative growth in the Riverina district particularly. The Riverina is a new almond production district, relative to the Riverland and Sunraysia. The early signs of bud failure in the Riverina were reported in spring 2007, and this corresponds to temperature extremes experienced in that area, in spring 2006 and the 2006/07 growing season. Some trees in this region show three years of abnormal growth. The mechanism for rough bark development and tiger-striping in affected trees is unknown. These signs are widespread in the Riverina's affected trees but not in other production regions where bud failure is widespread.

Management of bud failure requires economic as well as practical consideration. Although affected trees appear not to die, reducing or eliminating the advancement of bud failure, once it is triggered, is not possible. The advancement however will be slower in mature trees due to their reduced vegetative growth rates. Heavy pruning does not successfully stimulate productive wood, but top-working is effective (in California), if good buds can be identified. Bud failure in Australia appears to be the same as NBF in California and our recommendation is that trees in their fourth leaf or younger, be removed if more than 30% of their canopy is unproductive (bare wood).

Monash was unable to meet all budwood demands over the expansion period, and especially in 2006/07. The Monash trees are foundation trees or first generation from them. It is likely their original bud proximity (in genetic terms), their maturity and their management have limited the impact of recent temperature extremes on their bud health, as demonstrated in the dissections. Orchard performances of other 'original' Carmel trees in other locations should be reviewed as they may also be useful sources of buds.

The genetic bud failure *potential* of various bud sources other than Monash remains unclear. It is clear that Carmel budwood of unknown status, has been used in propagation, and this is particularly true for many young trees now in their third, fourth or fifth leaf. Until the bud failure potential of other source trees is known, it is reasonable to recommend that all Carmel buds for propagation, be sourced from Monash. If specific bud selection is possible, the basal or terminal buds on low budsticks (close to the original bud), are likely to have the lowest bud failure potential. Nursery practices and industry systems that increase budwood knowledge and traceability to source trees, would be valuable in investigating bud failure further in existing trees, and in new varieties that have Carmel parentage.

1 INTRODUCTION

1.1 Industry position

The Australian almond industry observed a widespread growth disorder in the Carmel variety, in spring 2008. Another widespread onset of the problem occurred in Carmel trees in spring 2010. The growth disorder has been reported in each of the major production regions, although the northern Adelaide Plains in South Australia has only reported it for the first time in spring 2010.

The growth disorder presents as extended areas of bare wood. Bare wood reflects failed vegetative (and/or floral) bud growth. The failure to 'leaf out' has been more apparent than the reduction in flowering, in affected trees. Vegetative growth in affected trees is sparse and often in tufts at the terminal end of shoots. In some areas, dark lesions around buds and rough bark are also associated with the disorder.

The growth disorder is most apparent as sparse canopies in young trees during spring, but it may also be evident as low nut loads in affected trees. The bud failure has been previously observed in some mature trees, although the distinction between 'bud failure' and the effects of drought on top growth is not always clear. In 2010, bud failure in mature tree has been confirmed in several orchards.

The specific cause of the 2008 growth disorder, and its potential to 'spread', could not be immediately determined. This project was initiated to investigate bud status in Carmel trees across the affected production districts. The presence of viruses in affected wood has also been tested and the potential to transmit the disorder through grafting has been investigated. Environmental conditions and the timing especially of high temperature extremes have been examined to determine their correlation with the observed Carmel bud failure.

This Final Report has been preceded by two comprehensive Milestone reports. These reports should be read in conjunction with the Final Report and they are attached to this report as Appendix 1 and 2.

1.2 Bud development

From international research it is known that bud initiation and development are influenced individually and in combination, by tree genetics, environmental conditions, nutritional, chemical and water status; and by biological organisms. The 'failure' of buds may be the result of single or multiple factors that cause a lack of chilling; or prevent bud formation, normal development, normal responses in dormancy and emergence from dormancy; bud death or abscission before emergence etc.

Our investigations have focussed on bud initiation and development and the factors that may have influenced them over the last three seasons in Australia. Buds from 'affected' and 'non-affected' wood have been investigated through systematic dissections. A review of the literature on bud initiation and development has been undertaken and a Fact sheet prepared for the almond industry.

2 MATERIALS AND METHODS

2.1 Surveys of growers and nurserymen

2.1.1 Growers' and nursery surveys

A grower survey was prepared and distributed to all registered almond growers, by the Almond Board Australia (ABA).

The nursery survey was delivered electronically through the ABA to all nurseries known to supply almond trees commercially. It was hoped the link to particular Carmel clones, bud sources and/or rootstock seed sources could be evaluated from information provided by nurseries.

2.2 Bud initiation and development Fact sheet

2.2.1 Literature review

A review of relevant bud development literature was undertaken and summarised in the form of a Fact sheet made available in hard copy (and electronically) to all growers.

2.2.2 Fact sheet

The Fact sheet provided detailed information on bud development to growers. It ensured growers had ready access to information on the events and factors that influence buds, and therefore tree growth and potential yields. An almond growth cycle was prepared to illustrate the bud development steps and influences on them. Growers have been made aware of the factors over which they have some control and the critical timing of activities and conditions on the next season's growth and yield.

2.3 Bud status testing

To investigate bud viability and health (and therefore tree growth and productive potential), the internal and external appearance of buds, and their relative positions (central or outside) within the bud cluster and on the budstick, were recorded. Lateral growth budsticks from Carmel trees in orchards in the Riverina (Murrumbidgee Irrigation District – MIA), Riverland (SA) and Sunraysia (NSW/VIC), were systematically cut and sent for inspection. Buds from the industry's budwood scheme (Monash) in SA's Riverland, were also investigated. Bud dissections were continued in the second year of the project, for Monash and one Riverland orchard.

2.3.1 Budstick cutting

Prior to cutting budsticks, '*affected*' and '*non-affected*' trees were tagged according to their symptoms in spring 2008. Co-operating orchards were provided with written and photographic instructions on how to determine which budsticks were relevant, how to cut and send them for dissections. The written and illustrated instructions are included as an appendix of Milestone 2 (Appendix 1).

Co-operators provided to the investigators, budsticks cut from 'affected' and 'non-affected' Carmel trees across three production regions, and from Monash. The budsticks were cut from the same trees at 2-3 week intervals from March-June 2009 and again from late July–August, 2009. Dr Kate Delaporte dissected each bud, or in situations of excessive bud numbers, the youngest 24 buds (not including the terminal cluster). Bud 1 was the first one below the apical tip cluster. The relative health and physiological status of each bud was recorded.

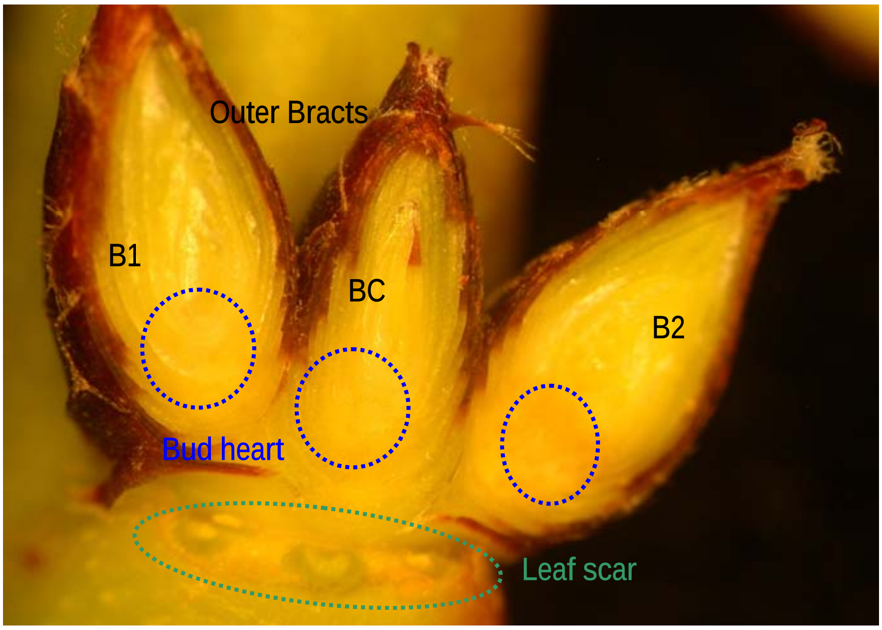
In the second season of this project, systematic budstick cutting continued from only one orchard and the cutting period re-commenced earlier than in the previous season. It commenced in early summer (late November-December) rather than autumn, so that the time of transition from healthy appearance to damaged, could be defined.

The Monash repository is the primary source of Carmel budwood. The trees are routinely pruned heavily or hedged to ensure ample supplies of fresh first-year budwood. These trees therefore are not usually allowed to bloom. It is therefore possible the pruning and management of mother trees could have masked bud failure, should it be present or triggered. By allowing the main scaffolds and branches on each of 20+ mother trees at Monash to remain unpruned in 2008/09, the opportunity to observe bud development (floral and vegetative) was provided (Photos - Appendix 2). The Monash trees from which sticks were cut are foundation trees or one generation removed. Significant resources were devoted to removal of flowers from them. The budsticks were cut from them as described above.

2.3.2 Dissections

The dissections were made longitudinally through the buds, so as to expose internal development status and health status (Figures 1, 2). As the season progressed, information on the transition to floral buds and missing buds was also recorded.

Figure 1: Almond bud set at a given node (longitudinally cut)

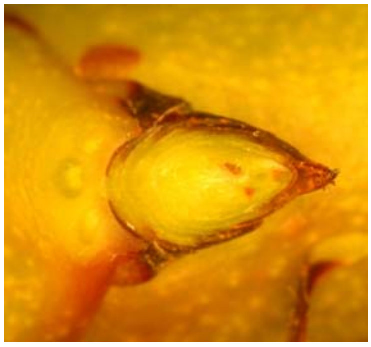
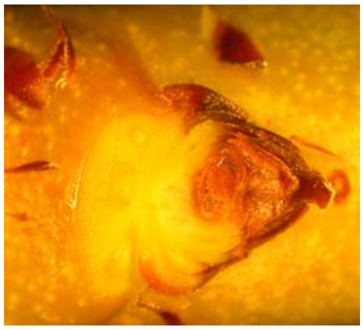
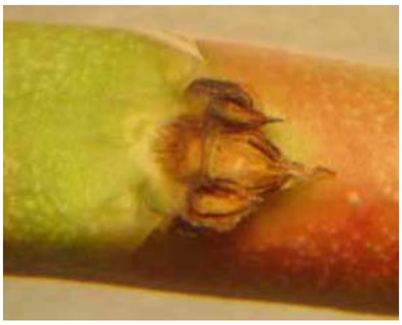
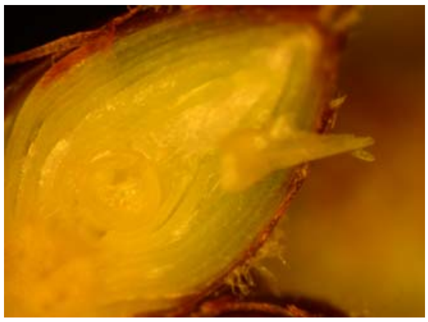
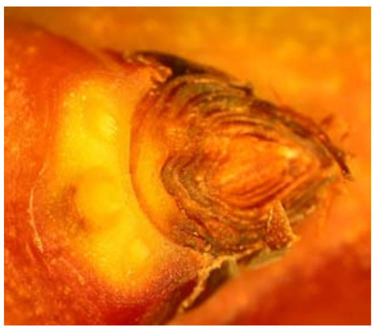


B1 = Bud 1 (Left side); BC = Centre Bud; B2 = Bud 2 (Right side)
Bud heart = the growing point of the bud
Leaf scar = the leaf attachment point
Outer bracts = the brown, lignified outer bracts that protect the bud heart

2.3.3 Damage criteria and descriptions

A visual damage code was prepared to allow consistent recording of results and comparison over time, of the type, location and severity of bud damage observed. The code developed and used is shown below in Figure 2.

Figure 2: Almond damage visual and descriptive scoring code

Observation description	1 = healthy green bud, 0% browning	1x = healthy green bud; with lignified section inside bud, but not at bud heart, usually tip section, 0% browning
Photo reference for Rating 1 and 1x		
Observation description	2 = bud heart brown/stained (<50%)	3 = bud heart brown/stained (>50%)
Photo reference for Rating 2 and 3		
Observation description	5 = bud heart brown/stained (> 50%) PLUS staining/scarring below bud	C = bud heart development advanced since previous observations (e.g. possible differentiation to floral bud)
Photo reference for Rating 5 and C		
Observation description	DC = bud heart is dead but still present	D = entire bud dead but still present
Photo reference for Rating DC and D		

2.4 Orchard visits

Participating orchards were visited prior to budstick cutting and again in the spring of 2009 in order to assess the predictive value of the summer-autumn bud dissections. One orchard was visited again in spring 2010. The specific trees (affected and non-affected) from which sticks had been cut were assessed for their leaf out and the percentage of canopy structure that was bare wood.

2.5 Environmental conditions and bud development

2.5.1 Growing period temperatures

There is little specific information on the range of temperatures (upper and lower thresholds) during which *Prunus* buds develop, as opposed to the hours of chill they may need. Degree days (DD) consider both time and temperature and as such provide some insight into physiological bud development. In California, accumulated DD above a lower threshold of 80˚F (27˚C) has been identified as critical in non-infectious bud failure (Kester, 2000, 1994; Kester and Gradziel, 1996; Kester *et al*., 1998). The rate of bud development differs in hot springs and autumns, and a bud's physiological state will influence its capacity to survive and/or continue development during environmental stress periods (e.g. extreme heat or cold, drought).

In order to assess the potential contribution of environmental conditions to the onset of the Carmel disorder, weather records for ten seasons were analysed and accumulated DD over 27˚C, were calculated[1] for the bud development period; spring-early summer (October-December) and summer-early autumn (Jan-March). Extreme heat (cumulative days over 35˚C) events in spring and over the growing (October-March) seasons, were also analysed.

2.5.2 Drought conditions

The growers were asked to detail their post-harvest irrigation practices in autumn 2008 and 2009.

2.6 Virus testing and grafting

It was unclear if the growth disorder was the result of a biological organism or if it was abiotic in nature. To assess this, specific viral testing was undertaken. The infectious potential of the disorder was also tested. Grafting/budding of affected, non-affected and Monash budwood onto clean, hybrid rootstocks was undertaken by the Department of Primary Industries, Victoria (DPIVic).

2.6.1 Virus testing of budsticks

The leaves from affected and non-affected trees (in spring 2008) were tested for the presence of known Prunus viruses. Leaf samples from each quadrant of both non-affected and affected trees were collected from four MIA orchards. At the time of collection, no typical symptoms of virus were evident in any of the trees, and no sampled leaves showed signs of problems, or infection by any biological entity.

Composite samples of 'affected' and 'non-affected' tree leaves were prepared and sent for Prunus Necrotic Ringspot Virus (PNRSV) and Prune Dwarf Virus (PDV) testing. Two rounds of

---

[1] The calculations in this report are an approximation based on daily maxima. Daily temperatures do not follow a uniform sine wave, no upper temperature threshold is identified for *Prunus* bud development and the lower limit of 27˚C has been determined from Californian research. As such our data over-estimate the DD, but they are relative and therefore provide indications of extreme heat within and between the investigated seasons. Data from 15 minute intervals would provide more accurate estimations.

testing were undertaken by Dr Michelle Wirthensohn (University of Adelaide). The second round employed 'nested' PCR tests which are more sensitive.

The DPIVic also undertook some virus testing during their woody indexing and graft transmission tests.

2.6.2 Graft transmission testing

Mr Mirko Milinkovic at DPIVic undertook virus testing also. The method of reverse transcription-polymerase chain reaction (RT-PCR) was used to test budstick material for the presence of four *Prunus* viruses – PNRSV, PDV, Apple Mosaic Virus (ApMV) and Apple Chlorotic Leafspot Virus (ACLSV).

Biological indexing to determine the graft transmissibility of the bud failure 'disorder', was also conducted from April 2009 - May 2010. Composites of budsticks (affected and non-affected) were submitted from each of two Riverland orchards (Orchards 2 and 3). Unrated material from two blocks at Monash was also provided as a composited 'candidate' for grafting.

Buds from each candidate group (affected, non-affected, Monash) were grafted onto Nemaguard rootstocks in April 2009, or Shirofugen indicators (on Sam Cherry rootstocks). In July 2009, two buds from the woody indicator GF305 were grafted onto the rootstocks above the candidate buds. Symptom expression in woody and herbaceous indicators requires callusing of indicator buds and candidate buds to ensure any potential transmissible agent had opportunity to transfer into the rootstock if present.

Observations of plants for symptom expression continued until May 2010. Symptomatic leaves on either indicator were re-tested using RT-PCR for viruses in the Ilarvirus group.

The methodologies utilised by DPIVic are briefly outlined in their report, which is attached in Appendix 3.

3 RESULTS

Most results and some discussion of them are provided in each of the Milestone reports, which are attached to this report as Appendix 1 and 2.

3.1 Surveys

The growers' survey, nursery survey, and the results from them, are included as appendices to the Milestone 2 report (Appendix 1). Twenty-three almond growers responded. Most included responses for multiple blocks. In total the investigators gained information on the growth disorder in 70 blocks that span the major production districts of Riverland, Sunraysia and MIA.

The key information revealed by the grower surveys was:

- The bud growth disorder was generally low in incidence but across a wide area
- The bud disorder severity ranged from minor to severe, in affected trees
- Carmel was the only widely affected variety, although one orchard reported some affected Non-pareil and Monterey
- Affected trees were on almond hybrid (peach-almond), Nemaguard (peach) or Bright's Hybrid® (peach-almond) rootstocks
- 'Affected' trees (as assessed by survey respondents) ranged in age from 2 to 20 years
- The majority of affected trees were reported to be young (fourth leaf or younger)
- Reported signs/symptoms consistently included bare wood; some tufted growth at terminal ends, and leafless shoots with some nuts
- Winter symptoms were reported by some MIA growers, as rough bark and horizontal, shallow lesions around affected buds
- Most orchards suggested their trees had suffered some water stress during the previous season

The survey, and further in-person discussions and field observations, confirmed that most affected trees were young, and had been planted since 2004. Some older trees originally reported to have the bud disorder in the top of canopies, have not continued to display such symptoms. It is likely these trees in spring 2008 were primarily displaying effects of water restrictions/drought, rather than the bud growth disorder.

Grower respondents named 21 different nurseries from which trees had been obtained. Many did not know the source of the buds used by their supplying nursery. Two different bud sources were identified by growers who knew their tree histories. Most growers assumed their trees had Monash-sourced buds, but had no evidence from the nursery to confirm their assumptions.

One grower had accurate records of the planting positions of Carmel trees from two different nurseries. One of the nurseries provided trees that subsequently developed 'growth failure'. Trees from the other nursery, despite being planted at the same time in the same orchard block, remain healthy.

Monash has annual records of those that have purchased budwood from the industry scheme. It is presumed that nurseries, for which there are no Monash sale records, sourced their Carmel budwood elsewhere. Three nurseries responded directly to the nursery survey, and three different sources of budwood were identified by them. It is known that during the almond planting boom, Monash could not meet all budwood demands. The survey responses support this knowledge. Our investigation has included Monash buds, but the quality of budwood from the other named sources remains untested.

None of the respondent nurseries indicated they had received feedback from grower clients on the growth of Carmel trees they had supplied; nor did any indicate there had been significant operational changes in grafting or budding in their nursery or changes in their seed or budwood sources in the previous year.

3.2 Bud initiation and development Fact sheet

The literature review underpinned the industry Fact sheet on bud initiation. Both of these documents are included in the Milestone 2 report which is attached as Appendix 1 to this Final Report.

The literature review exposed the factors known to affect *Prunus* flower and leaf bud initiation and viability. Although there is little information specific to the almond cultivar Carmel, it is known that the processes that result in leaf out and flowering (and therefore nut development) start a year or more before the harvest of current season nuts. Vegetative buds form the structure and bearing potential of trees and the capacity to sustain the tree through water uptake, nutrient capture and energy conversion.

Management decisions by growers and nurserymen influence bud formation and development, especially through pruning and post-harvest irrigation practices. The Fact sheet provides recommendations on how to maximise bud development potential. It also made clear why an investigation of ‘bud failure’ had to include some consideration of environmental, biological and genetic factors.

3.3 Bud status and dissections

Initially ten growers from three regions submitted budstick samples. Several however did not continue with the sampling, or believed their trees had “grown out” of the problem, as the season progressed. It is highly unlikely any trees had ‘grown out’ of the bud disorder but some older trees that were originally sampled (because of bare wood high in canopies) no longer displayed the symptoms and are thought to have suffered from general water stress, rather than specific bud failure.

Seven orchards were sampled throughout the March – August 2009 period. They were located in the Riverland (2) Sunraysia (3) and the Riverina/MIA (2). Monash and one Riverland orchard were sampled in both 2009 and 2010. Although bud failure is widespread in the Sunraysia region, no regular samples were submitted from the Robinvale area. Two participating orchards were located in the western part of Sunraysia (near SA border), and they generally experience Riverland conditions. Their results are more relevant to the Riverland and have been incorporated with others from that area in SA. The Wentworth orchard did not have typical bud failure and shading was believed to be the major cause of unproductive wood.

Region	Number of orchards participating	Total number of samples sent 2009
Riverland	3	6 sampling periods x 4 trees x 4 sticks = 96 sticks 4 sampling periods x 4 trees x 6 sticks = 96 sticks 5 sampling periods x 4 trees x 6 sticks = 120 sticks
MIA	2	4 sampling periods x 4 trees x 6 sticks = 96 sticks 3 sampling periods x 4 trees x 6 sticks = 72 sticks
Monash Budwood Repository (Riverland)	1	8 sampling periods x 10 trees x 4 sticks = 320 sticks

Bud dissections by Scholefield Robinson were undertaken to learn about bud appearance, quality and viability at various times of the year. ‘Healthy’ buds are those without necrotic areas either within or under the bud.

Floral buds emerge in almonds prior to leaf buds. Vegetative buds are usually central buds within a cluster. ‘Missing’ buds are buds that formed, but at the time of dissection were missing. This could have resulted from bud abscission, death, or being “knocked off” during transit. The transition to floral buds was most consistently observed during the dissection period May-June. Flower buds are a sub-set of ‘healthy’ buds as the floral transition appeared to occur only in undamaged buds. The ‘percentage’ value of buds with flower initiation was calculated by dividing the number of buds with flower initiation by the total number of buds recorded.

General observations included the on-going development of floral buds after their transformation. Vegetative buds appeared not to increase in size over the dormant season.

The commercial orchard trees from which budsticks were cut did not all have a known history. They were of various ages although the majority were young (second-fourth leaf) and had been exposed to slightly different post-harvest irrigation and temperature extremes during bud development stages. At every stage of testing, some degree of internal bud damage was found in buds removed from affected trees.

The results of bud dissections in autumn-winter 2009 are included in Milestone 3 report, which is attached to this report as Appendix 2.

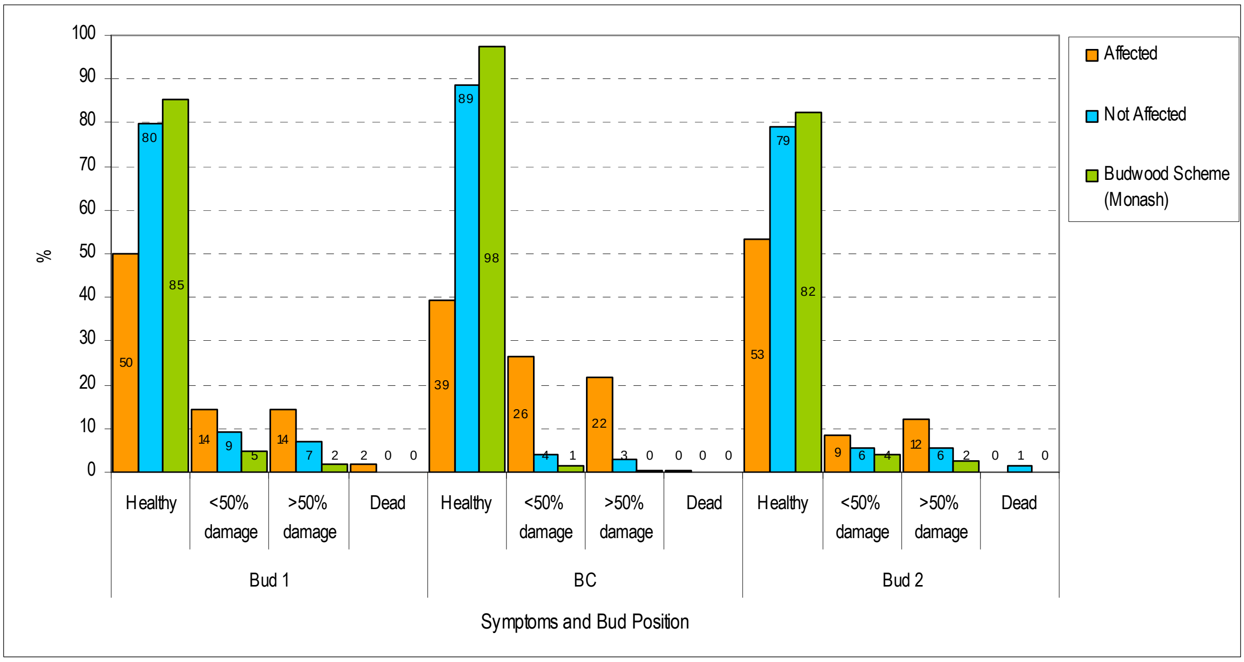
Damaged buds were visible by March 2009. These damaged buds provided the first indication that the orchard leaf out symptoms of 2008 had likely resulted from bud damage and necrosis, rather than a failure of buds to form or receive sufficient chill. Lateral shoots that arose from the basal buds of affected wood, had a high percentage of damaged buds. This percentage was far higher than that on laterals cut from non-affected wood, regardless of the location of the orchard. The buds from Monash trees were superior to all others and had the highest percentage of healthy buds.

Individual reports for each orchard were provided in April 2009 and some notes from these are included in Section 3.4.5 and 3.4.6. Although not all trees displayed bud failure to the same degree, an indication of the effect of bud failure across the sampled trees (March-April 2009) is provided below (Table 1). The results by bud position are presented in Figure 3. The effect on bud health, of the source wood (affected or non-affected) and bud position within a node, is clear. It should be noted that not all trees initially sampled continued to show, or be considered, as typical ‘bud failure’ trees.

Table 1: Average bud status across all sites - April 2009

Status - Outside Buds combined (averaged across regions)						
Trees across regions	Healthy buds (%)	Flower Initiation (%)	% Buds <50% damage	% Buds >50% damage	Missing buds (%)	Dead buds (%)
Affected	43.07	15.93	8.20	14.63	13.27	1.00
Non-affected	83.84	37.55	2.99	2.67	10.06	0.15
Monash	85.26	71.05	1.16	0.55	13.03	0.00
Status - Central Buds (averaged across regions)						
Affected	38.73	1.17	26.23	8.43	4.68	1.44
Non-affected	88.95	3.01	4.44	1.05	3.57	0.35
Monash	97.77	9.30	0.61	0.23	1.24	0.15

Figure 3: Bud status by position in node from all *affected*, *non-affected* and Monash trees - April 2009



Bud damage was not reversed over time and as the season progressed, the percentage of buds showing damage and the area discoloured or dead within damaged buds, increased. A general summary of results from the March-April 2009 dissections is given below.

- Damaged buds are evident by late autumn
- Some buds were dead by early March 2009
- External view of buds is not indicative of the internal health status
- All orchards had some budsticks with damaged buds
- Both *affected* and *non-affected* trees produced budsticks with some damaged buds
- Buds from *affected* wood (as of 2008) had the highest percentage of damaged buds
- Most damage appears as a dark (necrotic) area in the growing point region
- Within a bud cluster, more central buds (BC) were damaged, than lateral buds (B1, B2)
- Damaged central buds generally had more extensive damage than damaged lateral buds
- Damaged buds appeared to neither shrivel nor fall out at this stage.

The following observations are of buds removed from the Carmel mother trees at Monash, in the same period:

- Buds from the ABA budwood scheme consistently had little visible damage
- Almost all central buds (98%) appeared healthy
- A high percentage (82-85%) of lateral buds appeared healthy

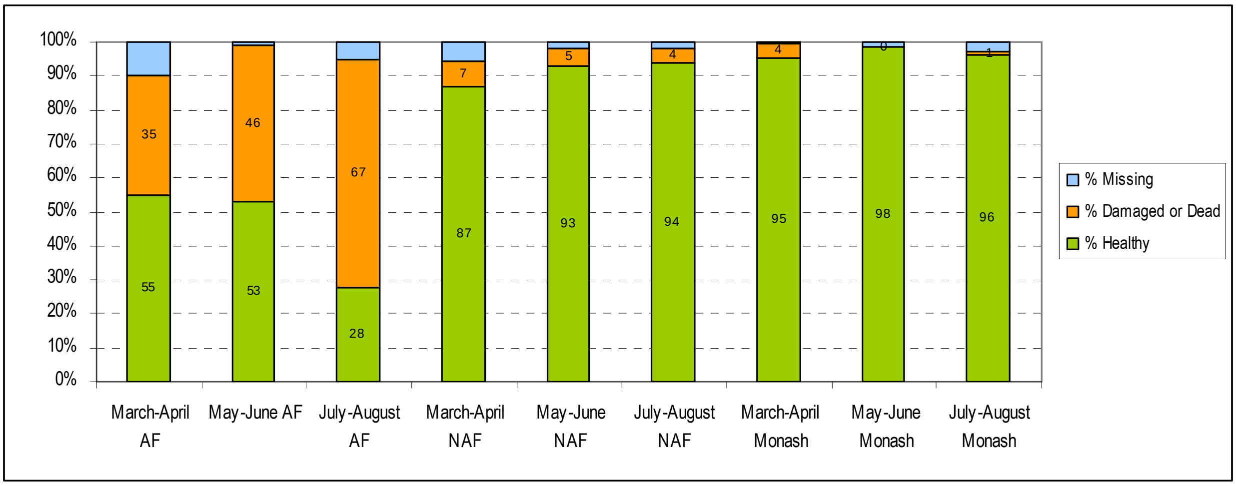
The combined results of *three* orchards that consistently submitted typical 'bud failure' samples is tabulated below and graphed for central buds, for March-August 2009 (Table 2 and Figure 4). It is clear that the extent of bud damage, especially in central buds, increased as the season progressed and that affected trees had more damaged buds. In contrast, the Monash trees produced a very high percentage of healthy buds.

Table 2: Bud status March – August 2009

Tree status	% healthy			% damaged or dead (ratings 2, 3, 5)		
	March - April	May - June	July - August	March - April	May – June	July - August
Affected trees						
All buds*	60	49	40	31	40	47
Central buds	55	53	28	35	46	67
Non-Affected trees						
All buds	86	88	90	8	4	4
Central buds	87	93	95	7	5	4
Budwood Repository (Monash)						
All buds	92	92	89	5	<1	<1
Central buds	95	98	96	4	<1	1

* regardless of bud position or collection location (MIA, Riverland).

Figure 4: Central bud damage status March - August 2009



3.3.1 Bud development May-June 2009

Bud development observations were also made at the time buds were dissected and assessed for damage. Observations over this period indicated that:

- Floral transition was limited and delayed in affected wood (Figures 5-11, Table 3)
- Lateral buds primarily transitioned to floral buds, if not damaged (Figure 5)
- Damaged buds (vegetative or floral) are smaller than undamaged buds
- Buds that show early damage do not recover
- Some affected trees had very few floral buds
- Affected Riverland trees had a higher percentage of floral buds than MIA affected trees
- Monash budwood consistently had a high percentage of healthy buds at each collection period
- A high proportion of Monash buds transitioned to floral buds by May

Figure 5: Damaged outside buds rarely transition to floral buds

Figure 6: Outside and central bud status, May-August, 2009

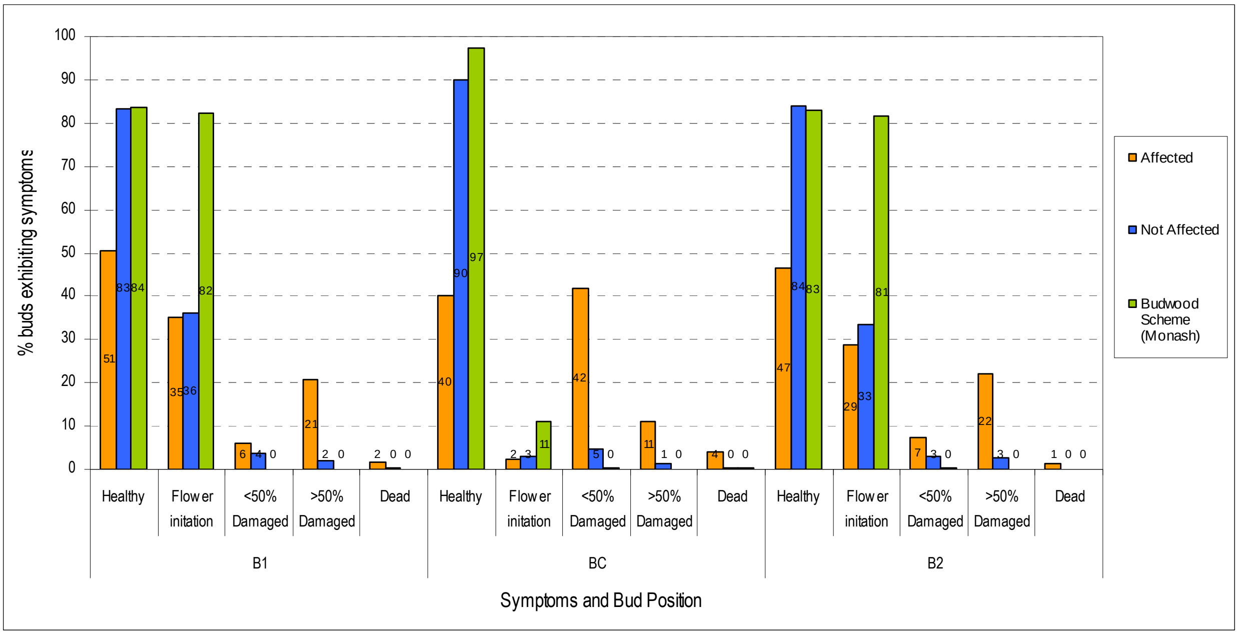
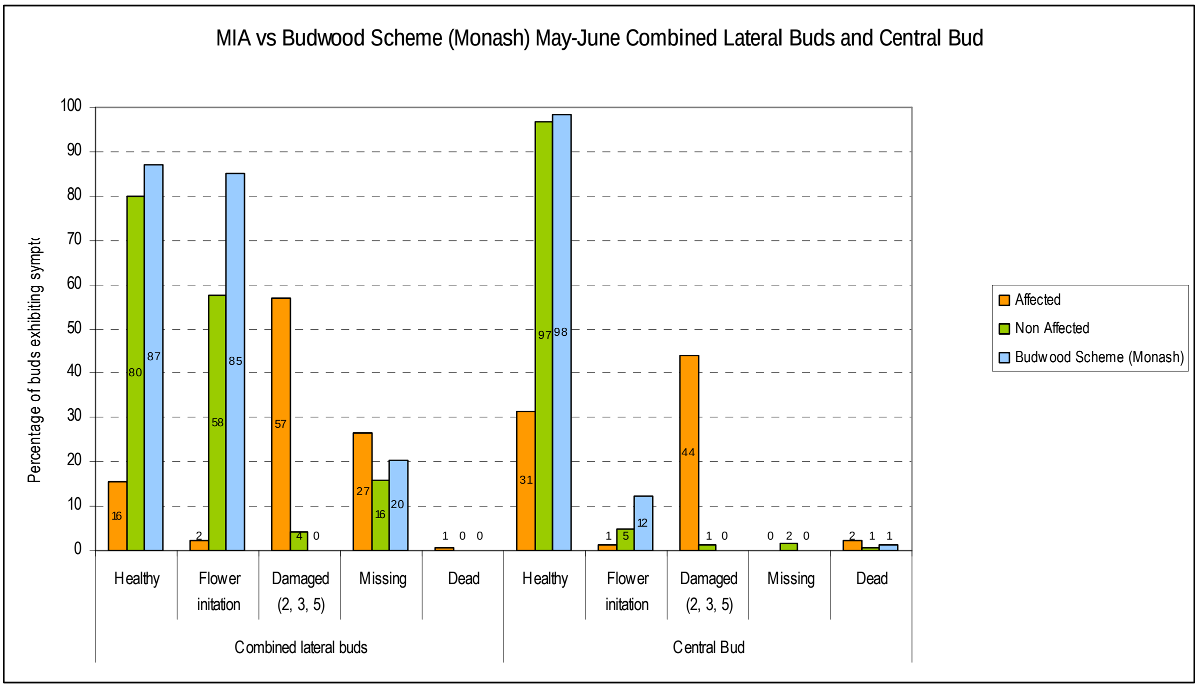


Figure 7: Floral transition and damage status in buds from MIA and Monash, May-June 2009



3.4 Bud status by production region

Two participating Riverland orchards (Orchards 1, 2) had young Carmel trees that displayed bud growth problems. Orchard 3 had mature trees which appeared only to have top of canopy symptoms, and that bare wood was not considered typical 'bud failure'. The two MIA orchards provided consistent bud failure samples (Orchards 4, 5).

Dissections from multiple trees revealed that there was consistency in budstick results from an orchard, within a sampling period. This, and the lack of recovery of damaged buds, meant March results could provide a reasonable indication of leaf out potential in the following spring, despite the extent of bud damage increasing as the season progressed.

3.4.1 Winter and Spring 2009

It did not appear by spring 2009 that any Carmel tree previously identified as '*non-affected*' had become *affected* during the 2008/09 investigation period. There was no indication that the disorder had 'spread' from neighbouring affected trees. There was also no indication that any *affected* trees had recovered or overcome their existing bud growth problems.

The MIA trial co-operators were visited in June, 2009. This winter visit confirmed that bark roughness and horizontal banding in wood two years or older, were characteristic of the disorder in severely-affected young Carmel trees (See photos in Appendix 2). Non-affected trees in the same block did not have the bark symptoms. These symptoms have since been observed in other locations, but rarely and inconsistently.

Visits to all production regions in spring-summer 2009, revealed that vegetative growth from *affected* trees was sparse, often stiff and at odd angles. Basal shoots arising from bare wood however appeared to grow to a 'normal' length and diameter. Leaves that emerged from them were neither wilted nor off-colour. Their bud health could only be assessed through close, internal examination. From the microscopic dissections and examination, the potential for unproductive growth in the next season can be predicted. In young, *non-affected* trees, canopies were full and nut loads in most cases were good. Non-affected and affected trees in all orchards were in close proximity. In no orchards were 100% of young Carmel trees affected.

Tufted growth at the terminal end of laterals, and the shoots that arise from basal buds of affected wood, suggest that not all buds are equally exposed to the bud failure cause. If the cause is temperature-related as proposed in California, it is true that basal and terminal buds would be formed in the Riverland and MIA areas, in the cooler temperatures of spring and autumn.

3.4.2 Spring 2010

Trees identified as affected in spring 2008 had an increased proportion of their canopy bare by September 2010. In Riverland Orchard 2 which was visited and inspected in both 2009 and 2010, the affected trees had up to 80-90% bare canopies, and for the first time some non-affected trees showed evidence of bud failure, as had been predicted from bud dissections in summer.

3.4.2.1 Riverland - Mature Monash Trees

The Carmel mother trees at Monash are of two generations – foundation and once removed. These are the industry source of buds 'closest to the original bud'. Based on our 2009 and 2010 observations, these mother trees have not suffered bud failure to a detectable level. This is despite their presumed exposure to temperatures that have been sufficient to trigger bud failure in other Riverland orchards. Leaf out in spring 2009 was complete in the Monash trees, albeit delayed in the basal buds. Leaf out in spring 2010 was also reported to be normal.

In other mature orchards in this region, most Carmel trees displayed little bud failure, however new onsets of bud failure high in canopies, were reported. In this season, bare wood at the top of canopies was more likely to reflect bud failure than water stress, given the wet 2010 post-harvest period.

3.4.2.2 Riverland - Young Trees

Young Carmel trees inspected and sampled in this region were on hybrid or Bright's® hybrid rootstocks. Both leaf out and bloom were poor in some affected trees, but the majority of trees had reasonable yields. Where the lack of crop was notable, a low percentage of bud transformation to floral buds had been observed in the bud dissections around May-June. In many cases flowers that did form were often delayed in their opening, and they may have missed pollination.

Many of the young affected trees appear not to have an economic future. Most were planted in 2004 or later but it is unknown if they share a similar bud history. All the plantings have been exposed to three consecutive seasons with extreme temperature events (See Section 3.6).

3.4.2.3 MIA - Young trees

The almonds inspected in the MIA were entering their fourth or fifth leaf. These young Carmel trees are the most severely-affected of those observed in this investigation, and they have had visible "crazy top" since their second leaf. Over a third of the trees in one location have significant bud failure with up to 90% of the canopy bare in the most severely-affected trees. Despite being unproductive, there is no indication the trees will die.

Rough bark is evident in most MIA affected trees. Investigations of, and isolations from, the bark have not revealed any organism with consistency. A *Botryosphaeria* sp. was isolated from necrotic areas but in most second-year wood the roughness is confined to bark only. Several trees had intense internal discolouration in larger scaffolds but this was not consistently associated with bud failure, and it is not reminiscent of *Botryosphaeria*-like discolouration. Its cause remains unknown.

The affected trees in MIA orchards appear not to have an economically-viable future as they have few nuts, poor structure and sparse canopies. The crazy growth and consecutive bud failure periods and possibly some pruning practices, have given rise to trees with poor framework. Attempted re-budding and hard pruning have not stimulated productive new growth, but top-working with a range of buds from different sources, is worthy of further investigation.

The traceability of budsticks to specific source trees is currently not possible. It is known that one of the nurseries that provided MIA trees has, at least once, purchased Monash buds. Another of the nurseries entered the almond business for only one season, that being the one in which trees were sold to the MIA orchard inspected. A great deal of knowledge useful in bud failure risk management, could be gained from a system that allowed bud traceability.

3.4.3 Riverland and MIA regions

There are some features of the bud failure disorder that are more consistent in some regions than others. Table 3 and Figure 7 indicate the effect of the disorder on floral bud transition. Average floral transition in buds from the MIA and Riverland orchards, and Monash, are shown in Table 3. The low transition (3%) in outside buds of affected trees was particularly notable for MIA orchards. This explained the low nut loads associated with the disorder in the MIA. In the Riverland some affected trees flower well but have little leaf area to support developing nuts.

Table 3: Bud transformation to floral buds May-June 2009

Region	Tree Status	% Bud transition to floral buds*	
		Outside Buds (combined)	Central Buds
Riverland	Affected	47%	5%
Riverland	Non affected	68%	5%
MIA	Affected	3%	1%
MIA	Non affected	58-74%	5%
Monash (Budwood repository)	not rated	71-85%	12%

* Averaged across orchards in the region that submitted budsticks

General comparisons of bud status between Riverland (Figures 8, 9), MIA (Figures 10, 11) and Monash (Figures 12, 13) orchards, are summarised below.

