Final Report

Management of Carpophilus Beetle in Almonds

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Delivery partner:

Department of Economic Development, Jobs, Transport and Resources Victoria

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Management of Carpophilus Beetle in Almonds – AL15004

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Summary

Since the harvest of 2013, Australia's almond industry has been suffering significant losses in its major production regions due to kernel damage by carpophilus beetles, a group of pests better known for damaging fresh stone fruit. Average damage levels as high as 5-10% are now not uncommon in almond orchards, and costs to industry from this insect have escalated well into the millions. Moreover, as infested nuts can be difficult to identify and reject during processing and sorting, the beetles pose a significant risk to export markets. In 2014, the almond industry and Agriculture Victoria Research began a collaboration to implement a mass trapping program against carpophilus, using commercially available attract and kill (A&K) traps that were developed to manage this group of pests in stone fruit. At the time, the likely efficacy of this A&K system in almonds was not known, but it was the only acceptable option available. This project's primary aim was to clarify which of the many species of *Carpophilus* was damaging almonds, and evaluate the success of an A&K strategy in reducing kernel damage by this pest.

Large scale field trials were conducted to collect and identify beetles from A&K traps across 12 orchards in three states. The study revealed at least 12 carpophilus species and other related beetle species were caught in traps in almond orchards, but that the vast majority of kernel damage was being caused by a single unidentified species. That species was temporarily named *Carpophilus* near *dimidiatus* due to its morphological and genetic similarity to the known species *C. dimidiatus*. Infestation and damage by *C*. nr *dimidiatus* was found to commence at hull split, and damage levels increase rapidly thereafter, indicating the need to harvest and disinfest crops as early as possible to minimise damage. Repeated sampling of nuts remaining in orchards after harvest showed these residual and mummy nuts to be a major source of the damaging carpophilus species, with potential to support massive population increases in the pest. Preventing these nuts or destroying them through orchard hygiene practices is therefore likely to be a critical factor for success in managing this pest, regardless of other management options being implemented.

Results from these comprehensive field trials revealed that whilst the stone fruit A&K system does trap the damaging species of carpophilus in almond orchards, trap catches do not reflect the high level of damage caused to kernels, suggesting that the current trapping system is not sufficiently effective to be of economic benefit to growers. The evidence gathered from this study strongly suggested the need for research to improve the current A&K system through tailoring the attractants specifically for *C.* nr *dimidiatus*, increasing longevity of these formulations, and making the trap more cost-effective to growers. This important knowledge gap is now being addressed as a key component of a new project AL16009 'An Integrated Pest Management program for the Australian almond industry'.

The high level of engagement with industry that was developed and maintained by the project team throughout its term, significantly improved producer awareness and understanding of the carpophilus issues in almonds, the acute need for improved orchard hygiene, and the necessity for an integrated pest management strategy to control the escalating populations of this major economic pest.

Keywords

Carpophilus; almond; pest management; mass trapping; attract and kill; diagnostic key.

Introduction

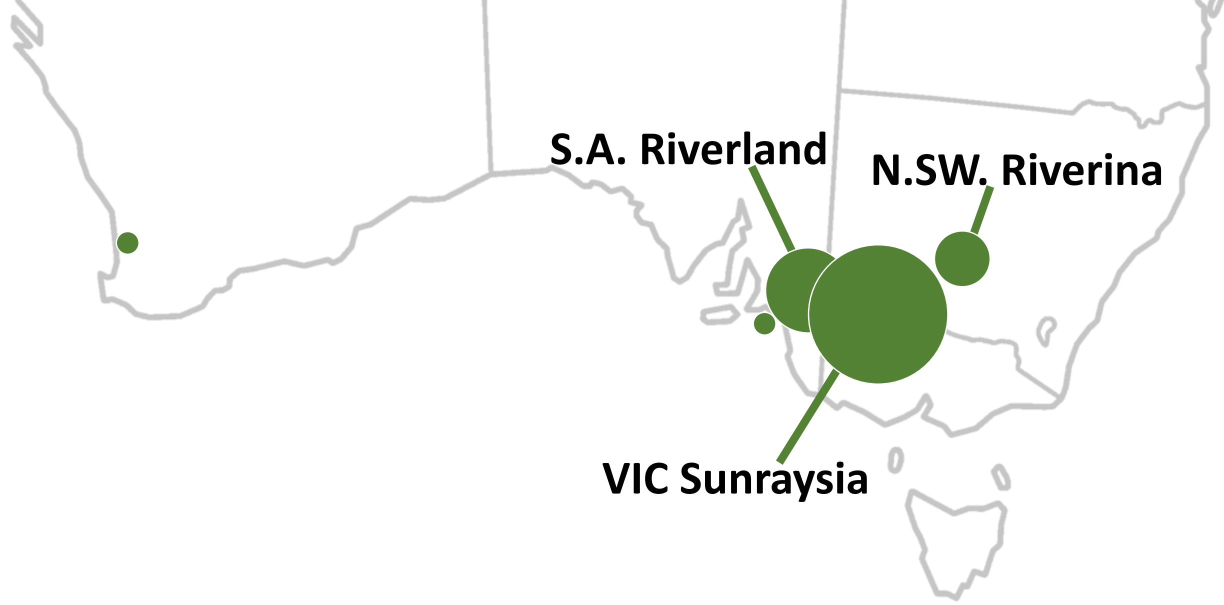
Australia's almond industry was alerted to carpophilus beetle as a new and potentially serious pest of almonds in 2013 when it was found damaging new season kernels during nut assessments for HAL Project AI12004 (Managing carob moth in almonds). During the 2014 harvest, carpophilus beetle was found damaging up to 10% of kernels in some districts. In response to industry concerns, Agriculture Victoria Research (AVR) staff collaborated with producers to help them implement a monitoring and 'attract and kill' (A&K) trapping program for the 2014/15 season. AVR also produced a set of guidelines ('Carpophilus – Preliminary guidelines: Monitoring and attract and kill') to assist producers. The trapping program was based on a system previously developed by AVR for use in fresh stone fruit (Hossain et al. 2006, 2007, 2009). At the time, it was not known why carpophilus was infesting almonds, but it was considered likely that volatile compounds from maturing or rotting nuts played a role, as they do in stone fruit.

Trapping during 2014/15 confirmed the presence of several species of carpophilus beetles across most almond orchards in the Victorian Sunraysia district, an area that accounts for almost 70% of Australia's plantings. The pest also infested almonds in the NSW Riverina and SA Riverland districts. While the species commonly found in other crops, *Carpophilus hemipterus*, *C. davidsoni* and *C. humeralis* were trapped in almond orchards, a fourth species, *Carpophilus* near *dimidiatus* (*C.* nr *dimidiatus*) was identified as the main species causing kernel damage. The cost of carpophilus damage to the Nonpareil crop in the 2015 harvest, across the Sunraysia district of Victoria alone, was conservatively estimated at almost $11 million. Apart from this direct loss of marketable crop, carpophilus-infested kernels can be difficult to separate from the crop during processing using optical sorters, and potential presence of this insect in exported nut and nut products posed a significant risk to the industry's reputation for quality.

To begin addressing the problem of carpophilus beetle as an emerging and major almond pest, AVR in consultation with industry, developed the project reported here. The key research objectives of the project were to: clearly determine the carpophilus species present in almond orchards, particularly those responsible for kernel damage; determine the distribution and behaviour of damaging species; investigate the effectiveness and optimal implementation of the stone fruit A&K system in almonds; investigate alternative hosts that may support the damaging species; and identify potential pesticide options for managing those species. This report presents the research approaches used to address those objectives, and the findings from that research.

Location map

Field research activities for this project were undertaken in the South Australian Riverland, Victorian Sunraysia and New South Wales Riverina districts.



Methodology

What do we know about managing carpophilus in stone fruit?

A literature review (Appendix 1) confirmed that carpophilus beetles are serious pests of stone fruit in Australia, not only because of the direct damage they cause to fruit, but also their role in distributing spores of fruit-rot fungi. Foliar and ground applications of broad-spectrum insecticides have tended to give unsatisfactory results due to the cryptic and migratory behaviour of the beetles. Both sexes of carpophilus are attracted to odours from ripe or rotting fruit, and both are also attracted to aggregation pheromones produced by males. A combination of fruit odours plus pheromone was found to be highly synergistic in attracting carpophilus beetles, and development of a concentrated synthetic fruit odour “co-attractant” facilitated the subsequent development of a compact ‘Attract and Kill’ (A&K) trap baited with fruit odour and pheromone. This mass trapping system allowed stone fruit growers to effectively manage carpophilus without pesticide applications. Successful implementation of a similar system in almonds will most likely require redesigning the attractants so that they are relevant to the damaging species of carpophilus, and are based on specific food (host-plant) odours from developing nuts as a co-attractant, and possibly modifications to the beetle pheromone used.

Which carpophilus species occur in almonds?

Large numbers of trapped adult carpophilus beetles from the major almond growing regions of the Riverland, Sunraysia and the Riverina, were provided to the project by almond producers who had implemented carpophilus trapping programs using the A&K trap developed for the fresh stone fruit industry. Combined morphological and molecular identification methods were used to analyse trap catches, and discovered at least 12 carpophilus and related species were common in almond orchards, including *Carpophilus hemipterus*, *C. davidsoni*, *C*. nr *dimidiatus*, *Urophorus humeralis* and species of *Brachypeplus* (Appendix 2). The first three species, and *C. hemipterus* in particular, were by far the most commonly trapped. Intensive sampling and inspection of new crop nuts consistently implicated only *C*. nr *dimidiatus* in causing kernel damage in almonds. Early season activity of *C. nr dimidiatus* adults was found to be very low, but increased in early January, around the time of hull split. Nut infestation and kernel damage by this species commenced at hull split and damage levels increased rapidly thereafter, indicating the need to harvest and disinfest crops as early as possible to minimise damage. *C. nr dimidiatus* makes up only a small fraction of the beetles caught in the current A&K traps, so the total trap catch is not a reliable measure of infestation levels or risk of kernel damage in almonds. Anecdotal evidence and results of industry trapping suggest that in some cases, the large numbers of non-damaging species of carpophilus trapped in almond orchards may originate in nearby areas of citrus and vineyards, especially where fruit residues are present.

How do we distinguish between species?

Live specimens of the five main species of Nitidulidae beetle commonly detected in almond orchards during the project (*C.* nr *dimidiatus*, *C. hemipterus*, *C. davidsoni*, *Urophorus humeralis* and species of *Brachypeplus*) were collected from almond orchards and used to establish laboratory colonies at the AVR Agribio Centre. From these colonies, adults and their matched egg, larval and pupal offspring were used to develop accurate morphological descriptions. Keys were then developed for adults and larvae of the important species by pinpointing their critical morphological differences, making use of diagnostic photographs where appropriate (Appendix 3). DNA barcoding was also used to assist with identification of immature stages. While the species causing damage to almonds was morphologically close to *C. dimidiatus*, molecular analysis showed it to be distinct. As a result, this species was temporarily labelled *C*. near *dimidiatus* until further analysis can confirm its identity.

Where is the damaging species coming from?

Potential alternative host plants and overwintering sites for carpophilus were investigated because of the role they may play in supporting populations of the beetles associated with almond crops, and the opportunities they may provide for improved pest management through, for example, management of alternative hosts, or destruction of overwintering beetle populations. Manual searches, extraction of beetles from samples of bark, leaf litter, fruits and nuts, and trapping, were used to investigate the host range and overwintering sites (Appendix 4). The very few carpophilus extracted from 45 species of fruits and nuts did not include *C*. nr *dimidiatus*. Also, *C*. nr *dimidiatus* was not detected in areas of native vegetation near infested almond orchards, suggesting that native vegetation in or near orchards is unlikely to be a routine source of this species infesting almonds. However, structured sampling of mummy nuts and residual crop within orchards found *C*. nr *dimidiatus* to be overwintering in these nuts and breeding large numbers in spring. The levels of infestation observed in those nuts show a massive potential for population increase, indicating that preventing or destroying residual crop and mummy nuts is likely to be a critical factor for success in managing this pest.

Can we manage the damaging species with the existing stone fruit A&K system?

The potential for the current stone fruit A&K system to protect almond crops from carpophilus damage was assessed by analysing and identifying beetle catches from A&K traps, and determining crop damage levels in relation to A&K programs implemented by orchard managers (Appendix 5). The project also commenced testing the food attractants (co-attractant) used in the current A&K traps, against the key species of carpophilus in almonds and stone fruit, to better understand the response of *C.* nr *dimidiatus* to the traps and guide the improvement of the A&K system. Whilst the current A&K traps do attract all the carpophilus species found in almond orchards to date, and the A&K system did appear to reduce kernel damage levels in some instances, it was not to the extent required for cost-effective pest management. Initial behavioural analysis in a laboratory wind tunnel demonstrated that the current co-attractant is significantly less attractive to *C.* nr *dimidiatus*, compared to carpophilus species causing damage to fresh stone fruit (for which the co-attractant was designed). It is now imperative to further explore the factors influencing beetle receptiveness to the traps, be they nutritional status of the pest or composition of the attractant (pheromone, co-attractant, or their combination). It seems clear that for A&K to be a cost-effective option for carpophilus management in almonds, effective orchard hygiene and an attractant that particularly targets *C.* nr *dimidiatus* are essential. Addressing these factors are key objectives of new Project AL16009 'An Integrated Pest Management program for the Australian almond industry'.

What is the optimal spacing for the existing A&K traps in almonds?

Cost-efficient management of carpophilus in almonds through mass trapping would require the use of trapping densities that adequately protect the crop while minimising unnecessary overlap of trapping spaces. The trapping radius of the current carpophilus A&K traps was investigated through a 400 ha trapping density trial in an almond orchard in Sunraysia (Appendix 6). Beetle samples collected during the 2016/17 season from 104 A&K traps were counted and analysed for species composition, and the data was then processed through spatial analysis software to determine the distance between traps at which their trapping spaces no longer overlap. For *C.* nr *dimidiatus*, the trapping radius of the current A&K traps was estimated as 319m, i.e. the traps appeared to have the potential to capture *C.* nr *dimidiatus* occurring within that distance. Effective A&K however, relies not just on correct trap spacing, but on a combination of factors that lead to beetles entering the traps, as discussed in Appendix 5.

What other management options are available?

We have shown that *C.* nr *dimidiatus* in almonds makes effective use of residual crop and mummy nuts in the orchard as a food resource and breeding habitat. Thus the destruction or removal of those nuts is highly likely to limit the potential for this species to maintain populations at economically damaging levels. Although the precise relationships between populations of residual and mummy nuts and carpophilus, and subsequent levels of kernel damage, have not yet been quantified, the industry has currently been striving to achieve as good a level of hygiene as possible. The effect of orchard hygiene on carpophilus and kernel damage, and the assessment of methods of mummy nut destruction are the subjects of research in project AL16009.

Some producers have applied synthetic pyrethroid pesticides in an attempt to protect almond crops from carpophilus damage, but these broad-spectrum pesticides have induced pest mite problems because of their impact on mite biological control. A small 'pesticide screening' component of this project sought to identify potential IPM-friendly pesticide options for carpophilus management, but the research focus was moved (via a contract variation) away from pesticide screening and towards improving the efficacy of A&K traps. The project did, however, continue to support the evaluation of pesticide options for the industry by assisting manufacturers with their field trials. The work been conducted via an agreement between DEDJTR and the company involved and no levy money was involved to conduct the work. Prior to the contract variation, a new pesticide (provided commercial in confidence) was assessed against *C.* nr *dimidiatus* in a preliminary unreplicated bioassay. The bioassay involved exposure of adult beetles to dry residues of the pesticide that had been applied to petri dishes via a Potter tower at the recommended field rate (Appendix 7). Although mortality due specifically to the pesticide appeared close to 60%, a high mortality amongst untreated beetles suggests that this result should be rejected and retested with adequate replication.

Technology transfer and project management

The critical nature of the pest issue addressed by this project necessitated a high level of engagement with industry from the beginning, to ensure the effective two-way flow of information between the project and industry. The many formats used to achieve this engagement included individual and small group discussions, district update meetings, orchard walks, R&D forums and industry conferences. A high level of direct industry participation in the project's research activities was also a major contributor to engagement and knowledge transfer. Guidelines for A&K were also published on the ABA website to ensure free access to the most up-to-date recommendations. Specific project outputs are detailed in the 'Outputs' section and Appendix 8.

A Project Steering Committee of industry, research and Hort Innovation representatives was used to track research progress, address stakeholder concerns, and in one instance redirect the focus of research through a contract variation, to better align with industry priorities. The Committee's terms of reference, membership and minutes are included in Appendix 8.

A monitoring and evaluation plan was also developed to allow some assessment of the project's effectiveness in its research and technology transfer activities. Although research towards an effective A&K system is still underway, the project was very effective in raising industry awareness of the importance of carpophilus as a major pest, and orchard hygiene as a component of carpophilus management. For details, see 'Monitoring and Evaluation' below.

Outputs

Formal and informal presentations by project officers

Project officers took part in over thirty events at which they provided the almond industry with technical information on carpophilus and its management options. These events, ranging from formal conference presentations to discussions with small producer groups or individuals (see Appendix 8), were all intended to improve producer knowledge and understanding of carpophilus as a serious almond pest. Presentations given to formal events are listed below.

- Hossain M. (2014). Carpophilus beetle: a new pest in almonds. Activated Almonds R&D Forum, 18/6/2014, McCormick Centre for the Environment, Renmark, S.A. http://growing.australianalmonds.com.au/wp-content/uploads/sites/17/2014/06/09-Carpophilus-beetle-a-new-pest-in-almond-orchards.pdf
- Hossain M., Madge D., Williams D. (2015). Carpophilus beetle (understand your enemy): an update. Activated Almonds R&D Forum, 28/10/2015. McCormick Centre for the Environment, Renmark, S.A. http://growing.australianalmonds.com.au/wp-content/uploads/sites/17/2014/06/06.-Carob-moth-Carpophilus-Presentation-Alm-Oct15Final.pdf
- Hossain M. (2014). Carpophilus beetle: a hungry pest. 16th Australian Almond Conference, 28-30 Oct, 2014. Glenelg S.A. http://growing.australianalmonds.com.au/wp-content/uploads/sites/17/2016/01/0830-Carpophilus-Beetle-A-Hungry-Pest-Hossain-M.pdf
- Hossain M.S., Madge D., Rako, L., Semeraro L. & Williams D. (2017). Control of *Carpophilus spp*. (Coleoptera: Nitidulidae) in almonds using attract and kill systems. Australian Entomological Society, 48th AGM and Scientific Conference, 17-20/9/2017, Terrigal, NSW. [see attached PDF 'AL15004 Abstract-Entomological Society Conference 2017']
- Hossain M.S., Madge D.,Rako L., Semeraro L., Williams D.G. & Lai D. (2017). Control of Carpophilus spp. (Coleoptera: Nitidulidae) in almonds using attract and kill traps. Poster presentation. VII International symposium on almonds and pistachios, 5-9/11/2017, Adelaide, S.A. [see attached PDF 'AL15004 Poster-Almond and Pistachios Symposium 2017']

Publications

The following publications were produced to improve industry knowledge of carpophilus beetle, and to maximise any benefits gained by industry and the project, from the deployment of A&K traps.

- 'Managing carpophilus beetle in almonds: Preliminary monitoring guidelines, attract and kill guidelines for 2016 - 2017 season. These guidelines were first produced in October 2014 to provide the information necessary for almond producers to begin monitoring carpophilus populations and attempt management of the pest through A&K. The guidelines were subsequently updated for the 2015/16 and 2016/17 seasons. [see attached PDF 'AL15004 Factsheet-Managing carpophilus beetle in almonds 2016-17.pdf']
- 'Tips for maintenance of traps and management of beetle samples for the Carpophilus project'. This document was provided to producers for use as a handy 'ute guide' by field staff who were responsible for trap maintenance and the collection of trap samples for the project. [see Appendix 7]
- 'Identification of adult Nitidulid beetles in almond orchards' and 'Identification of Nitidulid larvae'. Although the identification of carpophilus (Nitidulid beetles) can be a complex taxonomic task, these brochures were produced to provide a basic guide to the identification of key carpophilus species found in almond orchards. [see attached PDF files: 'AL15004 Identification brochure-Carpophilus adults' & 'AL15004 Identification brochure-Carpophilus larvae'].

Steering committee minutes

The Project Steering Committee met twice at the AVR office at Irymple (April 20, 2016 & February 15, 2017), and once at AVR's Agribio laboratory complex Bundoora (August 2, 2017). Minutes of those meetings are in Appendix 8.

Outcomes

This project's anticipated short-term outcome that 'Industry will have improved knowledge and expertise in management of Carpophilus beetle' has been achieved through:

- Improved producer knowledge and understanding of the underlying principles of trap-based A&K for carpophilus beetle management in almond orchards.
- Improved practical skills amongst industry technical staff in field implementation of A&K.
- A high level of awareness amongst producers, of carpophilus beetle, its destructive potential, the role of residual and mummy nuts in supporting carpophilus populations, and the importance of destroying or removing those nuts as a crucial aspect of carpophilus management.

Refer to the Monitoring and Evaluation report below, for the full evaluation data.

The project has also achieved the following, towards its medium-term outcome that 'Industry will have access to a cost-effective alternative to pesticides for Carpophilus management':

- Confirmation that (to date) *C.* nr *dimidiatus* is the only species known to be causing damage to almond kernels.
- Enhanced capability for future specimen identifications through establishment of a specimen reference collection and DNA sequence reference library.
- Improved support for producers and field staff, through development of guides to assist with identification of carpophilus and related species found in almond orchards.
- Producer awareness that current A&K traps are not reliable indicators of *C.* nr *dimidiatus* population levels or crop risk because they trap mostly non-damaging species.
- Confirmation that the current A&K co-attractant has relatively low attractiveness to *C.* nr *dimidiatus*, highlighting the need for lure components designed specifically to target the species.
- Confirmation that *C.* nr *dimidiatus* infests nuts at early hull split, with the implications that:
    - harvest should occur as early as possible to minimise crop losses,
    - almond volatiles at hull split may be a source of attractant for the species.
- Determination of a potential trap radius of over 300m for *C.* nr *dimidiatus* with the current A&K traps, with the understanding that actual trapping efficacy depends strongly on numerous factors including beetle receptiveness to the trap and its lures.
- Strengthened linkages with the almond industry at all levels which will facilitate future collaborations.