- MIA affected trees had extensive damage (<50% + >50% + dead) in both outside (30%) and central (54%) buds, by April
- Damage levels increased in MIA outside buds (to 54%) and central buds (to 67%), by August
- Riverland affected trees had damage levels of 27% in outside buds and 32% in central buds by April
- Riverland affected trees maintained the outside bud damage levels (at 26%) to August, but 57% of central buds were damaged by August (approx same as MIA)
- Riverland (37%) and MIA (30%) trees each had low levels of healthy central buds by end of winter in affected trees
- Riverland and MIA had similar levels of healthy buds in *non-affected* trees
- MIA affected trees had very few buds (2-5%) transform to floral buds

Figure 8: Riverland outside bud status during season

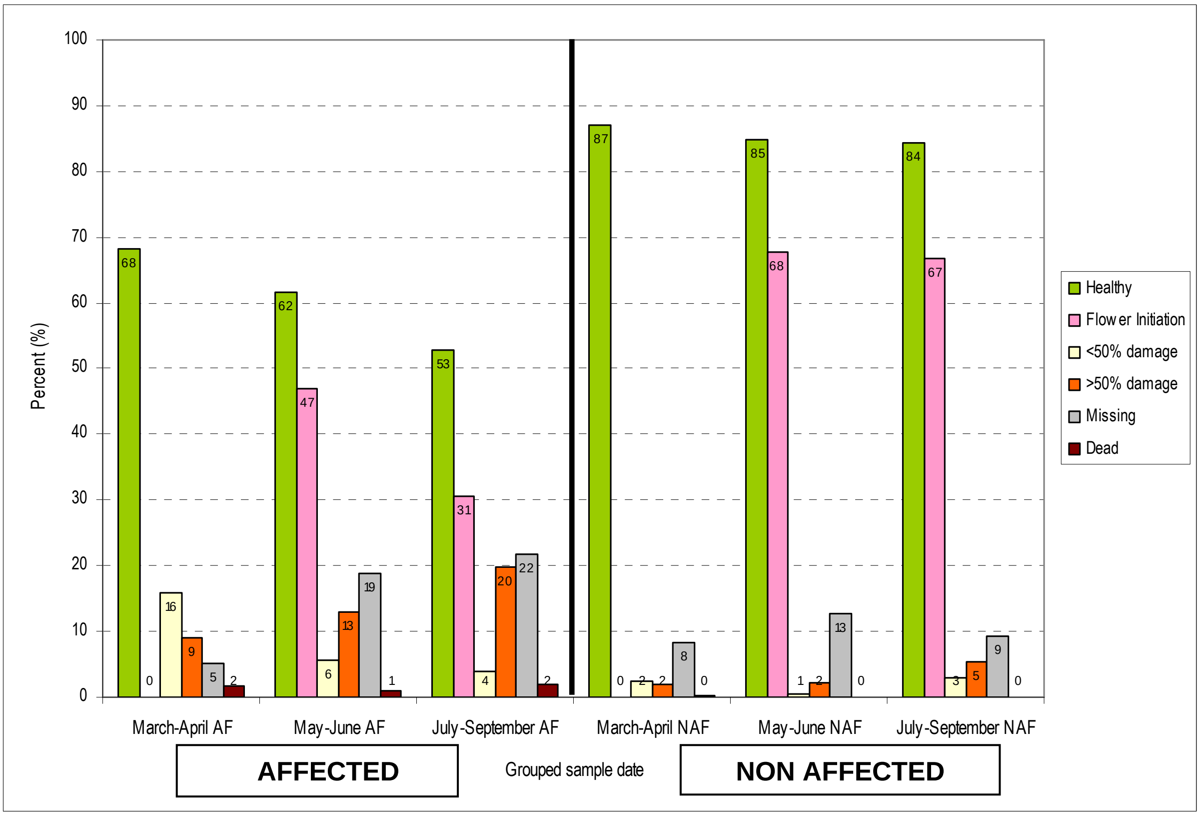


Figure 9: Riverland central bud status during season

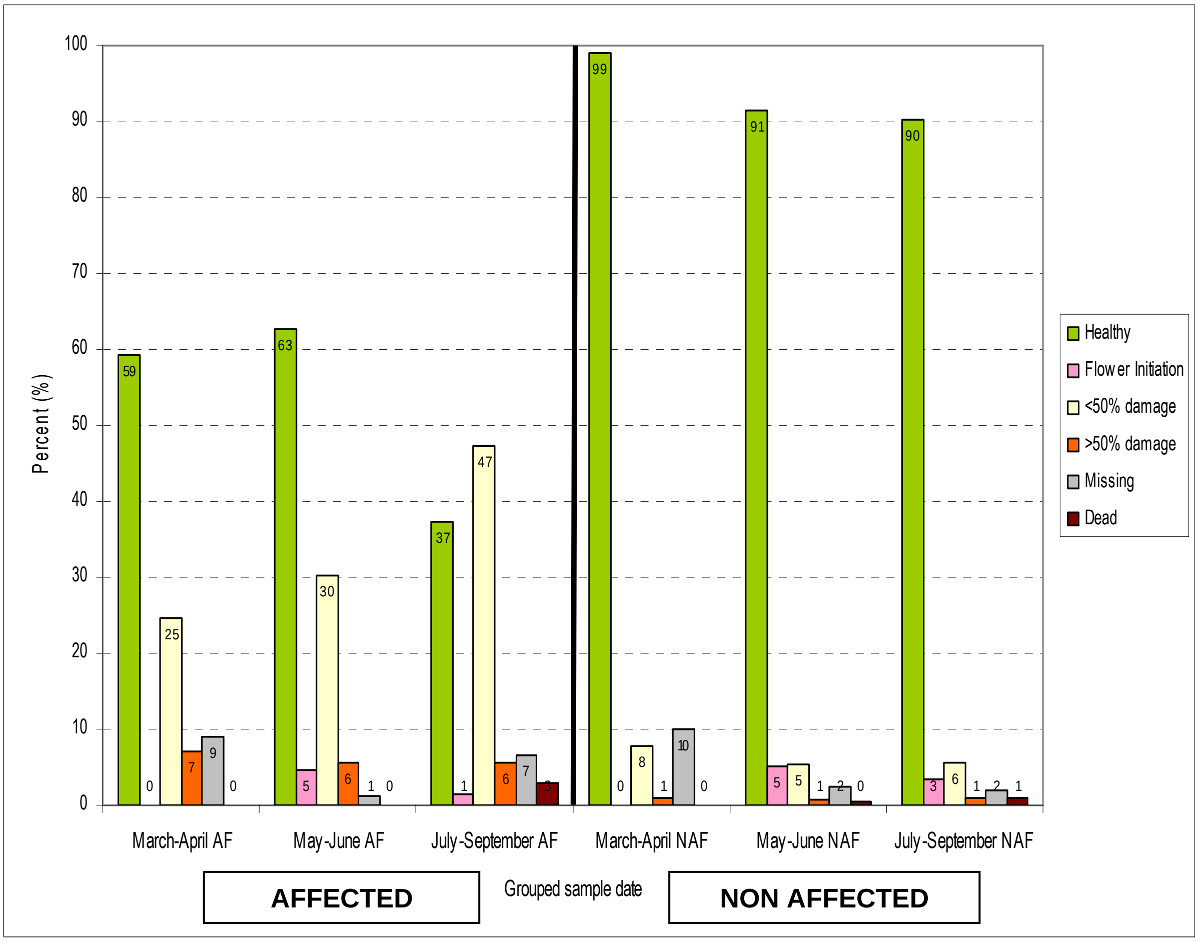
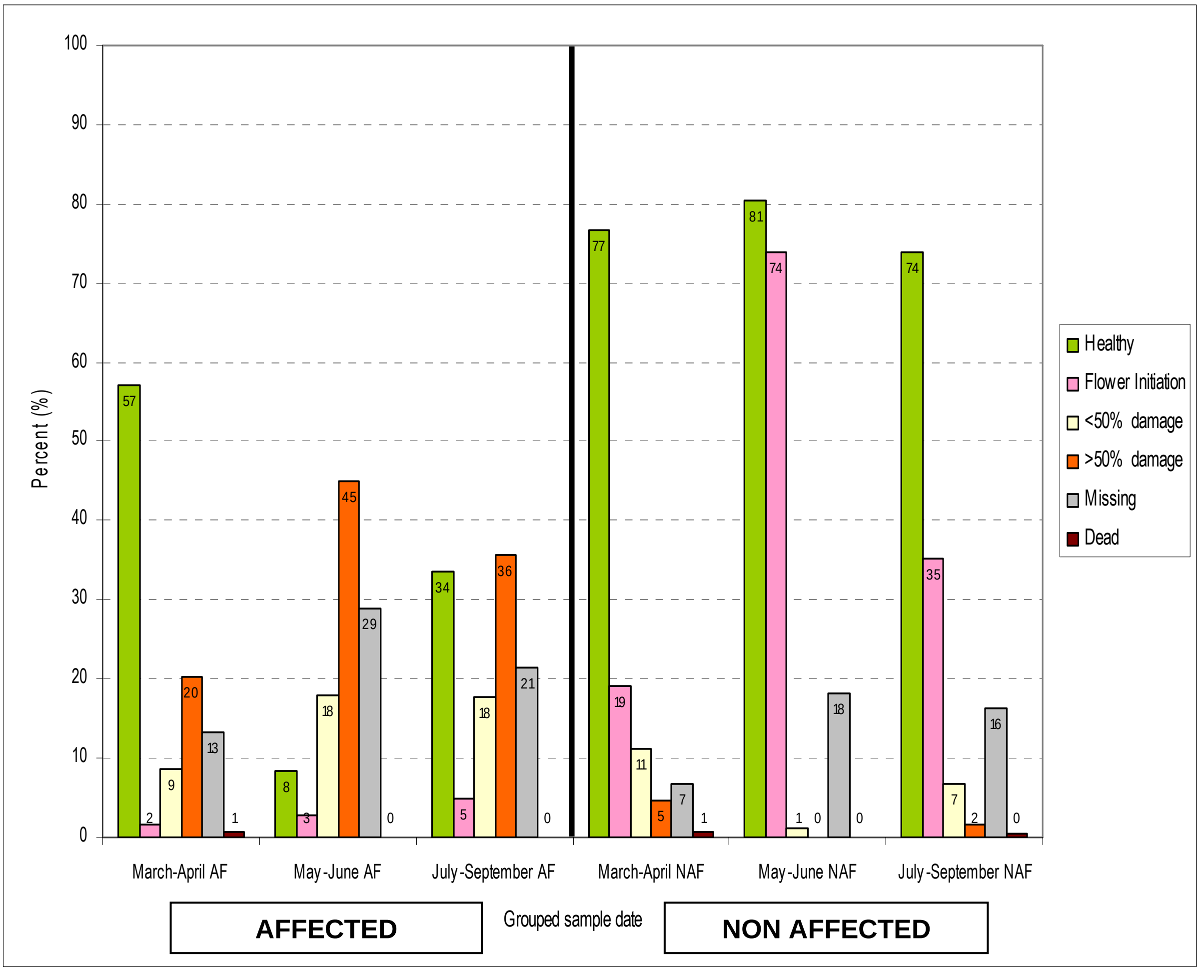


Figure 10: MIA outside bud status during season



3.4.4 Individual orchards – Monash mother trees

Some characteristics of the bud failure problem in individual orchards were notable.

In the first year of the investigation Monash trees were sampled on eight occasions. The dissections indicated the Monash trees had the highest proportion of healthy buds amongst all sampled trees, including those identified as "*non-affected*" in other orchards. Their suitability as providers of budwood appeared sound.

The Monash budwood repository trees showed consistently high percentages of healthy outside (avg. 92%) and central (avg. 97%) buds, with all values greater than 90% (Figures 12, 13). Correspondingly, they had consistently low percentages of damaged buds (<50% + >50% + dead), in every sampling period. The bud size, visual health status, and percentage that transitioned to floral buds was higher in Monash trees than any other trees from participating orchards (affected or non-affected trees), across the production regions.

The Monash trees could not be identified as 'affected' or 'non-affected' at the start of the sampling period (because their use and management as budwood trees may have masked areas of bare wood and problem flowering) but the results suggest Monash trees are not suffering the bud failure problems observed in many young Carmel trees in the Riverland and in other regions.

Figure 11: MIA Central Bud status during season

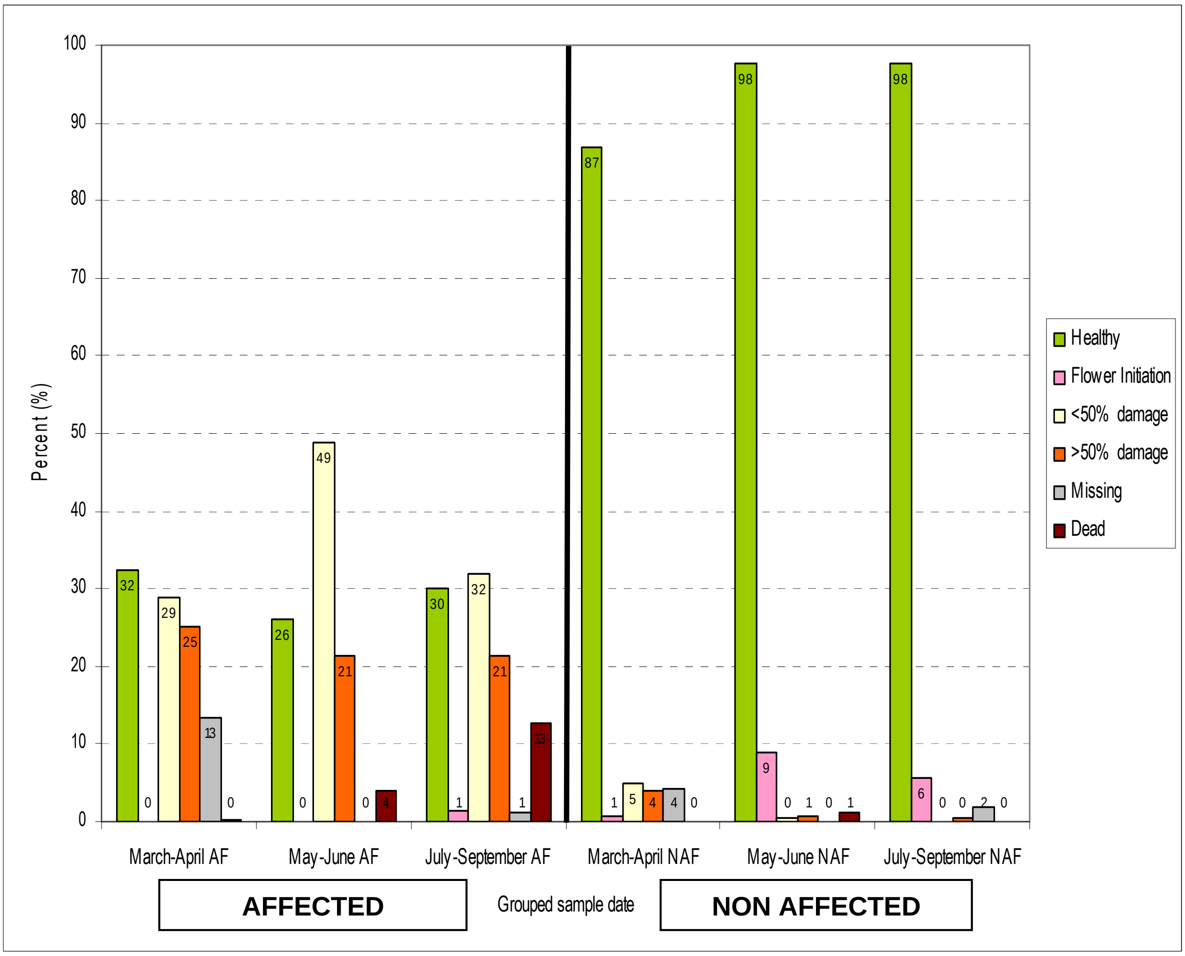


Figure 12 : Outside bud status on Monash trees March-September, 2009

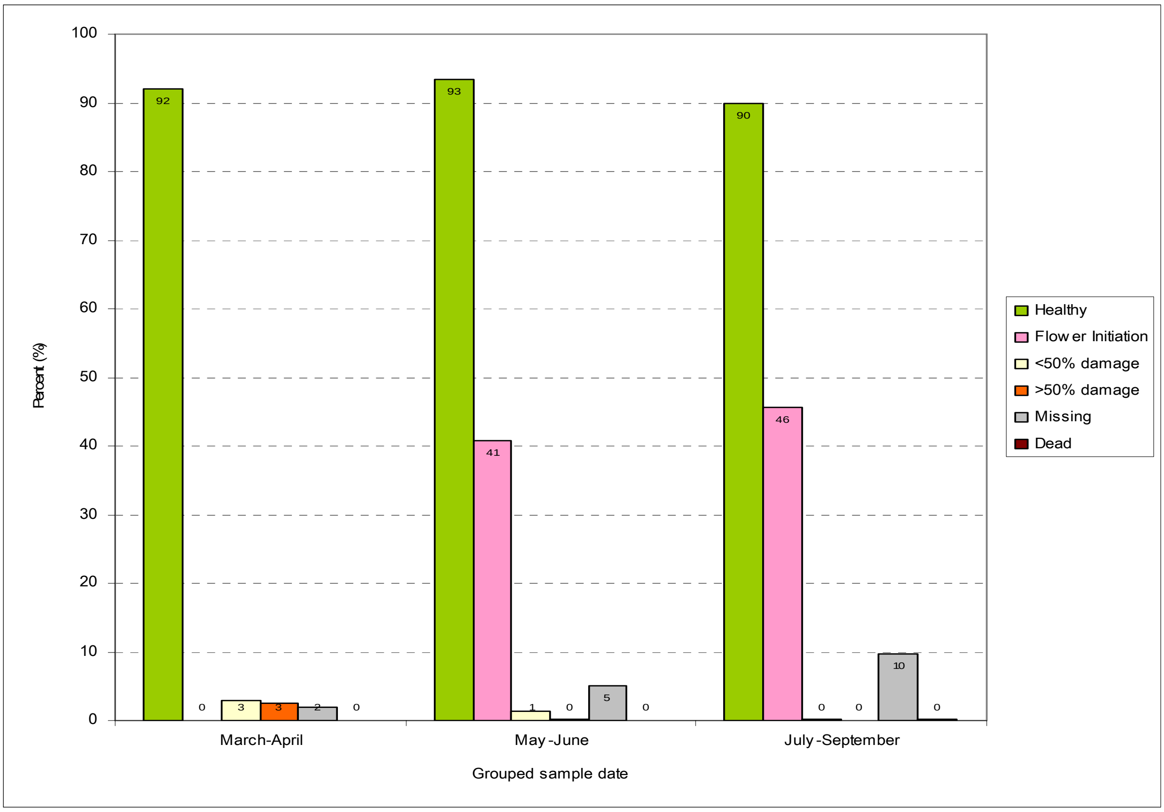
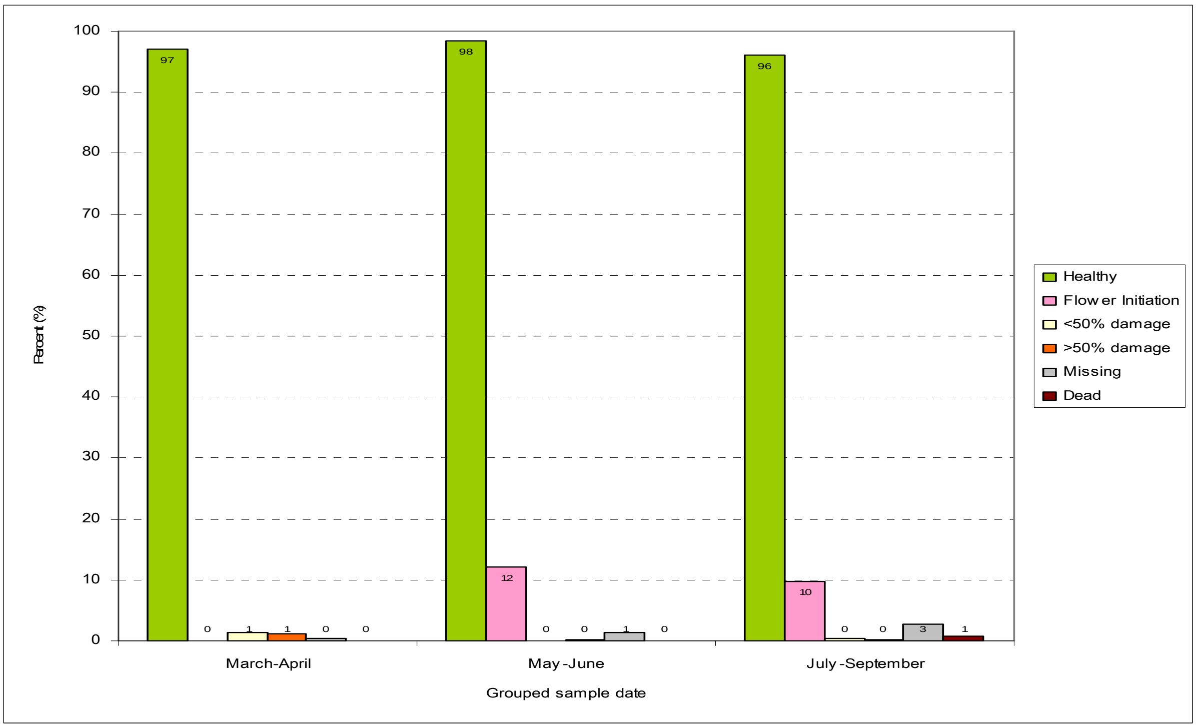


Figure 13: Central bud status on Monash trees March-September, 2009



3.4.5 Individual orchards – Riverland

Riverland Orchard 1

In this orchard, trees identified as *affected* in 2008 continued to display bud failure through 2009. Trees were planted in 2005 and in 2003/04, and in both plantings the proportion of the canopies that are unproductive, are increasing. The percentage of bare canopy in affected trees ranged from 30-50% in September 2009. At this time there appeared to be no trees previously identified as 'non-affected' that were showing new signs of bud failure (Figures 14, 15).

Levels of damage in affected buds when compared with those for Monash trees (same production district) were higher in this orchard in both outside and central buds. By the end of winter, only 13% of Orchard 1 central buds appeared healthy, whereas the central buds from non-affected trees had maintained good health in 87% of buds. These central bud observations were reflected in the poor leaf out. The bare canopy however did support some nuts as was predicted from the high level of floral transition in both outside buds.

Figure 14: Outside bud status in Riverland Orchard 1

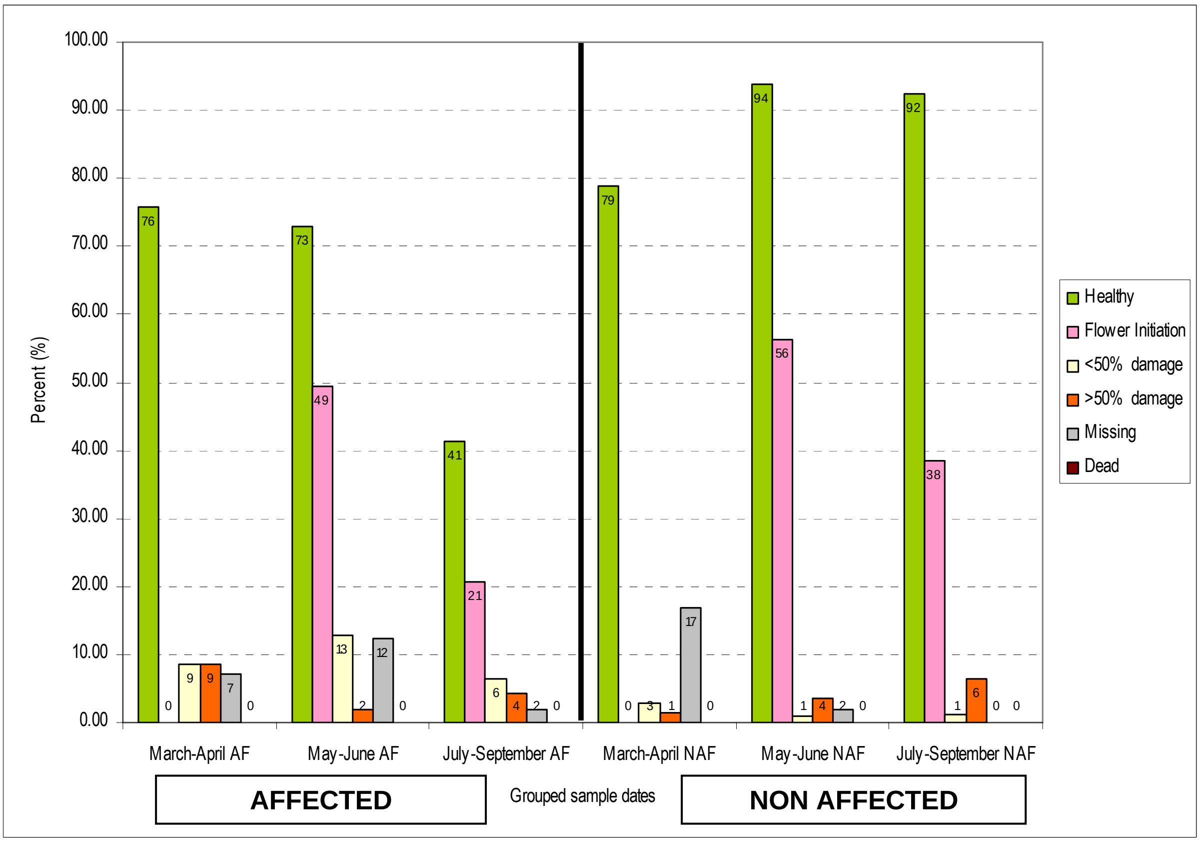
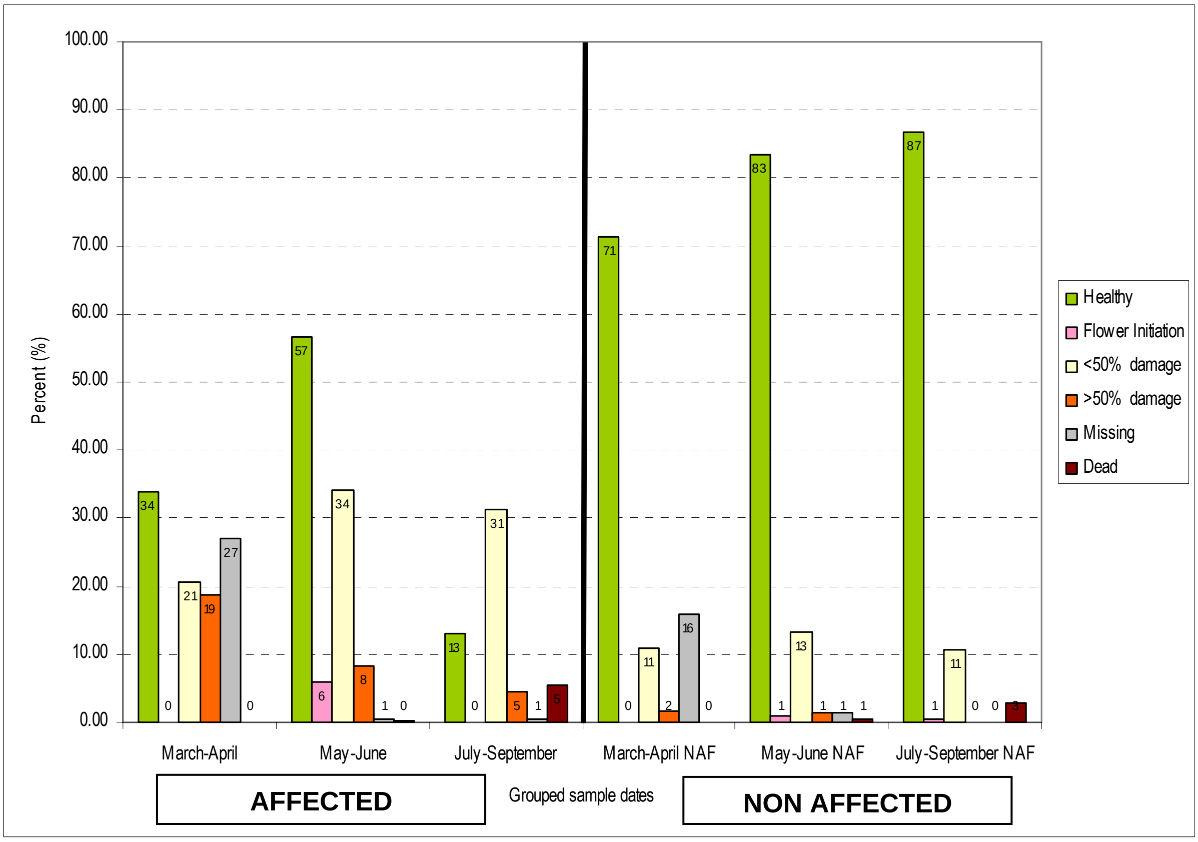


Figure 15: Central bud symptom development in Riverland Orchard 1



Riverland Orchard 2 – Season 1

The sampling of budsticks from this orchard continued for two seasons. The first period saw buds sampled from March-September 2009 (Figures 16, 17) and the second stage followed buds from December 2009-March 2010 (Figures 18, 19).

Throughout the first sampling period, the third-leaf trees identified as *affected* in 2008 continued to display bud failure in dissections and in spring leaf out. The proportion of bare wood in canopies in spring 2009 ranged from 40-60%. The affected trees had variable nut loads from negligible to moderate. In affected trees, fewer than 50% of outside buds transitioned to floral buds.

Some *non-affected* trees however had an exceptionally heavy blossom in spring 2009 with many flowers dropped. This was predicted from the dissections which found a high percentage (75-82%) of outside buds transformed to floral buds. The heavy flowering may have had an additional impact on leaf out as this was delayed, but not ultimately reduced, in non-affected trees. There was no evidence in spring 2009 of 'new' bud failure onset in previously non-affected trees. Data from the sampled trees confirmed March results were reliable in predicting potential leaf out and nut load in spring (Figures 16, 17).

When compared with other Riverland orchards in 2009, the affected trees in Orchard 2 had fewer healthy outside buds, but more healthy central buds. The comparison with Monash trees (Figures 12, 13) revealed this orchard had higher damage levels in outside buds of both affected and non-affected trees. The Monash trees and non-affected trees in Orchard 2, had a high percentage of healthy central buds.

Figure 16: Outside bud symptom development in Riverland Orchard 2 – Season 2009

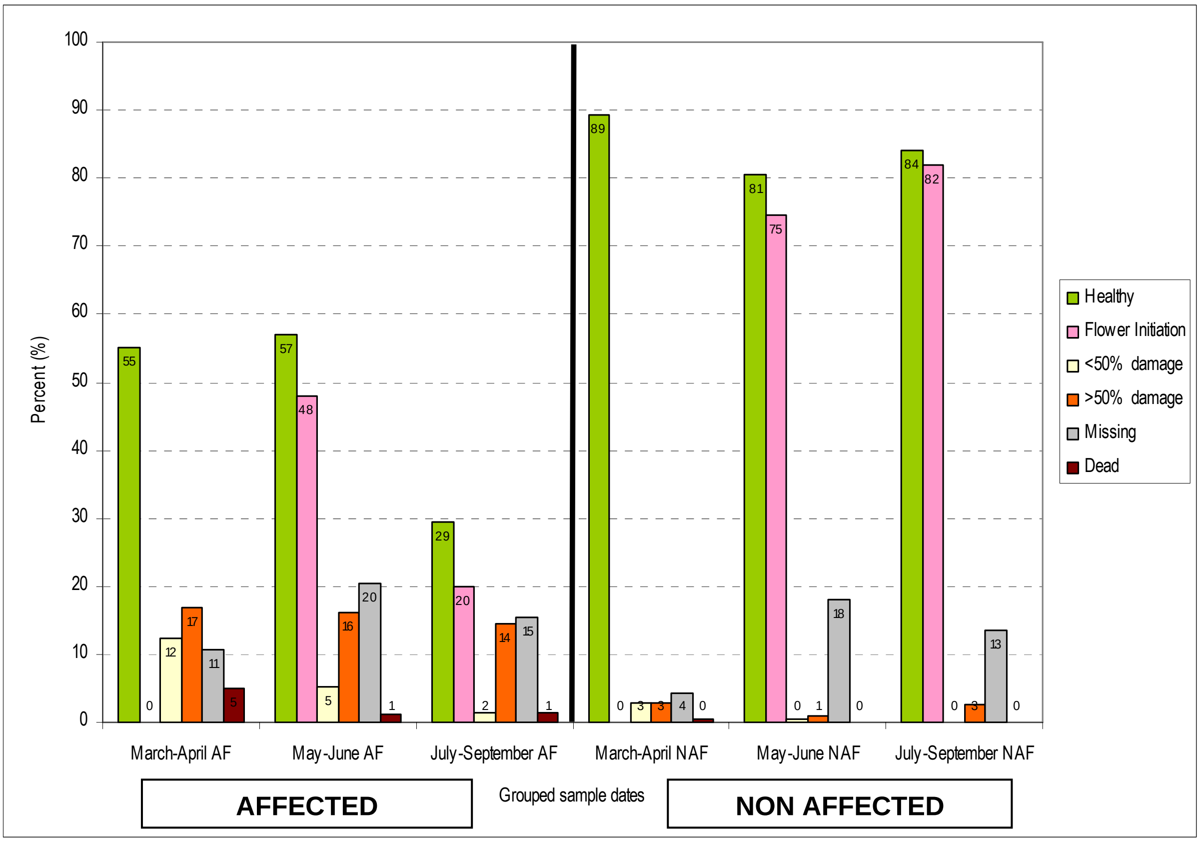
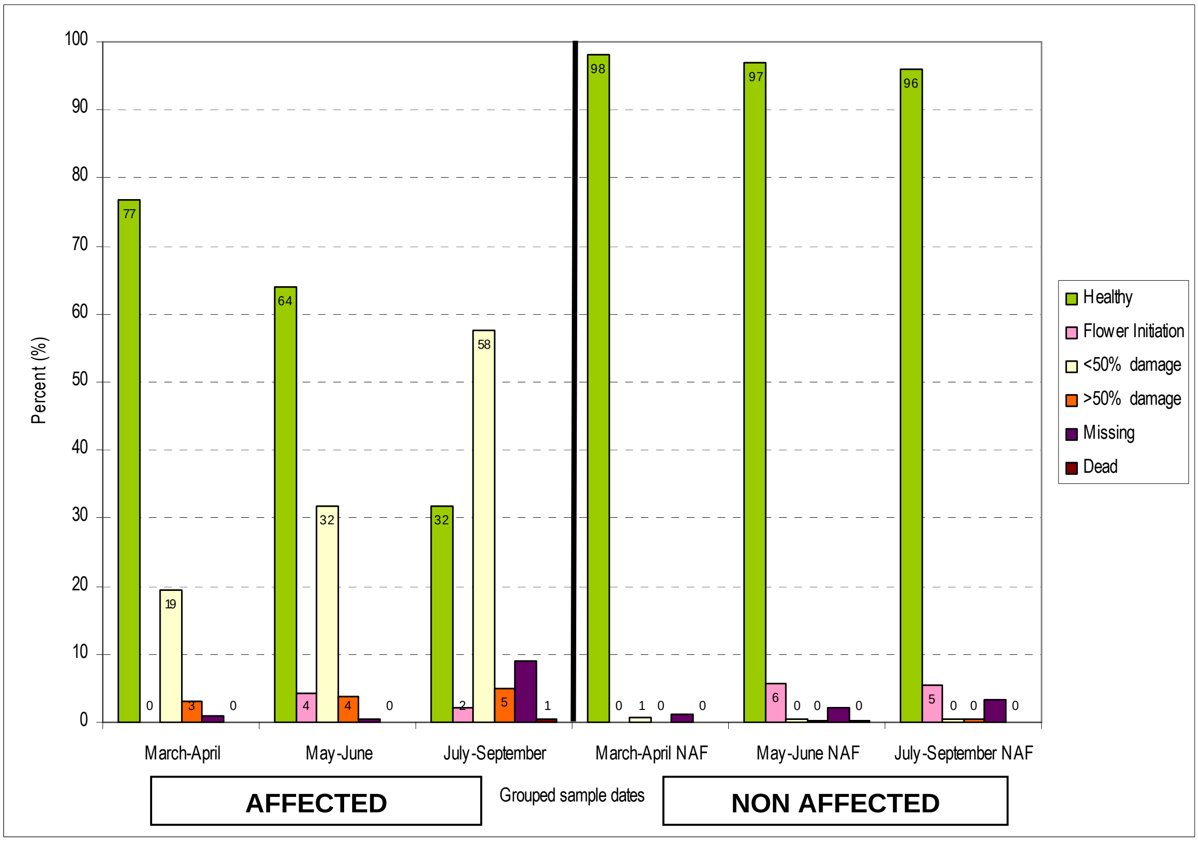


Figure 17: Central bud symptom development in Riverland Orchard 2 – Season 2009



Riverland Orchard 2 – Season 2

The sampling from this orchard continued from December 2009 through April 2010. In December-January, buds were very small and the nuts of the current season were not yet mature. The outside buds in both affected and non-affected trees appeared healthy in this orchard in December-January 2010 (Figure 18). However in February-March damage levels increased in the affected trees to reveal only 31% healthy buds by March 2010. In non-affected trees, damage also increased but 71% of buds remained of healthy appearance. In non-affected trees, central buds had no apparent damage from December-February.

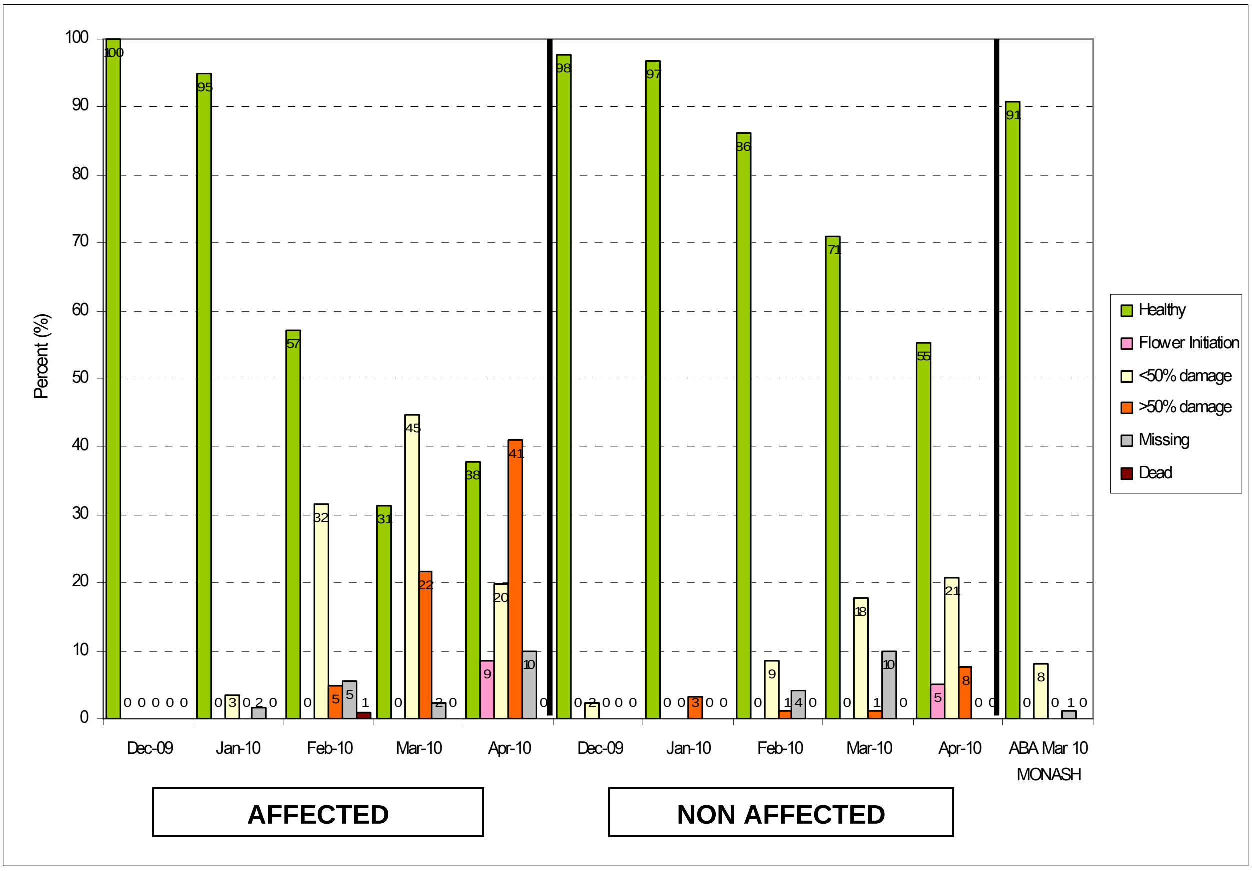
Sampling of Monash buds over the same period showed those trees to have 91% healthy outside buds. By March 2010 sampling of the Monash trees showed no major damage and 95% of buds to be healthy (Figure 12, 13).

In Orchard 2, the bud dissections revealed some central bud damage, by December (Figure 19). In affected trees, the majority of central buds (79%) remained healthy in January 2010, but by February this had declined to 23%. As such, it was predicted that leaf out in spring 2010 would be compromised. It was noted that the damage levels increased in central buds from both affected and non-affected trees. By March, only 13% of central buds from affected trees were rated as healthy, with 66% having minor damage and 13% major damage. The major damage (>50% of bud with necrosis) levels in central buds increased to 44% in affected trees, and 9% in non-affected trees, by April.

It was apparent from bud dissections that previously identified (in 2008 and 2009) "non-affected" trees had bud damage that would be reflected in leaf out problems. In September 2010, the time of leaf out inspections in the orchard, bud failure was evident in both *non-affected* and *affected* trees. Some affected trees had 80-90% of the canopy bare and non-affected trees had up to 20% bare in spring 2010.

The consecutive season sampling in this orchard supported the first year indications that March dissections provide useful indications of spring leaf out problems. Although it was possible to find damaged buds in December-January, damage levels (and the severity of damage) increase significantly in February, rendering the early dissection results to under-estimate the potential spring problems. While the disorder did not 'spread' to new buds in February, its effect within more buds became visible as they grew. The disorder progressively affects a greater proportion of buds, and therefore canopies, each year. The likely new onsets of spring bud failure in previously identified non-affected trees, was clearly indicated in bud dissections by March 2010.

Figure 18: Outside bud status in Riverland Orchard 2 and Monash – Season 2010



The decline in bud status in Orchard 2 trees from 2009 to 2010 is highlighted through comparison of data in Figures16 and 18, and Figures 17 and 19. In contrast, the stability of health status of Monash buds can be seen in Figures 12 and 13 and Figures 18 and 19. The data are summarised in Table 4. The decline in health was evident by March, in affected and non-affected trees in Orchard 2. Monash trees maintained a high level of healthy central (and outside) buds, despite their exposure to environmental conditions similar to those experienced in Orchard 2. The extent of annual growth and flowering allowed at Monash, and the proximity of Monash trees (and budsticks) to the original bud, may be key explanations.

It is presumed that the heatwave in November, 2009 (spring) alone – and/or in combination with the two previous heatwaves in March, 2008 (autumn) and January, 2009 (summer) may have triggered the new expressions of bud failure in orchard trees, in spring 2010.