Monitoring and evaluation

Introduction

Monitoring and evaluation is an important aspect of the development and management of a research project, as it allows for assessment of the project's effectiveness and efficiency in performing scientific research and informing industry of the outcomes of that research. The aim of evaluation of Project AL15004 was to identify successful approaches to research and knowledge transfer used during the project, along with those areas where improvement would be desirable, including potential solutions to issues encountered during the project. This report addresses the 'Activities and outputs' and 'Short-term outcomes' components of the project's monitoring and evaluation plan (Appendix 9). These components were due to be achieved by the completion of the project (May 2018). The evaluation may be used to improve the planning and implementation of similar projects in future, and may be of particular value in the implementation of the new project AL16009 'An Integrated Pest Management strategy for the Australian almond industry'. The primary audience for this evaluation report is the research team, Agriculture Victoria Research (AVR), Hort Innovation and the Australian almond industry.

Methods

Timeframe

AVR flagged carpophilus beetle as a cause of significant levels of almond kernel damage as early as the June 2013 ABA R&D Forum. However, to allow for a truer assessment of the impact of this current project, its activities and outputs were not considered to commence until April 2014 when the first industry information meeting was held to specifically address carpophilus as an almond pest, and the potential for 'Attract-and-Kill' (A&K) technology as a monitoring/management strategy. That meeting initiated considerable extension activity and collaboration between AVR and the almond industry, and predated the formal start of Project AL15004 by 14 months.

Short-term outcomes

The anticipated short-term outcome for the project was that '*Industry will have improved knowledge and expertise in management of Carpophilus beetle*'. Industry knowledge of carpophilus beetle and approaches to monitoring and management of this pest was assessed through a survey of independent growers and corporate technical managers (Appendix 9). Collectively, the growers/managers surveyed are responsible for pest management decisions for the vast majority of the Australian almond crop, so the findings are presented in terms of producers as well as the area of crop for which they are responsible. Information sources used by industry to develop their knowledge were also identified to allow links to be made with project outputs and activities. The survey was conducted via one-to-one phone interviews (Appendix 9).

Activities and outputs

Project activities such as presentations to industry and technical training sessions, and outputs such as publications, were quantified through project records.

Findings

Short-term outcomes

The survey of industry knowledge of carpophilus beetle found that amongst the 18 producers/corporate technical managers interviewed:

- **58% of respondents (covering 85 % of total bearing area)** were aware of carpophilus as an almond pest before the commencement of project activity (April 2014). Of that 58%, 37% had learnt of carpophilus in almonds from the AVR presentation at the 2013 R&D Forum, and 18% (3 growers) recall being notified by processors of beetle damage in their crop.
- **Respondents covering 99% of bearing area** are now aware of carpophilus as an almond pest.
- **79% of respondents (covering 93% of bearing area)** are aware of the existence of multiple carpophilus species which pose different levels of risk to almonds
- **53% of respondents (covering 90% of bearing area)** are aware of the project's guidelines on using A&K for carpophilus management, and where to source them
- **78% of respondents (covering 95% of bearing area in high-risk areas)** in high-risk districts, where carpophilus has been causing crop damage and producers have a more critical need for information are aware of the guidelines.

Although awareness of <u>potential</u> management options for carpophilus is high amongst producers (Appendix 9 Table 2), they made it clear during the interviews that they understand some options, in their current form, are undesirable and difficult to implement successfully (pesticide application) or are not sufficiently effective (A&K) to control the pest. In line with the message being promoted by the project for some time, the vast majority of producers are aware that orchard hygiene (destruction of mummy nuts) is likely to be a key aspect of carpophilus management in almonds.

Most of the interviewed almond producers obtained their information on carpophilus as an almond pest directly from the project (Appendix 9 Table 3). This was through direct communication with project officers, project presentations at conferences, R&D forums and orchard walks, and project fact sheets.

Activities and outputs

The achievements for each of the activities and outputs highlighted in the project's monitoring and evaluation plan are detailed in Appendix 9. These indicate the spread and intensity of activity during the project, from the collection and analysis of trap and nut samples, to engagement with industry in a variety of settings. In addition to these predetermined activities, the project implemented a Steering Committee recommendation to obtain a contract variation that would allow for a change of research focus away from pesticide screening and towards improvement of the A&K system through the investigation of new attractants. The project also initiated field assessments of industry hygiene practices (mummy nut destruction) when the opportunity arose.

Discussion

The numbers of traps (and associated technical labour) offered by industry to contribute to the project through carpophilus samples, the geographical spread of participating orchards, and the number of information sharing events to which the project contributed, indicate the high level of project engagement with the almond industry and the willingness of industry to assist the research effort. The project team endeavoured to make use of every opportunity to interact with industry, to build and strengthen the relationships that are necessary for successful collaboration.

The project has been effective in raising industry awareness of carpophilus beetles and potential management approaches, in particular orchard hygiene in the absence of an effective A&K system or appropriate pesticide option. The reliance on this project as the major information source for producers is not surprising, given that to date, it has been the only research effort investigating the damaging species of carpophilus as an economic pest.

Because of the nature of A&K technology and species diversity in carpophilus, the project was not able to easily adapt the stone fruit A&K system to realise its ideal of effective A&K within the project's life. The considerable amount of sampling and diagnostic effort did however confirm that to date, the vast majority of almond kernel damage is due to just one of the several species of carpophilus found in almond orchards. The project maintained its relevance to the almond industry by being flexible, and pursuing the critical objectives of improving A&K (by obtaining a contract variation and investigating alternative attractants to specifically target that species), promoting orchard hygiene, and commencing assessments of industry hygiene practices.

It was apparent during the interviews of producers that there is some confusion between monitoring and A&K, using the same traps, particularly amongst producers who have not yet used the traps. Also, only half of the interviewees volunteered awareness of the A&K guidelines (although they did account for 90% of the bearing area of almonds). These results suggest that a more concerted effort could have been warranted to get relevant information to, and understood by all producers, including those in low risk districts for whom CB is not yet a serious problem (forewarned is forearmed). For example, more use could have been made of ABA publications that reach all producers, or extension events in 'low risk' districts. As a counter argument to this, when it became apparent that the existing stone fruit A&K system was not providing an adequate level of protection to almond crops, the project placed more focus on researching improvements to A&K, and promoting the importance of orchard hygiene, of which most producers are now well aware.

Some aspects of the project, such as regular collection of trap samples, did not always go exactly according to plan. Regular communication between the research team and industry collaborators helped to minimise any disruption to the overall research and industry effort, but in hindsight, there were times when better communication or even direct intervention or assistance from the project may have helped to improve the outcomes.

The project evaluation itself would have been easier if the original M&E plan was more carefully structured with clearer goals set ('This many … achieved') rather than general questions asked ('How many…achieved?'). It would also have helped if we had collated evaluation data more consciously during project activities, but our focus was on achieving the research goals rather than monitoring the process of achieving those goals.

Conclusions

The high level of project engagement with the almond industry led to strong researcher-producer relationships which were invaluable for supporting the project's technical work in all areas, from field research sites and sample collection to provision of trap and crop quality data. These relationships also facilitated the process of obtaining a contract variation to realign the research work with current industry priorities.

This engagement resulted in high levels of producer awareness of carpophilus and management options, but knowledge transfer to producers for whom the focus of the research was not so relevant could have been improved, by for example, making better use of ABA extension publications and events.

Regular communication between producers and the research team was critical to minimise unforeseen disruptions to the research effort. A higher level of direct involvement/mentoring by the research team may be warranted at times when industry-based activities are being used to provide valuable data or resources to the project.

Recommendations

1. High levels of project engagement with industry should be developed and maintained, through direct contact between research teams and producers
2. Effective use should be made of industry extension networks/publications to achieve industry-wide awareness of key issues
3. Research and researchers should be flexible, and project Steering Committees emboldened, to consider contract variations to ensure that research efforts continue to maintain relevance to industry priorities.
4. More direct involvement in/mentoring of industry activities by project staff should be considered, to improve the outcomes of those activities, especially when they are contributing data or other resources to the project.
5. Project M&E plans should be clearly structured with clear goals and data requirements, and should be referenced frequently to facilitate the collection of evaluation data and reconfirm the direction of the research effort.

Lessons learned

Evaluation activities and data collection need to be integrated into most project activities to allow for 'real time' monitoring of progress towards project goals.

Recommendations

1. A cost-effective A&K trap to which *C*. nr *dimidiatus* is highly receptive should be developed as a priority. This may require identification of the species' aggregation pheromone, and does require identification of a co-attractant to which the species is highly responsive, along with appropriate dispenser technology.
2. Almond volatiles associated with nut maturation and hull split should be assessed for their potential role in a co-attractant, based on the apparent attraction of *C.* nr *dimidiatus* to nuts at the hull split stage.
3. Factors influencing *C.* nr *dimidiatus* dispersal behaviour and receptivity to aggregation pheromones and host odours should be identified, to inform the above development of an effective A&K trap for this species. These may include climatic conditions, beetle nutrition and availability of food resources.
4. New or improved methods to destroy or remove residual nuts from orchards should also be identified or developed as a priority, because of the role of those nuts in maintenance and growth of *C*. nr *dimidiatus* populations.
5. Until improved methods are available, industry should be strongly encouraged to achieve as high a level of hygiene as possible with existing technology, as good hygiene is likely to be a vital component of any management program for *C*. nr *dimidiatus* and other nut pests such as carob moth (*Apomyelois ceratoniae*).
6. Industry should also be strongly encouraged to remove and disinfest the new crop as early as possible, given the rapid increase in kernel damage by *C*. nr *dimidiatus* after hull split.

Refereed scientific publications

None to date.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

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Appendices

Final report: Management of carpophilus beetle in almonds
Project code: AL15004

Appendix 1. Literature review

Carpophilus as a pest of stone fruit

Several *Carpophilus* species are cosmopolitan and infest a variety of agricultural products, both before and after harvest. Affected crops include figs, dates, stone fruits and corn (Hinton 1945). Carpophilus beetles are also serious pests of ripening stone fruit in southern Australia (James et al. 1995, 1996, 1997). *Carpophilus davidsoni* Dobson, *C. mutilatus* Erichson and *C. hemipterus* (L) cause the greatest economic damage in ripening fruit. *Carpophilus* spp. are attracted to ripening stone fruit and will penetrate near the stem end. This is followed by rapid fruit breakdown (Hely et al. 1982), which can result in substantial fruit losses (James et al. 1993, 1997). Growers have reported annual losses of up to 30% of the crop (Hossain et al. 2000).

Carpophilus spp. also play an important role in transferring the spores of brown rot (*Monilinia* spp.) initiating the disease in apricots and peaches (Kable 1969). In seasons favourable for brown rot, fruit losses can exceed 50% in New Zealand (Elmer 1990; Spiers et al. 2003; Elmer et al. 2007). Losses of this magnitude occurred regularly for decades, despite the application of fungicides at recommended stages of fruit development and the introduction of new chemistry (Elmer & Gaunt 1993). There were also reports of substantial crop losses in Australian orchards during the 1980s-90s (Penrose 1998; Hossain et al 2000).

Management of carpophilus in stone fruit

Control of carpophilus using multiple foliar and ground applications of broad-spectrum insecticides is often unsatisfactory due to the cryptic nature of the beetles and their potential for mass migration. Spraying close to harvest when fruit is susceptible to damage by the beetles also increases the risk of exceeding permissible pesticide residue levels set by government agencies. In addition, global concerns over ground water pollution and insecticide resistance in certain crop systems increased the pressure to rethink insecticide use (Epstein et al. 2000).

The smell from ripening or fermenting fruit attracts *Carpophilus* spp., and fermenting fig baits and their synthetic chemical odour have been used in traps for beetle monitoring and control in California fig orchards (Warner 1961, Smilanick *et al.* 1978). James *et al.* (1998) and Hossain *et al.* (1999) demonstrated that Fermented Apple Juice (FAJ) could be used to monitor *Carpophilus* spp. populations in stone fruit orchards in Australia. Mansfield and Hossain (2004) found that fermented peach juice was a more effective attractant for carpophilus beetles than FAJ. However, fruit-based attractants alone are not effective in protecting fruit crops from *Carpophilus* spp. damage; poisoned fermenting fruit baits were not able to out-compete naturally ripening figs in Californian orchards (Smilanick 1979).

Identification and synthesis of the male-produced aggregation pheromones of *C. hemipterus* (Bartelt et al. 1990), *C. mutilatus* (Bartelt et al. 1993) and *C. davidsoni* (Bartelt & James 1994) made even more potent attractants available for *Carpophilus* spp. management. The fact that both sexes respond to the pheromones increases their practical and potential impact on *Carpophilus* spp. populations. Importantly, the effect of *Carpophilus* spp. pheromones is strongly synergised by various food odours, and the use of food scent as a co-attractant with the aggregation pheromone was recommended (Bartelt et al. 1992). Food type materials that have been used as synergists for *Carpophilus* spp. aggregation pheromone included fig juices (Bartelt et al. 1990, 1992), rotting grapefruit (Blumberg et al. 1993), rotting stone fruit (Hossain et al. 2005), whole-wheat bread dough (Bartelt 1997) and blends of synthetic compounds typical of yeast fermentation (Bartelt et al. 1992). James et al. (1998) demonstrated that FAJ was a very effective co-attractant for *Carpophilus* spp. in Australia and that it retained efficacy for at least two weeks. Hossain et al. (2006) demonstrated that ripening/rotting peaches plus fermented peach nectar was a very effective co-attractant. Subsequent trials to demonstrate the field activity of these materials (Bartelt et al. 1992, 1994a, 1994b, James et al. 1994, 2001) highlighted the potential of semiochemicals (aggregation pheromone and co-attractant) for *Carpophilus* spp. management in stone fruit orchards using attract and kill (A&K) strategies.

Proof-of-concept for mass trapping

James et al. (1996) demonstrated that perimeter-based A&K trapping (traps hung in perimeter trees) significantly reduced the incidence of *Carpophilus* spp. in ripe fruit in the centre of a one hectare apricot block. However, there was almost 100% infestation by *Carpophilus* spp. in fruit on the trees in which the traps were hung. To improve control in the perimeter trees, James et al. (2001) used A&K stations containing decomposing stone fruit as co-attractant plus aggregation pheromones, placed in an open area in the centre of an orchard, instead of perimeter traps. The percentage of damaged fruit (44%) within 200 m of the pheromone source was significantly greater than in trees located further away (200-500 m) from the pheromone source (14%). Reasons cited by James et al. (2001) to explain the apparent failure to protect trees within 200 m of the pheromone source included insufficient close range stimuli for *Carpophilus* spp. to enter the stations, poor quality of the food resources in the stations, and ineffective poisoning of the attracted *Carpophilus* spp. Timing of deployment of the stations also appears to have been a factor. Damage was already occurring when the stations were deployed. In unreplicated experiments James et al. (2001) used cordons of suppression traps 5-10 m away from the trees to suppress populations.

Hossain et al. (2001, 2005, 2006) continued development of the killing station concept. They found that three stations in the upwind corner of a 1-ha orchard block gave crop protection that was at least as good as the usual insecticide treatments (Hossain et al. 2006). Three A&K stations were placed 12-15 m outside the treated blocks in the north-west corner and 50 m apart from one another. Each A&K station consisted of six polystyrene boxes (48 cm x 34 cm x 19 cm) containing ripening peaches and fermenting peach nectar absorbed into polyacrylamide granules as co-attractant. Six pheromone septa were used for each station. The septa were normally used in the field for two weeks and then replaced. The co-attractant (fruit, nectar and granules) was replaced in all A&K stations weekly. This experiment clearly demonstrated that the pheromone stations could give excellent crop protection (maximum 0.33% fruit damage) even when the beetle populations in the area were high enough to cause unacceptable damage (up to 9.8%, when insecticide was not used). Timing of deployment of the stations was extremely important and had to be well before the fruit crop started to ripen. The idea was to reduce the beetle population as much as possible before the crop became susceptible (4-6 weeks before fruit harvest) and then to intercept remaining beetles before they were able to infest fruit. Beetles becoming established in peaches would emit pheromone of their own, which could compete with the bait stations, and every effort was made to prevent this from happening.

Development of cost-effective mass trapping

Despite their effectiveness, these stations were bulky, messy and time-consuming to service. The natural food attractants quickly lost their potency due to rapid decomposition and changes to the chemical composition under field conditions. Grower acceptance of the technique depended on eliminating the fruit and juice from the stations, which motivated the development of a synthetic food attractant that could take the place of the ripe fruit and fermenting juice. Bartelt and Hossain (2006) developed a six-compound water-soluble synthetic co-attractant. The co-attractant is highly attractive to *Carpophilus* spp. in the field and was an effective replacement for overripe fruit and fermenting fruit juice in the killing stations (Hossain et al. 2007). This high potential synthetic co-attractant overcomes the problem of high levels of fruit damage on trees adjacent to the A&K traps reported by James et al. (2001). Hossain et al. (2007) also developed a user-friendly compact A&K station based on a funnel trap baited with co-attractant and pheromone. It was generally believed that pheromone septa are effective in attracting carpophilus for up to two weeks and therefore common practice was to replace the septa every two weeks. However, Hossain et al. (2008) found that the pheromone septa are not fully depleted after four or even seven weeks in the field, but their attractiveness did decline dramatically over time. Therefore the current practice is not to discard old pheromone septa, but add new septa every two weeks (Hossain et al. 2007). Hossain et al. (2007) recommended the deployment of three traps per hectare to protect fruits from carpophilus damage, particularly in orchards where beetle populations are high. This trapping density is uneconomic for neighbouring areas with less susceptible varieties. Hossain et al (2010) demonstrated the potential for reducing trapping density in areas of lower pest pressure, in which case the gross cost could be reduced by as much as 33% (to approximately AU$100/ha).

Carpophilus emerging as a serious pest of almonds

In 2013, the high value Australian almond industry was alerted to carpophilus beetle as a potentially serious new pest of that crop, when it was found infesting new season kernels during nut assessments for HAL Project Al12004 'Managing carob moth in almonds' (Madge et al 2013). It is not known why this pest has become attracted to almonds, but it is likely that volatile compounds from maturing nuts play a role, just as they do in stone fruit, and as has also been found with another almond pest, navel orange worm, in USA (Beck et al 2012). This opens up the potential for development of an A&K system that effectively targets the species of carpophilus causing damage to almonds, by adapting the system successfully developed for stone fruit (Hossain et al 2006, 2009). More recent advances in volatile analysis and dispenser technology suggest that lure longevity could be improved by using Low Density Poly Ethylene (LDPE) sachets dispensers (as previously described in Torr et al 1997), in place of the current commercial solution. The results of a recent lab study suggest that an identical odour as that of the currently used commercial solution can be recreated using these dispensing devices. More importantly, sustained release rates obtained for some of the reportedly short-lived active components of the commercial solution (Hossain et al. 2006) using sachets, may help to maintain and/or increase lure efficacy over time in the field.

It should also be noted that apart from damaging kernels directly by feeding, carpophilus beetles may also vector aflatoxin-producing fungi (Aspergillus spp) (Chang and Jensen 1974; Tate and Ogawa 1975), which is a concern for the almond industry.

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Appendix 2. Species, distribution and behaviour

Introduction

In the 2014 harvest, high levels of kernel damage by carpophilus beetle became a major concern to the Australian almond industry. In an attempt to lower beetle populations in orchards, mass trapping of carpophilus was implemented by producers in the form of an Attract and Kill (A&K) program. With guidance from Agriculture Victoria Research (AVR), the A&K program was based on a trapping system developed (by AVR) in the early 2000's for carpophilus management in fresh stone fruit, although at the time little was known about how well the trap and trapping strategy would translate from stone fruits to almonds.

The almond industry and AVR collaborated in preliminary trapping studies and nut assessments during the 2014/15 season, which confirmed the presence of carpophilus beetle across almost 70% of Australia's almond plantings, with the pest causing an average of 2-5% kernel damage (and up to 30% damage noted in one case). These preliminary studies also provided evidence that at least four species of carpophilus were associated with almonds in Australia. The original stone fruit A&K system was designed to target three of these species (*Carpophilus hemipterus*, *C. davidsoni* and *C. mutilatus*), using a combined blend of aggregation pheromones from the three species, together with a synergistic co-attractant based on volatiles emitted from fermenting peaches. A fourth species, undescribed, was also commonly found in trap and almond samples collected during 2014/15. This species was morphologically similar to *C. dimidiatus*, a cosmopolitan species (Ewing & Cline 2005) known to attack a wide range of agricultural commodities such as corn, peanuts, cacao and various spices either in storage or in the field (Connell 1975). As the taxonomic identity of this fourth species could not be fully resolved at the time, it was temporarily referred to as *Carpophilus* near *dimidiatus* on the basis of its similarity to that species.

Clarifying the identity of carpophilus beetles found in almond orchards was an important goal, as not all *Carpophilus* species are agricultural pests. Project AL15004 therefore aimed to identify the beetle species caught in A&K traps across the almond production regions, and (through destructive sampling) those species that were residing in almond nuts and damaging the kernels.

During the planning and implementation of their A&K programs, major almond producers agreed to assist the project by providing beetle samples from their traps, and detailed data relating to trap catches and trap locations. Collectively, those producers managed almond orchards in Victoria's Sunraysia region, South Australia's Riverland and the New South Wales Riverina. Through subsequent analysis of trap samples, the project aimed to better understand the species composition of trap catches, and the succession and seasonal population dynamics of damaging *Carpophilus* species across Australia's major almond production regions.

However, whilst the A&K lure formulation (3-species beetle pheromones and fermenting stone fruit odours) is known to be highly attractive to *C. davidsoni*, *C. hemipterus* and *C. mutilatus* (Hossain et al. 2007), the relative effectiveness of this trapping system in attracting *C.* nr *dimidiatus* and any other species found in almond nuts had not been established. Moreover, a comprehensive survey of the species causing damage to almond kernels had not yet been carried out, so all species of carpophilus found in almond orchards were considered as potentially damaging. Project AL15004 therefore also aimed to conduct intensive sampling of new season nuts to determine just which species of carpophilus were responsible for kernel damage.

Methods

Carpophilus Attract and Kill system

The carpophilus beetle A&K mass-trapping programs were implemented on a trial basis by the almond industry, using the 'Carpophilus Catcha® Trapping System' (Insect Management Services P/L, Bacchus Marsh, Victoria) developed for carpophilus control in stone fruits. This system is comprised of a five-litre black plastic funnel trap; a three-species pheromone lure containing aggregation pheromones of *C. davidsoni*, *C. hemipterus* and *C. mutilatus*; a synthetic co-attractant mimicking odours of fermenting stone fruit; and an insecticidal strip to kill beetles that enter the trap (for details of trap components and maintenance, see attachment 'AL15004 Factsheet-Managing carpophilus beetle in almonds 2016-17.pdf').