Figure 19: Central bud status in Riverland Orchard 2 and Monash – Season 2010

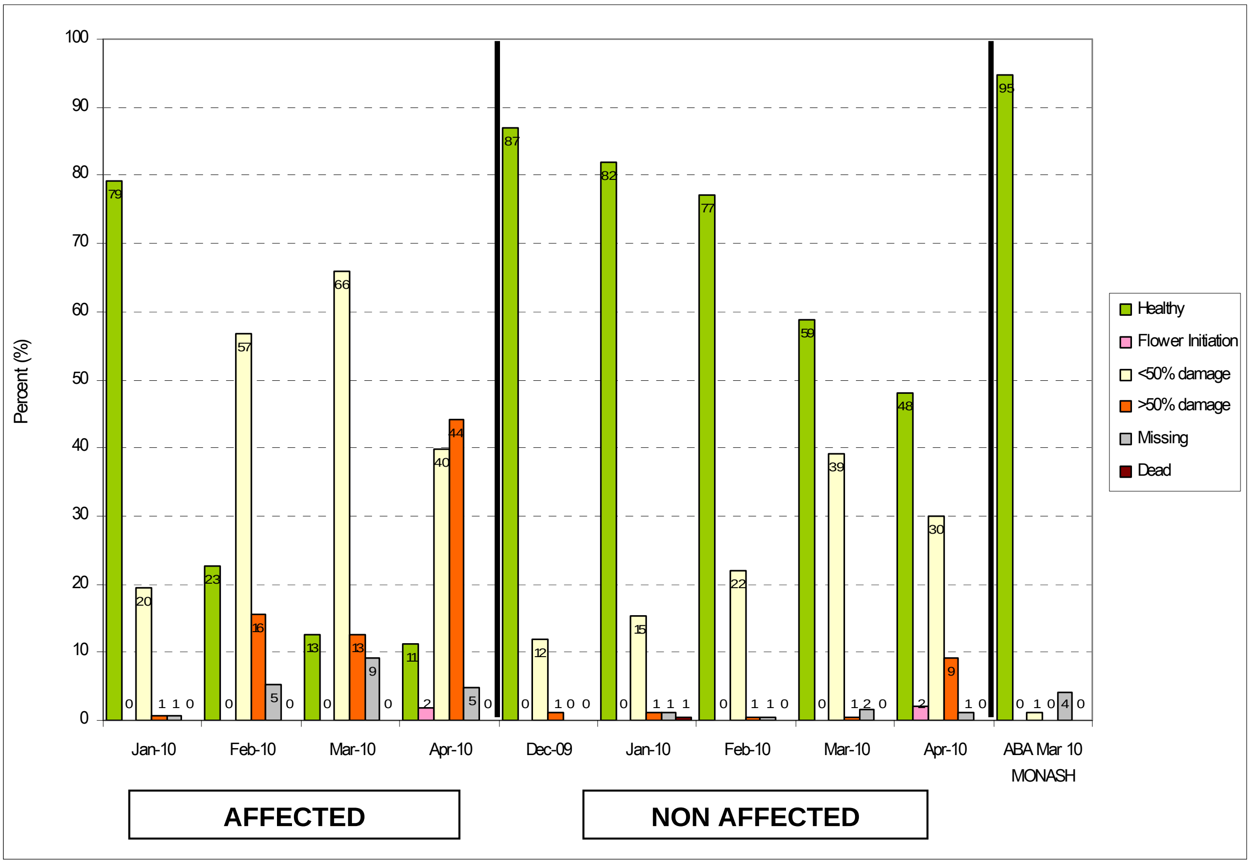


Table 4: Central bud comparison Orchard 2 and Monash, March 2009 and 2010

March 2009			
Bud status – (% buds)	Riverland Orchard 2		Monash budwood
	Affected	Non-affected	
Healthy buds	79	98	97
<50% damage	20	1	1
> 50% damage	1	0	0
Dead	0	0	0
March 2010			
Healthy buds	13	59	95
<50% damage	66	39	1
> 50% damage	13	1	0
Dead	0	0	0

Figure 20 : Bud failure 2010 Carmel

Riverland Orchard 3

In Orchard 3, the limited annual growth on the mature trees, the degree of shading and possibly water stress-caused dieback may have masked the failure of isolated buds to develop. There are however no extensive areas of bare wood on these trees today and it is unlikely these orchards have suffered typical 'bud failure'. Where some bare wood existed it was high in the canopy on these mature trees (Figures 21, 22).

In the initial sampling of Orchard 3, there was little difference in the percentage of healthy buds amongst affected and non-affected trees in the orchard. Some extensive damage (>50%) was observed in individual buds from each. Although it is unlikely the Orchard 3 trees have bud failure, comparison of their status with Monash trees still revealed they had fewer healthy buds and slightly higher damage levels.

Sampling was not continued in this orchard through 2009.

Figure 21: Outside bud status in Riverland Orchard 3

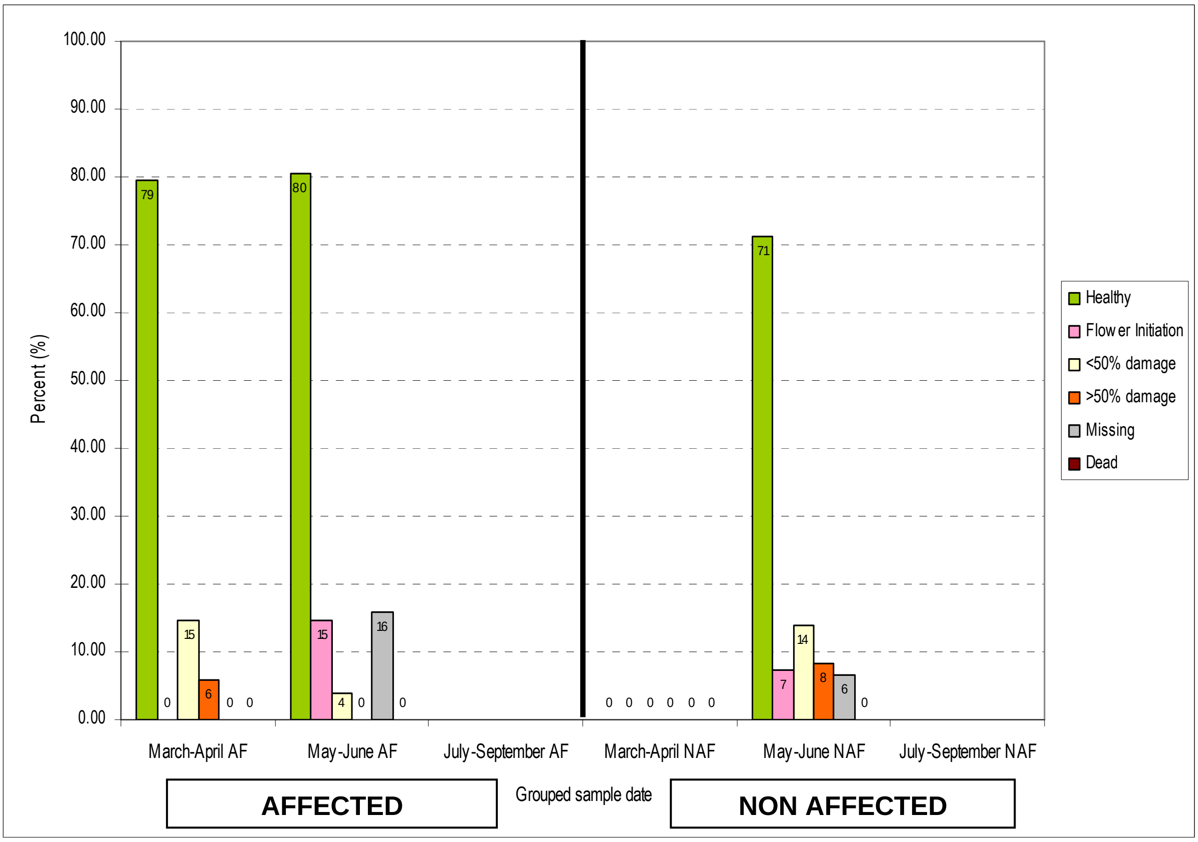
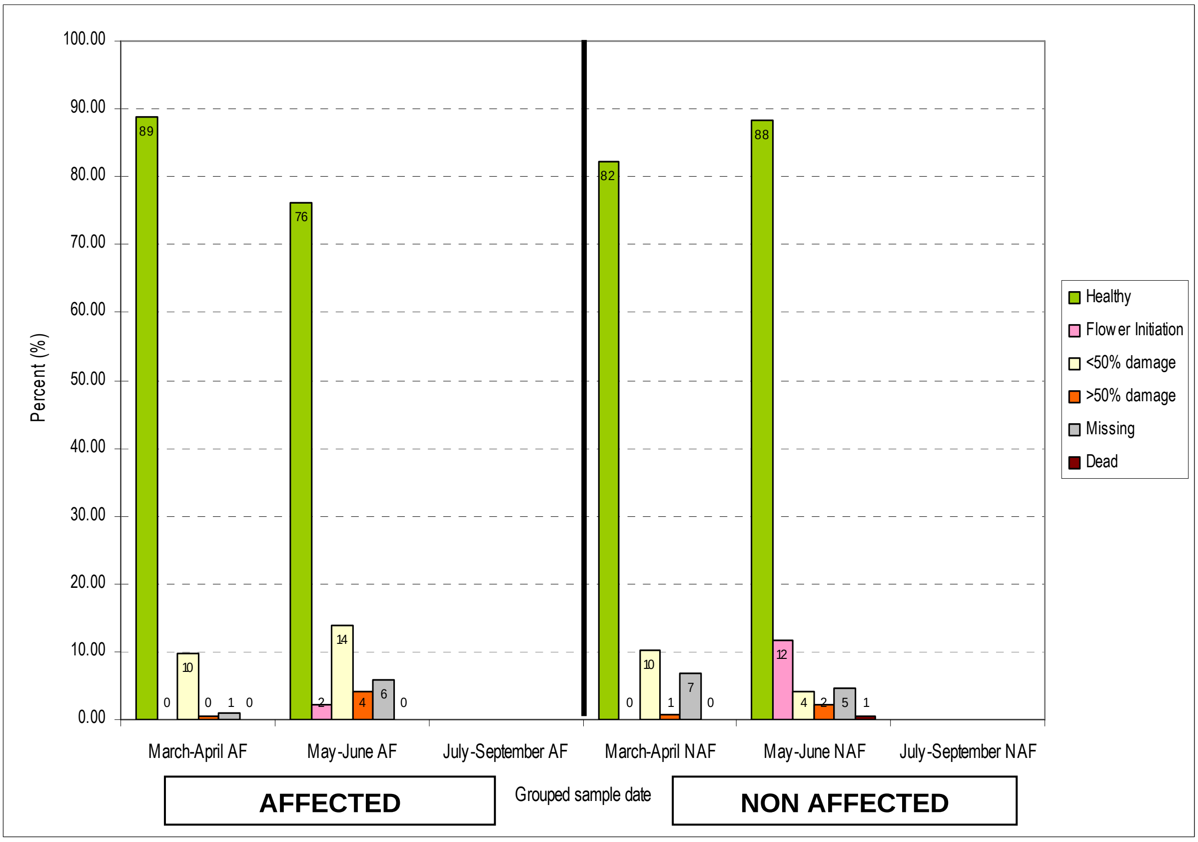


Figure 22: Central bud status in Riverland Orchard 3



3.4.6 Individual orchards – Riverina/MIA

Two orchards from the MIA were sampled. Each had serious growth problems in young Carmel trees and it appeared they had suffered abnormal pruning and early growth.

MIA Orchard 4

Dissection results for buds from affected trees were variable; but relatively consistent for those from non-affected trees. March damage levels however indicated flowering and leaf out problems were likely in affected trees. It was notable how few floral buds formed in affected trees. Also notable was the high percentage (44%) of outside buds that developed major (>50% necrosis) internal damage. Although fewer central buds had major damage, 63% displayed some minor damage. These damage levels are comparable with those found in the other MIA orchard sampled. When compared with buds from Monash trees, the percentages of healthy buds in this orchard on affected and non-affected trees, were considerably lower (Figures 12, 13 and 23, 24).

In September 2009, up to 90% of the canopy was bare in some affected trees. Many of these trees also displayed 'tiger striping'. The hot westerlies and the generally high summer and spring temperatures of the last two years have likely contributed to the extensive bud failure observed as the percentage of trees affected and the extent of their bud failure is greatest in the most westerly rows. In the two rows inspected, 33% of the trees were clearly displaying bud failure to the degree they were unlikely to be economically viable due to their low nut loads.

Despite the abnormal Carmel tree structure in many of the observed trees, there was no evidence in spring 2009 attributable to 'new' bud failure onset. Given the extreme heat in November 2009 this is unlikely to be the case in spring 2010. Field inspections during leaf out 2010 however have not been undertaken to confirm this prediction.

Figure 23: Outside bud status in MIA Orchard 4

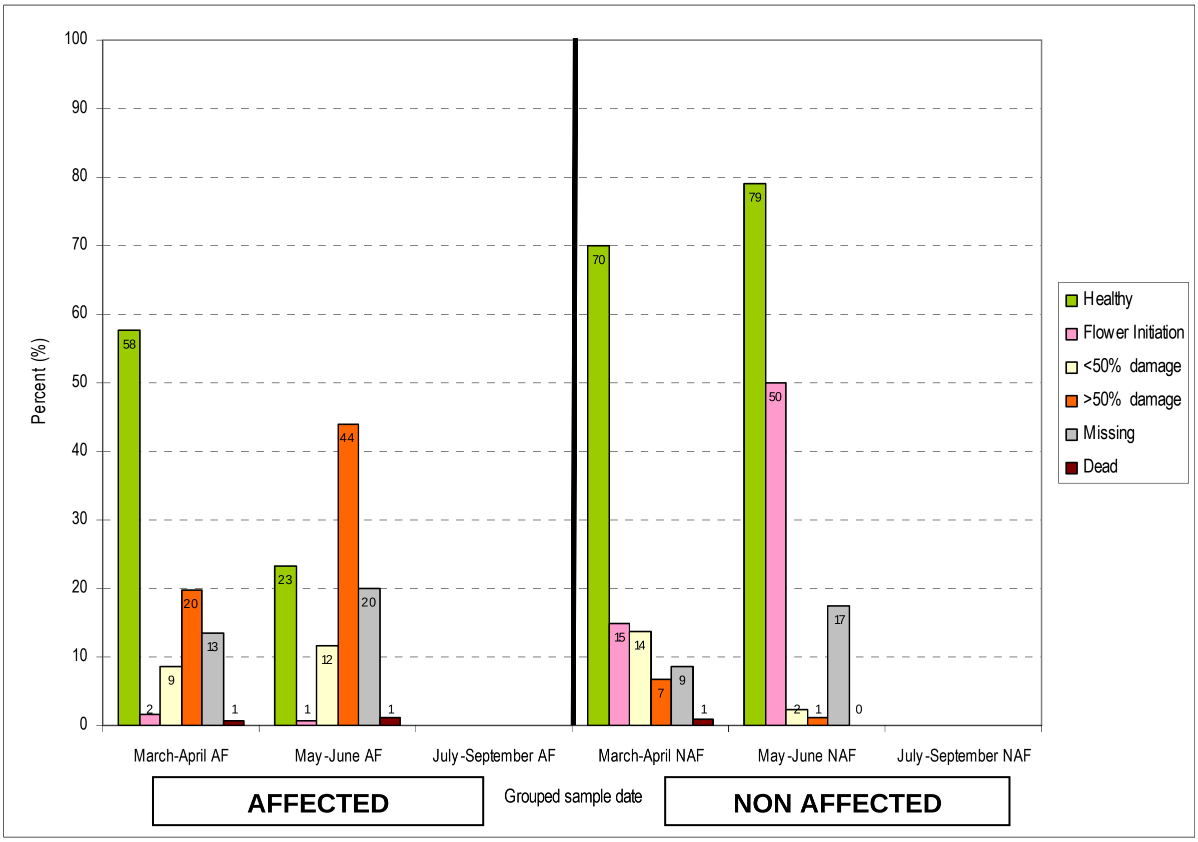
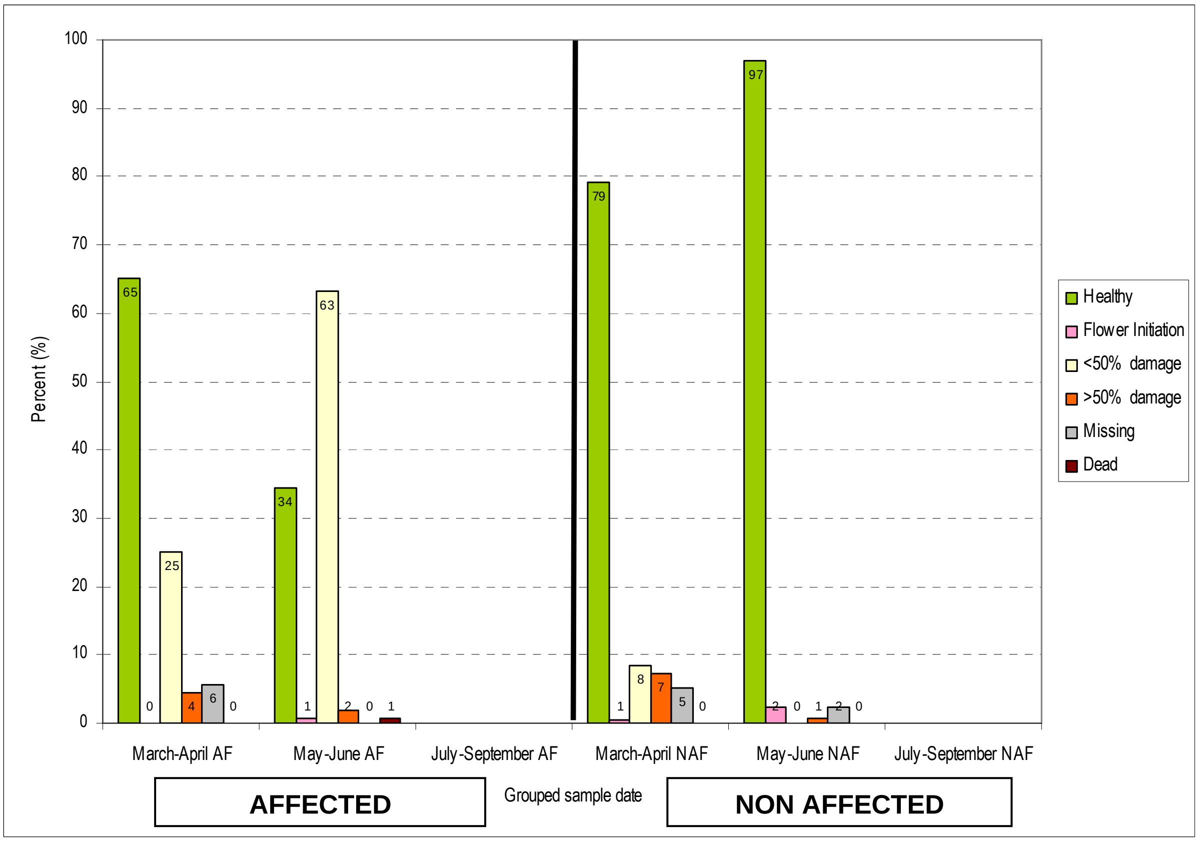


Figure 24: Central bud status in MIA Orchard 4



MIA Orchard 5

The second and third leaf trees in this orchard identified as *affected* in 2008, continued to display bud failure in 2009. An increased proportion of their canopies were unproductive both in vegetative (leaf) growth and yield (Figures 25, 26). In many trees the proportion of bare canopy in September 2009 was up to 70%. Many of the trees also displayed ‘tiger striping’ which appears to be a consistent symptom of the disorder in the Riverina, but is not consistently associated with affected trees in other production districts in Australia.

The data from dissections were variable across the three grouped sampling periods, but missing buds proved problematic and transit conditions may account for some of them. The number of single buds at nodes was also notable, as was the early, extensive damage seen in buds from affected trees.

There were significant differences in the formation of floral buds. In outside buds on affected trees only 6% transformed to floral buds, while 54% buds from non-affected trees made that transition. This explains the low productivity of many of the affected trees, and perhaps the cumulative impact of several previous seasons (2006 and 2007) of high summer and autumn temperatures.

There was no evidence from leaf out observations in spring 2009 that ‘new’ bud failure had developed in previously non-affected trees. The unseasonal spring 2009 conditions (heatwave November 2009) however were predicted to reduce bud health in both affected and non-affected orchard trees in the region. A 2010 orchard inspection was not undertaken, but there have been widespread reports of severe and new bud failure onsets in the MIA, in spring 2010.

When compared in 2009, with the Monash trees, the affected trees in Orchard 5 had far fewer healthy buds and significantly higher damage levels. In non-affected trees, the Monash and Orchard 5 results were similar (Figures 12, 13, 25, 26), but this may have changed in 2010.

Figure 25: Outside bud status in MIA Orchard 5

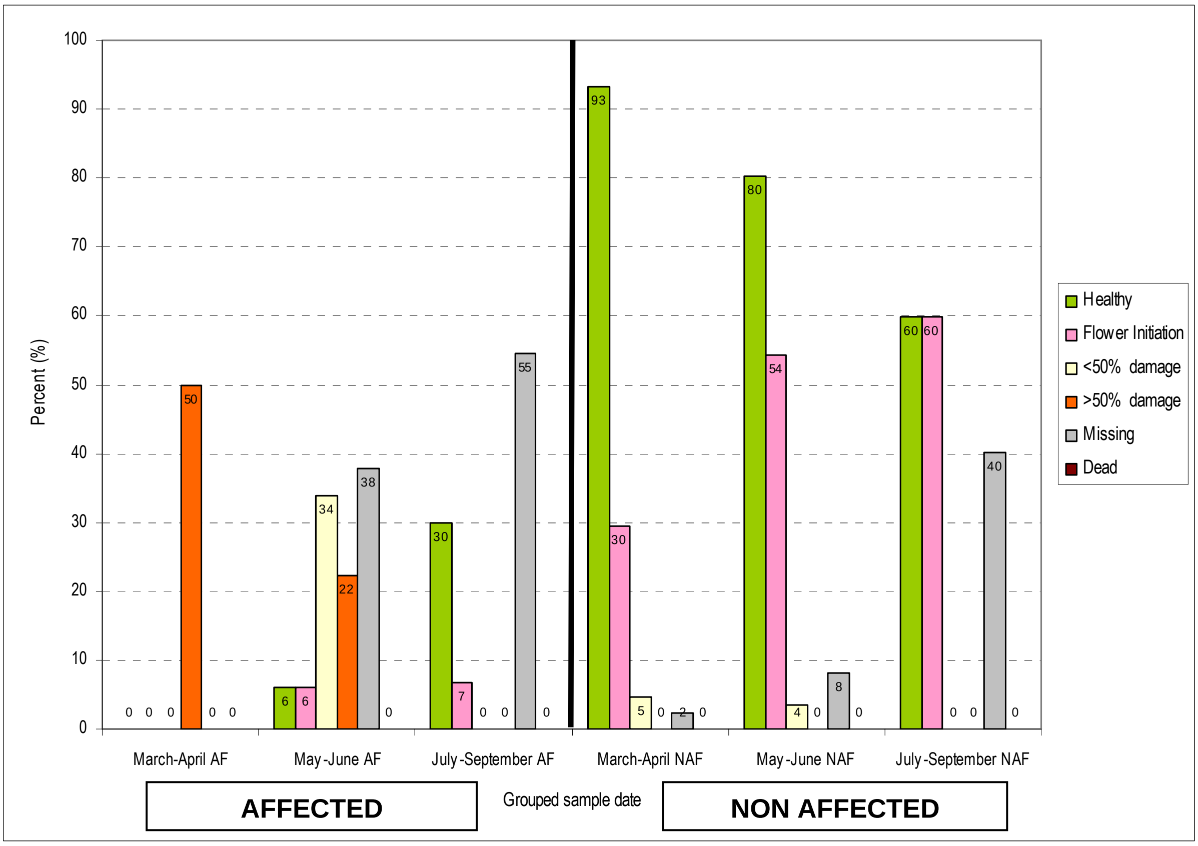
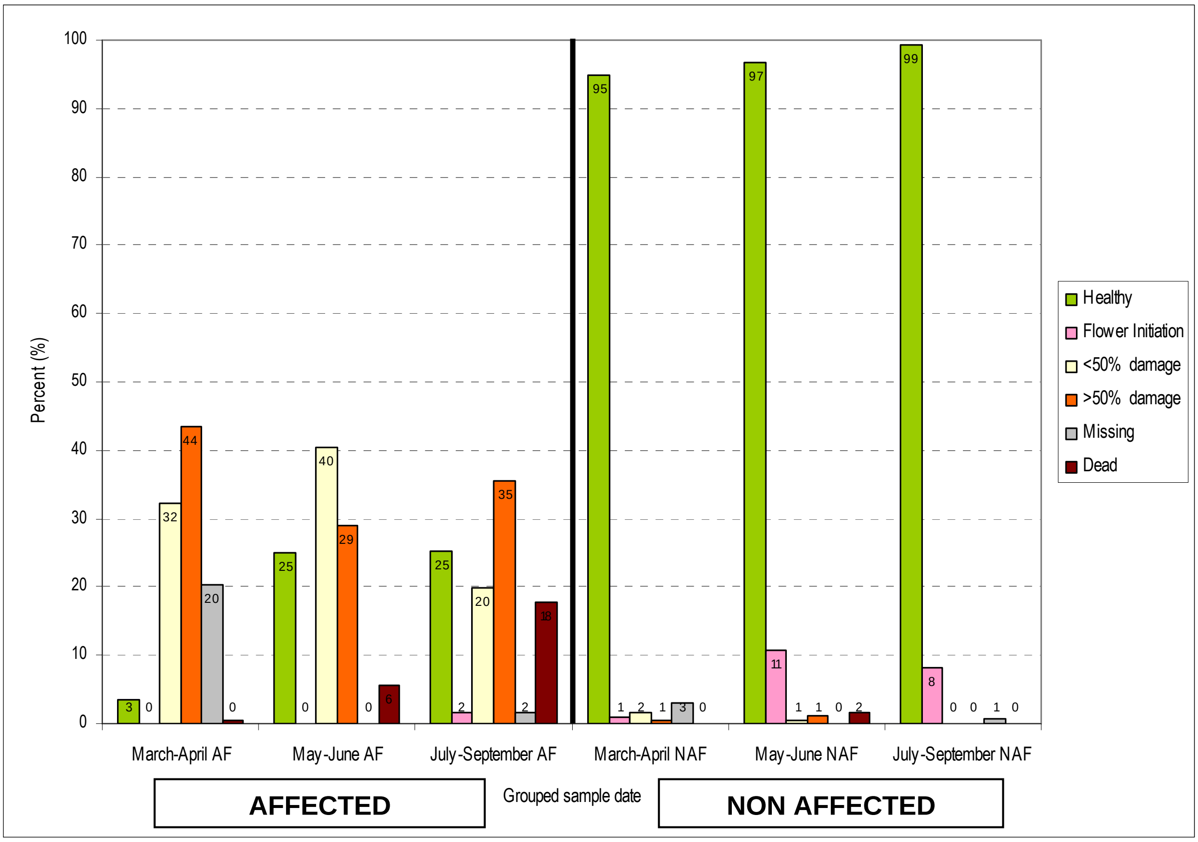


Figure 26: Central bud status in MIA Orchard 5



3.5 Orchard and bud failure summary

3.5.1 Bud health 2008/09 – Year 1

- Carmel is the only variety displaying widespread bud failure
- Young Carmel trees are more severely affected than mature trees
- Basal buds on otherwise bare wood, remain viable but produce laterals with a high proportion of damaged and unproductive buds
- Damaged vegetative buds do not leaf out
- Canopy sparseness is progressive because affected wood gives rise to affected buds that do not leaf out
- Once damaged, vegetative buds remain at an under-developed size and shape
- A higher proportion of damaged buds are central buds (especially on affected wood), than outside buds
- A higher percentage of floral buds (than vegetative buds) remain viable along the length of affected wood
- Affected wood has higher percentages of damaged floral and vegetative buds, than non-affected wood
- The negative correlation of early bud damage and leaf out the following spring is strong
- Spring vegetative bud emergence may be predicted by March (autumn), soon after harvest in the previous season
- Most terminal buds do not appear damaged

- Some terminal buds produce tufted spring growth
- Many young, affected trees in the MIA have up to 90% of canopy bare, but do not die
- Shoots in affected trees are neither wilted nor lacking in vigour
- Young affected MIA trees appear to have an abnormal framework, and compromised growth over at least three seasons
- Some young affected MIA trees have been exposed to consecutive and abnormally-hot growing seasons since they were planted
- Rough bark and striations in two+-year old wood is widespread in MIA's affected trees
- Hard pruning has not stimulated leaf out, or increased fruitful wood in affected trees
- Many affected trees do not appear economically viable
- Monash budwood has the highest percentage of undamaged buds
- Monash buds are larger than buds from any other orchard inspected
- Monash trees appear not to have bud failure
- Mature trees inspected had a negligible proportion of unproductive canopy
- In mature trees, factors other than bud failure (drought, shading, hull rot dieback) are more likely to have caused the minor stretches of bare wood observed from 2007-2009
- Bud history is incomplete in most orchards and the source of bud failure cannot be traced

3.5.2 Bud health 2009/10 - Year 2 (Orchard 2)

- Some *non-affected* trees (in 2008) had damaged buds and poor leaf out, in 2010
- Bud problems may be detected in dissections by early summer, but autumn dissections predict more reliably, bud emergence in the next spring
- Widespread Carmel bud failure was reported in September (spring) 2010
- The extreme November 2009 heatwave likely triggered the 'new onset' bud failure observed in spring 2010 (that was predicted from the December-March bud dissections)
- Spring/early summer heat affects more vegetative buds than floral buds.

3.6 Environmental conditions and bud development

The co-operating orchards in this project are located in production districts that have endured heatwaves in consecutive seasons – March (autumn) 2008, January (summer) 2009 and November (spring) 2009. Some young (fourth leaf and younger) orchards have been exposed to periods of extreme temperatures, in each of their growing seasons since planting. Some affected trees had by spring 2009 and 2010, up to 90% bare wood in the canopy.

3.6.1 High temperatures and bud development

Weather events, especially high temperatures during bud formation periods, directly affect bud development. NBF of Carmel in California is triggered in genetically-predisposed trees by high temperatures, especially those in May/June (USA). Specific data on the temperature contributions to NBF in California, as published in Fig 12.1 in Kester and Gradziel (1996), have been considered. This reference however does not identify either the data source/s or relevant time periods and it is unclear if the data are from a single Californian season and location, or if they are a 'representative' average from a number of NBF areas and seasons.

Of significance in the Australian situation may be the extreme temperatures themselves, duration of exposure and/or consecutive exposures during critical bud development periods, in conjunction with tree genetics. Our investigations have not included genetic analyses or water stress analyses, in association with the temperature review.

The review of temperatures in this project was initially focussed on comparisons of accumulated degree days (DD) over 27°C (Rubatsky and Yamaguchi, 1997) in three regions, with the 5-year and 10-year averages in the same regions. Consideration has also been given to extreme temperature events (days with maxima over 35°C) in the production regions centred on Griffith (NSW), Mildura (Vic) and Renmark (SA). Daily Australian weather data were sourced from the Bureau of Meteorology.

3.6.1.1 Growing season temperatures in production regions

Since 2005, in each production region there have been notable temperature events and deviations from the 5-year (2005-2009/10) or 10-year (1999/00-2009/10) averages. The production regions experienced significant heatwaves in March 2008, January 2009 and November 2009 and these abnormally hot months are clearly seen in Figures 27-29.

Australian high temperature data relevant to the growth development period in production regions are summarised in Tables 5-8 and Figures 27-29. Griffith data are representative of the Riverina/MIA region. Mildura details are representative of the Sunraysia region and Renmark is in the middle of the Riverland production area. The Californian data for the same period (as southern hemisphere equivalent months) are included in each of the figures for the purpose of comparison. Tables show notable heat periods for a region, in bold.

Griffith: The first signs of bud failure in the MIA, although not named as such, were in spring 2007 and this followed three consecutive abnormally hot periods (summer/autumn 2006 and 2007, and spring/summer 2006). In summer-autumn 2005/06, there were 330 accumulated DD > 27°C and this is comparable to the temperature profile Californian researchers believe capable of triggering NBF in susceptible cultivars. In 2006/07, Griffith also experienced conditions capable of triggering NBF. In spring-early summer, there were 186 accumulated DD > 27°C, and 301 in the summer-autumn period. The short-term averages for the region in these periods are 156 and 281 DD respectively. The long-term averages are lower.

Late season heat appears to be a feature of the Griffith region more often than in other regions, and this might explain the yield effects seen in affected trees in the MIA. The November heatwave in 2009 was extreme for the period and preceded widespread bud failure in spring 2010 (Table 5, Figure 27)

Mildura: The 5-year and 10-year averages for accumulated DD > 27°C in the Mildura region are 151 and 141 respectively for the early part of the bud development season (October-December). Extreme early season heat was experienced in this area in recent seasons 2007 and 2009. Later season extreme heat occurred in 2006 (Table 5, Figure 28).

Renmark: The summer-autumn period of 2006 (284 DD>27°C) was abnormally hot and was followed by three warm-extreme spring/early summers in 2006 (175 DD), 2007 (193 DD) and 2009 (210 DD). These temperatures are comparable with those known in California to trigger NBF. Leaf out problems observed in the Riverland, reflect this. Historical (5-year and 10-year) averages for the early part of the growing season are 166 and 153 accumulated DD > 27°C, respectively. These are higher than the spring/early summer averages for both Griffith and Mildura (Table 5, Figure 29). Griffith has the highest summer-autumn averages.

Table 5: Regional high temperature exposure 2005-2010

Spring-early summer								
Location		Accumulated DD >27°C (October 1- December 31)					Average	
	n/a	2005	2006	2007	2008	2009	5-yr	10-yr 2000-09
California	180							
Griffith		127	186	153	105	209	156	149
Mildura		127	158	173	97	202	151	141
Renmark		142	175	193	110	210	166	153
Summer-early autumn								
Location		Accumulated DD >27°C (January 1- March 31)					Average	
	n/a	2006	2007	2008	2009	2010	5-yr	10-yr 2001-10
California	330							
Griffith		330	301	243	280	249	281	267
Mildura		281	242	254	267	260	261	241
Renmark		284	251	266	275	270	269	249

Figure 27 : Griffith degree days >27°C for early bud development period

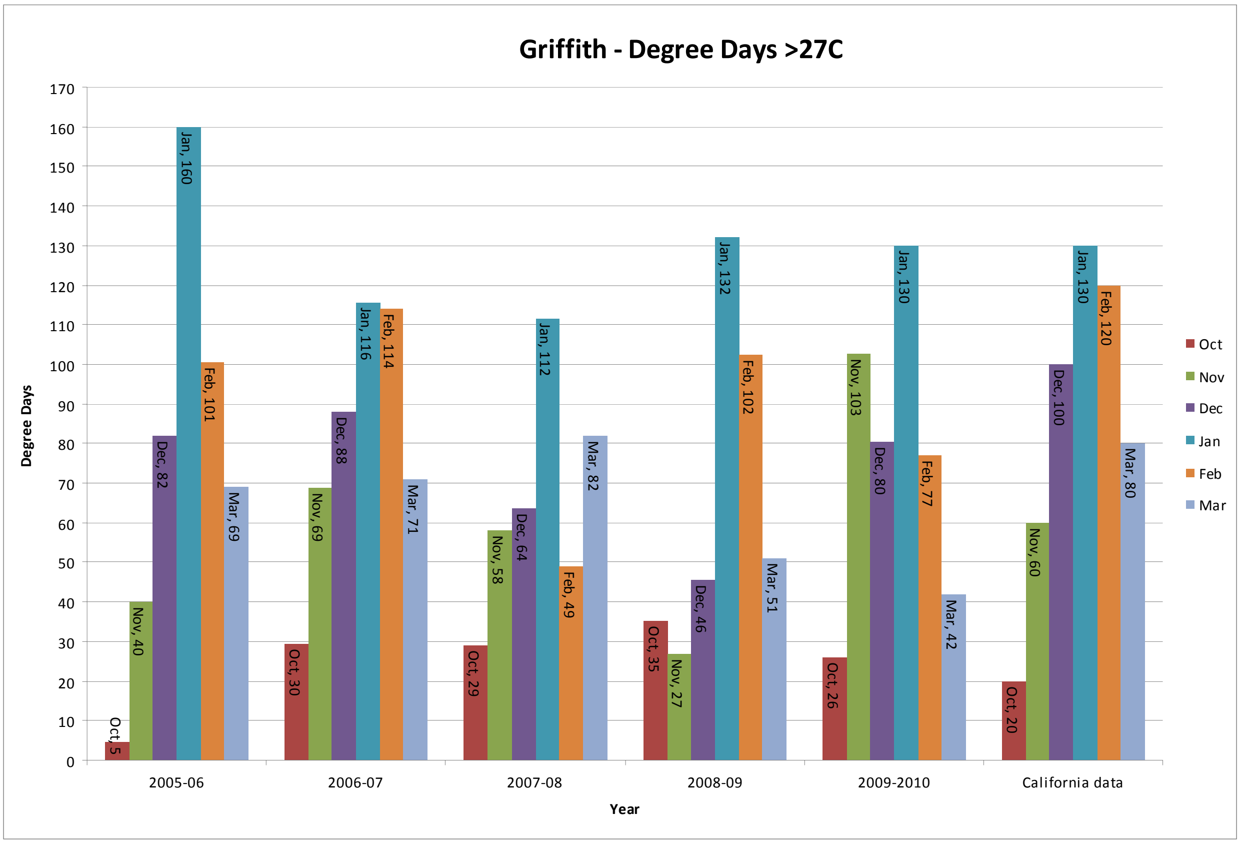


Figure 28 : Mildura degree days >27°C for early bud development period

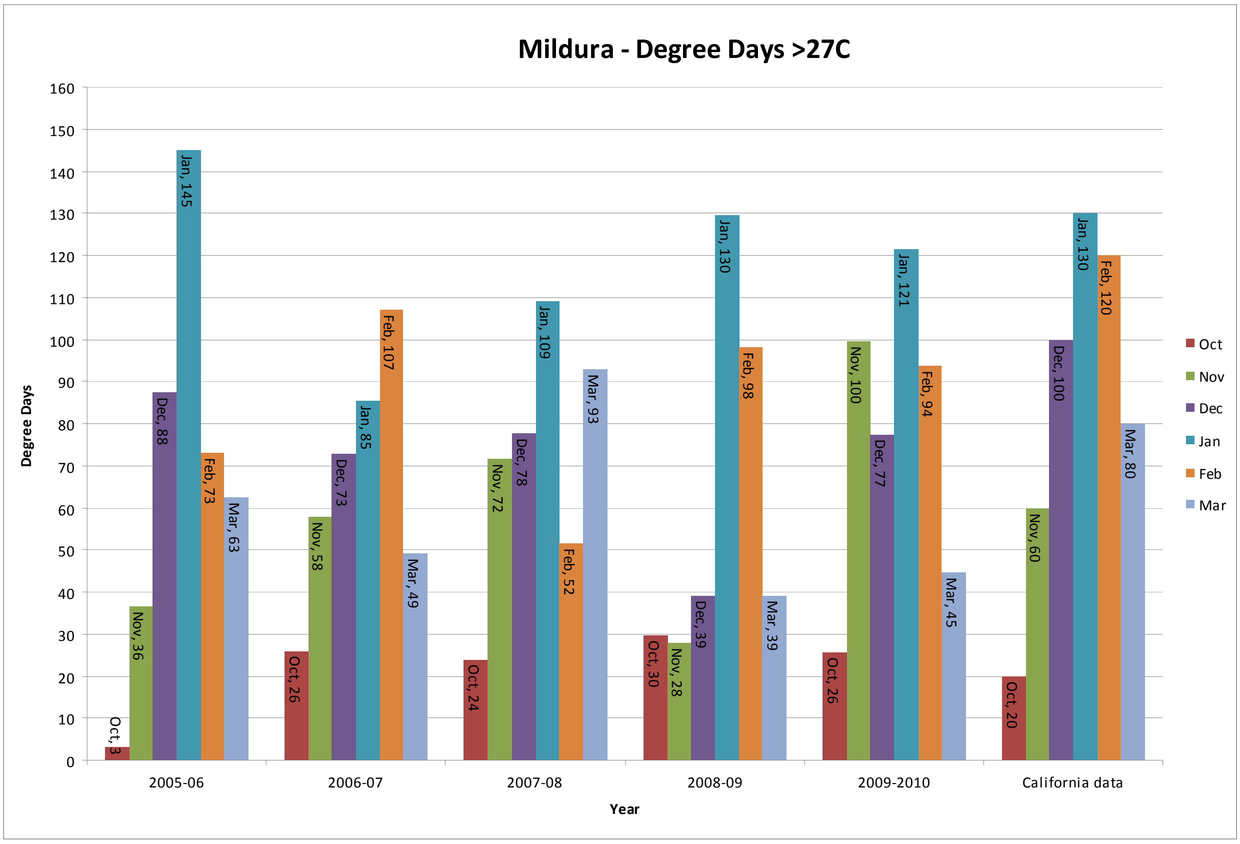
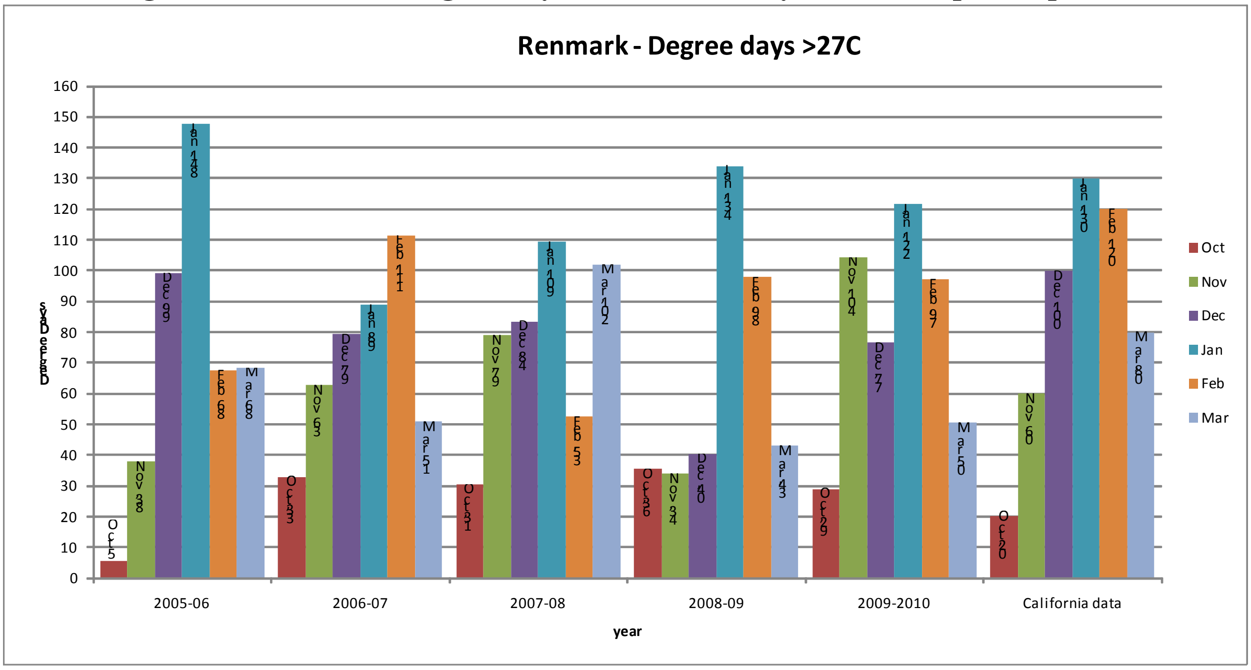


Figure 29 : Renmark degree days >27°C for early bud development period



In the 5-year period to 2005, the DD averages were lower than those for the 5-year period 2005-10 (Table 6). In only one season (2002 in Griffith and Renmark) extreme heat experienced in the early part of the bud development period. All regions however experienced a hot December and January in2000/01. In the summer/autumn part of the development period (January-March) the heat extremes in 2001 could have triggered bud failure. It is reasonable to expect that some mature trees (10+ years) have expressed bud failure but that it was not widely recognised as such at the time. The hot periods in Griffith (2001-2004) preceded most of the almond plantings in that region.

Table 6: Regional high temperature exposure 2000-2005

Spring-early summer								
Location		Accumulated DD >27°C (October 1- December 31)					Average	
	n/a	2000	2001	2002	2003	2004	5-yr	10-yr 1995-04
California	180							
Griffith		142	72	201	151	145	142	**
Mildura		145	57	165	153	130	130	121
Renmark		168	58	176	158	142	140	130
Summer-early autumn								
Location		Accumulated DD >27°C (January 1- March 31)					Average	
	n/a	2001	2002	2003	2004	2005	5-yr	10-yr 1995-05
California	330							
Griffith		303	204	256	281	226	254	**
Mildura		302	173	217	221	188	220	216
Renmark		318	181	229	224	188	228	216

The meteorological data for the period 1995-00 is incomplete but the available data suggest no extreme heat periods occurred in the regions during that time and that the 5-year averages were lower than in the subsequent 5-year periods.

Data from the review of daily maxima during the growing season (October-March) are presented in Tables 7-9. The data are presented for the early season (October–December), the summer-autumn period (January-March), and for the whole bud development period before dormancy.

Temperature maxima >27°C did not reveal significant events within or across regions, that might explain the widespread onset of the bud failure in Carmel in spring 2008 (Table 7).