Prevalence and diversity of *Carpophilus* species in A&K traps in almonds

The use of carpophilus A&K in almonds commenced in winter/spring 2014, when almond producers began to establish large-scale A&K programs in the major almond growing regions of Sunraysia (Victoria), the Riverland (South Australia) and Riverina (New South Wales). Since then, A&K has been applied in over 18,600 ha of almond orchards. Traps were installed on metal stakes at a height of approximately 1.5 m, within tree rows on the upwind side of orchard blocks, so that the pheromone and co-attractant odours would disperse downwind into the blocks. Wherever practical, the traps were placed in a shady position to protect them from direct sunlight, as excessive heat was considered very likely to shorten the longevity of co-attractant activity and to discourage beetles from entering the traps. Trapping density was a compromise between the high-density trapping used in intensive fresh stone fruit orchards (1-3 traps/ha) and that which was logistically and economically feasible in broad-scale almond orchards. The trapping densities trialled in almonds were in the range of one trap per 5-25 ha, and the programs generally operated from spring to autumn.

Because of the additional workload involved in establishment and maintenance of their large scale A&K programs, some producers employed dedicated field staff for the purpose. In an effort to maximise the effectiveness of these programs and the quality of trap samples collected for the project, guidelines and training were provided by the project for the industry staff responsible for installation and maintenance of the traps and sample collection (see 'Outputs' section of this report).

To identify the different species of carpophilus active in almond orchards, and study their distribution and seasonal population dynamics, beetles from traps across three states were counted and identified to species over the 2015/16 to 2017/18 seasons. Growers from twelve orchards collaborated with the project in this work, by providing beetles from their A&K traps. The samples represented carpophilus populations in the Riverland (1-3 orchards), Sunraysia (7-8 orchards) and Riverina (1 orchard). Within these orchards, 1-3 transects of 3-6 A&K traps were established for sample collection. While samples from at least three traps per transect were desired for analysis, six were requested. This provided a reasonable level of redundancy in an effort to ensure that in the event of sample loss or missing traps, there would always be at least three samples available from each transect. The 1.5-2 km long transects were generally oriented southwest-northeast. Two orchards maintained only three traps, so all three were selected for sample collection. The A&K traps were typically monitored and serviced by orchard staff each week, at which time the entire catch from selected traps was bagged, labelled and cool-stored (approximately 4°C) prior to despatch to AVR Bundoora where they were stored at 4°C until being examined. In this way, beetle samples from 87-139 traps were obtained for analysis each season.

Only the two end samples and one middle sample from each six-trap transect were selected for processing, to allow for more rapid analysis of representative samples from all transects. The remaining samples were kept in cool storage for later analysis if required. For each sample, total beetle numbers were estimated using the procedure described in Hossain et al. (2009). A subsample of approximately 300 beetles was then sorted into morpho-species by research staff, using a stereo-microscope and/or magi-lamp at 10X magnification. Identification to species was then performed under a stereo-microscope at 100X magnification using the descriptions of Dobson (1954, 1964). In order to cross-check and confirm research staff accuracy in species identification, sub-samples of the carpophilus specimens had their identity confirmed by an experienced taxonomist.

Carpophilus infestation of the new season nuts, 2015/16 season

The species identity, prevalence and behaviour of carpophilus beetles infesting new almond crops was determined through intensive nut sampling at two orchards in Sunraysia during the 2015/16 season.

Four sites were selected within each orchard, in areas that had a previous history of high catches of carpophilus in A&K traps, or high levels of kernel damage. Within each site, five sampling locations were marked, each consisting of three Nonpareil trees. At each sample time, nuts were collected from one of the three trees in each sample location. The nut collection rotated through the three trees week by week, to reduce the impact of nut removal on any one tree.

To gather information on the distribution of carpophilus within each tree, the trees were partitioned into the following sampling quadrats:

- Eastern side, Upper half of foliage, (EU)
- Eastern side, Lower half of foliage (EL)
- Western side, Upper half of foliage, (WU)
- Western side, Lower half of foliage (WL)

Samples of 13 nuts were collected from each of these quadrats, giving a total of 52 nuts/tree, 260 nuts/site and 1,040 nuts per orchard at each sample time. This sampling was conducted weekly for 16 weeks from 7/12/15 to 23/3/16, covering the period from well before hull split to after harvest. From 25/1/16 onwards, after the trees had been shaken in preparation for harvest, a single sample of 13 new crop nuts was also collected from the ground under each tree sampled.

Clusters of nuts were considered likely to be more attractive to carpophilus because of the shelter they provided, and thus clusters (3+ nuts) rather than single nuts were selected for sampling. To minimise the chance of beetles escaping from the nuts, each cluster was enclosed in a medium size Ziploc bag before being cut from the tree and quickly sealed in the bag. The bagged and labelled samples were kept in a cold esky for transportation to the laboratory for processing.

Each sample of 13 nuts collected from one of the four positions on a tree or from the ground, was processed separately as follows (see Figure 1):

1. A two-litre plastic container (large enough to allow for handling the nuts) was filled with enough water to ensure that the nuts would be fully submerged. A few drops of detergent were added to assist with wetting of the nuts and beetles.
2. The nut sample was emptied into the water, and the nuts were separated from the cluster. The sample bag was rinsed out into the water to remove any loose beetles and the nuts were left to soak for at least one hour.
3. The nuts and any leaves and twigs were then removed from the container, with care taken to wash any beetles back into the water. The water was then strained through a fine sieve/gauze to capture any adult beetles or larvae present.
4. Using a brush or forceps, the beetles and larvae were placed in a labelled tube with 70% ethanol.
5. Each nut was inspected externally for signs of chewing damage, and was then opened and inspected for internal damage and presence of adult beetles or larvae.
6. All adults collected from the wash water or nuts were identified as described above.

Numbers of adults and larvae removed from washed nuts prior to destructive sampling were recorded for each tree position (quadrat) within the tree. Adult and larval data were analysed on the log (X+1) scale, with X being the original count data, using a linear mixed effects model that was fitted with restricted maximum likelihood (ReML) in Genstat 19 software. The data were analysed on a log scale to satisfy the ReML assumptions of normality and constant variance for residuals. The model included two factors - quadrat, date - and the interaction between them as fixed effects. To assess efficacy of washing as a method for detecting infestations all nuts that had been washed were later destructively sampled and the numbers of adults and larvae recovered were recorded. No larvae were detected in nuts post-washing so only numbers of adults were compared. In this case, the model included three factors - quadrat, method, date - and the two-way and three-way interactions between these three factors as fixed effects. The random effects included in both models were "site within farm", "position within site within farm", "tree within position within site within farm", "quadrat within tree within position within site within farm", and "date within quadrat within tree within position within site within farm" which defined the study design.

Figure 1. Nut sampling: a) collecting nuts, b) nuts soaking, c) carpophilus larvae exiting a soaked nut, d) destructive inspection of nuts.

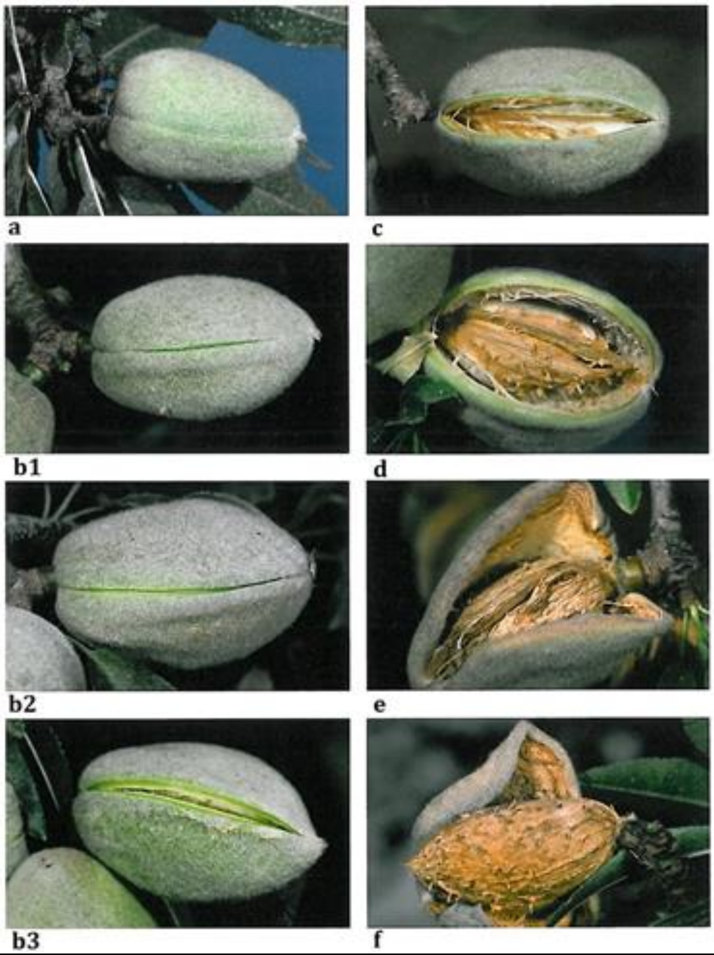
On 22/3/2016, two additional samples were collected for moisture testing, to determine if there was any marked difference in moisture content between nuts on the trees or ground. Twenty new season Nonpareil nuts from trees and 20 from dry ground under the trees were collected, sealed in plastic Ziploc bags and stored in a cold esky until processing. At the laboratory the nuts were hulled and shelled, the kernels and hulls + shells were weighed separately in bulk, then the samples were oven dried at 100°C for five days and reweighed.

Carpophilus infestation of hull split stages, 2016/17 season

The sampling of new nuts during 2015/16 provided an overall picture of the progression of infestation by carpophilus, but could not be segregated into nut developmental stages due to resource constraints. To determine the earliest stage at which carpophilus infests and damages almonds, nuts at different hull split stages were collected from trees in orchards in Sunraysia in January and February 2017, and destructively inspected for the presence of any life stages of carpophilus, and for kernel damage.

For this purpose, sampling was to begin when reasonable numbers of nuts were at hull split stages C and D (Table 1), and continue until kernel damage had been observed in at least two consecutive samples. The first sample was collected on 23/1/2017, with subsequent samples collected on 31/1/2017 and 16/2/2017. On each date, a total of approximately 1,000 nuts was collected, comprising of equal numbers of each hull split stage (Table 1: C, D, E & F) available on that date. The samples for each hull split stage were sealed separately in plastic Ziploc bags and stored in a cold ice box for transport to the laboratory. Until the nuts were destructively inspected, they were cool stored at 2-3°C to prevent any further development of carpophilus beetle or kernel damage. The destructive inspection of individual nuts for carpophilus beetle and kernel damage was performed under a dissector microscope. Beetle adults and larvae found during the inspection were stored in 70% ethanol for identification.

Table 1. Progression of hull split in almonds (Flint 2002).

a. Unsplit: No evidence of a separation at the suture.		c. Split, less than 1cm (⅜"): a visible opening of the suture less than 1cm at midsuture.
b1. Initial separation: 50% or more of a thin separation line visible.		d. Split, more than 1cm (⅜"): a visible opening of the suture greater than 1cm at midsuture.
b2. Deep V, unsplit: Beginning of splitting with a deep 'V' over at least 50% of the suture line, but the hull cannot be squeezed open at the suture.		e. Initial drying: The edges of the hull are beginning to dry.
b3. Deep V, split: A deep 'V' in the suture which is not yet visibly separated, but which can be squeezed open by pressing both ends of the hull.		f. Completely dry.

Carpophilus development in residual nuts, 2016/17 – 2017/18 seasons

The seasonal development of carpophilus in residual nuts (new season nuts remaining in the orchard after harvest) and mummy nuts, was followed for two seasons at two orchards in Sunraysia, and two in the Riverland. This research component is reported in Appendix 4.

Results & Discussion

Analysis of beetles in A&K trap catches, 2015/16 – 2017/18.

The following trap catch data is observational in nature, in that it reports what was found in samples collected by almond producers from their A&K traps. Because the trap maintenance routines, trapping periods and seasons of A&K application varied amongst producers for commercial reasons, continuity of trapping, and therefore of trap data, at consistent sites across the life of the project was not able to be achieved. The data does however achieve its aims in determining the presence and mix of carpophilus and related species in almond orchards, and in highlighting regional similarities and differences in seasonal population dynamics of important species.

Taxonomic analysis of beetle samples from A&K traps in almond orchards yielded specimens mostly (> 95%) of *Carpophilus hemipterus, C. davidsoni*, *C.* nr *dimidiatus*, *Urophorus humeralis* and species of *Brachypeplus*. Figure 2 shows an example of the contribution of each species to total trap catches. Another seven as yet unidentified

Carpophilus species were also detected at very low levels (<5%). *Carpophilus hemipterus* and *C. davidsoni* are the two primary carpophilus pests of fresh stone fruit in Australia (James et al. 1995, 1996, 1997), while *Urophorus humeralis* is a pest mostly of over ripe or rotting fruit (Brown 2009). *Brachypeplus basalis* was recently recorded as a pest of honey bee hives in the USA (Sagili et al. 2016). There are at least 15 species of *Brachypeplus* in Australia, but specimens in our samples were identified only to genus level.

Whilst all these species have been commonly trapped in almond orchards, it is important to note that to date, ***C.* nr *dimidiatus* is the only one of these species clearly implicated in causing damage to almond kernels**, as shown in the following sections relating to nut infestation. As Figure 2 indicates, *C.* nr *dimidiatus* typically contributes a very small proportion of the total catch in the current stone fruit A&K traps. **This highlights the need for caution when interpreting trap catches**, especially as the relative proportions of different species can vary markedly over time (Figure 3). The total catch of "beetles" does not necessarily relate to the risk or level of infestation by damaging species, and is therefore not a reliable monitoring tool without accurate diagnostics. Unless or until a trap is developed to be highly specific to *C.* nr *dimidiatus* (or any other damaging species), traps for monitoring purposes may only be useful if their catches can be rapidly assessed through such techniques as bulk DNA analysis. This approach is the subject of research in new Project AL16009.

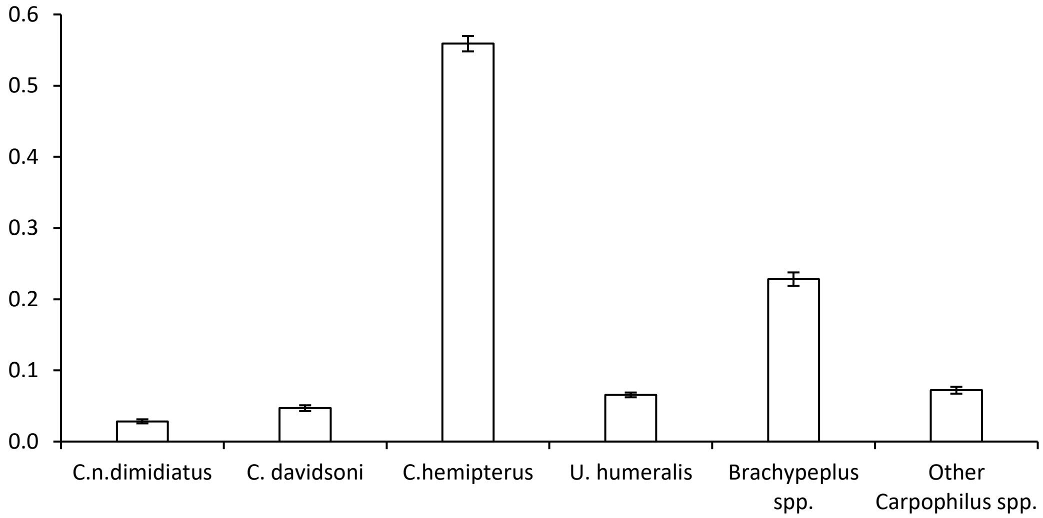


Figure 2. Mean proportion (±SE) of each species of beetle in subsamples104 A&K traps (N=104), Sunraysia, December 2016.

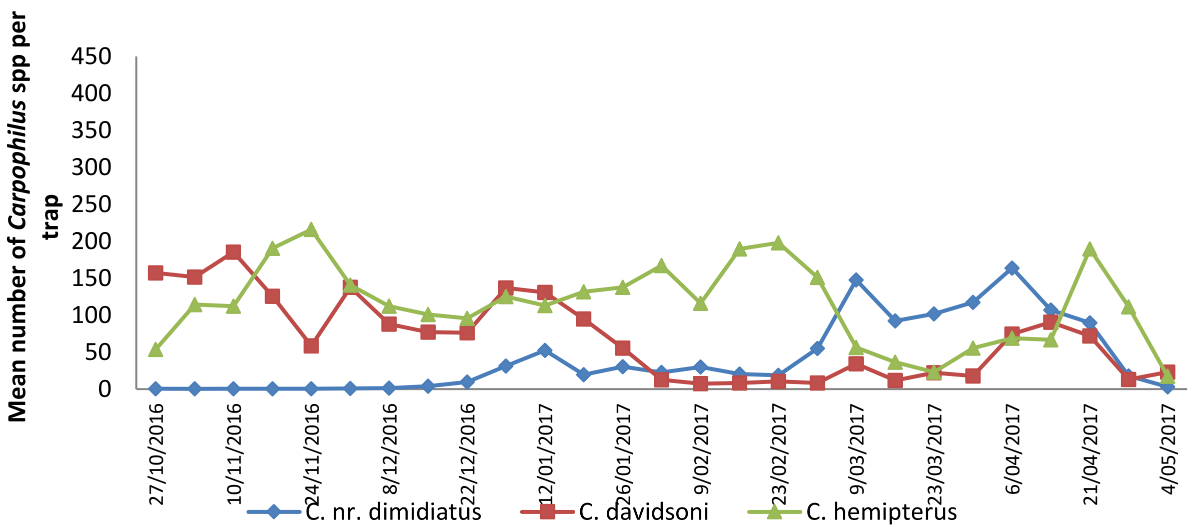


Figure 3. Mean numbers of key *Carpophilus* species in A&K traps, Riverland, 2016/17.

Because *C.* nr *dimidiatus* has to date been the only species found damaging almond crops, only data relating to that species are presented below.

Figure 4 shows catches of *C.* nr *dimidiatus* in selected almond orchards in the Sunraysia, Riverland and Riverina districts during the 2016/17 season (Trap data from other orchards and seasons are presented in Sub-appendix 2).

In all three regions, catches of *C. nr dimidiatus* were low early in the season, then increased through late December-early January, in the period leading up to hull split. This increase in catch of *C.* nr *dimidiatus* suggests an increase in mobility of the adult beetles, for which there could be several explanations such as the following:

- Maturing and splitting nuts may produce odours that are attractive to the beetles, drawing them out from their hiding locations such as the residual/mummy nuts in which they are commonly found at that time of the season (Appendix 4). The number of *C.* nr *dimidiatus* adults and larvae detected in residual nuts in an orchard in Sunraysia did increase during the same period as trap catches in that region (Appendix 4), supporting the possibility of those nuts being a source of the adults caught in traps.
- Climatic factors such as rainfall events or increases in temperature may provide some stimulus for beetles to become more active and mobile, and therefore more likely to be trapped.
- An increase in beetle activity could be driven by a drop in quality or availability of resources such as shelter or food that were being utilised by the beetles, although this seems unlikely to explain the almost synchronous increase in trap catches observed in the three different regions around hull split (Figure 4).

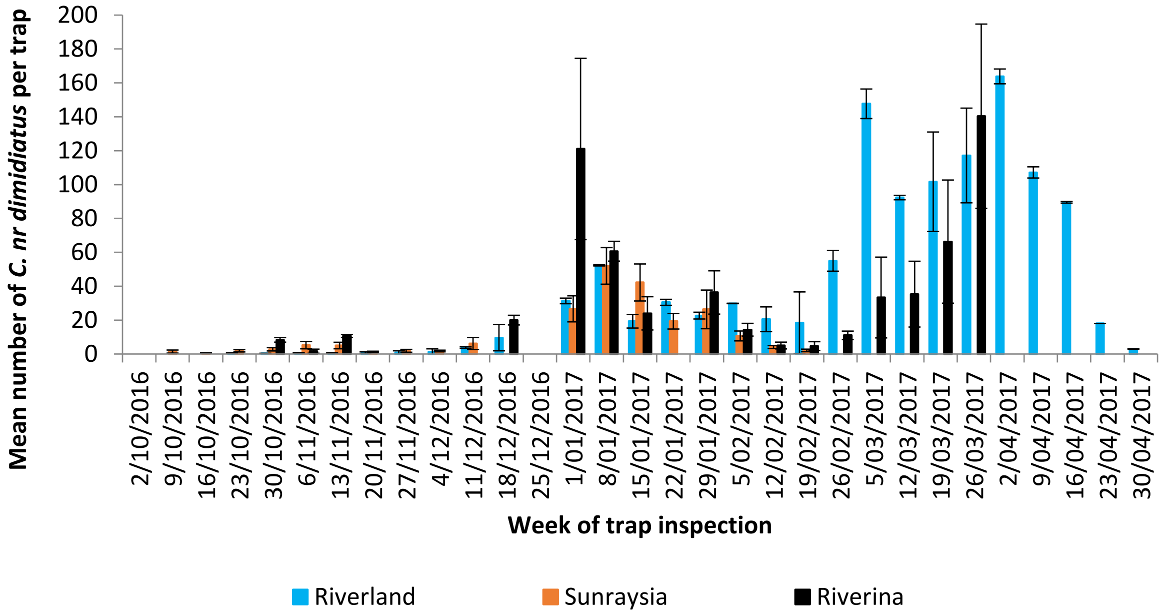


Figure 4. Mean number of *Carpophilus* nr *dimidiatus* (±SE) trapped in almond orchards in the 2016/17 season.

In all three regions during 2016/17, the peak in catches of *C.* nr *dimidiatus* around the time of hull split were followed by a drop to relatively low levels through February. Where trap data is available, a second substantial increase in catches can be seen in early March, and this is generally maintained until catches drop back to very low levels in early May. One possible explanation for the March-April peak, could be *C.* nr *dimidiatus* responding to the reduced availability of 'easy' food resulting from the bulk removal of the almond crop, by mobilising in search of new resources. In such a situation, increased hunger and mobility could make the beetles more receptive to, and likely to encounter, A&K traps.