Table 7: Regional high temperature daily maxima (> 27°C)

Spring-early summer							
Location		2005	2006	2007	2008	2009	5-yr average
Griffith	Average daily max (°C)	28.2	30.0	28.9	27.7	30.2	29°C
Mildura		28.0	29.1	29.2	27.4	29.4	29°C
Renmark		28.5	29.5	29.7	27.8	29.7	29°C
Griffith	No. days with max >27°C	56	63	61	54	61	59 days
Mildura		44	56	56	43	56	51 days
Renmark		53	53	59	48	57	54 days
Summer-early autumn							
Location		2006	2007	2008	2009	2010	5-yr average
Griffith	Average daily max (°C)	34.3	33.4	31.7	33.1	32.2	33°C
Mildura		32.9	31.8	31.9	32.5	32.4	32°C
Renmark		33.0	32.0	32.3	32.8	32.7	33°C
Griffith	No. days with max >27°C	85	82	73	72	78	78 days
Mildura		77	75	66	75	74	73 days
Renmark		77	75	67	80	74	75 days

For more extreme maxima (maxima 35°C+), the 10-year average of number of days in a season are similar across the regions with Mildura recording the least (38 days) and Renmark the most (42 days) (Table 8). In each region however in recent years these averages have been regularly exceeded. For the 2007/08 and 2009/10 growing seasons (October-March), Mildura and Renmark recorded significantly more extreme heat days than the long-term average for these regions. They preceded extensive expression of bud failure. The Griffith extreme maxima were more frequent in the 2005/06 and 2006/07 seasons and this may explain the earlier onset of bud failure in the MIA's young trees.

Table 8: Days with extreme maxima (> 35°C) 2005-2010

Location	Growing season days (total) with maximum >35°C					
	2005/06	2006/07	2007/08	2008/09	2009/10	10-yr avg to 2008/09
Griffith	55	58	47	36	43	41
Mildura	40	43	49	35	56	38
Renmark	45	47	57	38	57	42

Table 9 shows the seasonal timing of extreme maxima. Griffith had a significantly hotter spring than usual in 2006, as did Renmark in 2007. All regions had a high number of days with extreme maxima in spring 2009 (during the November heatwave). The hot summer/autumn of 2006 and 2007 in Griffith, and autumn of 2008 in Renmark are notable. The timing of extreme heat influences the degree of floral transition as well as bud damage and temperature data with seasonal relevance, is shown in Table 9.

Table 9: Days and seasons with extreme maxima (> 35°C) 2005-2010

Spring-early summer							
Location	Days with maximum >35°C (October 1- December 31)					Average	
	2005	2006	2007	2008	2009	5-yr	10-yr 1999-08
Griffith	12	22	16	8	21	16	13
Mildura	12	15	18	8	27	16	12
Renmark	14	19	22	9	26	18	14
Summer-early autumn							
Location	Days with maximum >35°C (January 1- March 31)					Average	
	2006	2007	2008	2009	2010	5-yr	10-yr 2000-09 2000-10
Griffith	43	36	31	28	22	32	28
Mildura	28	28	31	27	29	29	26
Renmark	31	28	35	29	31	31	28

In spring 2008, all regions recorded mild temperatures with the days exceeding 35°C maxima well below both the 5-year and 10-year averages. The heatwave of January 2009 saw a concentrated period of extreme temperatures, but across the period (January-March), neither the summer nor 'total days over 35°C' exceeded in the 5-year or 10-year averages, in any region.

The heatwave in November 2009 occurred during the period of bud development (before harvest of current nuts) and it was extreme in each region. The number of days with maxima above 35°C exceeded the 5-year spring/early summer average in Griffith by 5 days, Mildura by 11 days and Renmark by 8 days (Table 9).

3.6.2 Temperature review summary

- Almonds in each region, have been exposed during the last five seasons to more periods of extreme heat that exceed longer term averages (for days with extreme maxima, days in a season with extreme maxima, and accumulated degree days over 27°C)
- Temperature extremes have been recorded during both spring-early summer and summer-early autumn periods.

- The MIA and Riverland regions appear to have been exposed to extreme heat events, more often in recent seasons than the Sunraysia region
- MIA appears to have high temperatures in summer/autumn than other regions
- Specific, extreme high temperature exposures have preceded recent widespread expression of bud failure (spring 2008, 2010), particularly in young trees
- Temperature exposure contributes to expression and end trees and western rows usually express bud failure earlier than other trees of same age in an orchard
- Consecutive seasons of extreme and frequent high temperature exposure may explain NBF extent and severity, and/or abnormal tree structures in some young trees, in some regions
- The relative contributions of heatwaves (5 or more consecutive days), rather than total days or degree days at high temperatures, are unknown
- Temperature extremes 1995-2004/05 were less frequent, but the high temperature period in 2000/01 was capable of triggering bud failure
- Growing season 2008/09 was relatively cooler in all districts. This may explain the lack of new bud failure expression observed in spring 2009

3.6.3 Drought conditions and bud development

Although high temperatures at the time of bud initiation and development has been identified as the trigger for NBF expression, other stress such as drought, bud failure potential at planting, tree age, and annual growth influence the rate and extent of bud failure, its severity and economic impact. Water stress, especially post-harvest, is capable of disrupting bud development, as discussed in the bud development Fact sheet. As such it affects severity, rather than causes bud failure.

Water restrictions since 2007/08 have applied to most Australian almond orchards. In 2008, low volumes of water were available to many growers. The project co-operators however did not report that the young trees in our investigation had specifically suffered post-harvest water stress in 2008 or in previous seasons. The correlation therefore between observed bud failure in these trees, and water stress has not been adequately tested, and little can be concluded from available data about the contribution of post-harvest irrigation in 2007/08 to the observed widespread bud failure in spring 2008. During the heatwave in March 2008, the Riverland recorded 15 days over 35°C; Sunraysia, 14 days; and the MIA, 12 days. These temperatures may have accentuated bud failure and drought effects in orchards that had under-watered. In trees that did not have bud failure, drought stress was visible as sporadic dieback high in tree canopies, especially in mature trees. These symptoms were transitory and did not present again in 2009 or 2010. Post-harvest rain was received in all production districts in 2009 and 2010. It can reasonably be concluded that there was no post-harvest water stress in these seasons.

3.7 Virus testing and grafting

In work conducted by the University of Adelaide, no leaves from the *affected* or *non-affected* tree sources, tested positive (i.e. detection of virus) for Prunus Necrotic Ringspot Virus (PNRSV) or Prune Dwarf Virus (PDV) in initial molecular testing. With nested tests, all samples, except one from an *affected* tree, tested positive for PDV. Given the detections did not differ between affected and non-affected material, and that no sign of 'disease' was associated with the samples, virus presence appeared not to be a variable that could explain the onset or development of the Carmel growth disorder.

In the RT-PCR assays undertaken by DPIVic, there were no detections of PNRSV, PDV, ACLSV or ApMV.

When buds from "affected" and "non-affected" trees and Monash trees were grafted onto the Nemaguard rootstocks (induplicate) with GF305 woody indicators, and onto Shirofugen indicators (in duplicate), the results of callusing, subsequent growth, and symptom development on indicators, were highly variable. The results of grafting experiments conducted at DPI Victoria are included in Appendix 3 and summarised below (Table 9).

- Callusing was successful on all Shirofugen indicators
- Callusing was successful in 9 of 12 candidate buds on Nemaguard rootstocks
- Callusing of GF305 buds was successful in 7 of 12 cases
- Callusing of indicator GF305 buds was inconsistent; with 1 of 4 attempts successful with affected candidates and 3 of 4 with non-affected candidates
- No symptoms developed on Shirofugen, despite successful callusing
- Leaf growth of indicator GF305 occurred in only 4 grafted plants
- Symptoms developed in growth from two GF305 indicators
- Symptoms developed in only one (of the duplicate) indicator plants with Orch 3 and Monash B19-21 candidates
- The symptoms are not diagnostic in themselves.

Table 6 : Callus formation, growth and symptoms on indicators

Sample ID	Rootstock	Candidate (grafted 23/4/09)	Indicator (grafted 24/7/09)	Callusing of buds		Comment
				Candidate	Indicator	
Orchard 2	Nemaguard	Non-affected	GF 305	+	+	No visible growth
	Nemaguard		GF 305	+	-	
	Sam		Shirofugen	+		No symptoms
	Sam		Shirofugen	+		
Orchard 2	Nemaguard	Affected	GF 305	-	-	No growth
	Nemaguard		GF 305	-	-	
	Sam		Shirofugen	+		No symptoms
	Sam		Shirofugen	+		
Orchard 3	Nemaguard	Non-affected	GF 305	+	+	Growth - no symptoms
	Nemaguard		GF 305	+	+	
	Sam		Shirofugen	+		No symptoms.
	Sam		Shirofugen	+		
Orchard 3	Nemaguard	Affected	GF 305	+	+	Growth - symptoms on GF305 leaves
	Nemaguard		GF 305	+	-	No visible growth
	Sam		Shirofugen	+		No symptoms
	Sam		Shirofugen	+		
Monash B19-21	Nemaguard	Not rated	GF 305	+	+	Growth - symptoms on GF305 leaves
	Nemaguard		GF 305	-	+	No visible growth
	Sam		Shirofugen	+		No symptoms
	Sam		Shirofugen	+		
Monash A18-16	Nemaguard	Not rated	GF 305	+	+	No visible growth
	Nemaguard		GF 305	+	-	
	Sam		Shirofugen	+		No symptoms
	Sam		Shirofugen	+		

Source: Report DPIVic October 2010

The reported symptoms for the Orchard 3 candidate and Monash B19-21 candidate on GF305, were similar. The symptoms were detected in one of each duplicate, 7-8 months after grafting. They presented as leaf distortion, mild mosaic and red 'blotches' (Photo annexed to Report DPIVic – Appendix 3).

DPIVic suggests in their report a transmissible pathogen may exist within two candidates, however the results are inconsistent, and do not reflect the Shirofugen observations, nor prior virus testing. More work in this area may be warranted.

4 DISCUSSION

In California and now in Australia, the almond cultivar Carmel has displayed extensive bud failure. Although there are some minor differences in its expression, there is now sufficient evidence to suggest the Australian 'Carmel bud growth disorder' is non-infectious bud failure (NBF), as described in California. This is not surprising since, Australia's Carmel clones originated in California.

Californian researcher Dr Dale Kester and his colleagues (Kester, 2000; Kester, 1994; Kester and Gradziel, 1996; Kester *et al*., 1998) investigated the genetic component of NBF in Carmel, while also noting that Non-pareil was another cultivar that also displayed NBF in California. The researchers concluded that buds distant from the original bud through generations of vegetative propagation, had greater bud failure *potential*. Through experiments that followed specific single bud sources, they demonstrated that a bud's history (genetic) and location (source tree and position on it) predisposes trees propagated from it, to NBF. Bud failure potential varies bud-to-bud and therefore buds from the same tree may fail at different rates. Buds with inherited failure potential may remain latent in trees over the short- or long-term.

High temperatures and bud failure potential at planting explain the NBF variation observed in orchards. Once planted out in orchards, the factors that influence expression of bud condition (i.e. visible bud failure) include annual growth (year, age) and accumulated exposure to high temperatures at critical periods.

The Australian almond industry has considered its Carmel clones to have low bud failure potential and for a long period they have not expressed bud failure. However widespread bud failure in spring 2008 and 2010, have raised questions about the future of Carmel as a pollinator, the reliability of its performance and bud sources, and the influence of several heatwaves experienced in the last three years. At present Carmel accounts for 25 percent of trees in most Australian almond orchards, and therefore the status of Carmel budwood is particularly important to the Australian industry.

This project involved examination of bud status in orchard trees and in Carmel mother trees from the industry budwood repository at Monash, SA (Monash). The growing season environmental conditions to which these trees were exposed, and the subsequent expressions of vegetative and/or floral bud failure, were investigated. Despite having no knowledge of the bud failure potential (at planting) of the various inspected trees, our results and observations confirm that some bud sources in Australia have moderate to high bud failure potential and that recent environmental stresses during recognised bud development periods, have triggered its expression. The heatwaves in March 2008, January 2009, and November 2009 offer plausible explanations for the onset of widespread bud failure in young Carmel trees in spring 2008 and 2010.

In spring 2008 trees were marked as *affected* or *non-affected* based on the presence or absence of stretches of bare wood in their canopy. Field visits in spring 2009 revealed that these trees had maintained their status, and no new onsets of bud failure were reported. The temperatures were mild during the bud development period of the 2008/09 season. Extreme temperatures and a high number of days with maxima exceeding 35°C in seasons 2007/08 and 2009/10, preceded bud failure onset in spring 2008 and spring 2010. The initial onset of bud failure in the Riverina was reported in spring 2007 and this corresponded to temperature extremes in that area, in 2006/07.

In spring (September) 2010, orchard visits confirmed the bud dissection observations that central (and some outside) buds in affected trees were damaged, and that previously identified non-affected trees, had new onset vegetative bud failure. The November 2009 heatwave likely contributed to this.

Hot late summer/autumn periods, as widely experienced in March 2008 in all regions, and in 2006 in the Riverina, appear to affect floral and vegetative buds, as nut loads in affected trees were light in spring 2008, and have been light in Riverina trees for three consecutive seasons. Hot periods in spring/early summer appear to damage central buds predominantly, and therefore leaf out. Seasonal temperatures have implications for budwood cutting and more work on the timing of cutting based on high temperatures during the preceding bud development period, is warranted. It is reasonable to assume buds from first flushes (spring) under normal seasonal conditions, are less likely to fail than second flush summer budwood, due to the temperatures during which the buds have formed. Risk management however would recognise that each season is different and requires specific review of temperatures during the preceding bud development period.

In orchard trees, the bud damage results from March dissections were reliable predictors of leaf out in the following spring. The greater the proportion of damaged central buds, the poorer was the observed leaf out and the sparser the canopy. By June the effect on floral bud transition could also be predicted from the dissection of outside buds. Despite the exposure of Monash trees also to heatwaves, bud dissections showed Monash buds to be consistently larger than others dissected and of high health status in all sampling periods. The Monash trees that were left unpruned for this project, did not display bud failure.

Given equivalent bud failure potential, young trees growing vigorously in hot areas, will express bud failure earlier than mature trees in the same area. Although tree maturity in itself, may limit the effect of high temperature exposure by shading, it is more likely that mature trees are slower to develop bud failure (as a percentage of buds or canopy) due to their reduced annual vegetative growth (relative to reproductive growth). Mature Monash mother trees, because of their proximity to the original bud and management (hard hedging, restricted reproductive growth/flowering) are unlikely to express bud failure. Similarly, visible bud failure is unlikely to be seen in nursery trees. Their lack of annual growth delays the expression of bud failure.

The bare wood, tufted growth and ‘crazy top’ characteristic of NBF can be explained in terms of compromised bud development, but the mechanism for rough bark development and tiger-striping in affected Riverina trees is unknown. No other region with Carmel NBF consistently displays this symptom.

During the period 2004-2007 Australian almond plantings increased significantly and peaked in 2006-07. Over this time, the demand for budwood exceeded supply from Monash. It is possible that Monash budwood was cut from second flushes in late summer/autumn, during this period of high budwood demand. It is certain that some nurseries cut budwood from other sources, and that some Carmel trees in production today were established with buds distant from the original Monash mother trees.

The periods of high budwood demand appear to be correlated with the age of Carmel trees and orchards now expressing bud failure. The surveys conducted during this project and subsequent observations, indicate that some plantings from 2002/03 have signs of bud failure but the majority of affected trees were planted after 2004. However there are also orchards of similar age in the Riverland and Sunraysia, in which bud failure expression has been minor and their bud traceability and time of budding, is of considerable interest.

The impact of bud failure for growers is of economic and practical concern. Economically, the impact of bud failure declines as the tree age at onset increases. Affected trees appear not to die, but it is not possible to curb the advancement of bud failure, once triggered. Productivity in affected trees therefore declines with time, and the earlier the onset, the greater is the potential loss. Nut counts have not been taken, but it is our opinion that a young tree (fourth leaf or younger) with more than 30% bare wood throughout, is unlikely to be economically viable in the future, and should be replaced. Second year orchard trees with any NBF should be replaced as soon as possible.

Although it may appear within a season, that some trees ‘grow out’ of bud failure, this has not been demonstrated in this project. The viable shoots in affected trees lack competition and their continued vigorous growth may mask the bare shoots as the season progresses. In the subsequent season, affected trees remain affected and canopy bareness increases. However, visible canopy bareness may appear not to increase in situations where moderate late season temperatures in the previous season allowed extensive new shoot and terminal bud formation.

Heavy pruning does not stimulate productive growth from affected wood, nor does the application of nutritional supplements. Sun protection on young trees (either by created shade, or white coatings on the tree framework) has not been trialled, but trees at row ends and in western, outside rows, often show bud failure first and would be good candidates for such trials. Avoiding water stress (especially at harvest and post-harvest) and excessive annual growth may delay onset or slow the progress of bud failure, but in practice only top-working is an effective management option for orchards with NBF, given it is not a graft transmissible disorder. The success of top-working has not been broadly tested in Australia, but it will be dependent on the availability of buds with proven low bud failure potential.

Information is limited on the bud failure potential of Carmel clones in Australia. The Monash trees have been the primary industry source of Carmel budwood over a long period, and our investigations suggest the bud status of these trees is sound. It is not reasonable to conclude however that NBF potential exists in no Monash trees. To maximise the chance of selecting good buds, Monash buds on low, short, basal shoots with short internodes, should be cut in spring. This budwood is likely to be superior to any other budwood source, in Australia. The performance of the industry’s oldest Carmel trees in orchards (and of those propagated directly from them), is of considerable interest also as they could be a valuable source of ‘original’ buds, if virus-free.

Most orchards lack traceability of their trees to specific bud sources, however nurseries should have information on this recorded and readily available to growers. Nurseries need to be aware of the risks associated with budwood and they need to retain as much information as possible on the budwood they use, its source, appearance (e.g. vigour/internode length, girth), budding and grafting timing, and conditions. Bud traceability is currently lacking, and an industry system that requires nurseries to document such details and growers to document planting patterns, is needed if more specific understanding of budwood sources is to be gained. If growers reported to nurseries the age of trees at bud failure onset, useful information could be gained on bud sources, and risk associated with nursery locations.

5 CONCLUSIONS

See also Milestone reports 2 and 3 (Appendices 1, 2)

- Moderate and high bud failure potential exists within some Australian Carmel clones.
- Young Carmel trees planted since 2004/05 are the most severely affected.
- High temperature exposure (and budwood sources) induced the observed bud failure.
- Bud failure has not been traced to one bud source.
- Bud ‘damage’ is necrotic tissue and damaged buds do not recover.
- The choice of budwood predisposes trees to bud failure.
- Affected young (4^{th} leaf) trees, are unlikely to be economically viable.
- Buds from Monash are consistently larger and have negligible internal damage.

- Vegetative growth has been affected more extensively than flowering, but late summer/autumn heat appears to affect more floral buds, than spring heat.
- Rough bark and tiger striping is not consistently associated with Australian bud failure.
- Bud failure in Carmel in Australian orchards cannot be generally distinguished from NBF, but Australia has not seen NBF in Non-pareil.

6 TECHNOLOGY TRANSFER

6.1 Extension material

The surveys and Fact sheet were directed to all almond growers, by the Almond Board Australia. Other communication and extension material has been provided to the ABA and to the co-operating growers in the form of progress reports and Milestone reports.

Milestone reports and Industry Annual Report summaries were provided to Horticulture Australia Ltd in 2009 and 2010. The Milestone reports were comprehensive and have been attached as appendices to this Final Report (Appendices 1, 2). The HAL Annual Industry reports on this project have been included in a number of publications and they are attached as Appendix 4. Presentations to almond growers were made in 2009 and 2010 at the Almond Industry Conference and these are attached in Appendix 5.

7 RECOMMENDATIONS

7.1 Growers

- Keep planting maps and records of trees supplied by each nursery.
- Request bud source documentation from nurseries.
- Resist planting Carmel trees if budwood does not have a Monash origin.
- Inspect leaf out closely in 2nd - 4th leaf Carmel trees, especially if hot weather occurred during the previous spring or autumn.
- Map and document bud failure and canopy bareness in affected young trees.
- Provide specific feedback on NBF to relevant nurseries.
- Remove second leaf trees if NBF is present.
- Remove 3rd and 4th leaf trees if greater than 30% of canopy is affected.
- Top work affected trees if good scion buds (with low bud failure potential) are available.
- Check all Non-pareils for signs of bud failure.
- Avoid water stress at harvest and post-harvest.
- Trial the application of *Surround*® (or similar sunburn heat stress protection product) on several young, exposed end trees or outside rows.
- Review bud development and factors that affect it in the bud development Fact sheet.

7.2 Nurseries

- Increase knowledge of budwood sources and the features of suitable budwood.
- Increase understanding of seasonal heat effects and bud source history, on budwood suitability, bud failure potential and NBF expression.
- Specify the nature of budwood required for intended budding/grafting period – e.g. low, basal, thin, shoots in spring.

- Source Monash Carmel buds whenever possible.
- Document budding and grafting activities. Make budwood and budding activity records available to growers.
- Add to bud failure knowledge by trialling autumn (terminal buds) and spring (basal buds) budding from the same source trees.
- Request from growers feedback on NBF onset and tree performance in 2^{nd} and 4^{th} leaf; document feedback and link it to budwood source data.
- Evaluate Non-pareil sources, as outlined above.

7.3 Industry

- Evaluate new cultivars as pollinators.
- Evaluate all cultivars with Carmel (or Non-pareil) parentage for bud failure potential.
- Introduce new Carmel clones with low bud failure potential as determined in California.
- Increase knowledge of NBF potential amongst Monash clones and the oldest orchard Carmel clones (Nangiloc, Renmark).
- Consider comparative progeny testing, e.g. two budding periods; single-tree sources from Monash clones and oldest orchard Carmel trees; planting in hot and mild locations.
- Assist with acquisition of BF-potential information by introducing a traceability and feedback system between Monash, nurseries and Carmel growers.
- Trial top-working of affected young trees.
- Evaluate economics of tree canopy bareness to support (or otherwise) recommended affected tree removal criteria.

8 REFERENCES

1. Kester, D. 2000. *Final Report of the non infectious bud- failure project No.: 99-dk-00*. Almond Board of California, 25 pp. http://fruitsandnuts.ucdavis.edu/crops/Almond_Board_BF.pdf.

2. Kester, D.E. 1994. *Solving the problem of non-infectious bud-failure in California almond orchards*. International Congress on Almond. Acta Hort. 373: 35-40. http://www.actahort.org/books/373/373_3.htm

3. Kester, D.E., Shackel, K.A. Micke, W.C. Gradziel, T.M. Viveros M. 1998. *Variability in potential and expression of non-infectious bud-failure among nursery propagules of 'Carmel' almond*. Acta Horticulturae 470: 268-272 http://www.actahort.org/books/470/470_37.htm

4. Kester, D.E. and T.M. Gradziel. 1996 Chap 12 'Genetic Disorders' in *Almond Production Manual*. Ed. W. Micke. University of California. Publication 3364. 289 pp.

5. Rubatsky, V.E. and M. Yamaguchi. 1997. World Vegetables – Principles, Production and Nutritive Values. 2^{nd} edition. Chapman and Hall. 843 pp.

SCHOLEFIELD ROBINSON
HORTICULTURAL SERVICES PTY LTD

PA McMICHAEL
Principal Consultant/Plant Pathologist
F:\SRHSDATA\Clients\Almond Board of Australia\Bud Failure 2008\Reports\FINAL REPORT\Rpm1110HAL.doc

Appendix 1

Milestone Report 2 May 2009

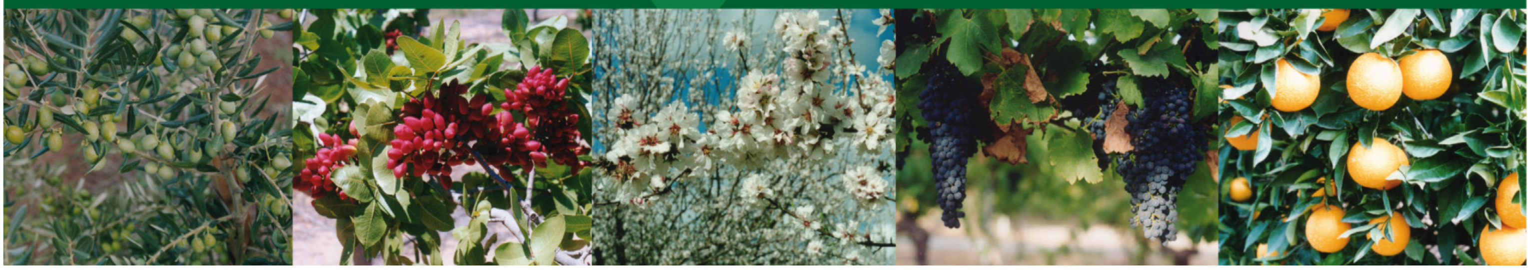
Scholefield Robinson
HORTICULTURAL SERVICES

Milestone 2 Report:
Project AL08015
Carmel Growth Disorder

Prepared for : Horticulture Australia Limited

By : Prue McMichael & Kate Delaporte

Date : 15th May 2009



1 SUMMARY

1.1 Survey Summary

The grower survey (Appendix 1) was distributed to all registered growers by the Almond Board Australia (ABA). There were 21 respondents, some from each of the major production districts of Riverland, Sunraysia and MIA. Nurseries were also surveyed through an ABA-delivered electronic survey (Appendix 1B).

The results of the surveys (Appendix 1C) were circulated to all growers and nurseries. They suggested that the incidence and severity of the growth disorder are not consistent across regions, and that the disorder is confined to the variety Carmel (except in one orchard which reported affected Non-pareil and Monterey). The spring symptoms reported were bare new growth with tufted leaf spurs at terminal ends, and/or leafless new shoots with few nuts. Symptoms from winter 2008 were also reported as rough bark and horizontal lesions around affected buds. Their association with the disorder will be determined later in the project.

The survey responses indicated that affected MIA trees were in their fourth leaf (or younger) but in the other regions, affected Carmel trees of 2-20+ years of age, were reported.

Growers in total named 21 different nurseries as their tree sources, and three known budwood sources. Respondents (3) to the specific nursery survey (Appendix 1C) indicated there had been no significant operational changes in grafting or budding, nor in their seed or budwood sources that may explain the field growth symptoms.

1.2 Literature Review

The literature review (Appendix 2) underpinned the Industry Fact Sheet on bud initiation (Appendix 3).

The literature review exposed the factors that are known to affect Prunus flower and leaf bud initiation and viability. Although there is little information specific to almonds of the variety, Carmel, it is known that the processes that result in leaf out and flowering (and therefore nut development) start a year or more before the previous harvest of nuts. Vegetative buds form the structure and bearing potential of trees and the capacity to sustain the tree through water uptake, nutrient capture and energy conversion. Growers and nurserymen management decisions also influence bud formation and development, especially through post-harvest irrigation and pruning practices. Weather (environmental) events, especially high temperatures during bud formation may directly affect bud development. Indirectly, high temperatures may also trigger bud failure. 'Non-infectious bud failure', a widely recognised growth disorder of Carmel in Californian trees, is triggered in genetically-predisposed trees by high temperatures in May (USA). Biological causes (especially pests and diseases) may also result in growth problems and loss of bud viability.

1.3 Fact Sheet on Bud Initiation and Development

The Fact Sheet (Appendix 3) on almond bud initiation and development was sent to all almond growers. It was written to ensure that growers had ready access to information on factors that influence bud development. It also aimed to increase growers' understanding of influences under their control, eg. post-harvest irrigation. An almond growth cycle chart was developed with bud development steps inserted. The Fact Sheet provided recommendations on how to maximise bud development potential. It was also used to explain the basis of the Carmel growth disorder investigation into its potential genetic, biological and/or environmental causes.

1.4 Virus Testing

Four co-operating growers from the MIA provided leaf samples (collected by project team member) from trees that had shown 2008 spring symptoms of 'growth disorder' (affected) and those from a similar growth stage and location that were asymptomatic (unaffected). At the time of collection, no symptoms of virus were evident in any of the trees, and no sampled leaves showed signs of problems, or infection by any biological entity.

For each orchard, composite samples from 'affected' and 'unaffected' trees were separately prepared and sent for virus testing. Two rounds of testing were undertaken by Dr Michelle Wirthensohn (University of Adelaide). The second round employed 'nested' PCR tests which are more sensitive.

The results of the tests indicated that 1) No leaves from the *affected* and *unaffected* tree sources, tested positive (i.e. detection of virus) for Prunus Necrotic Ringspot Virus (PNRSV) or Prune Dwarf Virus (PDV) in the first round of molecular testing; 2) Only one sample (from an *affected* tree) tested negative for PDV with 'nested' PCR; all others (from both affected and unaffected trees) were positive with nested PCR tests.

The conclusions drawn from this testing were: 1) Virus is not presenting as 'disease' in any of the trees; and 2) Virus presence is not a variable that can reasonably explain the onset or development of the Carmel growth disorder.

The results were circulated to all co-operators in the trial.

2 NEXT STEPS

The project is advanced beyond that indicated in the original timetable, in the following areas:

Cooperating grower properties have been visited by a member of the research team. All co-operators have been provided with comprehensive outlines of the project aims, activities and their involvement (Appendix 5). Trees have been tagged and budsticks cut progressively. Bud dissections have commenced with budsticks from 8-10 co-operators, covering the three production districts.

Grafting of budwood from affected and non-affected trees onto high health rootstocks has been completed (April 2009).

Progressive results from these, and the forth-coming activities (see below) will be reported on, in Milestone 3:

- Bud dissection results
- Summary of temperature data November – March 2007/08 and 2008/09
- Review of post-harvest irrigation information for 2007/08 and 2008/09
- Winter / Spring visits to co-operating orchards
- Assessment of Monash mother trees (to determine 'affected' or 'unaffected' status).

3 COMMUNICATIONS AND EXTENSION ACTIVITIES

The communication and extension material resulting from this project, even at this early stage, have been extensive. They are included in the Appendices to this report. The Literature Review was circulated to all growers in a grower-useful format, as a Fact Sheet. All growers and nurserymen have received the survey results summary, and explanation of the project aims. Co-operators have had detailed contact and correspondence regarding the project aims and activities and tree-marking and budstick cutting instructions. Bud dissection work will be reported progressively with the first co-operator results summary to be sent after six weeks of dissections (will be included in Milestone 3 report).

4 COMMERCALISATION/INTELLECTUAL PROPERTY ISSUES

There are no commercialisation or intellectual property issues associated with this project.

5 OTHER ISSUES

Post-harvest rain has been received in all production districts this year. This may affect the results of our desk top assessment of the potential effects of post-harvest water restriction, on the subsequent season's growth pattern in affected and unaffected Carmel trees.

SCHOLEFIELD ROBINSON
HORTICULTURAL SERVICES PTY LTD

PRUE McMICHAEL
Plant Pathologist\Principal Consultant
F:\SRHSDATA\Clients\Almond Board of Australia\Bud failure 2008\Summaries & Milestones\Rpmc0509Milestone2.doc

Appendices

Appendix 1 (a) Grower Survey
(b) Nursery Survey
(c) Grower survey – Results Summary

Appendix 2 Literature Review

Appendix 3 Fact Sheet

Appendix 4 Virus testing

Appendix 5 Grower Instructions - Sampling Budsticks

Appendix 1

(a) Grower Survey
(b) Nursery Survey
(c) Grower Survey – Results Summary

Appendix 1(a) – Grower Survey

BUD FAILURE PROBLEM IN CARMEL

1. **Make copies of blank table for later use or to share with neighbours**
2. **Review the attached photos and descriptions before completing the questionnaire**
3. **Please check Carmel trees for described symptoms**
4. **Please complete table/questions for your CARMEL plantings 2004-2007**
5. **RETURN this page by OCTOBER 30 - Electronically or fax: (08) 8373-2442**

Orchard Location or reference	Season Planted (2004-07)	% Carmel affected[w]	Symptoms and month/year first observed[x]	Nursery[y]	Bud source[z]

w Estimate **percentage** of Carmel trees showing <u>any</u> of the described symptoms
x Use the **coded description of symptoms on photos** (eg. A-F) to list symptoms seen and **circle** the most prevalent symptoms.
y Name of nursery that provided **trees**; or indicate if field budded
z Name bud **source** and **qualify** as '*certain*' (if you have documented* evidence); "*suspected* " (if you think you know the source but have no remaining evidence); or '*unknown secondary source*" (i.e. if you think came from another grower, nursery etc. without traceability)

* Attach copies of any documentation you think may be helpful in this initial scoping study.

1. **Please describe any significant weather events during seasons since affected Carmel was planted** (eg. frost, excessive heat; low chill; summer rain etc).

   2004/2005:

   2005/2006:

   2006/2007:

   2007/2008:

2. **Please describe any significant (for affected Carmel) production problems/changes encountered in same seasons** (eg. salinity; water/drought; changed irrigation method)

3. **Your name and contact details (optional) …………………………….…………..**

Appendix 1(b) Nursery Survey

BUD FAILURE PROBLEM

1. **Make copies of blank table for later use or to share with bud and seed suppliers**
2. **Review the attached photos and descriptions**
3. **Please check for symptoms, if appropriate, on-site Carmel mother trees**
4. **Please complete table/questions for CARMEL material released 2002-2007**
5. **RETURN this page by DECEMBER 3 - Electronically or fax: (08) 8373-2442**

Nursery Location or reference	Season released (2002-07)	Seed source*	Budwood source*	Mother tree symptoms? (use codes on photos)	Changes in budding/nursery practices ?	Feedback from growers? replacement requests?

* Attach copies of documentation that may be helpful in this initial scoping study; or to determine traceability.

1. **Please describe any abnormal Carmel bud or rootstock seed observations that may be relevant to the bud failure problem, and this initial scoping study** (i.e. poor seed; new seed source; buds from new source; bud discolouration; low take etc.)

   2001

   2002

   2003

   2004

   2005

   2006

   2007

2. **Please describe any significant changes to nursery, budding practices, or growing conditions that may be relevant** (eg. salinity; new budding or budwood storage techniques; new budding crew; water/drought shortages; more trees held-over **-** for second year sales etc.)

2001

2002

2003

2004

2005

2006

2007

3. **Please indicate if rootstock seed *and* budwood was 'virus-tested' to your knowledge -** by whom? when ? technique (if known)? for which viruses? **Indicate if supportive documentation/records are available?**

2001

2002

2003

2004

2005

2006

2007

4. **Your contact details (optional) ……………………….……………..**

F:\SRHSDATA\Clients\Almond Board of Australia\Bud failure 2008\Nursery Questionnaire 1008.doc

ALMOND GROWTH DISORDER - 2008

What we have seen

The 2008 growth disorder has not presented consistent symptoms across the major production regions, but Carmel has been the variety affected in each region.

Winter Symptoms
Rough bark
Horizontal lesions around affected buds

Spring Symptoms
Bare wood with tufted leaf spurs at terminal ends
Wood bearing nuts only – no leaves
Growth of laterals from below the affected wood

What we know

The ABA and Scholefield Robinson are grateful to the growers and nurserymen who returned the surveys and questionnaire. The results are summarised below. From these, we are able to conclude:

- Carmel trees in the MIA, Sunraysia, and Riverland are displaying *some* of the described symptoms
- No Carmel trees on the NAP are reported as having the symptoms
- Affected trees (except in the MIA) are greater than four-years-old
- MIA trees with winter 2008 symptoms, developed spring 2008 symptoms
- Some orchards (MIA) have observed similar problem over two consecutive seasons
- Some orchards have reported individual trees with up to 90% canopy affected
- Some orchards have reported up to 70% trees with some degree of symptom development
- Most orchards have reported low incidence of symptoms
- In no orchard are 100% Carmel trees affected
- Bare wood may carry nuts, suggesting viable flower buds but not vegetative buds
- Tufted terminal growth suggests viable terminal, vegetative buds
- Affected and non-affected Carmel trees were composed of budwood from two budwood sources

- Affected and non-affected trees were sourced from at least 21 different nurseries
- Most orchards reported environmental stress (excessive heat; irrigation with-holding etc.) in orchards in 2008 – but direct correlation with affected blocks/trees is unclear
- No nurseries have reported they have replaced Carmel trees with these problems, or been advised by growers of problems in new plantings.
- No nurseries have reported significant operational changes or changes in seed or budwood sources

Survey and questionnaire summary - for Carmel

Region	No. Of Replies	No. of Carmel Blocks	Planting Date (region range)	Most reported[x] 'first year of symptoms"	Percent trees affected[y] (region range)	(Max. % with symptoms)- Year Planted
Sunraysia (incl. Robinvale)	6	13	1987-2007	Spring 2008	<1% - 70%	2000
Riverland (incl. Lindsay Point)	11	48	1981-2007	Spring 2007	<1% - 90 %	2003
MIA	4	9*	2003-2007	Spring 2008	1% - 30%	2006
NAP	2	-	-	-	0	-
TOTAL	23	70				

[x] Year reported most often by respondents as that in which symptoms first seen

[y] Responses do not distinguish in some cases, between percentage of canopy affected (*severity*) and percentage of trees with any level of symptom development (*incidence*)

* One orchard with some Non-pareil symptoms also

Region	Nurseries	Bud Sources	Main Symptoms Listed*
Sunraysia (incl. Robinvale)	Seven named	Three sources named	All; A, B, C, D
Riverland (incl. Lindsay Point)	Eleven named	ABA; Flemings; unknown	Mostly B and D; some A, B, C
MIA	Six named	ABA; and several unknown	All; B

* As coded in photos circulated with survey and questionnaire: A = tufted growth at end of bare shoot; B = bare shoots, no nuts ; C = bare shoots, bearing nuts; D = no tufted growth, bare shoots with nuts; E = (winter) dark horizontal lesions around buds; F = rough, scaly bark

What we still need to find out

At this stage there is no certainty the problem is either 'on-going', increasing or spreading. Equally, there is no certainty about its cause/s. It is possible that environmental, biological and/or genetic factors have contributed to this year's symptoms.

Potential *biological* causes of blind wood include: viruses
Potential *environmental* causes include: drought stress, low chill, excessive heat at time of bud development (late Feb-early March is usual time for almond bud initiation)
Potential *genetic* causes include: non-infectious bud failure (NBF)

We do not know:

- If buds on bare wood are dormant, dead, or abnormal in their formation
- Why basal and terminal buds appear less affected
- Relative importance of shoot formation time on bud viability (eg. first or second flush)
- If 'blind' buds died, and if so -when that transition occurred
- The effects on bud development, of the significant hot period in March 2008 (the time that bud initiation naturally occurs)
- The relative susceptibility of flower and vegetative buds, to bud formation influences, like excessive heat, water restrictions post-harvest
- The relative susceptibility of flower and vegetative buds at the same node
- If mother plants at budwood sources also have (the potential to develop) similar symptoms - should they have been left to grow out, eg. remained uncut
- If this problem is transitory, temporary or permanent (with potential to increase)

What we are planning to do from this point

Because there are no immediate and specific tests to confirm a genetic or environmentally-caused growth disorder, investigations are needed to:

- Follow the development of buds over time (through dissections), eg. bud development on wood arising from this year's affected and non-affected wood from time of initiation (late-Feb/March) to flower and leaf emergence (July/August). Budwood mother trees included in investigations.
- Follow the presence, extent and distribution of symptoms over time, eg. tag branches and observe regularly, to determine "spread" or "recovery", during 2008/09 and 2009/10.
- Carry out diagnostic testing to determine virus presence; and attempt 'transmission' of the problem through budding or grafting of affected buds/wood, onto clean rootstocks.
- Clarify 'incidence vs. severity' of the symptoms, and determine correlations with environmental stresses to which severely-affected trees were exposed (eg. excessive heat and water stress post harvest).

Investigations will involve in-field visits in the Riverland, Sunraysia and MIA to tag trees for observations in winter and spring 2009, and wood removal for sequential bud dissections over time, starting late February, 2009.

Communication of results of investigations will be on-going and regular, as growers are in need of information on both the management of affected trees and the economic viability of affected, young trees. Details on the factors that influence bud development and timing (eg. heat, genetics, irrigation etc.) will be provided before harvest 2009.

Please call Prue McMichael at Scholefield Robinson or Ben Brown at ABA with any questions or observations in regard to this problem and the planned investigation.

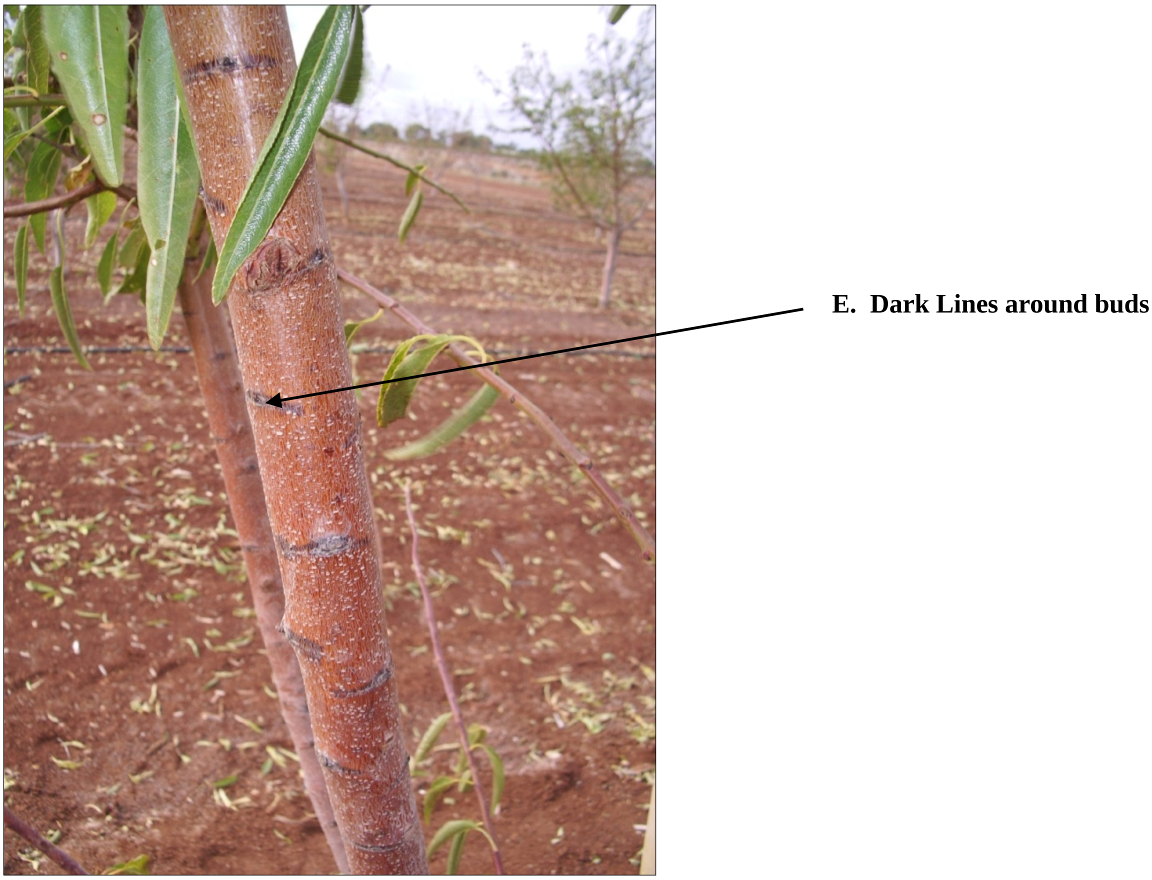
A. Tufted growth at end of bare shoots

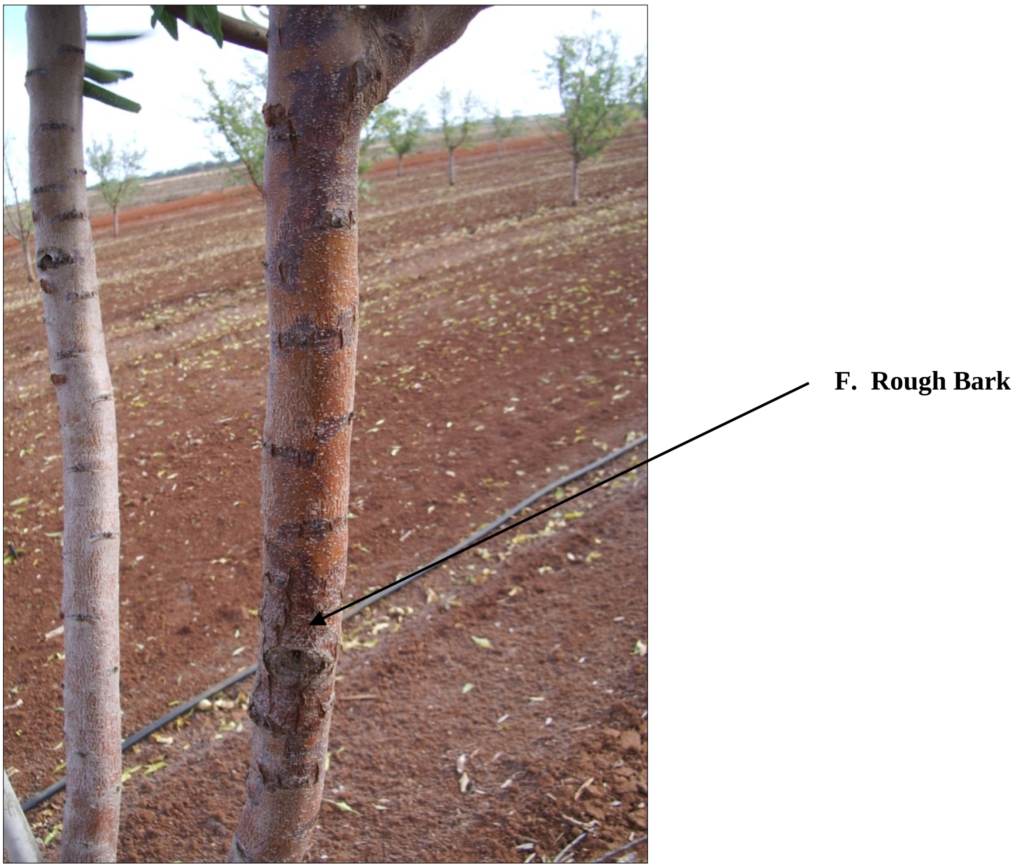
B. Bare shoots, no nuts

A. Tufted growth at end of bare shoots

C. Bare shoots, no nuts

D. No tufted growth on shoots - with nuts


E. Dark Lines around buds


F. Rough Bark

Appendix 2

Literature Review

Appendix 2 – Literature Review

Almond Bud Development and Influences on it

Flower bud initiation is the foundation of tree fruitfulness and therefore all factors that influence initiation and development of flower buds are of economic relevance. The processes that result in nut formation start a year or more before harvest. Vegetative buds form the structure and bearing potential of trees and the capacity to sustain the tree through water uptake, nutrient capture and energy conversion. It is important that growers and nurserymen understand the effects of management decisions and environmental events, on bud formation and development.