Infestation of the new season crop

Over 2,700 samples of new season almonds (over 41,000 nuts in total) were collected and assessed for carpophilus infestation by washing of nut samples and destructive inspection of individual nuts. No carpophilus beetles were observed in the samples until two adults were found on 5/1/2016 (Figure 5), at which time a small amount (<1%) of hull split had occurred at the sample site. The first kernel damage was observed two weeks later (19/1/2016). This suggests that carpophilus is similar to carob moth in that **no infestation or egg laying occurs until the hull has split**. The highest percentage kernel damage detected at the sample sites in the 2015/2016 season was 1%, although up to 44% of samples contained carpophilus (Figure 5). Almost 100% (99.9%) of the 2,078 carpophilus beetles found on or within the nut samples were confirmed as *C.* nr *dimidiatus*.

Washing consistently extracted 88% or more of the beetles present on or within bagged nuts . Overall, there were no significant differences (P = 0.207) in numbers of adults extracted from nuts from the four sampling quadrats of each tree . The relatively even height distribution of carpophilus adults gave us confidence in collecting subsequent nut samples from the lower part of the tree, avoiding the use of ladders and their associated OH&S concerns and logistical difficulties.

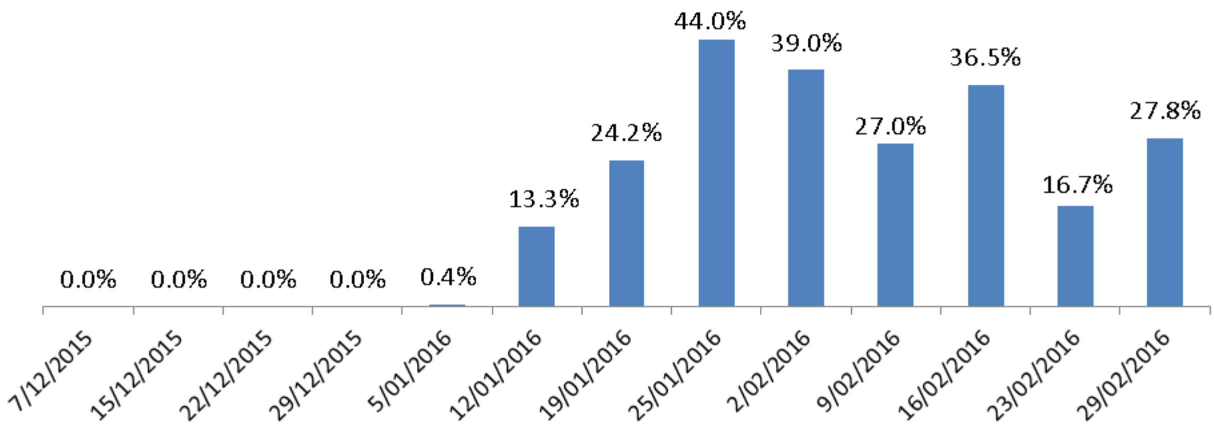


Figure 5. Timing of infestation of new nuts by *C.* nr *dimidiatus* during the 2015/16 season. Percentage of nut samples infested was derived by pooling samples from two properties in Sunraysia (1040 nuts from each property at each sampling date).

With beetle larvae, significant differences were found amongst the four sampling quadrats (Table 2). As indicated above, carpophilus appears not to be attracted to almond nuts until hull split, which begins in the upper and outer parts of trees (Flint 2002), taking around three weeks to reach 100% in Nonpareil (Connell et al 2010). It is likely therefore, that carpophilus would be attracted to, and start laying eggs in nuts in the upper canopy first, resulting in higher numbers of larvae detected in nuts from the upper quadrats overall, as is seen in Table 2.

Table 2 Mean number of larvae extracted from almonds by washing. Sampling quadrats are EU: East upper, EL: East lower, WU: West upper and WL: West lower). Values in the Mean (Q) column followed by the same letter are not significantly different (derived using log transformed data). A significantly higher number of larvae were extracted from the upper canopy.

Quad (Q)	Date (D)								Mean (Q)
	12/01/2016	19/01/2016	25/01/2016	2/02/2016	9/02/2016	16/02/2016	23/02/2016	29/02/2016	
EL	0.0174	0.0937	0.8247	0.4276	0.4259	0.2241	0.0000	0.3483	0.2706a
EU	0.0000	0.2215	2.6484	2.3931	0.9930	0.7140	0.0000	0.0447	0.6451b
WL	0.0000	0.0000	0.5660	0.5693	0.3213	0.3684	0.1363	0.0952	0.2382a
WU	0.0000	0.1540	1.4780	2.0082	0.8277	0.3014	0.2610	0.5290	0.5831b
Mean (D)	0.0044	0.1143	1.2542	1.1868	0.6185	0.3903	0.0914	0.2394	

F Prob: Q <0.001; D <0.001; QxD 0.002

LSD (5%): Q: 0.05858; D: 0.11113; QxD: 0.2131

Overall, more *C.* nr *dimidiatus* were extracted from new season nuts collected from the ground, when compared with nuts picked from the trees (Figure 6). This was also found in relation to infestation of residual/mummy nuts (Appendix 4). The kernel moisture content of a bulk sample of new season nuts collected from dry ground (5.14%) was 9% greater than that of a similar sample collected from trees (4.72%), and this difference may play a role in the beetle's preference for fallen nuts.

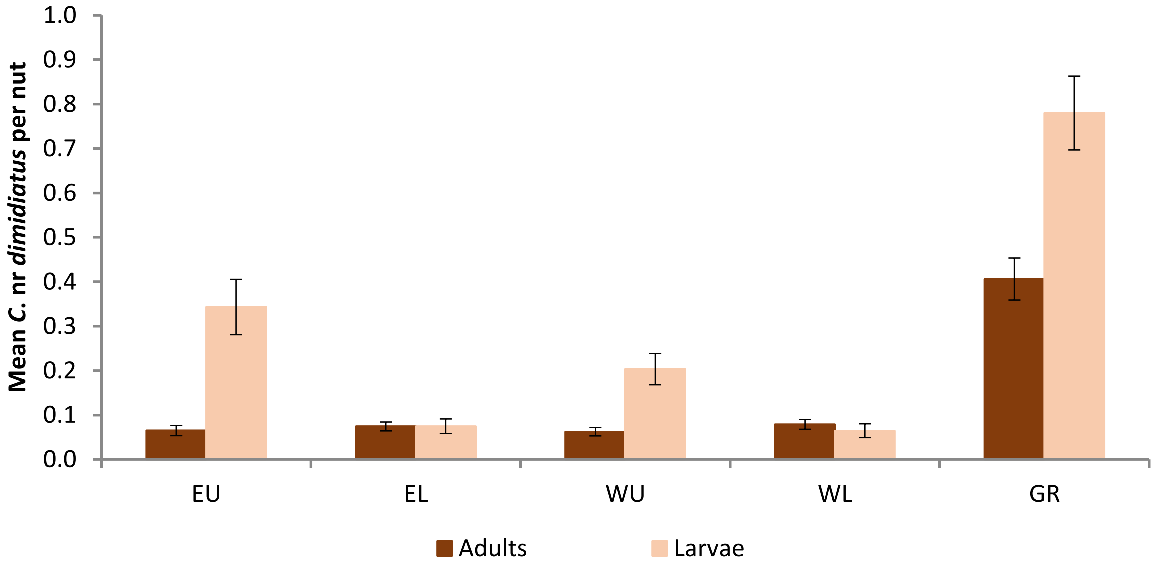


Figure 6. Mean numbers of *C.* nr *dimidiatus* (±SE) extracted from almonds from two orchards in Sunraysia, between 5/1/16 and 29/2/16. Sampling quadrats are EU: East upper, EL: East lower, WU: West upper and WL: West lower, GR=ground.

Hull split stages & infestation

The earliest nut developmental stage at which carpophilus beetle and kernel damage were detected, was the very early 'C' stage of hull split, when the hull suture had only just opened (Table 3), confirming that almonds are susceptible to damage by this pest as soon as hull split commences. In many instances, it appeared that adult beetles (Figure 7a) had chewed a small hole into the kernel before laying eggs singly or in clusters between the hull and shell around that hole (Figure 7b & c). Egg clusters soon resulted in heavily infested kernels (Figure 7d).

Once we had confirmed that infestation and damage was occurring as early as hull split stage 'early C', we decided not to process samples of stage F nuts.

Table 3. Presence of carpophilus beetle (CB) and kernel damage by carpophilus in nuts at different hull split stages.

			Number of nuts with:				
Sample date	Split stage	No. nuts	CB adults	CB eggs	CB larvae	Any CB stage	Kernel damage
23/01/2017	Very early C	325	0.3%	0.3%	0.0%	0.3%	0.3%
23/01/2017	mid-C	323	1.2%	0.6%	0.0%	1.9%	0.0%
23/01/2017	D/E	335	0.6%	0.3%	0.0%	0.9%	0.0%
31/01/2017	Early C	343	0.0%	0.0%	0.0%	0.0%	1.7%
31/01/2017	D/E	64	0.0%*	0.0%	0.0%	0.0%	10.9%
16/02/2017	Early C	491	7.1%	6.9%	2.4%	14.1%	8.8%
16/02/2017	D/E	350	21.4%	24.3%	19.7%	40.0%	18.9%

* Loose carpophilus adults were observed in the sample bags

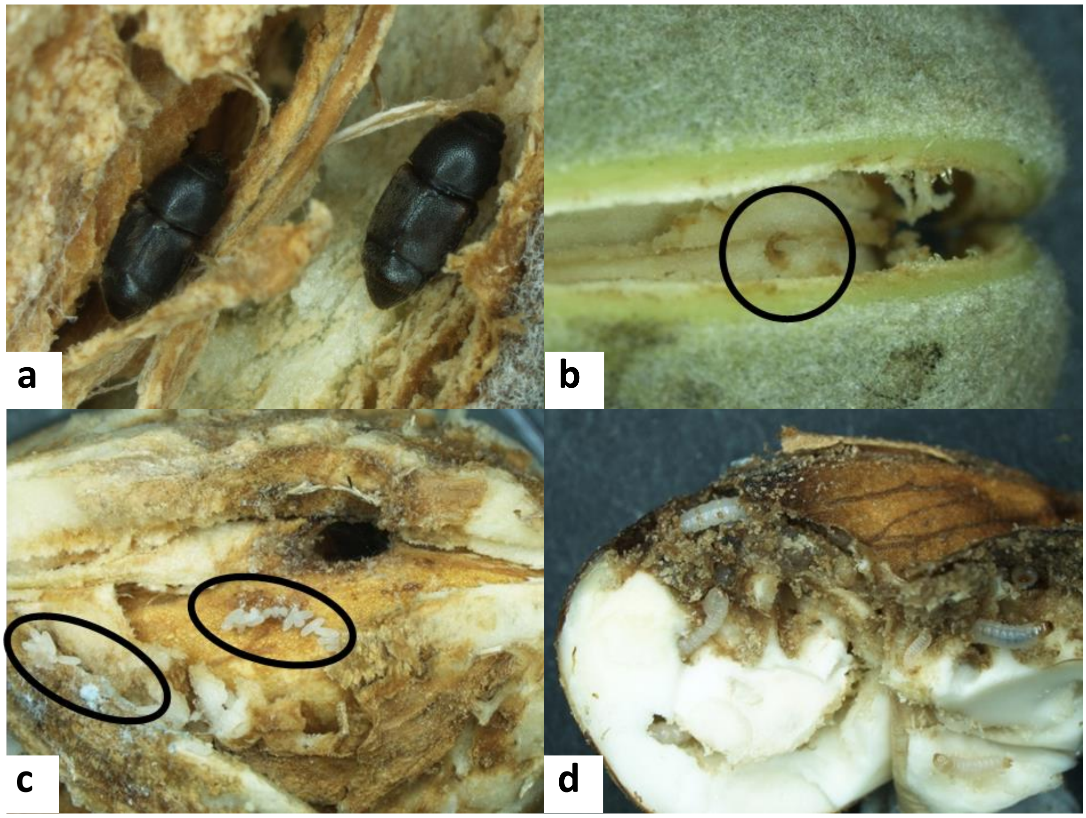


Figure 7. C. nr dimidiatus infestation of new crop almonds: a. Adults between hull and shell; b. Single egg on shell of newly hull split almond; c. Egg clusters and entrance hole in almond kernel (hull has been dissected away); d. Kernel infested with larvae.