Bud initiation and development as steps in the almond growth cycle

Through extrapolation from northern hemisphere research, a growth stage and development timeline relevant for Australia, is given below in Table 1.

Table 1. Almond bud initiation and development cycle

<table>
<tr><th>SEASON</th><th>MONTH</th><th colspan="2">TREE GROWTH STAGE</th><th>BUD DEVELOPMENT STAGE</th></tr>
<tr><td rowspan="2">WINTER</td><td>July</td><td colspan="2" rowspan="2">Bud burst – Full Bloom</td><td rowspan="2">Emergence of floral buds on shoots or spurs; pollination
Emergence of vegetative buds – leaves</td></tr>
<tr><td>August</td></tr>
<tr><td rowspan="3">SPRING</td><td>September</td><td colspan="2">Shuck Fall – Early Set</td><td rowspan="2">Leaf development and active growth of new shoots</td></tr>
<tr><td>October</td><td rowspan="4">Nut growth</td><td>Pit hardening</td></tr>
<tr><td>November</td><td rowspan="2"></td><td>Growth interruption; bud maturation</td></tr>
<tr><td rowspan="3">SUMMER</td><td>December</td><td>Bud scale formation</td></tr>
<tr><td>January</td><td>Hull split</td><td rowspan="2">Vegetative buds develop. Flower buds initiated</td></tr>
<tr><td>February</td><td colspan="2" rowspan="2">Harvest</td></tr>
<tr><td rowspan="3">AUTUMN</td><td>March</td><td rowspan="2">Flower buds continue to differentiate</td></tr>
<tr><td>April</td><td colspan="2">Post Harvest</td></tr>
<tr><td>May</td><td colspan="2" rowspan="2">Leaf Fall – Dormant</td><td rowspan="2">Vegetative buds in rest period. Flower buds continue to differentiate. Chilling occurs.</td></tr>
<tr><td rowspan="2">WINTER</td><td>June</td></tr>
<tr><td>July-August</td><td colspan="2">Bud burst</td><td>Emergence of flowers, followed by leaves</td></tr>
</table>

Adapted from Kester, D. 2000, Kester *et al*., 1996, Thomas, 2007

Almond buds

Almond kernels are the result of a two-year cycle of growth. Shoot, leaf, spur, flower/nut growth occur as a result of bud initiation and differentiation. Vegetative buds may remain vegetative, or transition into reproductive buds. Leaves are borne on shoots or spurs, as are flowers on wood more than a year old. In the first year, vegetative growth occurs in the spring. On short spurs or shoots, lateral vegetative buds in leaf axils, may differentiate into flower buds (usually observable by March). Buds develop over the next year but require certain temperature exposures to induce both external and internal dormancy periods, for the development to advance normally.

Managing trees and manipulating environmental conditions such that bud formation and health are maximised is important, complex, and at times in conflict with other management practices. Cultural practices (eg. pruning) for the development of tree structure and optimisation of light interception involves the removal of potentially fruitful buds. Pest and disease control measures maintain buds, flowers and fruit in a healthy condition. Weather events such as extreme cold or heat, dehydration and drought, frost, hail, rain and wind may physically kill or remove buds, flowers or pollen, or interrupt bee activity and pollination, or affect the rate of bud development. Tree genetics influence bud initiation and viability and the bloom period. Despite almonds requiring fewer chill hours than some other nut and *Prunus* spp., inherent cold resistance may affect the timing of almond flowering and leaf out, in some varieties and locations. Orchard design, through the choice of varieties and planting patterns, seeks to maximise pollination and the overlap of pollen receptivity periods in flowers. The nutritional status and water status of trees at particular times of the season, also affect bud initiation and development.

Flower buds

The floral buds encompass a terminal flower, but no leaves. More than one floral bud (up to six) may form on a single spur, or along the length of shoots more than a year old. Relative to other fruit (eg. peach and prune) and nut crops, almonds have late flower bud differentiation. In almonds it usually coincides with the post-harvest period, and therefore management decisions (as well as weather etc.) at that time, influence the next season's potential yield in terms of nut count. Potential kernel size is not influenced at this time.

Classic research on flower bud development was undertaken in 1925 (Tufts and Morrow, 1925) and more recently by Lamp *et al*. (2001). Polito *et al.* (2002) also investigated the effect of bud position and leaf area on the timing of flower differentiation, between and within spurs. Each investigation included the almond variety Nonpareil, while Lamp *et al.* (2001) also investigated Carmel and Butte with modern techniques and greater understanding of the early morphological events in flower development, and the influence of climatic conditions on floral development in varieties planted across production districts in California.

These researchers found that floral initiation in some varieties (Carmel and Butte) preceded hull split, but for Nonpareil they concluded floral initiation occurred after hull split. From their northern hemisphere research they reported that Nonpareil initiation in the two trial seasons was in mid-July (Australian equivalent, being mid-January) and first of August (February 1, Australia), with the majority of the Nonpareil bud differentiation occurring post-hull split and harvest, when compared with Carmel and Butte. Tufts and Morrow (1925) suggested differentiation in Nonpareil did not commence until mid-September in California (mid-March, Australia).

Lamp *et al.* (2001) photographed, numbered and described eight flower bud development stages (0-7). The stages 0-2 describe bud initiation, while stages 3-7 describe the flower formation.

0 = vegetative (apical meristem produces bud scales, before flower initiation)
1 = transition to reproductive state (evidenced as size increase at shoot apex)
2 = flower initiation (broadened apex top forms dome with bract primordia; move from vegetative to reproductive meristem)

3 = sepal initiation (five sepal primordia arise spirally)
4 = petal initiation (within spiral and arise alternately to sepals within the calyx)
5 = stamen initiation (within corolla stamens initiated sequentially)
6 = transitional/pre-carpel initiation (floral apex becomes concave), and
7 = carpel initiation (single primordium at periphery of meristem).

The male reproductive cells become pollen grains which are present in mid-December in California (mid-June, Australia).

Vegetative buds
Leaves are formed in vegetative buds, which are more pointed and covered with dark leaf bracts. The terminal bud of a spur is always vegetative and hence the capacity of almond trees for on-going shoot growth and canopy expansion. The vegetative terminal and lateral buds form during the previous year, then elongate and expand mid-summer. In spring, the emerged leaves are initially dependent on stored carbohydrates. The relative growth of terminal and lateral vegetative shoots determines the potential fruit-bearing capacity and structure of the different varieties.

In the process of bud formation, the vegetative bud development is temporarily halted by heat-induced dormancy in summer. Their development resumes when autumnal conditions appear, and buds then progress through a cold-induced internal dormancy, towards maturity. In conditions of excessive nutrition, or when mild conditions extend through autumn, the wood, leaf and bud maturation may be delayed and the duration of active growth extended. In contrast, water-stressed trees may lose leaves prematurely and have reduced vegetative growth, and compact canopies.

Influences on bud initiation and development
There have often been seasons in which growers have reported “patchy bloom”; bare shoots; witches’ broom growth; or limited extension growth etc. Each of these reflect in part, effects on bud initiation and development. Bare shoots for example may result from failed bud formation, bud death before emergence, abnormal development or extended dormancy. The causes of disrupted or abnormal bud initiation and development are not all understood, but it is known that buds are influenced individually or in combination, by their genetics; environmental conditions; nutritional, chemical and water status; and by biological organisms.

Genetics
A bud development disorder attributed to varietal genetics, is prevalent in California in the Carmel variety (but also documented in Nonpareil, Price and several other American varieties) and has curbed the planting of Carmel in California. Non-infectious bud failure (NBF) may first appear in the spring of the second leaf. It presents as leafless lengths of one-year old wood that may or may not carry nuts. On occasions a tuft of vegetative growth appears at the ends of bare shoots suggesting that growing points in basal and terminal leaf buds (which are formed early or late in the season and therefore often in cooler conditions), remained viable despite the other lateral, vegetative buds between, dying or failing to develop the previous summer or autumn. It has been concluded that the vegetative buds absent in NBF trees, initially formed and were viable. Their death however occurs prior to spring, and most likely during the previous winter and autumn.

Where nuts appear in NBF shoots, it is evidence that the floral buds have survived despite often being at the same node as a failed vegetative bud. In NBF trees, the bloom period may be delayed (Connell, 2007; Kester, 2000). The tendency for almond kernel doubles has also been reported from NBF trees (Connell, 2007).

Key NBF researcher Dale Kester found that the failure of vegetative (and sometimes floral) buds to develop was an inherited characteristic with the potential to increase in propensity with repeated vegetative propagation, and in severity with cumulative exposure to high summer temperatures (see below). Once present, NBF cannot be eliminated. Replacement (or top-working) of young (second-fourth leaf), affected trees is often justified economically, but in older trees with symptoms in the upper canopy, the yield decline (through reduction in fruiting wood primarily) often does not justify the removal of the tree unless the framework of the tree has been affected severely.

In order to manage risk and improve planting material quality in NBF susceptible varieties, it is reasonable today for registration, certification and breeding programs, and nursery operations to identify low bud-failure potential trees and clones. The NBF-potential in each clone is dynamic, with temperature exposures, drought stresses, fruit load, tree pruning and management, and re-propagation contributing to NBF expression. Once identified there should be commitment to the selection of single tree sources of buds for mother trees, and to observe progeny (from traceable bud sources and positions on scaffolds) growth in areas conducive to NBF expression (eg. warm-hot areas). The mother trees of bud sources identified with low NBF-potential require management and maintenance such that NBF is minimised (eg. hedge pruning to ensure budwood is from trees and canopy locations closest to the original buds). In California, no Nonpareil or Carmel sources of budwood are considered NBF-free. Despite their programs and those practised in most budwood schemes (eg. Monash), it is reasonable to expect NBF will increase over time in genetically predisposed clones and in situations of repeated vegetative propagation.

Non-productive syndrome (NPS) is also reported from the USA. This presents as vigorous, non-productive trees, with nuts if formed being elongated and mis-shapen. The cause is believed to be genetic through environmentally-induced mutation, although viruses have also been implicated.

Environmental

Temperature: Although flower initiation is thought to be controlled by naturally-occurring plant hormones, the timing and duration of the floral development stages is directly affected by tree genetics and temperature exposures at critical periods. The temperature triggers for normal bud development (eg. heat-induced dormancy, chilling hours, and the subsequent warm temperatures to break dormancy and trigger growth etc.) are well-documented.

The cumulative effect of high-temperature (above 27°C) exposure in the previous summer, affects NBF onset and expression. It is proposed that NBF may also be indirectly triggered in stressed trees since the premature defoliation increases canopy temperatures. In contrast, frosts may kill buds depending on their developmental stage, and the duration and severity of the frost.

Water status. Almond is generally considered a drought-tolerant tree, however it has been demonstrated that almond trees pass through annual development stages in which water-stress sensitivity varies. The stages most sensitive to water-stress in flowering deciduous trees are flowering, fruit set and early stages of fruit growth (Fereres and Goldhamer, 1990). Although almonds are irrigated in Australia, water status remains critical in terms of almond production levels. The almond harvest coincides with the floral bud initiation period. The post-harvest period coincides with the floral bud development. The usual practice of ‘holding off’ the water in preparation for, and during harvest and drying, must be managed carefully because pre-harvest water deprivation affects current season nuts, and post-harvest water stress directly affects the subsequent yield. The benefits (minimised trunk damage from shakers, hull rot, and ground moisture and humidity, for drying) of pre-harvest deprivation must be balanced alongside the less desirable effects (reduced kernel weight, increase in ‘partial splits and/or ‘hull-tight’ nuts, reduction in late season leaf function, and stress presenting as wilt and/or premature leaf drop and biomass reduction).

The pre-harvest to post-harvest water deficit period varies in length each season and across orchards for a number of reasons: the staggered maturity of pollinators and Nonpareil; orchard size and equipment capacity and availability; and prevailing environmental conditions that may either hasten or delay harvest or drying (and therefore the resumption of irrigation). The severity and duration of the water deprivation affect the varietal responses and capacity to compensate and/or recover. It is also noted that irrigation not only allows growers to manipulate the water status of trees, but also the micro-environmental conditions of canopy heat and humidity. It is also a tool for crop protection chemical and nutrient delivery.

Pre-harvest water stress
Water stress at harvest and pre-harvest has less effect on bud development (and therefore subsequent flowering and leaf out) than do post-harvest drought conditions. However, it has been demonstrated in California that the negative effect on annual vegetative growth, may be cumulative over successive seasons of pre-harvest drought conditions. Fruiting spur growth (and therefore potential productivity) may be significantly reduced (Esparza *et al*., 2001a).
Although other researchers have looked at pre-harvest water stress (Goldhamer *et al*., 2006; Esparza *et al*., 2001b; Klein *et al*., 2001), it is post-harvest water stress and regulated deficit irrigation (RDI) that have been thoroughly investigated in almonds because of their effect on the subsequent productivity of trees.

Post-harvest water stress
It has been clearly shown that post-harvest deficit irrigation or water stress reduces fruitfulness the following season (Goldhamer *et al*., 2006; Girona *et al*., 2003; Lamp *et al*., 2001; Goldhamer and Viveros, 2000; Goldhamer and Smith, 1995). Goldhamer and colleagues have done the most comprehensive research on RDI, its timing and magnitude and have demonstrated the primary effect of post-harvest water deprivation is yield reduction, because of its direct effect on floral bud differentiation. The yield decline is founded primarily in the reduced flower number. However a negative effect on fruit set also is reported. This may be explained by the published observations that the stigmas may emerge before petals in post-harvest stressed tress. Given the fixed period of pollen receptivity, early stigma emergence might result in senescing stigmas by the critical time of pollination. Post-harvest water stress presenting as premature defoliation affects carbohydrate reserves. Low leaf retention through autumn results in weaker vegetative growth and reduced fruiting capacity the following season.

Goldhamer and Smith (1995) concluded that the application of a limited (less than optimal) water volume over a shorter duration in the early part of the season was less effective than irrigating at lower volumes over a longer period (especially when it extended through the post-harvest period), in sustaining production in the subsequent season. In periods of restricted water access, it is particularly important for growers to understand the water-sensitive stages in almond development throughout the season, and to ensure post-harvest water availability.

Biological
Viruses may cause infectious bud failure in several Prunus hosts. In almonds, such bud failure is associated with the calico strain of PNRSV, and is characterised by both floral and vegetative bud failure. This virus (and its bud failure effects) is graft transmissible, unlike NBF. Non-productive syndrome may also be associated with the presence of viruses.

While mites and aphids may be associated with almonds during some seasons, in general they do not affect bud development. However it is agreed that minimised stress and the maintenance of good tree health tree health are beneficial to all tree functions, including bud development.

Nutritional

Tree nutrition influences tree vigour, growth rates, bud size, and the retention of leaves in autumn. Autumn is the period when carbohydrate accumulation occurs in the perennial parts of almond trees (roots, branches and trunk). This carbohydrate is used at the start of the next growing season to support early shoot growth, until the new leaves are functioning and contributing to the tree. It is therefore important to retain leaves on trees as far into autumn as possible (ideally into May). Post-harvest irrigation also assists with leaf retention and the period of carbohydrate accumulation prior to dormancy.

Since nutrition affects functions throughout the tree and season, it is presumed that not only is the leaf functional period important, but also the relative growth of new shoots, leaves, canopy size, fruit load etc. The correlation with spur leaf area and floral bud intensity has been demonstrated. The spur leaf area is positively correlated with the number of lateral buds that transition into floral buds per spur, on non-fruiting spurs (Polito *et al*., 2002). The Californian researchers provided evidence suggesting spurs with low leaf area carried fewer floral buds and that these floral buds had a slower developmental rate.

Summary

In summary, bud initiation and development determine the potential production capacity of an almond tree. Given the effects on bud initiation and formation, of seasonal conditions and water stress, genetics, nutrition and growth capacity, and the presence of viruses, it is important that growers and nurserymen make management decisions throughout the season that are mindful of all stages of bud formation, and the sensitivities of each to the above influences. Detailed record keeping of all cultural practices (pruning, fertilisation, irrigation, pest and disease control etc.), budwood history and sources, and weather events assist investigations of bud growth disorders should they arise.

REFERENCES

Connell, J.H. 2007. *Non-infectious bud failure Management*. Nickels Field Day, May 2007. 2 pp.

Esparza, G., DeJong, T.M., Weinbaum, S.A. and Klein, I. 2001a. *Effects of irrigation deprivation during the harvest period on yield determinants in mature almond trees*. Tree Physiology 21: 1073-1079.

Esparza, G., DeJong, T.M. and Weinbaum, S.A. 2001b. *Effects of irrigation deprivation during the harvest period on nonstructural carbohydrate and nitrogen contents of dormant, mature almond trees*. Tree Physiology 21: 1081-1086.

Fereres, E and Goldhamer, D.A. 1990. *Deciduous fruit and nut trees*. In: Stewart, BA. Neilson, DR. (eds). *Irrigation of agricultural crops (Monograph 30),* American Soc. Agronomy. Wisconsin. pp.987-1017.

Girona, J., Mata, M., Arbonès, A., Alegre, S., Rufat, J. and Marsal, J. 2003. *Peach tree response to single and combined regulated deficit irrigation regimes under shallow soils*. J. American Society of Horticultural Science 128: 432-440.

Goldhamer, D.A. and Smith, T.E. 1995. *Single-season drought irrigation strategies influence almond production*. California Agriculture 49: 19-22.

Goldhamer, D.A. and Viveros, M. 2000. *Effects of preharvest irrigation cutoff durations and postharvest water deprivation on almond tree performance*. Irrigation Science 19: 125-131.

Goldhamer, D.A., Viveros, M. and Salinas, M. 2006. *Regulated deficit irrigation in almonds: effects of variations in applied water and stress timing on yield and yield components.* Irrigation Science 24: 101-114.

Kester, D. 2000. *Non-infectious Bud Failure*. Final Report to the Almond Board of California. Project 99-DK-00. 25 pp.

Klein, I., Esparza, G., Weinbaum, S.A. and DeJong, T.M. 2001. *Effects of irrigation deprivation during the harvest period on leaf persistence and function in mature almond trees.* Tree Physiology 21:1063-1072.

Lamp, B.M., Connell, J.H., Duncan, R.A., Viveros, M. and V.S. Polito. 2001. *Almond Flower Development: Floral Initiation and Organogenesis*. J. Amer. Soc. Hort. Sci. 126(6):689-696.

Polito, V.S., Pinney, K., Heerema, R. and S.A. Weinbaum. 2002. *Flower differentiation and spur leaf area in almond*. J. Hort Sci. and Biotech. 77(4): 474-478.

Thomas, B.T. 2007. *Drought Strategies for the Australian Almond Industry*. Almond Board of Australia. 14 pp.

Tufts, W.P. and E.B. Morrow. 1925. *Fruit-bud differentiation in deciduous fruits*. Hilgardia 1 (1): 1-26.

Other reading

Micke, W.C. 1996. (ed.) *Almond Production Manual*. University of California. 289 pp.

Romero, P., Botia, P. and Garcia, F. 2004a. *Effects of regulated deficit irrigation under subsurface drip irrigation conditions on water relations of mature almond trees*. Plant and Soil 260: 155-168.

Romero, P., Botia, P. and Garcia, F. 2004b. *Effects of regulated deficit irrigation under subsurface drip irrigation conditions on vegetative development and yield of mature almond trees.* Plant and Soil 260: 169-181.

Rosati, A., Metcalf, S.G., Buchner, R.P., Fulton, A.E. and Lampinen, B.D. 2006. *Physiological effects of Kaolin applications in well-irrigated and water-stressed walnut and almond trees.* Annals of Botany 98: 267-275.

Teviotdale, B.L. and Michailides, T.J. 1995. *Reduction of almond hull rot disease caused by Rhizopus stolonifer by early termination of preharvest irrigation.* Plant Disease 79: 402-405.

F:\SRHSDATA\Clients\Almond Board of Australia\Bud failure 2008\Almond bud development Lit review 0109.doc

Appendix 3

Fact Sheet

All About Almonds
Fact Sheet 07 – Almond Bud Initiation and Development

Author: Dr Prue McMichael, Scholefield Robinson Horticultural Services

Welcome to the seventh edition of "All About Almonds", Almond Bud Initiation and Development. Fact sheets are distributed to almond growers via email and fax, in addition to being made available for download from the levy payers' access page on the ABA website: www.australianalmonds.com.au (follow links to the login section of the "industry" page).

The information provided in these fact sheets should be kept confidential.

Summary
Bud initiation and development determine the potential productive capacity of an almond tree. Bud initiation and formation, are directly affected by seasonal conditions (especially temperature extremes and water stress), genetics, nutrition, tree structure and the presence of viruses. Since some of these influences are within the control of growers and nurserymen, it is critical that managers understand the stages of bud formation, and the relative sensitivities of each stage, to the influential factors. This fact sheet describes these influences and the management decisions that may affect their impact.

Background
There have often been seasons in which growers have reported "patchy bloom"; bare shoots; witches' broom growth; or limited extension growth etc. Each of these reflect in part, effects on bud initiation and development. Bare shoots for example may result from failed bud formation, bud death before emergence, abnormal development or extended dormancy. The causes of disrupted or

abnormal bud initiation and development are not all understood, but it is known that buds are influenced individually or in combination, by their genetics; environmental conditions; nutritional, chemical and water status; and by biological organisms.

Several of the above symptoms were visible in many almond orchards during spring 2008. In particular, patchy bloom and a vegetative growth disorder with bare shoots and witches' broom growth on Carmel, were observed. The specific contribution of recent and on-going water restrictions, pre- and post-harvest water stress, and the record high temperatures in March 2008, to these symptoms, are not known. Given the timing of bud initiation (Feb-March) in almonds, it is possible the extreme events of early 2008 contributed to the onset and extent of the spring 2008 symptoms.

This fact sheet has been developed to summarise the information available on bud initiation and flower development, and the influences on them. It is important that growers and nurserymen understand the effects of management decisions and environmental events, on bud formation and development.

The ABA has also initiated a bud growth disorder project that includes: systematic bud development assessments from trees that were affected or unaffected in recent seasons, and from mother trees at Monash; review of weather conditions over the period of bud development in several regions and their potential correlation with bud growth disorder onset in 2008 and/or 2009; review of post-harvest irrigation practices and the distribution of growth disorder symptoms in 2008 and 2009; and recommendations for seasonal management decisions that influence bud development.

Bud Initiation and Development as Steps in the Almond Growth Cycle

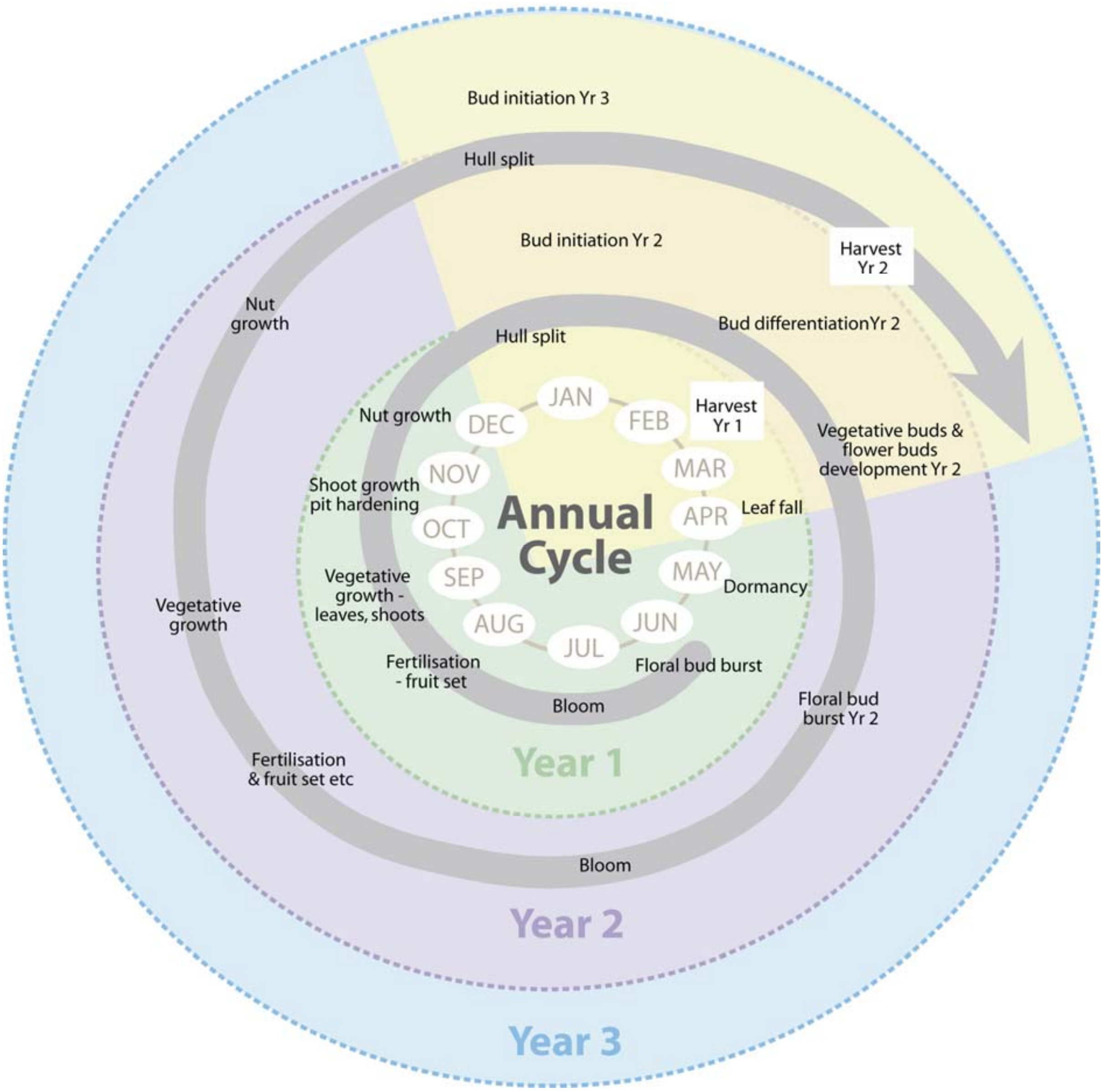
Flower bud initiation is the foundation of tree fruitfulness and yield potential. Therefore, all factors that influence initiation and development of flower buds are of economic relevance. The processes that result in nut formation start before the previous crop has been harvested. Table 1 and **Error! Reference source not found.** illustrate the overlapping developmental stages. The processes are complex and often in conflict with other management practices.

Table 1. Almond bud initiation and development cycle

<table>
<tr><th>SEASON</th><th>MONTH</th><th colspan="2">TREE GROWTH STAGE</th><th>BUD DEVELOPMENT STAGE</th></tr>
<tr><td rowspan="2">WINTER</td><td>July</td><td colspan="2" rowspan="2">Bud burst – Full Bloom</td><td rowspan="2">Emergence of floral buds on shoots or spurs; pollination
Emergence of vegetative buds – leaves</td></tr>
<tr><td>August</td></tr>
<tr><td rowspan="3">SPRING</td><td>September</td><td colspan="2">Shuck Fall – Early Set</td><td rowspan="2">Leaf development and active growth of new shoots</td></tr>
<tr><td>October</td><td rowspan="4">Nut growth</td><td>Pit hardening</td></tr>
<tr><td>November</td><td rowspan="2"></td><td>Growth interruption; bud maturation</td></tr>
<tr><td rowspan="3">SUMMER</td><td>December</td><td>Bud scale formation</td></tr>
<tr><td>January</td><td>Hull split</td><td rowspan="2">Vegetative buds develop. Flower buds initiated</td></tr>
<tr><td>February</td><td colspan="2" rowspan="2">Harvest</td></tr>
<tr><td rowspan="3">AUTUMN</td><td>March</td><td rowspan="2">Flower buds continue to differentiate</td></tr>
<tr><td>April</td><td colspan="2">Post Harvest</td></tr>
<tr><td>May</td><td colspan="2" rowspan="2">Leaf Fall – Dormant</td><td rowspan="2">Vegetative buds in rest period. Flower buds continue to differentiate. Chilling occurs.</td></tr>
<tr><td rowspan="2">WINTER</td><td>June</td></tr>
<tr><td>July-August</td><td colspan="2">Bud burst</td><td>Emergence of flowers, followed by leaves</td></tr>
</table>

Adapted from Kester, 2000; Kester *et al*., 1996, and Thomas, 2007

Figure 1. Cycle of fruitfulness potential in almonds



Source: Adapted from Krstic *et al,* 2005.

Flower buds

The floral buds encompass a terminal flower, but no leaves. More than one floral bud may form on a single spur, or along the length of shoots more than a year old. Relative to other fruit (eg. peach and prune) and nut crops, almonds have late flower bud differentiation (i.e. the transition from vegetative to floral buds). In almonds bud initiation and development (for the following season's nuts) usually coincides with the current season's hull split-to-post-harvest period. Management decisions over that time, influence the tree's capacity and potential yield in the subsequent season), in terms of bloom density and therefore nut count (Figures 1 - 3). Management decisions that affect crop load, carbohydrate reserves and general nutritional status, also influence fruit set. However the percentage fruit set is influenced primarily by the variety and weather conditions during bloom and fertilisation.

Classic research on flower bud development was undertaken in 1925 (Tufts and Morrow, 1925) and more recently by Lamp *et al*. (2001). Polito *et al.* (2002) also investigated the effect of bud position and leaf area on the timing of flower differentiation, between and within spurs. Each investigation included the almond variety Nonpareil, while Lamp *et al.* (2001) also investigated Carmel and Butte with modern techniques and greater understanding of the early morphological events in flower development, and the influence of climatic conditions. These researchers found that floral initiation in some varieties (Carmel and Butte) preceded hull split, but for Nonpareil they concluded floral initiation occurred after hull split.

Lamp *et al.* (2001) photographed, numbered and described eight flower bud development stages (0-7). The stages 0-2 described bud initiation and transition, while stages 3-7 described the flower formation. Almond flowers undergo continuous development (even during tree dormancy), once their transition commences. Vegetative buds however have a rest and maturation period.

Vegetative buds

Vegetative buds form the structure and bearing potential of trees and the capacity to sustain the tree through water uptake, nutrient capture and energy conversion. Leaves are formed in vegetative buds which are pointed in shape. Flowering buds are plumper, and flat-domed at the apex. The terminal bud of a shoot or spur is always vegetative and hence the capacity of almond trees for on-going shoot growth and canopy expansion. The relative growth of terminal and lateral vegetative shoots determines the potential fruit-bearing capacity and structure of the different varieties. The vegetative terminal and lateral buds form during the previous year, then elongate and expand mid-summer. In conditions of excessive nutrition, or when mild conditions extend through autumn, the wood, leaf and bud maturation may be delayed and the duration of active growth extended. In contrast, water-stressed trees may lose leaves prematurely and have reduced vegetative growth and compact canopies. Each of these scenarios have an effect on carbohydrate accumulation at the end of a season, and its availability for leaves emerging from second year wood, in the subsequent spring.

Influences on Bud Initiation and Development

Some factors that affect bud initiation and development may be manipulated while others are inherent or result from external factors over which a grower has little control.

Difficult to manage influences on bud initiation and development

Genetics

A bud development disorder attributed to varietal genetics (and environmental triggers), is prevalent in California in the Carmel variety (but also documented in Nonpareil, Price and several other American varieties) and has curbed the planting of Carmel in California. At its worst, non-infectious bud failure (NBF) may first appear in the spring of the second leaf. It presents as leafless lengths of one-year-old wood that may or may not carry nuts. On occasions a tuft of vegetative growth appears at the ends of bare shoots suggesting that leaf buds (which are formed early or late in the season and therefore often in cooler conditions), remained viable despite the other lateral, vegetative buds between dying or failing to develop the previous summer or autumn. It has been concluded that the vegetative buds which fail in NBF trees, formed and were initially viable. However the affected buds die prior to spring in the following season, and most likely during the previous autumn and winter.

Nuts on NBF shoots,] are evidence that the floral buds have survived despite often being at the same position as a failed vegetative bud. In NBF trees, the bloom period may be delayed (Connell, 2007; Kester, 2000) and the tendency for almond kernel doubles has also been reported (Connell, 2007).

Key NBF researcher, the late Dale Kester found that the failure of vegetative (and sometimes floral) buds to develop was an inherited characteristic with the potential to increase in propensity with repeated vegetative propagation, and in severity with cumulative exposure to high summer temperatures (see below). Once present, NBF cannot be eliminated. Replacement (or top-working) of young (second-fourth leaf), affected trees is often justified economically, but in older trees with symptoms in the upper canopy, the yield decline (through reduction in fruiting wood primarily) often does not justify replanting, unless the framework of the tree has been affected severely.

In order to manage risk and improve planting material quality in NBF susceptible varieties, it is reasonable today for registration, certification and breeding programs, and nursery operations to identify low bud-failure potential trees and clones. The NBF-potential in each clone is dynamic, with temperature exposures, drought stresses, fruit load, tree pruning and management, and re-propagation contributing to NBF expression. Once identified there should be commitment to the selection of single tree sources of buds for mother trees, and to observe progeny (from traceable bud sources) performance and growth in areas conducive to NBF expression (eg. warm-hot areas). The mother trees of bud sources identified with low NBF-potential require management and maintenance such that NBF is minimised (eg. hedge pruning to ensure budwood is from trees and canopy locations closest to the original buds). In California, no Nonpareil or Carmel sources of budwood are considered NBF-free.

Temperature exposure

Although flower initiation is controlled by naturally-occurring plant hormones, the timing and duration of the floral development stages is directly affected by tree genetics and temperature exposures at critical times. The temperature triggers for normal bud development (eg. heat-induced dormancy, chilling hours, and the subsequent warm temperatures to break dormancy and trigger growth etc.) are well-documented.

Almonds rarely have insufficient chill, but in some *Prunus* spp. (eg. apricots, peaches) floral buds may drop before budswell if the winter has been mild. Although the vegetative buds may not drop under similar conditions, they often develop abnormally with an extended leaf out period and weaker shoots. Frosts may kill buds, flowers or cause early abortions, depending on their developmental stage, and the duration and severity of the frost.

Cumulative high-temperature (above 27°C) exposure in the previous summer, affects NBF onset and expression. It is proposed that NBF may also be indirectly triggered in stressed trees since premature defoliation may result in increased canopy temperatures.

Manageable Influences on bud initiation and development

Water status

Almond is generally considered a drought-tolerant tree, however it has been demonstrated that almond trees pass through annual development stages in which water-stress sensitivity varies. The most water-stress sensitive stages in flowering deciduous trees are flowering, fruit set and early stages of fruit growth (Fereres and Goldhamer, 1990). Although almonds are irrigated in Australia, water status still remains critical in terms of almond production levels.

The almond harvest coincides with the floral bud initiation period (Table 1, Figure 1). The post-harvest period coincides with the floral bud development. The usual practice of 'holding off' the water in preparation for, and during harvest and drying, must be managed carefully because pre-harvest water deprivation affects current season nuts, and post-harvest water stress directly affects bud initiation and development, and therefore the subsequent season's yield (See Figure 1 – yellow sector). The benefits (minimised trunk damage from shakers, hull rot, and ground moisture and humidity for drying) of pre-harvest deprivation must be balanced alongside the less desirable effects (reduced kernel weight, increase in 'partial splits and/or 'hull-tight' nuts, reduction in late season leaf function, and stress presenting as wilt and/or premature leaf drop and biomass reduction).

The pre-harvest to post-harvest water deficit period varies in length each season and across orchards for a number of reasons: the staggered maturity of pollinators and Nonpareil; orchard size and equipment capacity and availability; and prevailing environmental conditions that may either hasten or delay harvest or drying (and therefore the resumption of irrigation). The severity and duration of the water deprivation, affect the varietal responses and capacity to compensate and/or recover.

Pre-harvest water stress. Water stress pre-harvest has less effect on bud development (and therefore subsequent flowering and leaf out) than does post-harvest water deprivation. However, it has been demonstrated in California that the negative effect on annual vegetative growth, may be cumulative over successive seasons of pre-harvest drought conditions. Fruiting spur growth (and therefore potential productivity) may be significantly reduced (Esparza *et al*., 2001a).

Post-harvest water stress. It has been clearly shown that post-harvest deficit irrigation or water stress reduces fruitfulness the following season (Goldhamer *et al*., 2006; Girona *et al*., 2003; Lamp *et al*., 2001; Goldhamer and Viveros, 2000; Goldhamer and Smith, 1995). See Figures 2 and 3. Goldhamer and colleagues have done the most comprehensive research on regulated deficit irrigation (RDI), its timing and magnitude. They demonstrated the primary effect of post-harvest water deprivation is yield reduction, because of its direct effect on floral bud differentiation and reduction in flower number (Figure 2). A negative effect on fruit set, kernel yield and fruit load were also reported (Goldhamer and Viveros, 2000). See Figure 3. Post-harvest water stress may also promote premature defoliation, and therefore lower carbohydrate reserves. Low leaf retention through autumn results in weaker vegetative growth and reduced fruiting capacity the following season.

Goldhamer and Smith (1995) concluded that the application of a limited (less than optimal) water volume over a shorter duration in the early part of the season was less effective than irrigating at lower volumes over a longer period (especially when it extended through the post-harvest period), in sustaining production in the subsequent season. In periods of restricted water access, it is particularly important for growers to understand the water-sensitive stages in almond development throughout the season, and to ensure post-harvest water availability.

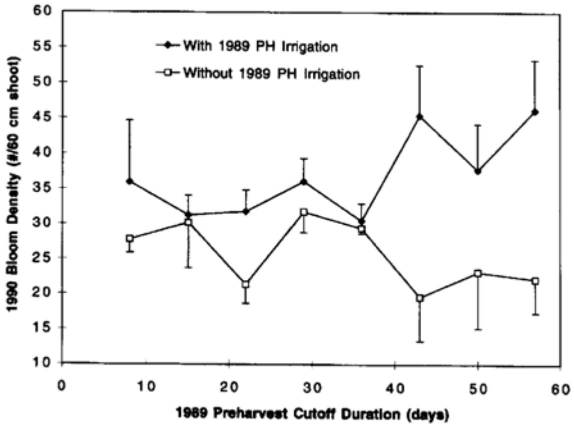


Figure 2. Bloom density related to previous season's preharvest cutoff duration and presence or absence of postharvest (PH) irrigation. Vertical bars represent one standard error (Goldhamer & Viveros, 2000)

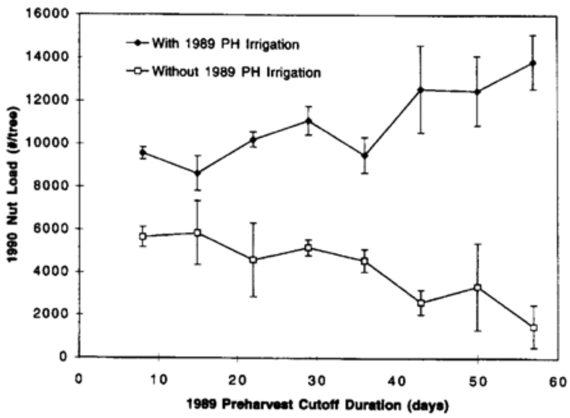


Figure 3. Nut load as related to previous season's preharvest cutoff duration and presence or absence of postharvest (PH) irrigation. Vertical bars represent two standard errors (Goldhamer & Viveros, 2000)

Biological

Viruses may cause infectious bud failure in several Prunus hosts. In almonds, such infectious bud failure is associated with the calico strain of Prunus Necrotic Ringspot Virus (PNRSV), and is characterised by both floral and vegetative bud failure. This virus (and its bud failure effects) is graft transmissible, unlike NBF. This virus is present in Australia and it is recommended that all imported budwood and rootstock material, mother trees and any other local budwood supply trees be routinely tested for its presence.

Nurserymen need to develop a schedule for testing and a system of record-keeping such that Prunus tree and/or budwood purchasers may be able to trace their budwood to its source, access its diagnostic test timeframe and the relevant results, and be assured that hygiene practices within the nursery have not contributed to the transmission of any infectious agent.

Nutritional

Tree nutrition influences tree vigour, growth rates, bud size, and the retention of leaves in autumn. Autumn is the period when carbohydrate accumulation occurs in the perennial parts of almond trees (roots, branches and trunk). This carbohydrate is used at the start of the next growing season to support early shoot growth, until the new leaves are functioning and contributing to the tree. It is therefore important to retain leaves on trees as far into autumn as possible (ideally into May). Post-harvest irrigation also assists with leaf retention and the period of carbohydrate accumulation prior to dormancy.

Since nutrition affects functions throughout the tree and season, not only is the leaf functional period important, but also the relative growth of new shoots, leaves, canopy size, fruit load, etc. Polito *et al.* (2002) provided evidence suggesting spurs with low leaf area carried fewer floral buds and that these floral buds had a slower developmental rate.

Record-keeping

Detailed record keeping of all cultural practices (pruning, fertilisation, irrigation, pest and disease control, etc.), budwood history and sources, and weather events, assist investigations of bud growth disorders when they arise.

Recommendations for Optimal Bud Initiation and Development

- Use budwood and rootstocks that have been regularly virus-tested *and* found free of detectable levels
- Use Carmel budwood from mother trees known to be propagated from original (or as close to original as possible) buds
- Minimise the pre-harvest-to-post-harvest water stress duration
- For current season crops, minimise pre-harvest water stress
- Manipulate nutrition where possible, to ensure leaf retention into May (and an extended period of carbohydrate accumulation)
- Record cultural practices and weather events

REFERENCES and RECOMMENDED READING

Connell, J.H. 2007. *Non-infectious bud failure Management*. Nickels Field Day, May 2007. 2 pp.

Esparza, G., DeJong, T.M., Weinbaum, S.A. and Klein, I. 2001a. *Effects of irrigation deprivation during the harvest period on yield determinants in mature almond trees*. Tree Physiology 21: 1073-1079.

Esparza, G., DeJong, T.M. and Weinbaum, S.A. 2001b. *Effects of irrigation deprivation during the harvest period on nonstructural carbohydrate and nitrogen contents of dormant, mature almond trees*. Tree Physiology 21: 1081-1086.

Fereres, E and Goldhamer, D.A. 1990. *Deciduous fruit and nut trees*. In: Stewart, BA. Neilson, DR. (eds). *Irrigation of agricultural crops (Monograph 30),* American Soc. Agronomy. Wisconsin. pp.987-1017.

Girona, J., Mata, M., Arbonès, A., Alegre, S., Rufat, J. and Marsal, J. 2003. *Peach tree response to single and combined regulated deficit irrigation regimes under shallow soils*. J. American Society of Horticultural Science 128: 432-440.

Goldhamer, D.A. and Smith, T.E. 1995. *Single-season drought irrigation strategies influence almond production*. California Agriculture 49: 19-22.

Goldhamer, D.A. and Viveros, M. 2000. *Effects of preharvest irrigation cutoff durations and postharvest water deprivation on almond tree performance*. Irrigation Science 19: 125-131.

Goldhamer, D.A., Viveros, M. and Salinas, M. 2006. *Regulated deficit irrigation in almonds: effects of variations in applied water and stress timing on yield and yield components*. Irrigation Science 24: 101-114.

Kester, D. 2000. *Non-infectious Bud Failure*. Final Report to the Almond Board of California. Project 99-DK-00. 25 pp.

Kester, D.E., Martin,G.C. and J.M. Labavitch. 1996. *Growth and Development,* in Almond Production Manual 1996. ed W. C. Micke, University of California. pp. 90-97

Klein, I., Esparza, G., Weinbaum, S.A. and DeJong, T.M. 2001. *Effects of irrigation deprivation during the harvest period on leaf persistence and function in mature almond trees*. Tree Physiology 21:1063-1072.

Krstic, M., Clingeleffer, P., Dunn, G, Martin, S. and Petrie, P. 2005. Grapevine growth and reproduction: On overview. ASVO Seminar 2005 “Transforming Flowers to Fruit”, Mildura July 2005.

Lamp, B.M., Connell, J.H., Duncan, R.A., Viveros, M. and V.S. Polito. 2001. *Almond Flower Development: Floral Initiation and Organogenesis*. J. Amer. Soc. Hort. Sci. 126(6):689-696.

Micke, W.C. 1996. (ed.) *Almond Production Manual*. University of California. 289 pp.

Polito, V.S., Pinney, K., Heerema, R. and S.A. Weinbaum. 2002. *Flower differentiation and spur leaf area in almond*. J. Hort Sci. and Biotech. 77(4): 474-478.

Romero, P., Botia, P. and Garcia, F. 2004a. *Effects of regulated deficit irrigation under subsurface drip irrigation conditions on water relations of mature almond trees*. Plant and Soil 260: 155-168.

Romero, P., Botia, P. and Garcia, F. 2004b. *Effects of regulated deficit irrigation under subsurface drip irrigation conditions on vegetative development and yield of mature almond trees.* Plant and Soil 260: 169-181.

Rosati, A., Metcalf, S.G., Buchner, R.P., Fulton, A.E. and Lampinen, B.D. 2006. *Physiological effects of Kaolin applications in well-irrigated and water-stressed walnut and almond trees.* Annals of Botany 98: 267-275.

Thomas, B.T. 2007. *Drought Strategies for the Australian Almond Industry*. Almond Board of Australia. 14 pp.

Teviotdale, B.L. and Michailides, T.J. 1995. *Reduction of almond hull rot disease caused by Rhizopus stolonifer by early termination of preharvest irrigation.* Plant Disease 79: 402-405.

Tufts, W.P. and E.B. Morrow. 1925. *Fruit-bud differentiation in deciduous fruits*. Hilgardia 1 (1): 1-26.

Published by Almond Board of Australia, *PO Box 2246, Berri, South Australia 5343*
Telephone (08) 8582 2055 Facsimile (08) 8582 3503

ACKNOWLEDGEMENTS
The input and suggestions made by Dr Ben Robinson.

Appendix 4

Virus Testing

Appendix 4 - Virus Testing and Results

Virus testing

Leaf samples were collected from four MIA orchards. Samples were collected from both ‘unaffected’ and ‘affected’ trees, in each orchard. None of the trees or leaf samples displayed any “typical” viral symptoms at the time of sample collection.

For each orchard, composite samples from ‘affected’ and ‘unaffected’ trees were separately prepared and sent for virus testing. Only leaves (from either source) of ‘healthy appearance, were collected.

Two rounds of testing are done to detect viruses. The second round uses ‘nested’ PCR tests which are very much more sensitive.

Results

- No symptoms of virus were detected in any of the trees observed in the MIA.
- No sample from either the affected and unaffected tree sources, tested positive (i.e. detection of virus) for Prunus Necrotic Ringspot Virus (PNRSV) and Prune Dwarf Virus (PDV) in the first round of molecular (PCR) testing.
- Only one sample (from an affected tree) tested negative for PDV with ‘nested’ PCR.
- All samples (except one – as above) tested positive for PDV in “nested” PCR tests.

Conclusions

- Viral disease has not been observed any sampled trees
- Virus was not detected through regular PCR molecular tests.
- PDV was detected through nested PCR tests, in both affected and unaffected trees
- Virus presence is not a variable that can reasonably explain the onset or development of the Carmel growth disorder.

Appendix 5

Grower Instructions - Sampling Budsticks

Scholefield Robinson
HORTICULTURAL SERVICES

Appendix 5 – Grower Instructions: Sampling Budsticks

2008 CARMEL GROWTH DISORDER
Investigation 2008/09 – 2009/10

BUD AND SYMPTOM DEVELOPMENT COMPONENTS
In 2008 the ABA was alerted to a range of symptoms (patchy bloom, witches' broom, bare shoots) that suggested abnormal bud development and viability. An initial survey of growers and nurseries suggested the symptoms were predominantly on Carmel; were not unique to 2008 but were more widespread in the 2008 spring; appeared on Carmel in most production districts, in trees with budwood from the two major sources, and from 21 nurseries.

Viruses, high temperatures, genetics and post-harvest water stress are known to affect bud initiation and development. Excessive heat, post-harvest water stress and below optimal irrigation were widespread features of the 2007/08 season but their contribution to the observed spring symptoms have not been determined. Further investigations into bud development in 2009 will provide information on the likelihood of these symptoms being observed again in 2009/10, or increasing in severity.

BACKGROUND
At this stage there is no certainty the 2008 Carmel growth disorder is either 'on-going', increasing or spreading. Equally, there is no certainty about its cause/s. It is possible that environmental, biological and/or genetic factors have contributed to the symptoms.

Potential *biological* causes of blind wood include: viruses
Potential *environmental* causes include: drought stress, low chill, excessive heat at time of bud development
Potential *genetic* causes include: non-infectious bud failure (NBF)

Because there are no immediate and specific tests to confirm a genetic or environmentally-caused growth disorder, investigations are needed to:

- **Follow the development of buds over time**. This will require comparison of bud development on shoots arising from this 2008's affected and non-affected wood, from time of initiation (late-Feb/March) to flower and leaf emergence (July/August). (It is possible the comparison could also distinguish between the performance of buds arising from bare shoots and those from shoots that carried nuts only).
- **Follow the presence, extent and distribution of symptoms over time.** This will require the tagging and regular observation of branches to determine "spread" or "recovery" during 2008/09 and 2009/10. This will also allow observation of the effects of stress removal on productivity and 'recovery', i.e. minimal vs extended post-harvest water stress.
- **Determine virus presence** in symptomatic and asymptomatic trees of same age and source, across several orchards.
- Attempt transmission of the disorder through **budding or grafting buds** from **affected and unaffected trees**, onto clean Shirofugen or GF305 rootstocks. This will involve a commercial nursery and DPIVic at Knoxfield.

SCHOLEFIELD ROBINSON HORTICULTURAL SERVICES PTY LTD
118A Glen Osmond Road, Parkside SA 5063 Australia
Ph: (08) 8373 2488
Email: srhs@srhs.com.au
ACN 008 199 737
ABN 63 008 199 737
PO Box 650, Fullarton SA 5063
Fax: (08) 8373 2442
Web Site: www.srhs.com.au
Offices in Adelaide and Mildura

BUD INVESTIGATIONS

- **Six trees** in your orchard that **showed symptoms** of the growth disorder in 2008 (or earlier), and six that showed **no symptoms** in 2008 should be **tagged** in order to follow 1) **symptom development** next season, and 2) **bud development from initiation to flowering and leaf out**, next season.
- Several scaffolds on each of **four** (of the six) tagged trees from the affected and non-affected groups will be **photographed** and assessed for **distribution** and **extent of canopy symptoms** in **winter** (rough bark, raised bark lesions, necrosis around buds)**,** and **spring** (flowering, leaf out, bare shoot presence etc.) and summer (if warranted).
- The remaining two (of the six) tagged trees from each group will be used for the **bud development** component. **Buds on laterals arising from affected and non-affected wood in spring 2008** will be removed at two-week intervals (from after hull-split, i.e. late February 2009 until leaf out) and sent to Scholefield Robinson for bud dissection (to look for bud presence, abnormal buds, staining, necrosis, or abscission layers etc.)*.

* Do not send any budsticks on a Thursday or Friday as they risk being left in the mail over a weekend. Overnight mail bags will be provided.

Guidelines for tagging trees

1. Choose Carmel trees of same age and source – 6 with and 6 without the 2008 Carmel growth disorder. Four of each group will be followed during season visually. Two of each will be used as sources of budsticks.
2. Tag with **flagging tape around trunk or butt paint** and prepare rough **map** of location, **row number and tree numbers.** Use same colour flags for affected (eg red/pink) and a different colour for the unaffected (eg. blue).
3. **Distinguish** the two trees in each group to be used as **budstick sources,** by using **an additional tag (eg white flagging tape)** on those trees.
4. **Identify scaffolds*** (after leaf drop) to be followed visually (photographically) in each of the four other trees per group. These will be observed through winter, spring/summer. Tag these scaffolds with **orange (or something bright) flagging tape** so they may be readily seen, even after leaf out. (* this can be done by Scholefield Robinson on the first winter visit, if necessary).

Guidelines for cutting budsticks from tagged trees

1. Remove sticks from the **two** (previously tagged), *unaffected* trees before removing them from the **two** *affected* trees, in each collection period.
2. Clean secateurs between trees.
3. **Remove 6 budsticks** per tagged tree, from **around the canopy** (north, south, east and west quadrants) at **mid-high canopy height**, on each cutting occasion, i.e. 24 sticks will be sent every two weeks from late February.
4. Choose budsticks that are not excessively long. (We need to look at all the buds along the length).
5. From **affected trees**: choose one-year-old budsticks that have **arisen from the terminal end or basal end of bare shoots (or those that carried nuts only)** (SEE PHOTOS)

6. From **unaffected trees**: choose one-year-old budsticks that have arisen from **shoots that were productive** (flowers and leaves) in 2008.

7. Bundle the 6 sticks from each tree and **label each bundle, as either A, B, C or D** - and with **orchard name** and the **date of collection** - on every cutting occasion. Please also record the source (basal or terminal) of laterals from the **affected shoots that had** ii) **tufted growth** at end of bare shoots. Only basal laterals will be available from i) **bare shoots** or iii) **shoots that carried nuts, but no leaves.** SEE PHOTOS.

Please be consistent – i.e. every bundle from a particular tree retains the ***same letter code*** throughout the cutting period. *Remember to keep a record of which trees the codes refer to, and provide this to Prue at Scholefield Robinson*. (The Technical Officer doing bud dissections will not know the codes).

8. **Cool the budsticks** after cutting (but do not freeze); **maintain moisture** in them by either wrapping base in moist paper towel or newspaper. Do not wet/soak them as they'll rot. Enclose the four bundles in a plastic bag and send* in the provided **overnight (pre-addressed) delivery bags**, as soon as possible after cutting.

* Do not send any budsticks on a Thursday or Friday as they risk being left in the mail over a weekend. Overnight mail bags will be provided.

NOTE: There are no quarantine issues/restrictions on the movement of almond budwood into SA for dissection work.

OTHER HELPFUL INFORMATION

Tree histories of tagged, Carmel trees

To assist with interpretation of results, we may need information on the tree histories – planting date, rootstock, nursery source, site history, any particular stress suffered in the last two-three seasons; season of first observed Carmel growth disorder and some seasonal information as indicated below.

Tree records (draft template attached for reference) **for Carmel** tagged trees **AND Non-pareil** of comparative age - **in same orchard**

- pre-harvest water cut-off 2008 and 2009
- the first post-harvest irrigation for 2008 and 2009
- number of post-harvest irrigations 2008 and 2009
- dates of (majority) leaf fall in summer/autumn 2009
- bud burst 2009
- full bloom 2009

Thank you very much for considering cooperation with this investigation.

Please contact Prue McMichael at (08)-8373-2488 or prue.mcmichael@srhs.com.au, with any questions or suggestions.

EXAMPLES OF RECORD-KEEPING TEMPLATES

Tagged trees: Record template - Example

ORCHARD BLOCK NAME: R/L 804

Tree* number	Row number	Planting date; rootstock	Source: Budwood/ rootstock	2008 affected	Scaffold (visual) orange tags	Budstick source (white tag)	Budstick bundle code
1	24	2000 /pch	ABA/Ausbud	+	✓	-	
2	25			+	✓	-	
3				+	✓	-	
4				+	✓	-	
5				+	-	✓	A
6				+	-	✓	B
7				-	✓	-	
8				-	✓	-	
9				-	✓	-	
10				-	✓	-	
11				-	-	✓	C
12				-	-	✓	D

* as numbered in the orchard

Budstick source trees - Background information template – Example

ORCHARD BLOCK NAME: R/L 804

Tree* number	Row number	2008 affected	2008 symptoms*	Pre-harv. irrig'n cut–off 2008	Post-harv. Re-start irrig'ns 2008	Pre-harv. Irrig'n cut-off 2009	Post-harv. Re-start irrig'ns 2009
5	24	+	i; ii	Jan 18	March 7 (3x)	Jan 14	Mar 17 (2x)
6	25	+	iii.				
11		-					
12		-					

* identify 2008 symptoms in tagged trees as **i)** bare wood – no leaves or nuts; or **ii)** bare, but with terminal growth tufts; or **iii)** nuts only, no leaves

Budstick samples - Information – Example

ORCHARD BLOCK NAME: R/L 804

Tree* number	Sample code	2008 affected*	Laterals source**	Cutting dates									
5	A	+	terminal	Feb 18									
6	B	+	basal	"									
11	C	-		"									
12	D	-		"									

* Symptoms in affected, tagged trees **i)** bare wood – no leaves or nuts; or **ii)** bare, but with terminal growth tufts; or **iii)** nuts only, no leaves
** For trees with symptoms **i)** or **iii)**, only *basal* laterals will be available for cutting in 2009. In trees with symptoms **ii)**, identify if *basal* and/or *terminal* laterals are included in the budstick bundle.

Budstick bundles labels:

Include : **Orchard block number**; **sample code (A,B, C, D); cutting date; basal or terminal laterals in sample.**

There are no quarantine restrictions on the movement of almond bud wood into SA for dissection.

CO-OPERATOR RECORD SHEETS

Tagged trees

ORCHARD BLOCK NAME:

Tree* number	Row number	Planting date; rootstock	Source: Budwood/ rootstock	2008 affected	Scaffold (visual) orange tag	Budstick source white tag	Budstick bundle code

* as numbered in the orchard

Budstick source trees - Background information

ORCHARD BLOCK NAME:

Tree* number	Row number	2008 affected	2008 symptoms*	Pre-harv. irrig'n cut–off 2008	Post-harv. Re-start irrig. 2008	Pre-harv. Irrig'n cut-off 2009	Post-harv. Re-start irrig. 2009

* 2008 symptoms: **i)** bare wood-no leaves or nuts; **ii)** bare, but with terminal growth tufts; or **iii)** nuts only, no leaves

Budstick samples - Information

ORCHARD BLOCK NAME:

Tree number	Sample code	2008 affected*	Laterals source**	Cutting dates									
5	A												
6	B												
11	C												
12	D												

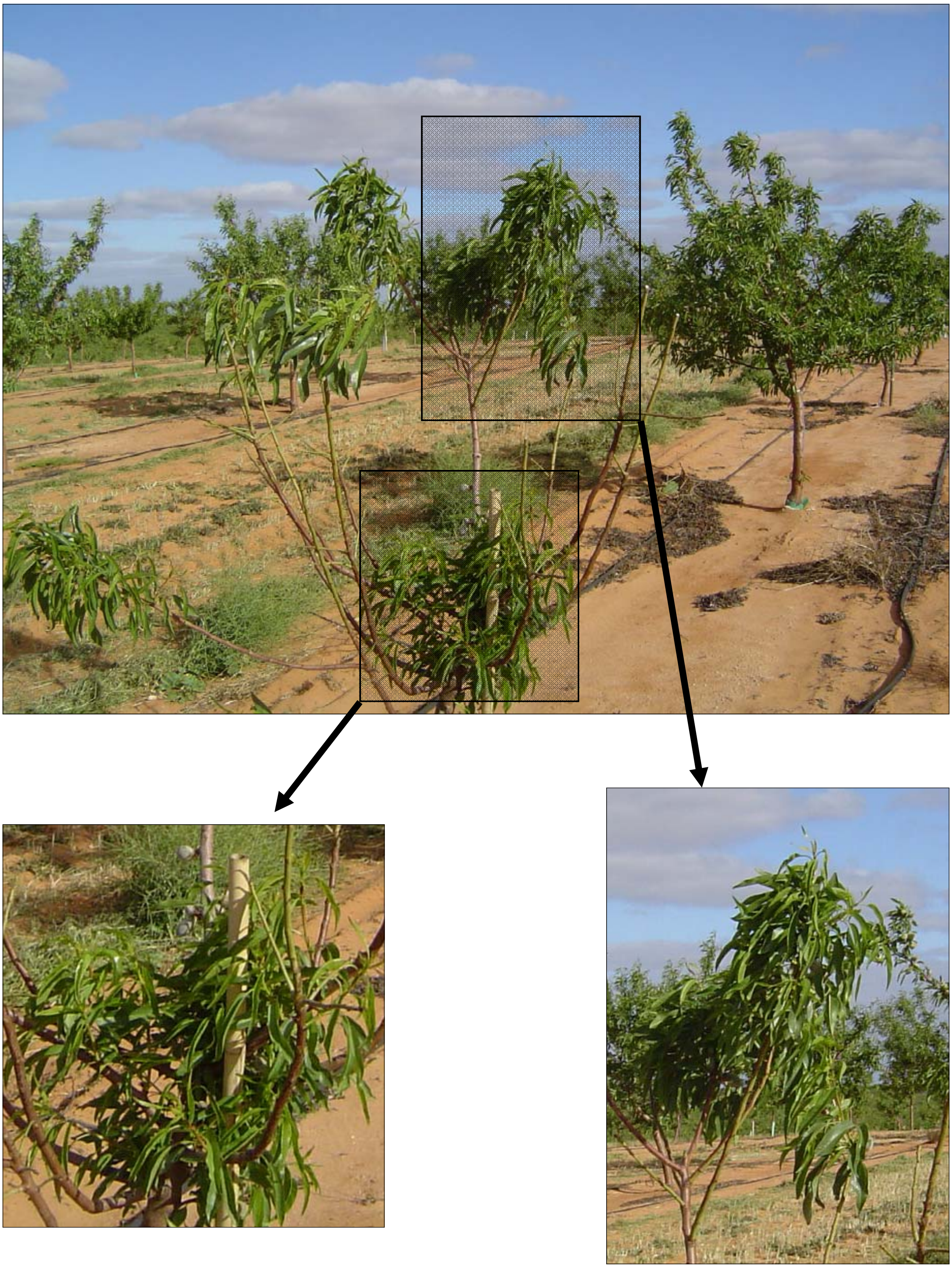
* 2008 symptoms: **i)** bare wood-no leaves or nuts; **ii)** bare, but with terminal growth tufts; or **iii)** nuts only, no leaves

****** For tagged trees with symptoms **i)** or **iii)**, only *basal* laterals to cut in 2009; trees with **ii)**, indicate if *basal* and/or *terminal* laterals included in the budstick bundle.

Other general information for the Orchard Block

Variety	2008 bud burst	2008 full bloom	2008/09 leaf fall	
Carmel				
Non-pareil				
Variety	2009 bud burst	2009 full bloom	2009/10 leaf fall	
Carmel				
Non-pareil				

SHOOTS WITH TUFTED GROWTH AT END



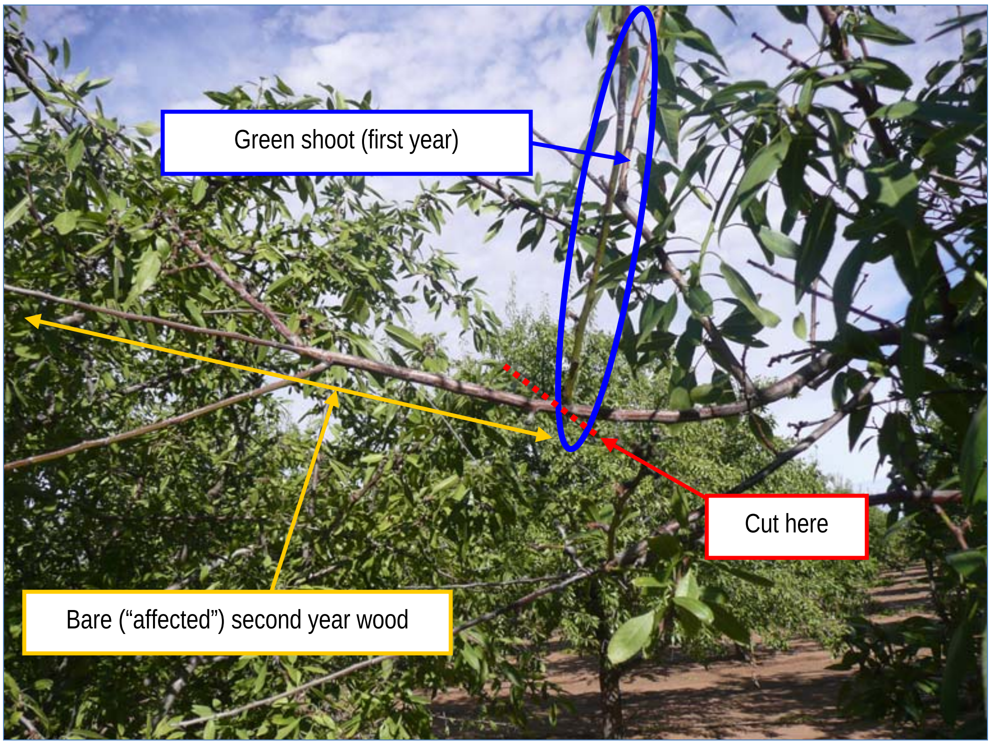
Collect lateral shoots that have grown in 2008 from the tufted end and below the blank wood

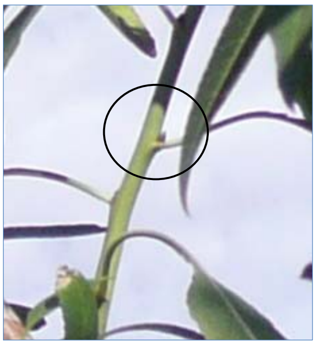
BARE SHOOTS WITHOUT TUFTED GROWTH AT END

Collect lateral shoots that have grown in 2008 from below the blank wood

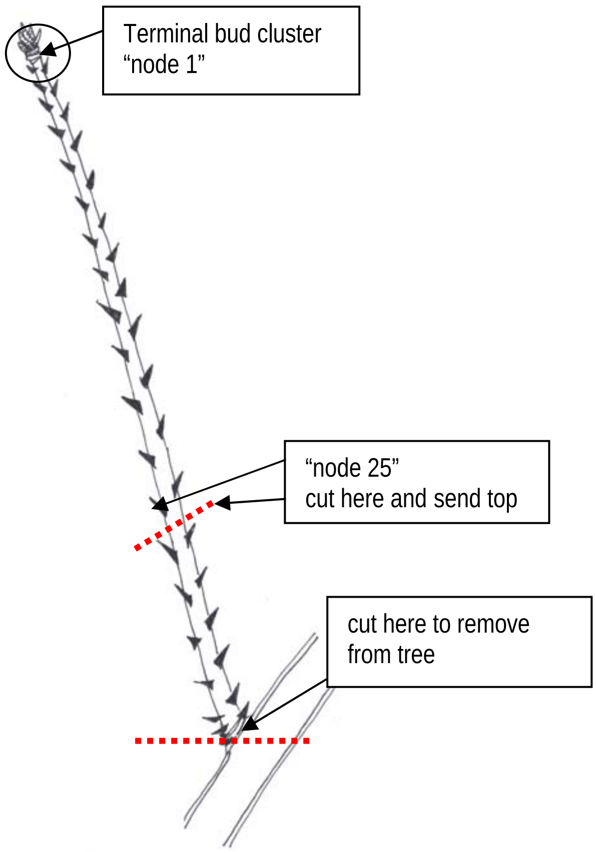
Almond Budstick Harvest Instructions

Preferred shoots: green (first year) laterals arising from bare ("affected") second year wood. Cut only the green shoot and send it. Only the first 25 nodes will be assessed, so if the shoot is too long for the postbag, count backwards from the terminal bud cluster (counted as "node 1") to "node 25" and cut there (refer to diagram below). Leave the leaves on if they are still there as they protect the buds during transit.





A node = a bud or group of buds at a leaf point.



Appendix 2

Milestone Report 3 October 2009

Scholefield Robinson
HORTICULTURAL SERVICES

Milestone 3 Report:
Project AL08015
Carmel Growth Disorder

Prepared for : Horticulture Australia Limited

By : Prue McMichael & Kate Delaporte

Date : October 2009

Milestone 3 Report : Carmel Growth Disorder

HAL Project No. AL08015

Prepared by Dr Prue McMichael & Dr Kate Delaporte

Scholefield Robinson
Horticultural Services Pty Ltd
PO Box 650
Fullarton SA 5063
Ph (08) 8373 2488
Fax (08) 8373 2442
www.srhs.com.au

October 2009

TABLE OF CONTENTS

LIST OF APPENDICES

1 SUMMARY

This stage of the project aimed to determine if bud failure was perpetuated in shoots arising from affected wood. Investigations were focussed on the development and internal condition of buds from affected and non-affected trees, from late summer until spring. These buds were also compared with those from Carmel mother trees at Monash. Bud dissection results were correlated with leaf out in spring. A review of high temperature exposure during recent seasons was conducted. The results and other observations are reported in this Milestone 3 report.

1.1 Temperature Review - Background

The purpose of reviewing temperatures in various ways in this investigation was to compare recent high temperature events in our almond production regions with those published as being influential in triggering non-infectious bud failure (NBF) in California. Californian research has demonstrated that high temperature events in one season affect vegetative bud viability in the subsequent season.

Kester and Gradziel (1996) reported that high temperatures are a stress trigger rather than the direct cause of bud failure. They correlated high temperatures (as degree days over 28°C) during late spring/early summer with a greater incidence of bud failure in the following spring in the Carmel cultivar. The best correlation was with the late-May-early June temperatures (in California). Buds that failed to leaf out in spring had been visibly damaged by the previous autumn. The researchers also concluded that high temperature exposure triggering bud failure could be experienced in mother trees, the nursery or orchard, and that NBF would eventually appear in any 'susceptible cultivar'. They noted that 'susceptible cultivars were those that had genetic NBF-potential. In California these include clones of Non-pareil, Carmel, Jordanolo and Price.

It is evident from field inspections that failed buds are generally in the mid-section of a shoot. Basal and terminal buds failed less frequently. There is reason to believe this relates to the time during which the buds have developed, e.g. basal buds are formed earlier than those 'above' it. They form during cooler weather. Shoots that grow late in the summer are at the terminal ends of shoots and presumably they grow during cooler autumn weather. Evidence of their survival is the tufted growth that appears at terminal ends of some bare shoots. In between are the buds that are exposed to long periods of high temperatures and it is these that fail most often.

Related also is the extent of vegetative growth in young trees. Affected, young trees display a greater proportion of failed buds than mature trees. Mature trees have more reproductive than vegetative growth. It might also be proposed that trees pushed hard from a young age, if susceptible, will show bud failure sooner. Californian researchers have established that location (and therefore heat exposure) and the source of buds (bud failure potential), interact to determine the onset of bud failure. Moisture stress may affect the rate and severity of bud failure.

The Australian almond industry underwent rapid expansion during the last decade, and at times the demand for planting material outstripped supply from the industry's budwood scheme (Monash). It is known that not all orchards established in the last decade were done so with budwood from Monash. Many nurseries provided almond material for the first time over that period and traceability to actual bud sources is very limited. During the last five seasons, almonds have been established for the first time, in the Riverina.

With extrapolation from the Californian information and knowledge that the Carmel clones utilised widely in Australia's almond industry originated from California, we have presumed that high temperature exposure of Carmel during the southern hemisphere months of November – December may similarly result in buds that fail to grow and develop normally.

1.2 Temperature Review – Australian Production Regions

Our review of temperatures was initially focussed on comparisons of accumulated degree days (DD) over 27°C in three regions, with 4-year and 10-year averages. Consideration has also been given to extreme temperature events (days recording maxima over 35°C) in the production regions centred on Griffith (NSW), Mildura (Vic) and Renmark (SA). A similar review of minimum temperatures will be conducted to confirm (or otherwise) that required chilling hours have been met in recent seasons.

Data were sourced from the Bureau of Meteorology and DD were calculated according to Rubatsky and Yamaguchi (1997). Californian data, as published in Fig 12.1, in Kester & Gradziel (1996), has been considered. This reference however does not identify either the data source/s or relevant time periods. It is unclear if the data are from a single season and location or if they are a 'representative' average from a number of areas and seasons.

Relevant Australian high temperature data are summarised in Tables 1-4. Figures in bold indicate a notable increase over the average for region, or time period.

Table 1a – Regional high temperature exposure (DD over 27°C) - spring-early summer

Location		Accumulated degree days (DD) >27°C (October 1- December 31)				Average	
	?	2005	2006	2007	2008	4-yr	10-yr
California	180						
Griffith		127	186	151	108	143	128
Mildura		127	157	173	97	138	129
Renmark		142	175	193	110	155	140

Table 1b – Regional high temperature exposure (DD over 27°C) - summer-early autumn

Location		Accumulated degree days >27°C (January 1- March 31)				Average	
	?	2006	2007	2008	2009	4-yr	10-yr
California	330						
Griffith		330	301	243	286	290	243
Mildura		281	242	254	267	261	236
Renmark		284	251	264	275	268	238

Table 2a - Regional high temperatures (> 27°C) - spring-early summer

Location		2005	2006	2007	2008	4-yr average
Griffith	Average daily max (°C)	28.2	30.0	28.9	27.7	28.7
Mildura		28.0	29.1	29.2	27.4	28.4
Renmark		28.5	29.5	29.7	27.8	28.9
Griffith	No. days with max >27°C	56	63	61	54	59
Mildura		44	56	56	43	50
Renmark		53	53	59	48	53

Table 2b - Regional high temperatures (> 27°C) - summer-early autumn

Location		2006	2007	2008	2009	4-yr average
Griffith	Average daily max (°C)	34.3	33.4	31.7	33.1	33.1
Mildura		32.9	31.8	31.9	32.5	32.3
Renmark		33.0	32.0	32.3	32.8	32.5
Griffith	No. days with max >27°C	85	82	73	72	78
Mildura		77	75	66	75	73
Renmark		77	75	67	80	75

Table 3a – Extreme (> 35°C) heat events - spring-early summer

Location	Days with maximum >35°C (October 1- December 31)				Average	
	2005	2006	2007	2008	4-yr	10-yr
Griffith	12	22	16	8	15	13
Mildura	12	15	18	8	13	12
Renmark	14	19	22	9	16	14

Table 3b – Extreme (> 35°C) heat events - summer-early autumn

Location	Days with maximum >35°C (January 1 – March 31)				Average	
	2006	2007	2008	2009	4-yr	10-yr
Griffith	43	36	31	28	34	28
Mildura	28	28	31	27	28	26
Renmark	31	28	35	29	31	28

Table 4 – Extreme (> 35°C) seasonal exposures (October – March)

Location	Growing season days (total) with maximum >35°C				Average	
	2005/06	2006/07	2007/08	2008/09	4-yr	10-yr
Griffith	55	58	47	36	49	41
Mildura	40	43	49	35	42	38
Renmark	45	47	57	38	47	42

1.3 Temperature Review – Results Summary

Although the production regions had notable heatwaves in March 2008 and January 2009, it cannot be assumed from our investigation to-date, they alone have triggered bud failure. Of greater significance may be consecutive seasons of high temperature exposure or early season high temperature exposure.

Discussion: Data (Tables 1a and 1 b) of the accumulated degree days over 27°C, and daily maxima are discussed below.

Griffith: Season 2006/07 was notable in terms of high temperature exposure, when compared with other seasons in the past four years. In spring-early summer, there were 186 accumulated degree days over 27 C, and another 301 in the summer-early autumn period. These exposures exceed the average of those experienced over the decade starting 1999/00, for the Griffith region (128 and 243 respectively). In summer-autumn 2005/06, there were 330 accumulated degree days over 27°C and this is comparable to the temperature profiles Californian researchers believe capable of triggering NBF in susceptible cultivars.

The spring-summer average daily maximum in 2006 was 30°C, not far below the summer-autumn average of 33°C. These results suggest the hot summer of 2005/6 was followed by a hot spring and summer in 2006/07. During the 2006/07 growing season (October –March) a total of 145 days had a maximum > 27°C.

Mildura: The 4-year and 10-year averages for accumulated degree days over 27°C in the Mildura region are 138 and 129 respectively for the early part of the growing season (October-December). Unusual, early season heat exposure was experienced in both 2006 (157 degrees days) and 2007 (173 accumulated degree days). However the latter parts of these seasons were not extreme in terms of maximum temperatures.

Renmark: Historical (4-year and 10-year averages) early season accumulated degree days over 27°C are 155 and 140, respectively. These are higher than averages for both Griffith and Mildura. The Riverland spring-early summer exposures in both 2006 and 2007 were high, with 175 and 193 accumulated degree days over 27°C recorded. These are comparable with Californian data for the same (equivalent) seasonal stage.

The summer-autumn heat exposure in 2006 was 284 accumulated DD - considerably higher than the 10-year average of 238. The summer 2008/09 had 284 accumulated DDs over 27°C. The hot summer of 2005/2006 in this region was followed by abnormally warm springs in 2006/07 and 2007/08.

Discussion: Data (Tables 2-4) from the review of extreme temperature (over 35°C) exposure are discussed below.

The 10-year averages for number of extreme heat days in a season (October – March) are similar across the regions with Mildura and Griffith (38) recording the least and Renmark the most (42). For both the spring-early summer and the summer-early autumn periods, days over 35°C have exceeded the 10-yr averages in each region, as has the 4-year average in each region.

In spring-early summer 2008, all regions recorded milder temperatures with days exceeding 35°C maxima below both the 4-year and 10-year averages. However the heatwave of March 2008 significantly increased the 'total days over 35°C' in Mildura and Renmark, to well above the 4-year and 10-year averages.

The heatwave of January 2009 saw a concentrated period of extreme temperatures, but neither the summer nor seasonal 'total days over 35°C' exceeded in that period, the 4-year or 10-year averages in any of the three regions.

Griffith: The summer period of 2006 was extremely hot with 43 days over 35°C recorded. The 4-year average for this time of the growing season is 34 days and the 10-year average, 28 days. This hot summer was followed by a hot spring in 2006, in which 22 days exceeded 35°C. This was 'extreme' given the 4-year average for this period being 15 days and the10-year average, 13 days. The 2005/06 and 2006/07 seasons had 55 or more days over 35°C compared with the 10-year average of 41 days.

Mildura: Spring 2007 was hot with 18 days over 35°C, compared to the 10-year average of 12 days. This period contributed to a season (2007/08) during which the maximum temperature exceeded 35°C on 49 days – well above the10-year average of 38 days.

Renmark: The spring-early summer period of 2007 included 22 days in which the maximum temperature exceeded 35 C. This was followed by a summer-early autumn period during which another 35 days exceeded this temperature. The season (2007/08) therefore had 57 extreme heat days, compared with a 4-year average of 47 days and 10-year average of 42 days.

1.4 Temperature Review – Conclusions

- Almonds in each region, have been exposed during the last four seasons to higher (than longer term average maxima and/or accumulated degree days over 27°C) temperatures. This is likely to be of most significance to bud failure in young and new plantings.
- Temperature extremes have been recorded during both spring-early summer and summer-early autumn periods. Some are comparable with those considered capable of triggering NBF in California.
- It cannot be determined from our investigation if short periods of extreme temperatures (heatwaves) result in fewer bud failures than longer exposures to higher temperatures (eg accumulated degree days over 27).
- The MIA region has experienced more frequent and significant increases over 'average' high temperatures and accumulated degree days, than the Sunraysia or Riverland regions, during the last four seasons.
- Consecutive seasons of extreme and frequent high temperature exposure in the Griffith (eg summer 2005/06; spring-summer 2006/07) may have contributed to the extensive bud failure and abnormal vegetative growth in young Carmel trees in this region.
- Periods of extreme and frequent high temperature exposure in the Mildura and Renmark regions during season 2007/08, may yet result in more observed bud failure in young Carmel trees in this region.
- Season 2008/09 overall was relatively cooler. This may be relevant to the lack of 'new' onset bud failure observed this spring.

1.5 Bud Dissections and Bud Status - Background

Kester and colleagues (Refs 1-4) investigated the genetic contribution to NBF in Carmel. He concluded that buds distant to the original bud (through generations of vegetative propagation), had greater bud failure potential. As such, the source of the bud used in propagation, is critical.

In Australia, the industry's mother trees at Monash have been the primary source of Carmel budwood over a long period, however during the planting boom over the last decade it is known that Monash buds have not been used by all nurseries. It is also known that nurseries that previously had not provided almond planting material, entered into almonds as the demand for them increased. It is likely that some Carmel trees have been established with budwood distant to the Monash mother trees, and therefore with genetically unstable material. If our observed bud failure disorder is in fact NBF, high temperature exposures as observed over the last four seasons, and water stress due to restrictions, become more relevant.

Almond orchards lack traceability to the specific source (tree) of their budwood. Most orchards have traceability to the nursery that provided their trees and in turn nurseries should have traceability to their budwood source location. There is however no industry system in place to identify specific trees (or rows) from which budwood is cut. Monash has annual records of those who have purchased budwood. It is presumed that those nurseries for which there are no Monash sale records, sourced their Carmel budwood elsewhere.

Bud dissections by Scholefield Robinson were undertaken to learn about bud quality and viability at various times of the year. Healthy buds are uniformly coloured inside, lack necrotic areas either within or under the bud. Floral buds grow consistently after their transformation, whereas vegetative buds tend not to increase in size over the dormant season. Floral buds emerge prior to leaf buds.

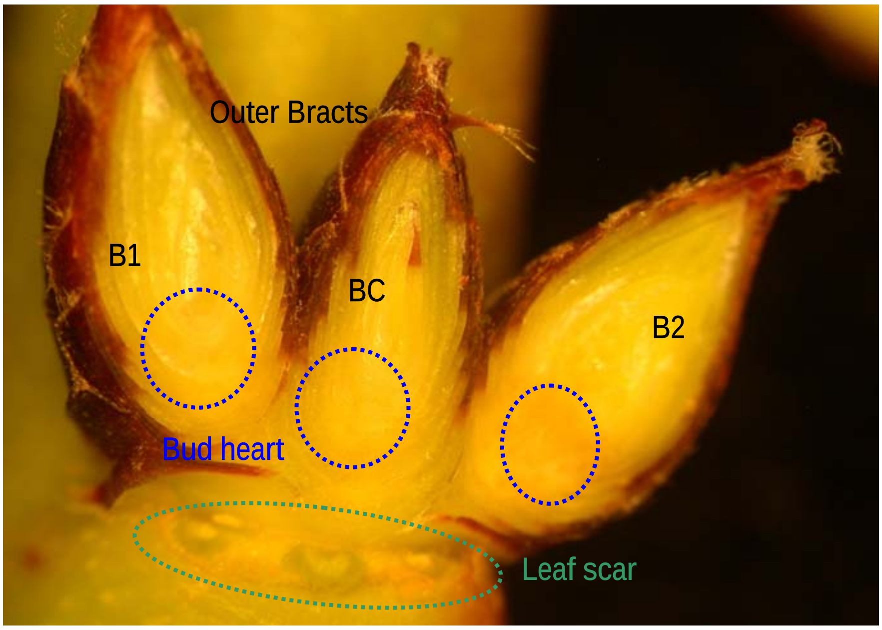
Co-operators provided to the investigators, budsticks cut from Monash Carmel mother trees, and ‘affected’ and ‘non-affected’ Carmel trees across three production regions. The budsticks were cut from the same trees at 2-3 week intervals from March-June and again late July–August. Dr Kate Delaporte dissected every bud, or in situations where more were provided, the youngest 24 buds and those in the terminal cluster. Bud 1 was identified as the first one below the apical tip cluster. The relative health and physiological status of each bud was recorded.

1.6 Bud Dissection and Bud Status – Methodology Summary

The budstick cutting instructions for grower co-operators are included in Milestone 2.

Buds were dissected longitudinally to expose internal developmental status and health status (Figure 1). The damage location, types and severity were assigned a number or letter code to ensure consistency in recording observations. Four of the 10 categories and their codes are shown in Figure 2.

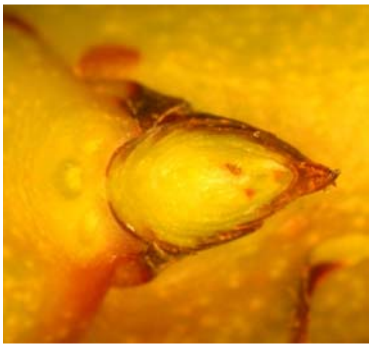
Figure 1: Almond bud set at a given node (longitudinally cut)



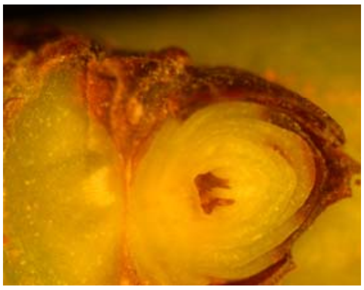
B1 = Bud 1 (Left side); BC = Centre Bud; B2 = Bud 2 (Right side)
Bud heart = the growing point of the bud
Leaf scar = the leaf attachment point
Outer bracts = the brown, lignified outer bracts that protect the bud heart

Figure 2: Visual description of symptoms and codes (excerpt)

1 = healthy green bud, 0% browning



1x = healthy green bud; with possible lignification inside bud, but not at bud heart, usually tip section, 0% browning



DC = bud heart is dead but still present

D = entire bud dead but still present

1.7 Bud Dissection and Bud Status – Results Summary

Grower co-operators were provided with a summary report on the dissection results in April 2009 (six weeks after commencement of dissections). This report is attached as Appendix 1 and the results are summarised below. Bud dissections in March-April provided the first indication that the field symptoms of 2008 likely resulted from bud damage and necrosis, rather than the failure of buds to form.

1.7.1 March-April Observations

The observations from three participating and representative orchards, one from each of the MIA, Riverland and Sunraysia, are summarised below and in Table 5.

- Damaged buds are evident by late summer
- External view of bud is not indicative of the bud's internal health status
- All orchards had some budsticks with damaged buds
- Both *affected* and *non-affected* trees produced budsticks with some damaged buds
- Buds from (2008) *affected* wood had more buds that were damaged
- Most damage appears as a dark area in the growing point region
- Some buds were dead by early March 2009
- Within bud cluster at a node, more central (BC) than lateral buds (B1, B2), were damaged
- Damaged central buds generally had more extensive damage than damaged lateral buds.

The following observations are of buds removed from the Carmel mother trees (at Monash) in the same period:

- Buds from the ABA budwood scheme (Monash) consistently had the least damaged buds (Figure 3)
- Almost all central buds (98%) appeared healthy
- Most (82-85%) of lateral buds appeared healthy

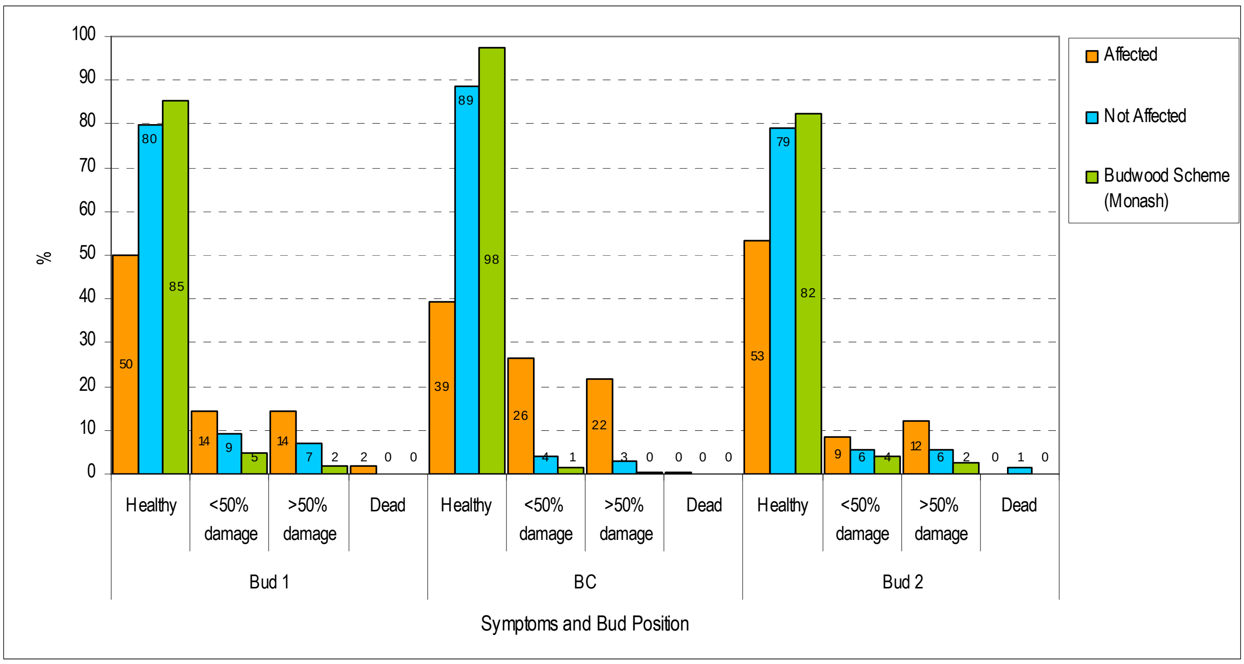
The summary data from the March-April and subsequent dissection periods (May-June and July-August) are provided in Table 5 and Figures 3-5. Graphical presentation of this data set is included as an Appendix (Appendix 2). Throughout the season 'missing buds' were recorded. It is possible that transit conditions contributed to their loss, especially as the buds became larger later in the season.

Table 5 - Bud damage over the growing season

Tree status	% healthy			% damaged or dead (ratings 2, 3, 5)		
	March - April	May -June	July - August	Mar-Apr	May –June	July - August
Affected						
All buds*	60	49	40	31	40	47
Central buds	55	53	28	35	46	67
Non-Affected						
All buds	86	88	90	8	4	4
Central buds	87	93	95	7	5	4
Budwood Repository (Monash)						
All buds	92	92	89	5	<1	<1
Central buds	95	98	96	4	<1	1

* regardless of bud position or collection location (MIA, Riverland or Sunraysia).

Figure 3: April 2009 - Relative levels of early damage in central and lateral buds from *affected*, *non-affected* and ABA Budwood Scheme (Monash) trees



1.7.2 May-June Observations

General observations from May-August from three participating and representative orchards, one from each of the MIA, Riverland and Sunraysia follow:

- Transition to floral buds was evident by May-June dissections
- Affected MIA trees had few floral buds (Figure 4)
- Damaged buds did not recover during dormancy
- More central buds are damaged than lateral buds
- Buds damaged by late summer appeared not to have shrivelled or fallen out
- External view of buds is not indicative of a bud's internal damage
- Some floral buds are damaged (Appendix 3 - Photos 1, 2)

Buds from Monash were collected and dissected over the same period. Their appearance is discussed below.

- Monash budwood had a very high percentage of healthy buds at each collection period
- A high proportion of Monash buds had transitioned to floral buds by May

1.7.3 July–August Observations

General observations over this period indicated that:

- Floral transition was delayed in affected wood (Figures 4, 5)
- Lateral buds primarily transitioned to floral buds if not damaged
- Damaged buds (vegetative or floral) are smaller than undamaged buds
- Buds that show early damage do not recover

Figures 4-6 illustrate that damage may occur in lateral and central buds and that affected wood has delayed transition to floral buds, and fewer buds make the transition. Central buds primarily are the vegetative buds and damage levels in these may be used to predict the potential leaf out in the spring. Central bud data for the whole dissection period (March-August) is presented in Figure 6.

Figure 4: May-June - Level of transition to floral buds in affected and non-affected MIA trees and Monash mother trees

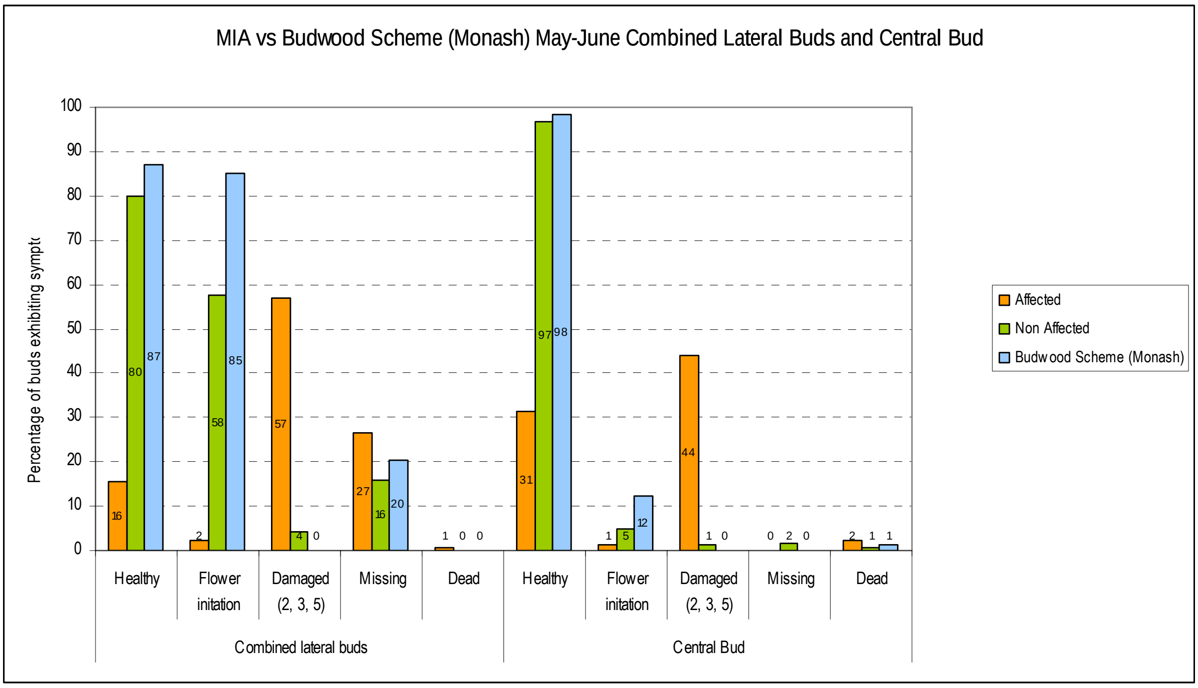


Figure 5: May-August, 2009 - Relative damage in central and lateral buds from *affected*, *non-affected* and ABA Budwood Scheme (Monash) trees

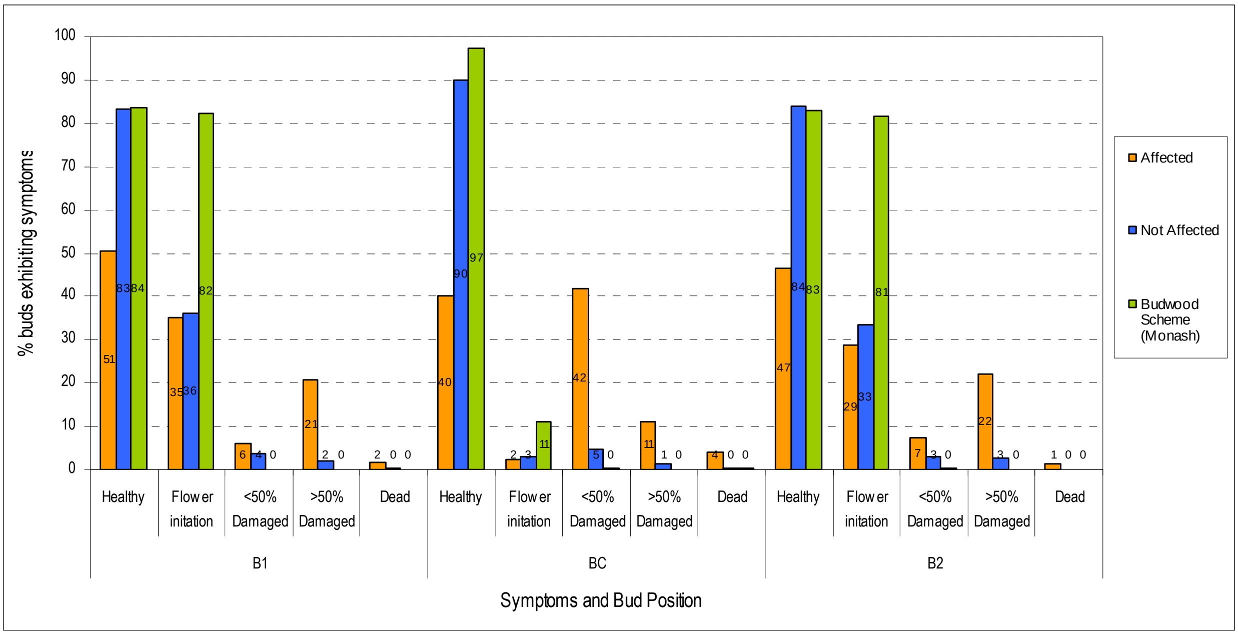
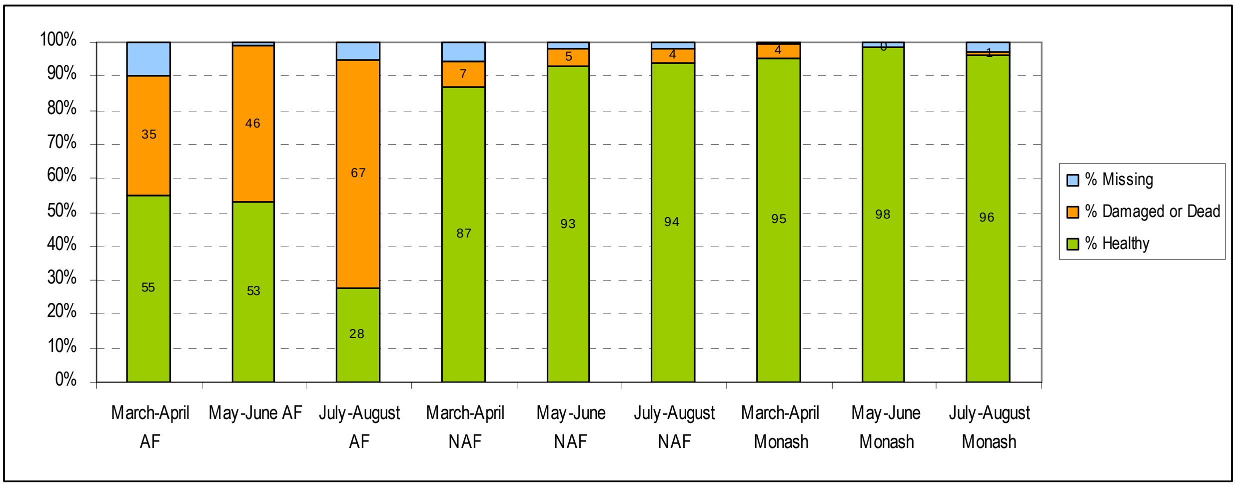


Figure 6: Central bud damage status March- August 2009



1.8 Bud Health - Conclusions from Bud Dissections

- Shoots from basal buds (on otherwise bare wood), have high proportion of damaged buds
- Affected wood has a higher percentage of damaged floral and vegetative buds than non-affected wood
- Spring bud health, may be predicted by late summer of the previous season
- At a single node on affected or non-affected wood, both central and lateral buds may become damaged
- The higher percentage of buds damaged are in the central position (especially on affected wood) at a node
- Once damaged, vegetative buds remain at an underdeveloped size and shape.
- Some terminal buds did not appear damaged
- Monash budwood produces a high proportion of floral buds
- Monash budwood has the highest percentage of undamaged buds

1.9 Field Visits and Leaf Out

The MIA trial co-operators were visited in June, and all co-operators (including Monash) were visited in September 2009 by a member of the research team. Tagged trees and bud dissection trees were examined. The correlation of observed leaf out and bud dissection results were determined during the September visits. A summary of the observations follows.

The winter field visit to the MIA confirmed that bark roughness and horizontal banding in wood two years or older, were characteristic of the disorder in severely-affected young trees (Appendix 3 - Photos 3 to 5). These symptoms have since been observed in other locations. Non-affected trees in the same block did not have these bark symptoms.

During the spring field visits to co-operating orchards in the Riverland, Sunraysia and MIA, it was clear that trees identified as affected in spring 2008 remained that way. There was little, if any leaf out on the shoots that had developed as basal laterals from bare wood in spring 2008. There was strong correlation between the observed bud damage in late summer and the leaf out in September 2009. The greater the proportion of damaged buds, the poorer was the observed leaf out and the sparser the canopy. It also appeared that bloom (and leaf out) had been delayed in affected trees. Some leaves were recently emerged, in the last week of September.

In some young, affected trees (entering their third or fourth leaf) up to 90% of the canopy was bare. It was also noted that the nut loads in these trees were low, indicating that both vegetative and floral buds had been affected – or that the limited vegetative growth had limited the volume of potentially fruitful wood. Vegetative growth that had developed was often stiff and at odd angles.

In mature trees, the bare wood was generally infrequent, and high in the canopy. This could have reflected moisture stress rather than bud failure alone.

Some young affected trees had been heavily pruned in the previous season in an attempt to stimulate new, productive wood. The growth from these trees also showed bud failure. Where re-budding with another variety had been attempted on affected trees, the buds had not taken. It is unclear if this reflects another aspect of the disorder, or the budding technique in this particular case.

It did not appear that any Carmel tree previously identified as non-affected, had become affected during the investigation period. There was no indication that the disorder had 'spread' from neighbouring affected trees. In young, non-affected trees, the canopies were full and nut loads in most cases were good. These trees in many cases were in close proximity to severely-affected trees of the same age.

In September 2009, the mother trees in the Monash collection were also inspected. Because these trees are the primary source of Carmel budwood, they are routinely pruned heavily or hedged to ensure ample supplies of fresh first-year budwood. These trees therefore are not usually allowed to bloom. As such, it is possible the pruning and management of mother trees could have masked bud failure, should it have been triggered. By allowing the main scaffolds and branches on each of 20+ mother trees at Monash to remain unpruned last season, the opportunity to observe bud development (floral and vegetative) was provided (Appendix 3 - Photos 6, 7).

Both the dissection results and the field observations indicated that none of the new growth from Carmel mother trees at Monash displayed failure to leaf out. Although there were some individual, isolated buds that hadn't leafed out or had delayed emergence, they were very infrequent. When dissected in the laboratory it was found these buds were still viable and likely to leaf out. There were no areas of extended bare wood on the Monash trees. Unlike the young trees observed in other orchards, mature trees at Monash and at several other orchards showed no evidence of bud failure.

1.10 Conclusions from Field Observations and Bud Dissections

- Damaged vegetative buds do not leaf out.
- The negative correlation of bud damage in late summer and leaf out the following spring is strong. Bud dissections may predict spring leaf out.
- Affected wood in 2008 gave rise to laterals bearing no or very few leaves in spring 2009.
- Canopy sparseness due to bud failure will be on-going because affected wood gives rise to affected buds that do not leaf out.
- The percentage of 'undamaged' lateral buds in autumn is indicative of the potential blossom.
- Some terminal buds did not appear damaged and these resulted in tufted 2009 spring growth.
- Buds from the ABA budwood scheme (Monash) showed little damage and developed leaves and flowers as expected.
- Monash buds transitioned to give a high percentage of large, healthy floral buds.
- A greater percentage of floral buds (than vegetative buds) remained viable along the length of affected wood.
- Fruit load was low in many young, affected trees. This may have resulted from damage, delayed transition and bloom.
- No previously 'non-affected' trees displayed onset of bud failure, in spring 2009.
- Mature trees have a lower proportion of their canopy showing bud failure.
- Many young, affected trees in the MIA have up to 90% of canopy bare.
- Hard pruning has not resulted in full leaf out and fruitful new wood.
- Rough bark and striations can been seen in two-year and older wood, even in winter.
- Rough bark (tiger striping) is associated with severely-affected trees.
- Some young affected trees in the MIA appear not to have had a normal growth season since planting. There is evidence of three seasons of bud failure and poor vegetative growth.
- Tree framework has been compromised in trees with evidence of several seasons of bud failure.
- Young affected trees are not economically viable.

1.10.1 Notes on Each of the Regions Investigated and Bud Failure

1.10.1.1 MIA - Young trees

The almonds in the MIA are entering their fifth leaf or are younger. The young Carmel trees observed in two MIA orchards are entering their third or fourth leaf and are the most severely-affected of those observed in this investigation to-date. Over a third of the trees in one location displayed significant bud failure and a range of 60-90% canopy affected. Many of the shoots are stiff and arise from branches at an odd angle.

In both orchards the first sign of bud failure was in the second leaf when trees displayed 'crazy growth'. In each of the orchards non-affected trees had full canopies and had leafed out as expected by late September.

The traceability to specific bud sources is lacking, but it is known that one of the providing nurseries has purchased Monash buds on a prior occasion.

Rough bark is evident in most affected trees. Investigations of, and isolations from, the bark have not revealed anything with consistency. A *Botryosphaeria* sp. has been isolated from necrotic areas but in most second-year wood the roughness is confined to bark only. Several trees had intense internal discolouration in larger scaffolds (Appendix 3 - Photo 8) but this was not consistently associated with bud failure and is not reminiscent of Botryosphaeria-type discolouration. Its cause remains unknown.

The leaves present are not wilted and there appears to be no indication trees with this disorder may die, even when severely-affected from an early age. In one location the most severely-affected trees are those most exposed to hot westerly winds.

The affected trees in these orchards appear not to have an economically-viable future as they have few nuts, poor structure and sparse canopies. The crazy growth and consecutive bud failure periods and possibly some pruning practices, have given rise to trees with poor framework. Attempted re-budding and/or hard pruning have not been successful in stimulating viable new growth.

1.10.1.2 Riverland - Mature Trees

The Carmel mother trees at Monash are of two generations – foundation and once removed. These are the industry source of buds 'closest to the original bud'. Based on our observations this season, these mother trees haven't suffered bud failure to a detectable level. This is despite their presumed exposure to California trigger temperatures over many years. Leaf out in spring 2009 was complete albeit delayed in the basal buds.

In other cooperating orchards in this region, mature 'affected' trees displayed little bare wood. It was confined to the top of canopies and is unlikely to have any economic effect. In these locations it is possible the small extent of bare wood at the top of trees is a reflection of water stress rather than bud failure. The same extent of bare wood was observed in varieties other than Carmel.

1.10.1.3 Riverland - Young Trees

Young trees in another Riverland orchard were observed. The trees were on hybrid rootstock. Those planted in 2005 are displaying less bud failure than those planted in 2003. Both plantings would have been exposed to high temperatures in the last four seasons. Rough bark was not observed in these trees. The non-affected trees in the 2003 planting had leafed out well by mid-September.

The nursery sources were not the same for each planting, but the specific bud sources remain unknown.

1.10.1.4 Sunraysia

Three orchards in Sunraysia have been observed and have contributed samples for bud dissection. Two are in Lindsay Point while the third is in Wentworth. Two are mature orchards and these have shown very little bud failure. The limited annual growth, degree of shading and possible water stress-caused dieback may have masked the failure of isolated buds to develop. There are however no extensive areas of bare wood on these trees.

The young planting coming into its fourth leaf is on Bright's hybrid. Both leaf out and bloom in affected trees were consistently poor. The lack of crop was notable, and suggested floral bud transition was poor, and/or bloom was delayed such that pollination did not occur. In this orchard, some dieback was evident also.

The young affected trees in this block appear not to have an economic future. In contrast, the bloom in the non-affected trees in the same orchard was heavy and the nut load was very good.

See Photos 9 to 13 (in Appendix 3) of relative leaf out during September 2009.

1.11 Post-Harvest Irrigation Review

Although high temperature exposure at the time of bud initiation and development has been identified as the trigger for NBF in California, the bud source remains critical. Moisture stress and tree age may further influence the rate and extent of bud failure, its severity and economic impact.

Post-harvest rain has been received in all production districts in 2009. It can reasonably be concluded that this season there has not been post-harvest water stress.

In 2008, 'less than optimal' volumes of water were available to many growers, due to water restrictions. Few growers however specifically reported the trees being observed in this investigation, suffered 'post-harvest water stress'. Some growers who retained little water available for post-harvest irrigations, chose to irrigate young blocks in preference to old trees. During the heatwave of March 2008, the Riverland recorded 15 days over 35°C; Sunraysia, 14 days; and the MIA, 12 days. These likely accentuated the effects in orchards that had under watered. Where bud failure was not present some evidence of drought could still be found as sporadic dieback high in trees.

Despite under-irrigation across some orchards in 2007/08, bud failure has been reported only in Carmel. It is not occurring uniformly down rows or through orchards, and it is more extensive on young rather than older trees.

MIA co-operators will be asked more specifically to identify particular trees that were stressed in seasons 2005/06 and 2006/07 to see if any correlation exists with the observed bud failure onset in their young trees.

1.12 Conclusions about the Influence of Post-Harvest Irrigation

At this time there is little that can be concluded from available data about the contribution of post-harvest irrigation in 2007/08 and 2008/09 to the observed bud failure. It is however accepted that water stress, especially post-harvest, is capable of disrupting bud development, as discussed in the bud development fact sheet (Milestone 2), and it may therefore contribute to the severity of bud failure.

1.13 Grafting Update

Grafting of budwood from affected and non-affected trees onto high health rootstocks has been completed (April 2009). The intention is to confirm (or otherwise) that bud failure is not transmitted through grafting.

There are no indications to-date of success of the grafting/budding or of resultant growth disorders.

1.14 Conclusions from 2008/09 Investigations of Bud Failure

- Young trees planted in the last five seasons are the most severely affected.
- Bud failure has not been traced to one bud source.
- MIA Carmel trees may have been exposed to temperatures capable of triggering bud failure in every year since they were planted.
- Young severely-affected Carmel trees also have rough, scaly bark in second-year (and older) wood.
- Buds from Monash Carmel trees show very low level damage; trees had normal leaf out and full canopies.
- Affected young trees are unlikely to be economically viable.

It is however too early to conclude:

- That bud failure observed in Australian orchards is/is not NBF.
- The cause and mechanism of bud failure (in Australia).
- Specific temperature (minima and duration) triggers and their predictive value.
- If the bud sources for affected trees were distant from Monash original buds.
- The mechanism and conditions for rough bark development.
- If the disorder is transmissible.

2 OTHER ISSUES

2.1 Potential Breeding and Propagation Implications

The work carried out on inheritance in California has demonstrated the genetic nature of NBF. The same experiments have not been carried out here. However it is reasonable to assume that any new cultivar or tree with Carmel parentage has higher bud failure potential than other cultivars. The choice of buds is very important. In general buds for used for autumn budding have greater potential to fail because of the heat during which they have developed. Regardless of the timing of bud collection, the chance of new trees or seedlings derived from affected trees displaying early bud failure, is high. Evaluations of new varieties should identify the specific bud source, time of budding and observations for bud failure. The industry should soon undertake increased trialling of new pollinators.

Budwood source trees should show no sign of bud failure. Even symptomless trees in California may have a NBF potential that increases with age and/or exposure to high temperatures. It is recommended that a system of identifying bud source trees be developed and for nurseries and growers to retain this traceability evidence for all new Carmel plantings.

3 NEXT STEPS

The intended 2009 project activities have been completed. In 2010 the bud dissection work will continue for one property and bud dissections will start in December 2009 in order to identify a specific time damage is initiated.

Management of bud failure will be considered in economic terms, eg when tree removal or top-working is recommended based on percentage of canopy affected and yield at a specified tree age.

4 COMMUNICATIONS AND EXTENSION ACTIVITIES

The communication and extension material resulting from this project, has been directed to the co-operating growers primarily.

A presentation at the annual Almond Industry Conference (October 29-30, 2009) is planned and a report summary has been included in HAL Annual Industry Report 2008/09. These will alert all almond growers to the status of the project and the potential causes of the growth disorder.

5 COMMERCIALISATION/INTELLECTUAL PROPERTY ISSUES

There are no commercialisation or intellectual property issues associated with this project.

SCHOLEFIELD ROBINSON
HORTICULTURAL SERVICES PTY LTD

PRUE McMICHAEL
Plant Pathologist\Principal Consultant
F:\SRHSDATA\Clients\Almond Board of Australia\Bud Failure 2008\Reports\Milestone 3\Rpmc231009Milestone3.doc

REFERENCES

1. Kester, D. 2000. *Final Report of the non infectious bud- failure project No.: 99-dk-00*. Almond Board of California, 25 pp. http://fruitsandnuts.ucdavis.edu/crops/Almond_Board_BF.pdf.

2. Kester, D.E. 1994. *Solving the problem of non-infectious bud-failure in California almond orchards*. International Congress on Almond. Acta Hort. 373: 35-40. http://www.actahort.org/books/373/373_3.htm

3. Kester, D.E., Shackel, K.A. Micke, W.C. Gradziel, T.M. Viveros M. 1998. *Variability in potential and expression of non-infectious bud-failure among nursery propagules of 'Carmel' almond*. Acta Horticulturae 470: 268-272 http://www.actahort.org/books/470/470_37.htm

4. Kester, D.E. and T.M. Gradziel. 1996 Chap 12 'Genetic Disorders' in *Almond Production Manual.* Ed. W. Micke. University of California. Publication 3364. 289 pp.

5. Rubatsky, V.E. and M. Yamaguchi. 1997. World Vegetables – Principles, Production and Nutritive Values. 2^{nd} edition. Chapman and Hall. 843 pp.

Appendix 1

(a) Results summary April 2009 (provided to co-operators)

(b) Excerpt from HAL industry Annual Report 2008/09

Carmel Growth Disorder Project
Bud Dissections and Bud Status

SUMMARY AFTER SIX WEEKS OF ASSESSMENTS

The following early observations and information are provided to our cooperating growers in appreciation of their efforts. All levy-payers will receive result summaries at a later date, in-line with the Horticulture Australia Limited milestone reporting requirements.

Eight participants have sent in samples to-date. They have arrived from MIA, Riverland and Sunraysia orchards. We are expecting four more orchard participants to send their samples again soon. While no conclusions can be drawn from the bud dissections at this stage, on the cause of the Carmel growth disorder, some trends are emerging, and worth noting.

All our observations are recorded. Any change over time, as the season progresses can therefore be recognised and analysed. Later in the season we will understand more about the significance of each type of damage observed, and what it potentially results in.

PROCESS OF BUD DISSECTION

Staff at SRHS are assessing the budsticks under dissecting microscopes, as they arrive. The participating orchards had their 'bud dissection' trees tagged in advance of cutting, and they are sending 6 sticks from each (1 or 2 trees) of the tagged *affected* trees and 6 from each of the *non affected* trees. On each cutting occasion, the same trees are cut and the bundles are labelled as before. The cutting has taken place at 2-3 week intervals.

Observations are recorded for each bud at each of 24 nodes. Starting at the terminal end, node 1 is the first node beneath the apical tip bud cluster. Buds are sliced longitudinally in several sections from the outer bract layer through to the stem, to ensure that all levels and parts of the bud are inspected.

Nodes may have 1, 2 or 3 buds, rarely 4 (Refer to **Figures 1a and 1b**). The damage types and severity have each been assigned a number or letter code to ensure consistency in recording observations. Photographic reference has also been prepared (Refer to **Figure 3**). The damage codes are shown below in **Table 1**. At this stage of the season we are most interested in internal symptoms at the growing point – eg. 'heart' damage; ill-defined staining in or below the buds; and buds that appear to have formed normally but have already died. 'Missing' buds are recorded but interpretation is difficult, since they may either have fallen in transit or at some earlier point in the field due to mechanical or wind damage, or death of the whole bud.

Table 1: Damage code and rating description

Rating	Observation : Internal Description
1	healthy green bud
1x	healthy green bud; lignified(?) section inside bud, but not at bud heart, usually tip section
2	bud heart brown (<50%)
3	bud heart brown (>50%)
4	below bud scarred/stained, bud heart healthy
5	bud heart brown/stained (>50%) PLUS staining/scaring below bud
C	bud heart development advanced since previous observations (e.g. possible differentiation to floral bud)
M	bud missing (recent loss - knocked out during transit ?)
DC	bud heart is dead but still present
D	entire bud dead but still present

Figure 1a: Representation of Almond bud set at any given node (longitudinally cut)

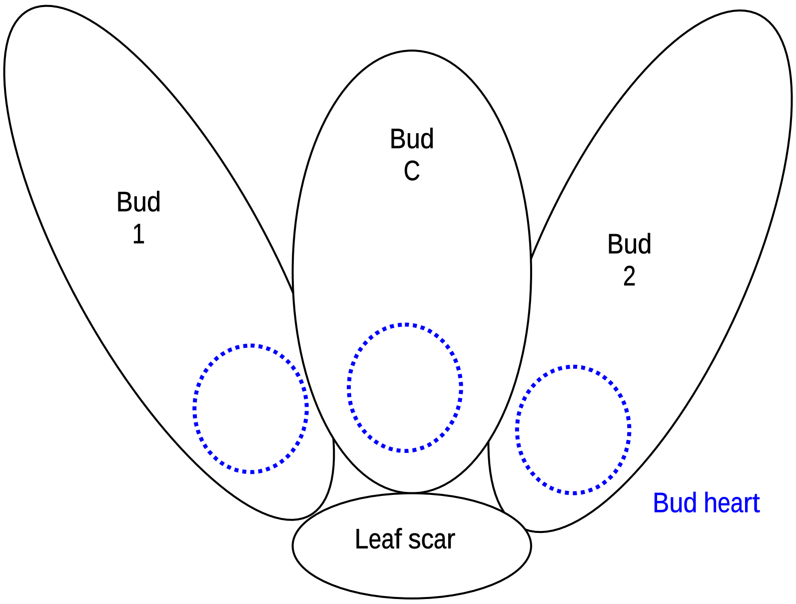
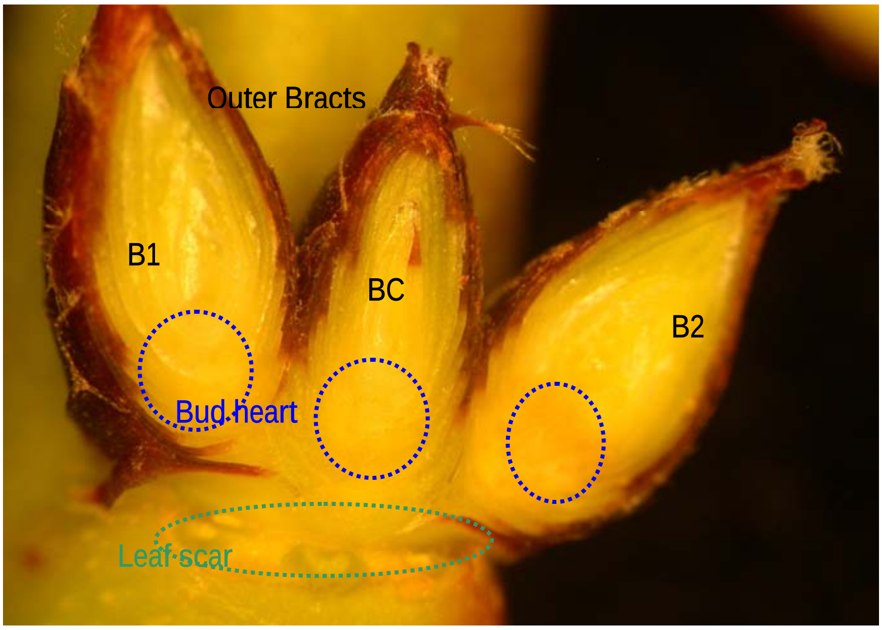


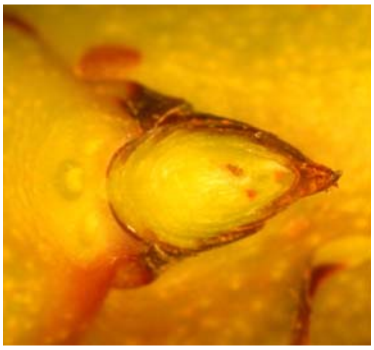
Figure 1b: Almond bud set at a given node (longitudinally cut)



B1	= Bud 1 (Left side)
BC	= Centre Bud
B2	= Bud 2 (Right side)
Bud heart	= the growing point of the bud
Leaf scar	= the leaf attachment point
Outer bracts	= the brown, lignified outer bracts that protect the bud heart

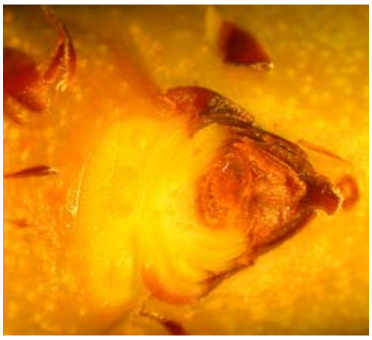
Figure 3: Photographic observation reference: Visual description of symptoms and codes

1 = healthy green bud, 0% browning



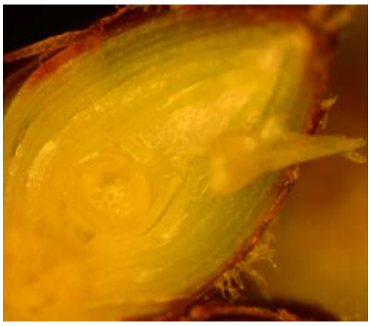
1x = healthy green bud; with lignified(?) section inside bud, but not at bud heart, usually tip section, 0% browning

2 = bud heart brown/stained (<50%)

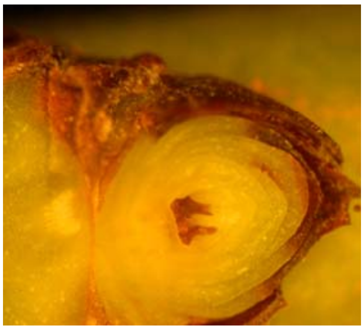


3 = bud heart brown/stained (>50%)

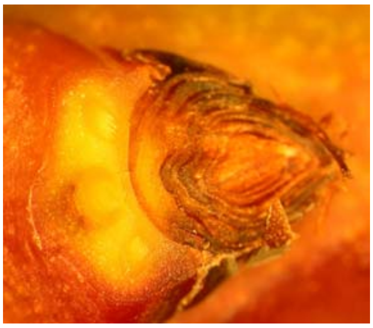
5 = bud heart brown/stained (> 50%) PLUS staining/scarring below bud



C = bud heart development advanced since previous observations (e.g. possible differentiation to floral bud)



DC = bud heart is dead but still present



D = entire bud dead but still present

RESULTS – WEEK 6

The composite results discussed below are those from three participating and representative orchards, one from each of the MIA, Riverland and Sunraysia.

General Observations

- All orchards have had some damaged buds in each sample.
- Some damage has been observed in buds from both *affected* and *non affected* trees.
- Damage in buds from *affected* trees is more prevalent and extensive and than in buds from *non affected* trees (**Figure 2** and **Table 2**).
- Within bud clusters, more central buds (BC) are damaged, than lateral buds (B1, B2) (**Figure 2** and **Table 2**).
- Damaged central buds have more extensive symptoms that damaged lateral buds.
- Buds from the ABA budwood scheme (Monash) have consistently had the least damaged buds (**Figure 2** and **Table 2**).

Results

- *Affected* tree buds (average data, regardless of bud position):
  - 47% are healthy (no damage, rating 1)
  - 32% have some damage (rating 2, 3, 5)
    - of damaged buds, 55% have greater than 50% damage or are dead (ratings 3 and 5)
- *Non affected* tree buds (average data, regardless of bud position):
  - 83% are healthy (no damage, rating 1) and
  - 12% have some damage (rating 2, 3, 5)
    - of damaged buds, 47 % have greater than 50% damage or are dead (ratings 3 and 5)
- Central buds (BC) from *affected* trees:
  - 48% have damage ratings of 2 or 3 – e.g. growing point/'heart' damage
- Central buds (BC) from *non affected* trees:
  - 7% have damage ratings of 2 or 3 – e.g. growing point/'heart' damage
- Lateral buds (B1 and B2) from *affected* trees:
  - 12.5% have damage ratings of 2 or 3.
- Lateral buds (B1 and B2) from *non affected* trees:
  - 7% have damage ratings of 2 or 3.
- Monash: 98% central buds (BC) have no damage, and 82-85% of lateral buds (B1 and B2) have no visible damage (rating 1).
- Multiple buds at nodes: *non affected* (27% nodes) and *affected* trees (26%); 31% of nodes on Monash budsticks carry multiple buds

Conclusions: Bud Dissections – Week 6

- Buds from 2008 *affected* wood, have more visible damage than buds from *non affected* wood.
- Most damage is in the growing point region.
- Some buds have died.

It is too early to draw conclusions about:

- The cause of Carmel growth disorder
- The significance of the minor bud damage, in terms of future bud viability, bud retention, and normal development later in the season
- When the damage was caused/triggered
- How the damage was caused/triggered.

It may be necessary to continue budstick sampling throughout the year to determine exactly when change from healthy to "damaged" buds occurred, if we believe the trigger preceded our first sampling dates.

Figure 2: Symptoms observed in buds from *affected, non affected* and ABA Budwood Scheme (Monash) trees

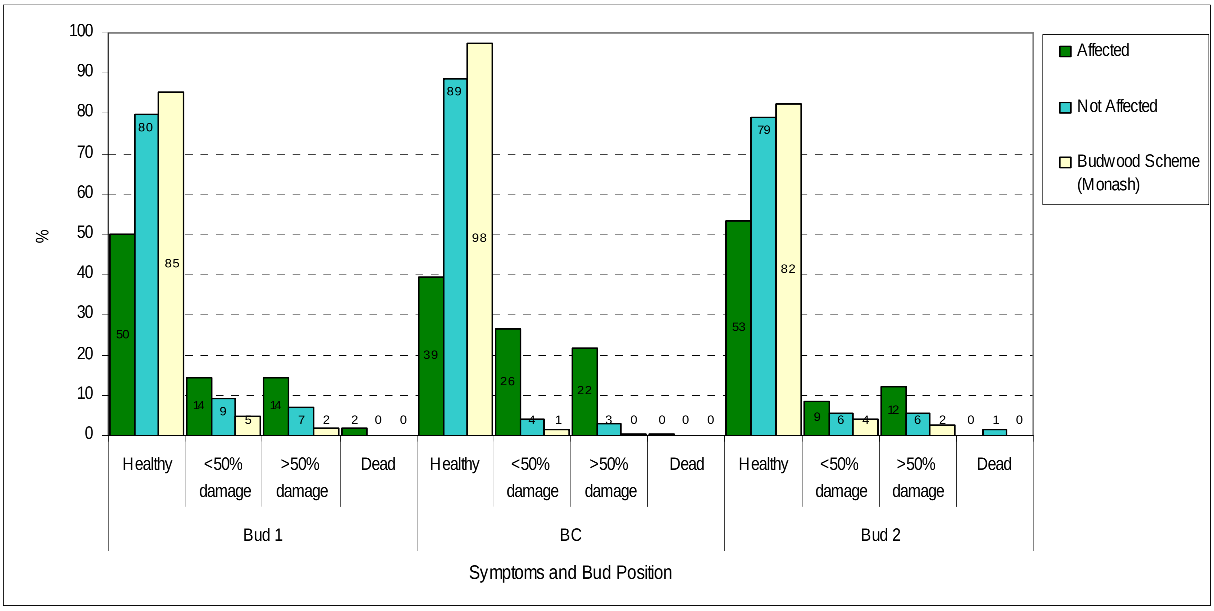


Table 2: Percentage of buds showing different symptoms at each bud location

Location	Rating	%	Affected	Non Affected	Budwood Scheme (Monash)
Bud 1	*1*	Healthy	50	80	85
	2	<50% damage	14	9	5
	3	>50% damage	14	7	2
	M	Missing	9	4	7
	D	Dead	2	0	0
BC	*1*	Healthy	39	89	98
	2	<50% damage	26	4	1
	3	>50% damage	22	3	0
	M	Missing	11	4	0
	D	Dead	0	0	0
Bud 2	*1*	Healthy	53	79	82
	2	<50% damage	9	6	4
	3	>50% damage	12	6	2
	M	Missing	22	8	10
	D	Dead	0	1	0

The data have not been statistically analysed as yet, so these results should be viewed as 'indicative' rather than "statistically significant".

VIRUS TESTING

Leaf samples were collected from four MIA orchards. Samples were collected from both 'unaffected' and 'affected' trees, in each orchard. None of the trees or leaf samples displayed any "typical" viral symptoms at the time of sample collection.

For each orchard, composite samples from 'affected' and 'unaffected' trees were separately prepared and sent for virus testing. Only leaves (from either source) of 'healthy appearance, were collected.

Two rounds of testing are done to detect viruses. The second round uses 'nested' PCR tests which are very much more sensitive.

Results - Virus presence

- No symptoms of virus were detected in any of the trees observed in the MIA.
- No sample from either the *affected* and *unaffected* tree sources, tested positive (i.e. detection of virus) for Prunus Necrotic Ringspot Virus (PNRSV) and Prune Dwarf Virus (PDV) in the first round of molecular (PCR) testing.
- Only one sample (from an *affected* tree) tested negative for PDV with 'nested' PCR.
- All samples (except one – as above) tested positive for PDV in "nested" PCR tests.

Conclusions

- Viral disease has not been observed any sampled trees
- Virus was not detected through regular PCR molecular tests.
- PDV was detected through nested PCR tests, in both *affected* and *unaffected* trees
- Virus presence is not a variable that can reasonably explain the onset or development of the Carmel growth disorder.

F:\SRHSDATA\Clients\Almond Board of Australia\Bud Failure 2008\Reports\Milestone 3\A1a Summary combined - 6weeks.doc

Investigating almond growth disorder

The appearance of a widespread growth disorder in the Carmel variety during spring 2008 caused significant concern across almond production districts.

The disorder results in a failure of bud growth, which is very apparent in spring as reduced vegetative growth and extended areas of bare wood. Leaf buds are more extensively affected than flower buds, but reduced nut production especially in younger trees, has been noted in some orchards.

This research project is focused on bud initiation and development and the factors influencing these processes. To investigate bud viability and health, lateral growth budsticks from orchards in the MIA, Riverland and Sunraysia have been systematically cut from affected and unaffected trees since late February 2009.

The buds have been dissected and their internal and external appearance recorded, with the type and severity of damage coded to allow for comparison between samples and sampling times. Early research also involved virus testing to ensure viruses were not a potential cause of bud failure. Initial molecular testing of leaf samples from four MIA orchards did not detect the presence of Prunus Necrotic Ringspot Virus (PNRSV) or Prune Dwarf Virus (PDV), and it appears these viruses do not explain the onset or development of the disorder.

A literature review of bud development noted the potential role of post-harvest water deprivation. A review of post-harvest watering and pre-harvest temperature extremes has been made to determine if there is any correlation with the observed 2008 disorder. The contribution of environmental factors has not been determined but the heat waves of March 2008 and January 2009 are likely to have affected bud development to some degree. Post-harvest water deprivation in 2008 may have been a contributory factor, but rains during autumn 2009 were widespread and few orchards suffered post-harvest water stress this season.

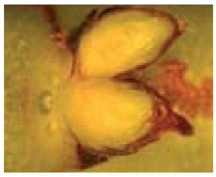
The research team has made orchard visits in September 2009, to observe leaf out in previously marked affected and unaffected trees. There is no evidence to suggest the disorder has spread between trees. However, the correlation between autumn bud dissection results and spring leaf out is strong. Trees with significant levels of damaged buds in autumn, have sparse canopies, poor leaf out and extensive areas of bare wood. Within affected trees, the results to date suggest 2007/08 affected wood has given rise to 2008/09 affected wood.

The role of genetics cannot easily be determined but the predictive value of bud dissections in late summer is being further investigated. It is too early in the research program to determine the cause of the Carmel disorder, or its trigger.

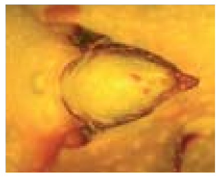
To help the researchers assess the extent of the disorder and the potential contributing factors, growers and nurserymen were surveyed about their orchard and the history of their budwood. To assist almond growers' understanding of the project, and of bud initiation and development and the factors that influence them, all were sent a fact sheet on bud development, a summary of the survey data, virus test results, and bud dissections.

Project AL08015

For more information contact:
Prue McMichael, Scholefield Robinson Horticultural Services
T 08 8373 2488
E prue@srhs.com.au

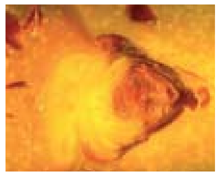


1 = Healthy green bud, 0% browning



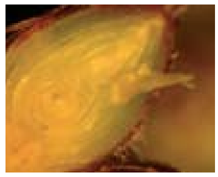
1x = Healthy green bud; with a potentially lignified section inside bud, but not at bud heart, usually tip section, 0% browning

2 = Bud heart brown/ stained (<50%)

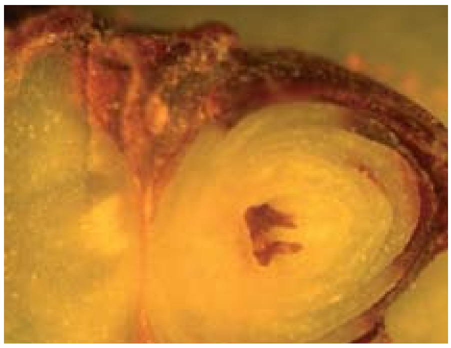


3 = Bud heart brown/ stained (>50%)

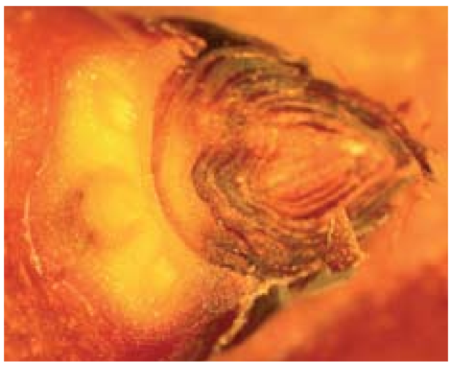
5 = Bud heart brown/ stained (> 50%) PLUS staining/scarring below bud



C = Bud heart development advanced since previous observations (e.g. possible differentiation to floral bud)



DC = Bud heart is dead but still present

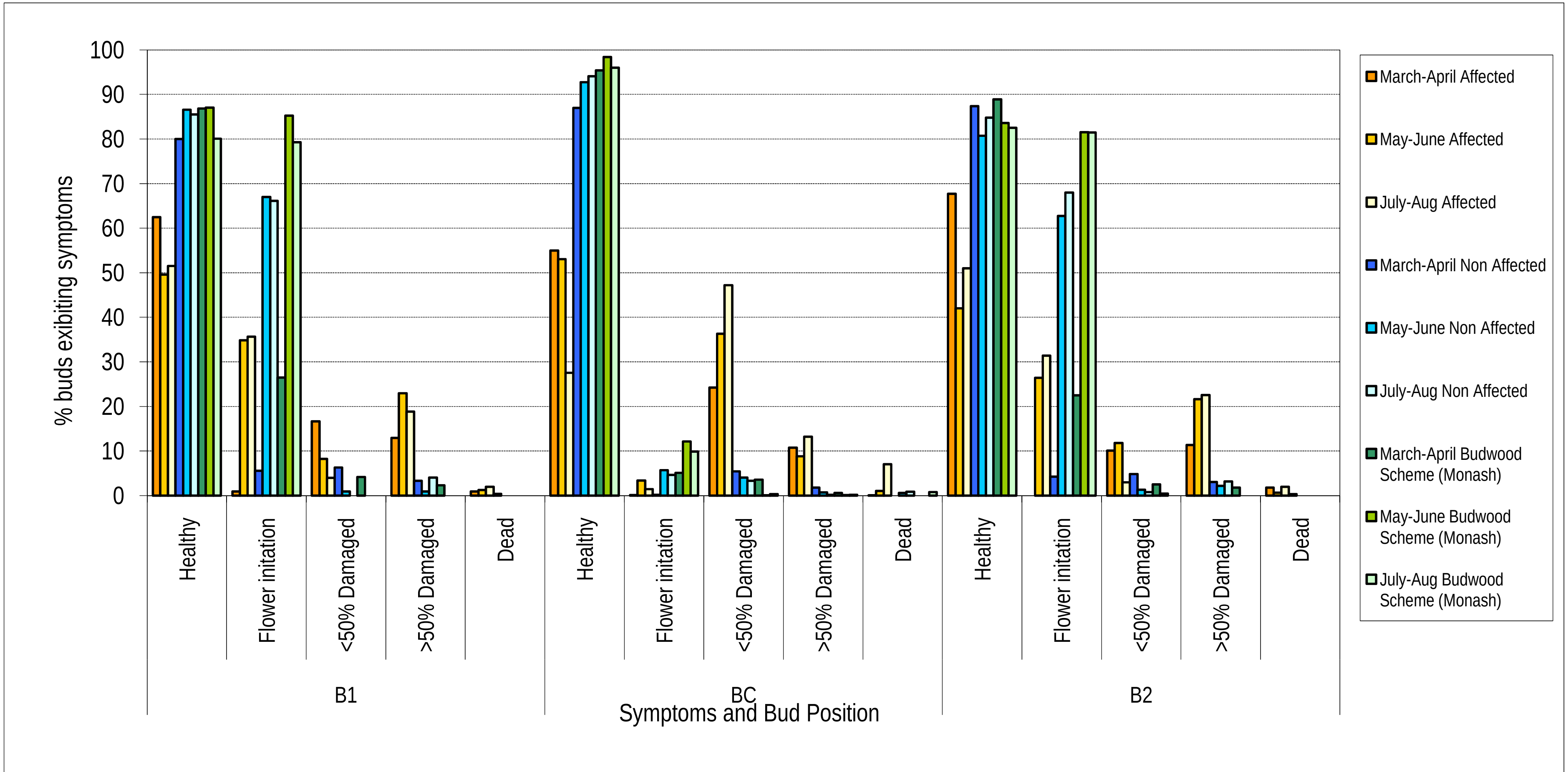


D = Entire bud dead but still present

Appendix 2

Graphical presentation of bud dissection data MarchApril, May-June and July-August 2009

Appendix 2 : Symptoms observed in buds from *affected, non-affected* and ABA Budwood Scheme (Monash) trees. March-August 2009



Appendix 3

Photographs

Photo 1 - Healthy lateral buds transitioned to floral buds.

Photo 2 - Damaged lateral buds unlikely to transition to floral buds.

Photos 3 & 4 - Horizontal lesions around buds on two year old (and older) affected wood.

Photo 5 - Rough scaly bark on severely affected young Carmel tree.

Photo 6 - Heavily pruned mother tree at Monash (normal practice).

Photo 7 - Unpruned mother trees left to flower (blossoms removed) and grow in spring 2009.

Photo 8 - Intense internal discolouration in older wood of affected young tree.

Photo 9 - Young tree with bud failure, Riverland September 2009.

Photo 10 - Non-affected young tree with full canopy, MIA September 2009.

Photo 11 - Young, severely affected tree with crazy top, MIA September 2009.

Photo 12 - Non-affected, young productive tree, MIA September 2009.

Photo 13 - Leaf out September 2009. Affected (2 top sticks) and non-affected trees.

Appendix 3

Grafting Experiment DPI VIC

Almonds Woody Indexing
Consultancy Services for Dr Prue McMichael
Principal Consultant/Plant Pathologist
Scholefield Robinson Horticultural Services Pty Ltd

Report October 2010

In April 2009 Ben Brown from Almond Board of Australia submitted the budwood samples from the 6 almond trees for biological indexing on woody indicators.

The material sent to us in April 2009 was tested by reverse transcription-polymerase chain reaction (RT-PCR) for the presence of the four endemic viruses (*Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple mosaic virus* (ApMV) and A*pple chlorotic leafspot virus* (ACLSV), which can infect almonds in Australia. Two different RT-PCR assays were used for PDV detection. PNRSV, PDV, ACLSV and ApMV were not detected by RT-PCR

The biological indexing trial was conducted from April 2009 to May 2010 to determine if a disease affecting almonds was associated with a graft transmissible agent. Three groups of candidates were indexed and included:

- material from the varieties Omega and Lacton that were unaffected by disease
- material from the varieties Omega and Lacton that were affected by disease
- material from the varieties B19-21 and A18-16 Monash.

In April 2009 we grafted buds from each “candidate” on the two *Nemaguard* rootstock plants and two Shirofugen indicators (grafted on Sam Cherry rootstock). In July 2009 two buds from GF305 (woody indicator) were grafted on the *Nemaguard* rootstock just above candidate buds.

In both groups of indicator plants, most of the grafted buds did callus, including the indicator (GF305) buds (Table 1). Symptom development and expression on both groups of indicator plants was monitored until May 2010.

Table 1. Callus formation and growth of “diseased” and “healthy” almond buds that were grafted onto the *Nemaguard* rootstock with GF305 woody indicators and on the Shirofugen indicators.

Sample ID	Rootstock	Candidate grafted 23/4/09	Indicator grafted 24/7/09	Comment
Omega	Nemaguard	Unaffected	GF 305	Both, the candidate and indicator buds callused. No symptoms on GF305.
Omega	Nemaguard	Unaffected	GF 305	
Omega	Sam	Unaffected	Shirofugen	The candidate buds callused. No symptoms.
Omega	Sam	Unaffected	Shirofugen	
Lacton	Nemaguard	Unaffected	GF 305	Both, the candidate and indicator buds callused, no visible growth.
Lacton	Nemaguard	Unaffected	GF 305	Only the candidate buds callused, no visible growth.
Lacton	Sam	Unaffected	Shirofugen	The candidate buds callused. No symptoms.
Lacton	Sam	Unaffected	Shirofugen	
Omega	Nemaguard	Affected	GF 305	Both, the candidate and indicator buds callused. The GF305 indicator showed symptoms on the grown leaves.
Omega	Nemaguard	Affected	GF 305	Only the candidate buds callused, no visible growth.
Omega	Sam	Affected	Shirofugen	The candidate buds callused. No symptoms.
Omega	Sam	Affected	Shirofugen	
Lacton	Nemaguard	Affected	GF 305	Neither, the candidate or indicator buds callused and did not grow.
Lacton	Nemaguard	Affected	GF 305	
Lacton	Sam	Affected	Shirofugen	The candidate buds callused. No symptoms.
Lacton	Sam	Affected	Shirofugen	
B19-21	Nemaguard	Monash	GF 305	Both, the candidate and indicator buds callused. The GF305 indicator showed symptoms on the grown leaves.
B19-21	Nemaguard	Monash	GF 305	The candidate buds did not callus and did not grow. The indicator buds did callus, but did not produce any growth.
B19-21	Sam	Monash	Shirofugen	The candidate buds callused. No symptoms.
B19-21	Sam	Monash	Shirofugen	
A18-16	Nemaguard	Monash	GF 305	Both, the candidate and indicator buds callused, no visible growth.
A18-16	Nemaguard	Monash		Only the candidate buds callused, no visible growth.
A18-16	Sam	Monash	Shirofugen	The candidate buds callused. No symptoms.
A18-16	Sam	Monash	Shirofugen	

From the 10 month observation of grafted buds performance we made following comments:

1. Omega (unaffected candidate) buds callused both on the *Nemaguard* rootstock and Shirofugen indicator plants. Neither of the two indicators produced visible symptoms. A graft transmissible agent was not detected.

2. Lacton (unaffected candidate) buds callused both on the *Nemaguard* rootstock and Shirofugen indicator plants. There were no symptoms on the Shirofugen indicator. However, the indicator (GF305) buds did not callus or did not produce sufficient growth for the symptoms observation. This candidate should be monitored for another 12 months before a conclusion on it's health status is made.

3. Omega (affected candidate). Both the candidate and indicator buds callused on the *Nemaguard* rootstock and Shirofugen indicator plants. After 7 to 8 months GF305 indicators grafted above the candidate buds showed symptoms on the grown leaves including leaf distortion, mild mosaic and red blotches, suggesting the presence of a pathogen in the candidate buds. No symptoms were observed on the Shirofugen indicator grafted with buds from this candidate.

4. Lacton (affected candidate). Neither, the candidate or indicator buds callused when grafted onto *Nemaguard* rootstock. It is possible that the failure of the buds to callus was caused by a pathogen present in the candidate material. The buds from the non-affected Lacton tree callused when grafted onto indicators indicating that the grafting time and methods that we used in this experiment were correct. However these non-affected Lacton buds failed to grow and both the affected and unaffected Lacton candidates should be re-grafted on the same indicator for the further observation to confirm our observations.

5. B19-21 (Monash). The candidate and the GF305 indicator buds callused on one of the two *Nemaguard* rootstock plants. The GF305 indicator showed similar symptoms on the grown leaves to the affected Omega candidate including leaf distortion, mild mosaic and red blotches. On the second *Nemaguard* rootstock plant the candidate buds did not callus and did not grow and the GF305 indicator buds did callus, but did not produce any growth. From these observations we can suspect that a pathogen was present in buds taken from the B19-21 candidate. No symptoms were observed on the Shirofugen indicator grafted with buds from this candidate.

6. A18-16 (Monash). Both, the candidate and indicator buds callused on one of the two *Nemaguard* rootstock plants, but only the candidate buds callused on the second rootstock plant. No visible growth was produced by either candidate or indicator buds. No symptoms were observed on the Shirofugen indicator grafted with buds from this candidate. This candidate should be re-grafted on the same indicator if the budwood available.

Biological indexing is used to assess candidates for the presence of graft-transmissible agents. If symptoms are observed on the developed growth of the woody indicators the presence of a graft transmissible agent is suspected. As almost all candidate buds from the "affected" material have callused a transmissible agent has had opportunity to transfer into the rootstock tissue if present. From the above observations we could suspect that a graft transmissible agent associated with the “diseased almonds” was present in the two varieties (Omega and B19-21).

In February 2010 we tested a number of Almond plants expressing similar symptoms on the woody indicator GF305 using an RT-PCR test for the generic detection of virus species in the Ilarvirus genus. A PCR amplicon was produced in some samples however further work is required to determine if it is associated with an Ilarvirus species.

We will continue to monitor any symptoms expression on the woody indicators in the following season. If the candidate buds produce any growth during this trial, we will inoculate our herbaceous indicators with sap from the candidate bud growth. We will also re-test symptomatic herbaceous and woody indicators with the generic Ilarvirus RT-PCR test. In addition, we will re-graft GF305 buds on the seedlings where these buds failed to grow and completely re-graft the Lacton (affected candidate) candidate. From this candidate we will need more budwood from ABA.

If you would like to continue or repeat the grafting experiment or if you require further testing of your almond material we will be glad to assist your business.

MM
October 2010

Symptoms on indicator GF305

Appendix 4

HAL Annual Industry Reports on Project AL08015

HAL ANNUAL INDUSTRY REPORT 2008-2009

Investigating almond growth disorder

The appearance of a widespread growth disorder in the Carmel variety during spring 2008 caused significant concern across almond production districts.

The disorder results in a failure of bud growth, which is very apparent in spring as reduced vegetative growth and extended areas of bare wood. Leaf buds are more extensively affected than flower buds, but reduced nut production especially in younger trees, has been noted in some orchards.

A research project was commenced and is focussed on bud initiation and development and the factors influencing these processes. To investigate bud viability and health, lateral growth budsticks from orchards in the MIA, Riverland and Sunraysia have been systematically cut from affected and unaffected trees since late February 2009.

The buds have been dissected and their internal and external appearance recorded, with the type and severity of damage coded to allow for comparison between samples and sampling times. Early research also involved virus testing to ensure viruses were not a potential cause of bud failure. Initial molecular testing of leaf samples from four MIA orchards did not detect the presence of Prunus Necrotic Ringspot Virus (PNRSV) or Prune Dwarf Virus (PDV), and it appears these viruses do not explain the onset or development of the disorder.

A literature review of bud development noted the potential role of post-harvest water deprivation. A review of post-harvest watering and pre-harvest temperature extremes has been made to determine if there is any correlation with the observed 2008 disorder. The contribution of environmental factors has not been determined but the heat waves of March 2008 and January 2009 are likely to have affected bud development to some degree. Post-harvest water deprivation in 2008 may have been a contributory factor, but rains during autumn 2009 were widespread and few orchards suffered post-harvest water stress this season.

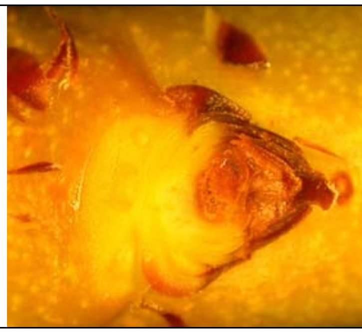
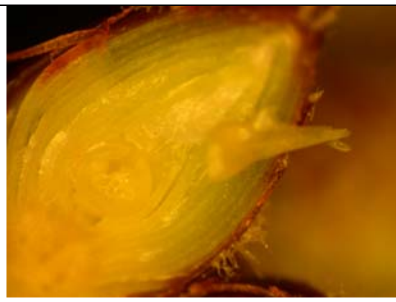
The research team has made orchard visits in September 2009, to observe leaf out in previously marked affected and unaffected trees. There is no evidence to suggest the disorder has ‘spread’ between trees. However the correlation between autumn bud dissection results and spring leaf out is strong. Trees with significant levels of damaged buds in autumn, have sparse canopies, poor leaf out and extensive areas of bare wood. Within affected trees, the results to-date suggest 2007/08 affected wood has given rise to 2008/09 affected wood.

The role of genetics cannot easily be determined but the predictive value of bud dissections in late summer is being further investigated. It is too early in the research program to determine the cause of the Carmel disorder, or its trigger.

To help the researchers assess the extent of the disorder and the potential contributing factors, growers and nurserymen were surveyed about their orchard and the history of their budwood. To assist almond growers’ understanding of the project, and of bud initiation and development and the factors that influence them, all were sent a fact sheet on bud development, a summary of the survey data, virus test results, and bud dissections.

Project AL08015

For more information contact:
Prue McMichael, Scholefield Robinson Horticultural Services
T 08 8373 2488
E prue@srhs.com.au

Observation description	1 = Healthy green bud, 0% browning	1x = Healthy green bud; with lignified(?) section inside bud, but not at bud heart, usually tip section, 0% browning
Photo reference for Rating 1 and 1x		
Observation description	2 = Bud heart brown/stained (<50%)	3 = Bud heart brown/stained (>50%)
Photo reference for Rating 2 and 3		
Observation description	5 = Bud heart brown/stained (> 50%) PLUS staining/scarring below bud	C = Bud heart development advanced since previous observations (e.g. possible differentiation to floral bud)
Photo reference for Rating 5 and C		
Observation description	DC = Bud heart is dead but still present	D = Entire bud dead but still present
Photo reference for Rating DC and D		

HAL ANNUAL INDUSTRY REPORT 2009-2010

Almond growth disorder

The Australian almond industry observed a bud growth disorder in the pollinator, Carmel, in orchards across three production districts that had experienced heatwaves in March 2008 and January 2009. Extensive areas of bare wood, sparse canopies, poor leaf out and, in some cases, poor or delayed flowering occurred.

Leaf out on the same trees in the following spring showed evidence that the damaged buds did not recover, and most damage was in vegetative, rather than floral, buds.

Spring and summer temperatures influence bud development, but it is unlikely that temperatures alone *cause* bud failure as no other cultivar has developed this disorder, suggesting that genetic predisposition is also involved. High temperatures influence bud failure expression.

In affected trees, the disorder is progressively affecting a greater proportion of the canopy each year.

In early summer (December-January), buds are very small and the nuts of the current season are not yet mature. However by this time, dissections of buds from some affected trees demonstrated that internal damage was visible. Although 79% of vegetative buds from affected trees appeared healthy in January 2010, only 13 % of central buds were healthy by March. At the same time in the same orchard, 39% of trees identified as non-affected in 2008 and 2009 also had damaged buds. It is presumed that the heatwave in spring 2009 may have triggered the bud damage in these trees. March is a reliable time to conduct bud dissections as autumn bud health is indicative of bud emergence problems in the following spring.

During the last four seasons, young (fifth leaf and younger) trees have been exposed to consecutive seasons with extreme temperatures. Some of these trees have up to 90 per cent bare wood in the canopy. Management of non-infectious bud failure in such trees is not possible. Once bud failure develops in young trees, they are unlikely to remain economically viable.

Spring 2010 is expected to reveal another wave of bud failure onset due to the heatwave of November 2009.

Project AL08015

For more information contact:
Prue McMichael, Scholefield Robinson Horticultural Services
T 08 8373 2488
E prue@srhs.com.au

Leaf-out 2009. Top two sticks - laterals from ‘affected’ Carmel; bottom two sticks from ‘non-affected’ Carmel

Young tree displaying growth characteristic of bud failure. Spring 2009

Appendix 5

Presentations to Almond Conference, October 2009 and 2010

Almond Bud Disorder
HAL Project AL08015

Dr Prue McMichael
Dr Kate Delaporte

Background

- ***What we saw***: Spring 2008
  - Carmel affected
  - Bare wood
  - Tufted growth
  - Variable nut loads
  - Poor structure
  - Rough bark, banding

Tufted growth at end of bare shoots

Bare shoots, no nuts

Dark Lines around buds

Background

- ***What we knew:***
  - Heatwaves
  - Water restrictions
  - California's history of non-infectious bud failure (NBP)
- ***What we didn't know:***
  - Cause – environmental? Genetic? Biological? Chemical?
  - How widespread
  - Other affected cultivars
  - Affected tree ages
  - Source of all buds
  - Health status of trees
  - Monash tree status
  - Could it spread
  - Were affected buds dormant or dead
  - Why terminal or basal buds grew

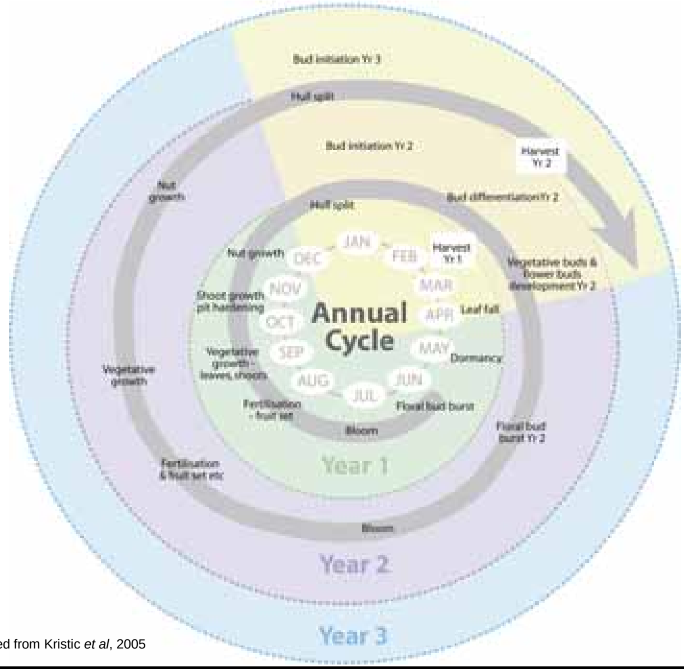
Our Approach

- Information from industry – Survey of growers, nurseries ☑
- Information to industry – Fact Sheet on bud development ☑
- Find willing co-operators – Riverland, Sunraysia, MIA ☑
- Investigate bud health - sequential dissections ☑ *
- Correlations of dissections with leaf out ☑ *
- Review temperature exposures ☑ *
- Review post-harvest irrigation ☐
- Biological testing ☑
- Graft transmission? – on-going ☐
- Recommendations – on-going ☐

Survey Summary

- Some Carmel trees in all regions (except NAP) had bud failure
- Most orchards reported low incidence
- Some individual trees had majority canopy affected
- Some winter symptoms
- No orchard had 100% of Carmel trees affected
- Budwood - sources other than Monash
- Trees sourced from > 21 nurseries
- Age of affected trees*
- Unlikely to have chemical cause

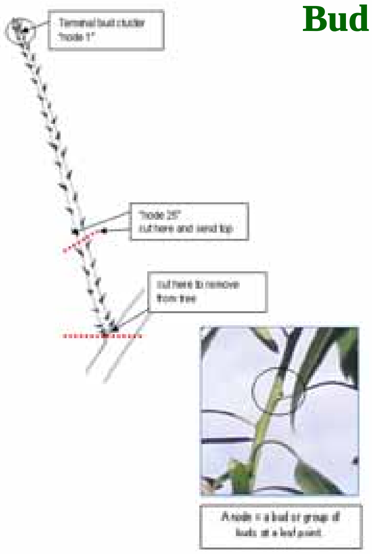
FACT SHEET *Cycle of Fruitfulness Potential in Almonds*

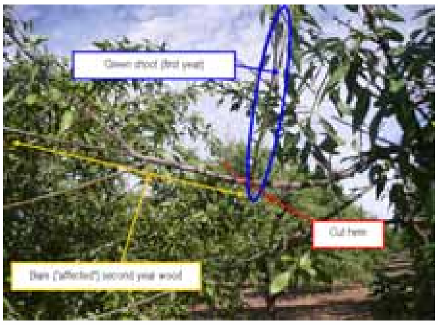


Source: Adapted from Kristic *et al*, 2005

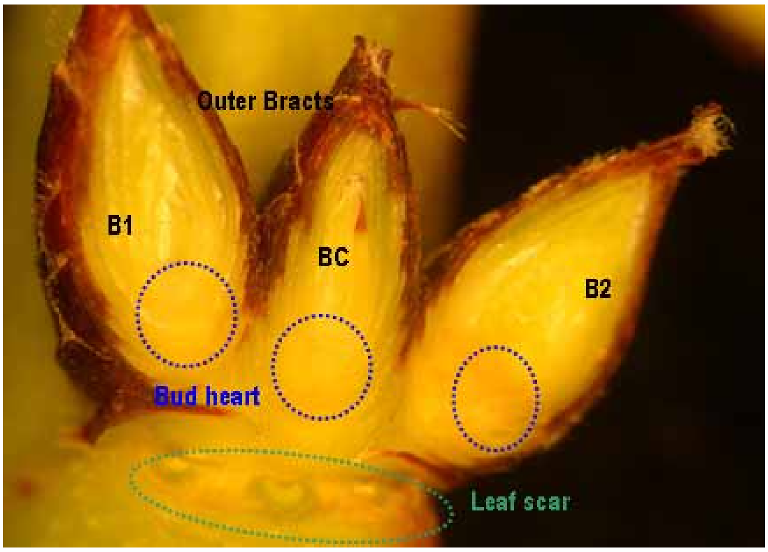
Bud Dissections

- Budsticks cut from *affected* and *non-affected* trees
- Collections from same trees March-September
- Leaf out correlation – September field visits

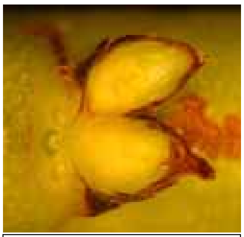




Scholefield Robinson



Visual Rating of Damage



1 = healthy green bud, 0% browning

1x = healthy green bud; with possible lignification inside bud, but not at bud heart, usually tip section, 0% browning

DC = bud heart is dead but still present

D = entire bud dead but still present

Schofield Robinson

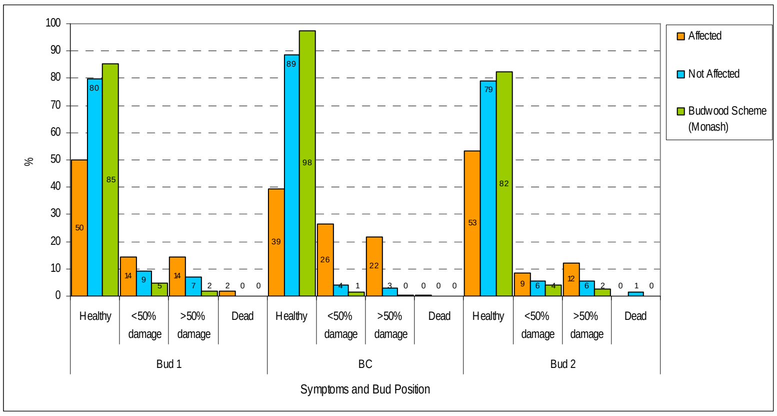
Bud Dissection Results – April 2009

- Damaged and dead buds evident
- External view is not indicative of bud health
- *Affected* and *non-affected* trees have some damaged buds
- Buds from *affected* wood have most damage
- At a node, central buds have more damage than laterals
- Bud position effects
- **Monash** buds look good – health , size

Schofield Robinson

Bud Damage from *Affected, Non-affected* and ABA Budwood Scheme (Monash) Trees

April 2009



Schofield Robinson

Bud Dissection Results – August 2009

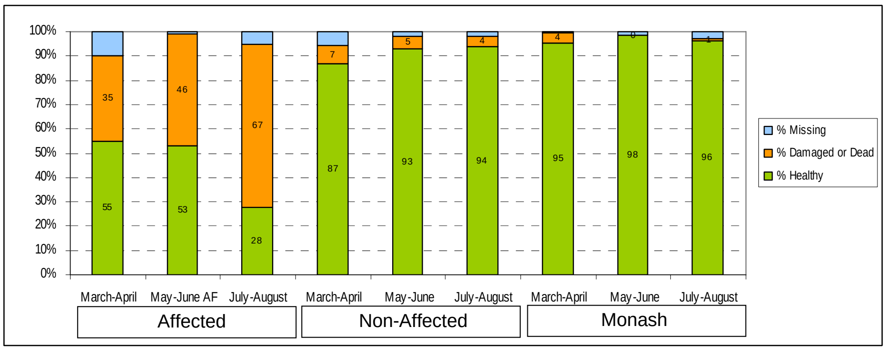
- Spring bud viability can be predicted late in the previous summer
- Damaged buds do not recover
- Bare wood laterals have damaged buds
- Vegetative and floral buds may display bud failure
- Central buds (mostly vegetative) from affected trees, have most damage*
- Damaged, vegetative buds remain underdeveloped
- Damaged/dead buds still attached
- **Monash buds -** Very high percentage of healthy buds
- **Monash buds -** High proportion of floral buds

Schofield Robinson

Bud Health & Damage Summary

	All buds		Central buds	
March-April	Healthy	Damaged/dead	Healthy	Damaged/dead
Affected trees	47%	33 %	39 %	48 %
Non-affected	83	12	89	7
Monash	88	5	98	1
May-August				
Affected trees	46	39	40	57
Non-affected	86	6	90	6
Monash	88	0	97	0

Central Bud Status: March - August 2009



Spring Field Visits - Monash

Spring Field Visits - MIA

Spring Field Visits - Riverland

Spring Field Visits - MIA

Conclusions - 2008/09 Bud & Field Investigations

- Damaged vegetative buds do not leaf out
- Bud dissection results reflect leaf out
- Young affected trees have high percentage of canopy affected (up to 90%) (3 seasons)
- Most young, affected trees also have low nut loads
- Severely-affected trees may also have rough bark
- Young affected trees - not economically viable*

Conclusions - 2008/09 Bud & Field Investigations (continued)

- Tree framework has been affected in some young trees
- Mature trees have a low proportion of their canopy showing bud failure
- Non-affected (2008) trees have not 'developed' bud failure in 2009
- Heavy pruning has not reversed the problem
- Monash trees and buds appear not to carry this disorder*

Temperature Review

California suggests May-June temperatures critical (Aust equivalent November – December)

Regional high temperature exposure

Spring-early summer

Location	Accumulated degree days (DD) >27°C (October 1- December 31)					Average	
	?	2005	2006	2007	2008	4-yr	10-yr
California	180						
Griffith		127	186	151	108	143	128
Mildura		127	157	173	97	138	129
Renmark		142	175	193	110	155	140

Summer-early autumn

Location	Accumulated degree days >27°C (January 1- March 31)					Average	
	?	2006	2007	2008	2009	4-yr	10-yr
California	330						
Griffith		330	301	243	286	290	243
Mildura		281	242	254	267	261	236
Renmark		284	251	264	275	268	238

Temperature Review

Extreme heat events

Spring-early summer (days over 35°C)

Location	Days with maximum >35°C (October 1- December 31)				Average	
	2005	2006	2007	2008	4-yr	10-yr
Griffith	12	22	16	8	15	13
Mildura	12	15	18	8	13	12
Renmark	14	19	22	9	16	14

Extreme events in recent seasons (October – March)

Location	Growing season days (total) with maximum >35°C				Average	
	2006	2007	2008	2009	4-yr	10-yr
Griffith	55	58	47	36	49	41
Mildura	40	43	49	35	42	38
Renmark	45	47	57	38	47	42

Temperature Review - Conclusions

- Last 4 seasons - higher temperature exposure and extremes than last decade (averages)
- Spring-early summer extremes
- Some exposures similar to those in California
- MIA region has had more frequent and significant deviations from high temperature averages
- Young trees planted in the MIA since 2004/05 have been consistently exposed to above average durations of high temperatures, and some extreme temperatures
- Temperatures alone unlikely to explain (or predict) bud failure

Biological Testing

- Viruses ☒
- *Botryosphaeria* spp. ?
- Saprophytes ☒

Slide 25

Thoughts

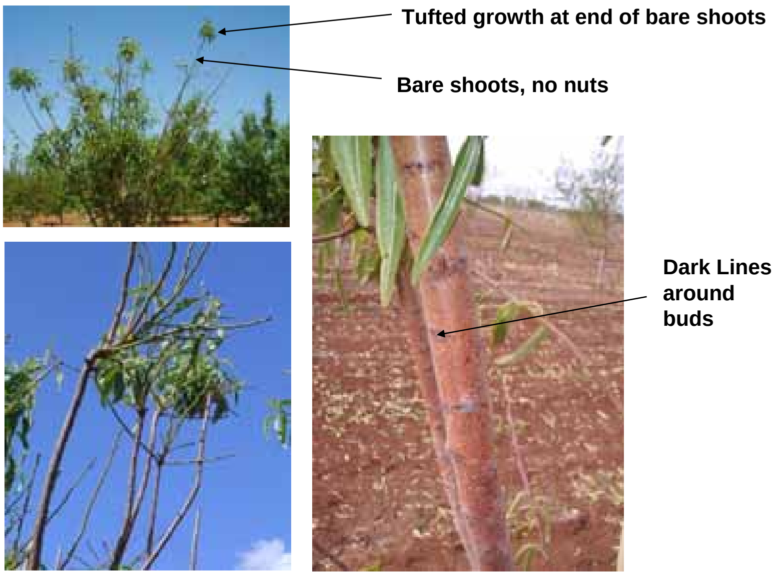
- Cannot be sure it is/is not NBF
- Consider bud sources – time of development?
- Use budwood closest to original source
- Record budwood source – ask same of nurseries
- Minimise water stress
- New pollinator needed
- Decision point – probably 5-year-old trees

Slide 26

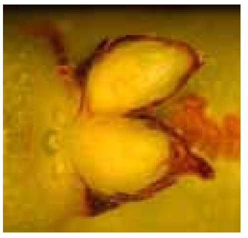
Carmel Bud Failure

HAL Project AL08015

Dr Prue McMichael
Dr Kate Delaporte
ABA & Trial Co-operators

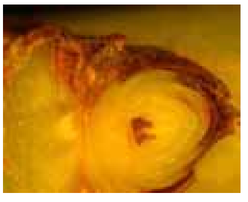


Visual Rating of Damage



1 = healthy green bud, 0% browning

1x = healthy green bud; with possible lignification inside bud, but not at bud heart, usually tip section, 0% browning



DC = bud heart is dead but still present

D = entire bud dead but still present

Scholefield Robinson
HORTICULTURAL SERVICES

Bud Dissection Results – Spring 2009

- External bud appearance – not indicative
- Spring bud viability predicted in previous autumn
- Damaged buds do not recover
- Affected and non-affected trees have some damaged buds
- Bare wood laterals have most damaged buds
- Vegetative and floral buds may display bud failure
- Central buds (mostly vegetative) from affected trees, have most damage
- Damaged/dead buds still attached
- Monash buds - High percentage of healthy buds
- Monash buds - High proportion of floral buds

Scholefield Robinson

Bud Transformation to Floral Buds
May-June 2009

Region	Status	Outside Buds (combined)
Riverland	Affected	47%
Riverland	Non affected	68%
MIA	Affected	3%
MIA	Non affected	74%

Scholefield Robinson

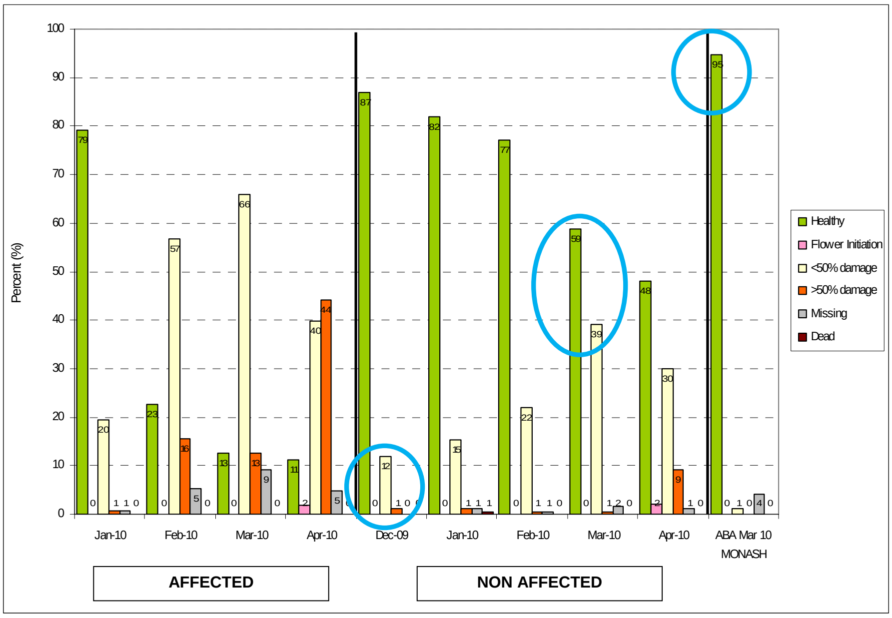
Summary – 2008 / 2009

- Only Carmel affected
- No recovery from 2008 onset – affected trees
- ***No new bud failure in non-affected trees**
- Damaged vegetative buds don't leaf out
- ***Damaged floral buds – delayed flowering or no flowering**
- Young affected trees with up to 90% canopy affected
- Severely affected trees – low nut load
- Heavy pruning has not reversed situation
- Temperatures in March 2008, January 2009 - extreme

2009/10 Investigations

- Followed trees in one orchard
- Started bud dissections in late November
- Continued to review temperatures

Central Bud Status Season 2010



Schofield Robinson

Central Bud Comparison March 2009 & 2010

Bud status – (% buds)	Riverland Orchard 2 Affected	Riverland Orchard 2 Non-affected	Monash budwood
March 2009			
Healthy buds	79	98	97
<50% damage	20	1	1
> 50% damage	1	0	0
Dead	0	0	0
March 2010			
Healthy buds	13	59	95
<50% damage	66	39	1
> 50% damage	13	1	0
Dead	0	0	0

Schofield Robinson

Key Observations 2010

- **By January 2010**
  - Damaged buds in affected AND non-affected trees
- **By March 2010**
  - Leaf out predictions – problems in both
- November 2009 heatwave contributed?

Scholefield Robinson

Regional High Temperature Exposure (DD > 27 °C)

Spring-early summer						
Location		Accumulated DD >27°C (October 1- December 31)			Average	
	?	2007	2008	2009	4-yr	10-yr
California	180					
Griffith		151	108	209	143	128
Mildura		173	97	202	138	129
Renmark		193	110	210	155	140

Scholefield Robinson

Regional Extreme (> 35 °C) Heat Events

Spring-early summer							
Location	Days with maximum >35°C (October 1- December 31)					Average	
	2005	2006	2007	2008	2009	4-yr	10-yr
Griffith	12	22	16	8	21	15	13
Mildura	12	15	18	8	27	13	12
Renmark	14	19	22	9	26	16	14

Scholefield Robinson

Extreme (> 35°C) Seasonal Exposures (Oct-March)

Location	Growing season days (total) with maximum >35°C					Average	
	2005/06	2006/07	2007/08	2008/09	2009/10	4-yr	10-yr
Griffith	55	58	47	36	43	49	41
Mildura	40	43	49	35	56	42	38
Renmark	45	47	57	38	57	47	42

Scholefield Robinson

Technical Knowledge

- Non-infectious bud failure – California
- Bud failure potential:
  - genetic, inherited
  - Varies bud to bud on shoots
  - Progressive, persistent
  - Increases with successive generations
  - Increases once planted

Scholefield Robinson

Technical Knowledge (cont’d)

- Bud failure onset:
  - interactions, variable
  - high temperatures DD >27°C
  - annual growth
- Severity and rate;
  - bud failure potential
  - age of onset
  - % canopy affected
  - seasonal temperatures

Scholefield Robinson

Technical Knowledge (cont’d)

- Bud failure patterns:
  - growth management
  - reflect bud failure at planting
  - bud source & cutting time
  - budding time
  - Temperatures
  - yield vs veg effects

Practical Responses - Nurseries

- Know budwood sources, cutting strategy
- Use only basal buds, low shoots, spring
- Understand temperature effects;
  - hot autumn - floral, veg buds affected
  - hot spring - veg buds affected
  - 1 flush vs 2 flushes
- Seek grower feedback
  - number of affected trees, onset age

Practical Responses - Growers

- Assess impacts
- Record onset, tree age, % canopy
- Options;
  - Remove
  - Block viability
  - Top-work;
    - Affected bud on non-affected tree – NBF
    - Good bud on affected tree – OK
- Check Non-pareils
- Traceable budwood – ask nursery

Practical Responses - Industry

- New Carmel, Non-pareil clones
- Find oldest Carmel orchard trees
- System for Monash and nursery bud traceability – ID high and low bud failure potential source trees
- Evaluation – include bud failure, regional, new pollinators

Why now in Australia?

- Successive generations?
- 2 flushes – autumn cuts?
- High temperatures – autumn 2008, spring 2009
- Unknown budwood sources