Once hull split had begun, around mid-January, levels of carpophilus damage to kernels increased rapidly (Figure 8), highlighting the importance of early and rapid harvest and disinfestation to keep damage levels to a minimum.

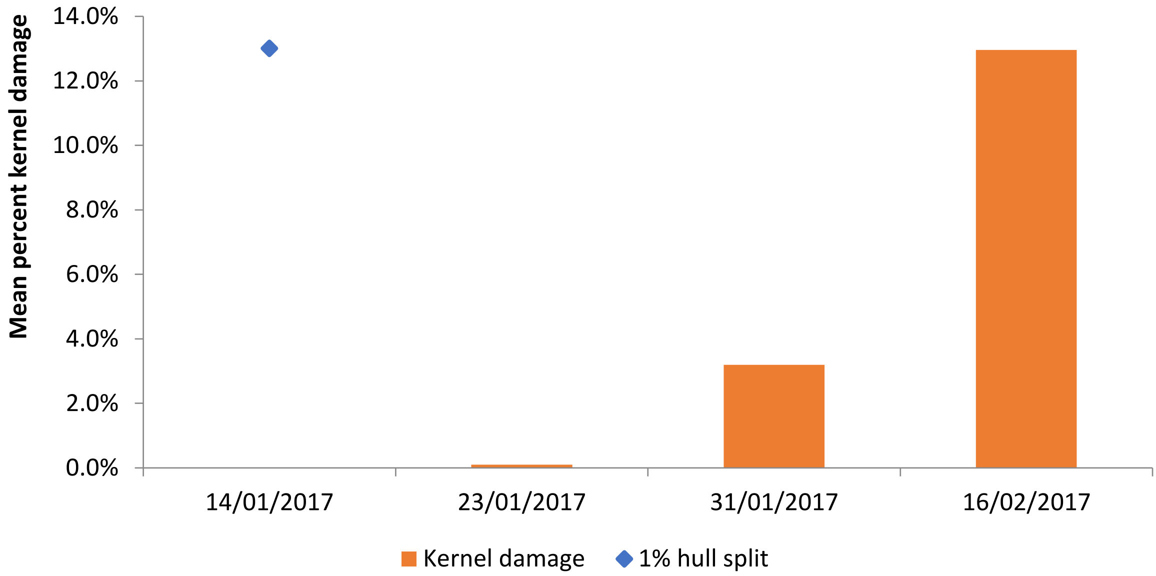


Figure 8. Increase in kernel damage by *C.* nr *dimidiatus* after hull split. No. of nuts inspected 23/1/2017=983; 31/1/2017=407; 16/2/2017=841.

Conclusions

At least 12 carpophilus and related species are found in almond orchards, but only one, *Carpophilus* near *dimidiatus* has thus far been clearly implicated in damaging almond kernels. This species typically makes up only a small proportion of total beetle catch in the current A&K traps, and the proportions of different species varies greatly over time, so total trap catch is not a reliable indicator of the population level of this species or of the risk of crop damage. This issue may be addressed by a system of rapid bulk DNA analysis, which is currently being investigated by AVR.

Patterns of adult *C.* nr *dimidiatus* activity as determined by A&K trap catches appear generally similar between the Riverland, Sunraysia and Riverina regions. Catches of this species remain very low until around the time of hull split, then increase markedly, creating a peak in activity that extends over approximately one month. The timing of this increase suggests that hull split nuts may be attractive enough to mobilise the beetle. If this is the case, volatile compounds released by nuts during maturation and hull split may be a potential source of attractants for the beetle – a possibility being explored in project AI16009. This and other possible explanations for the increase in catch around hull split, such as environmental factors or changes in resource availability warrant further investigation.

Infestation of new crop nuts by *C.* nr *dimidiatus* appears relatively evenly distributed throughout the tree, so there is probably no need for sampling regimes to take height or aspect into account. Higher rates of infestation have however been found in nuts collected from the ground, highlighting the importance of removing or destroying fallen nuts as part of orchard hygiene practices.

C. nr *dimidiatus* begins to infest almond nuts at early hull split, as soon as the suture has opened, and often lays multiple eggs, leading to heavy larval infestation and kernel destruction. Levels of kernel damage increase rapidly after hull split, so harvest and disinfestation or processing should be completed as early as possible to limit crop damage as much as possible.

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Sub-appendix 2. A&K trap catches of *Carpophilus* near *dimidiatus*

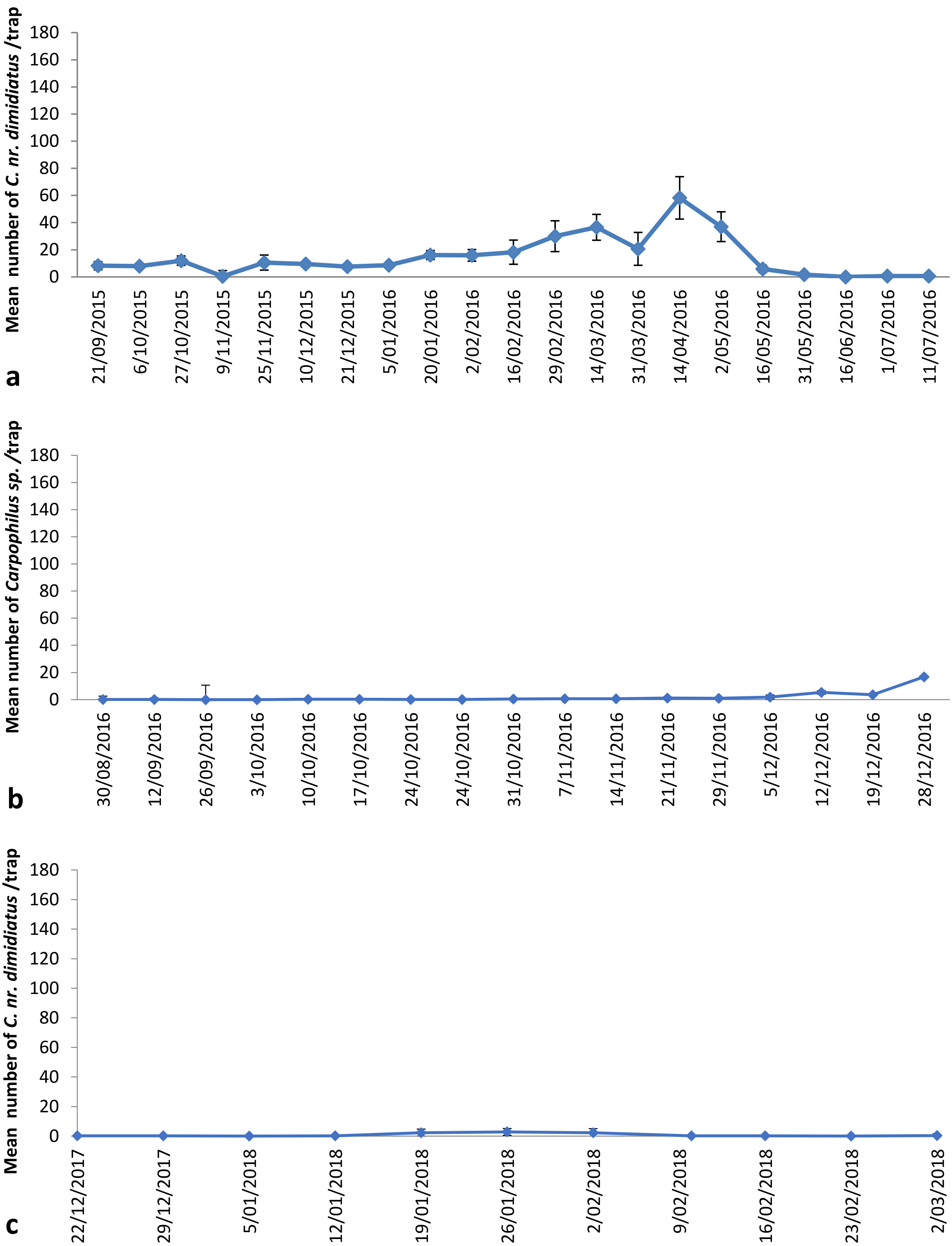

Figure 9. Mean number of *Carpophilus* nr *dimidiatus* (±SE) trapped in Sunraysia almond orchard No. 3 during the 2015/16 (a), 2016/17 (b) and 2017/18 (c) seasons.

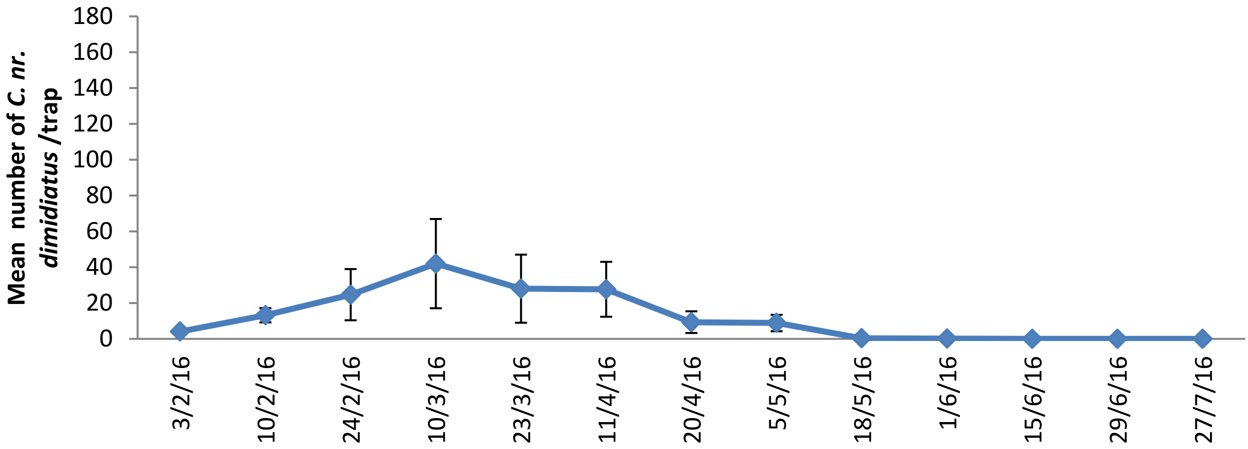

Figure 10. Mean number of *Carpophilus* nr *dimidiatus* (±SE) trapped in Riverland almond orchard No. 5 during the 2015/16 season.

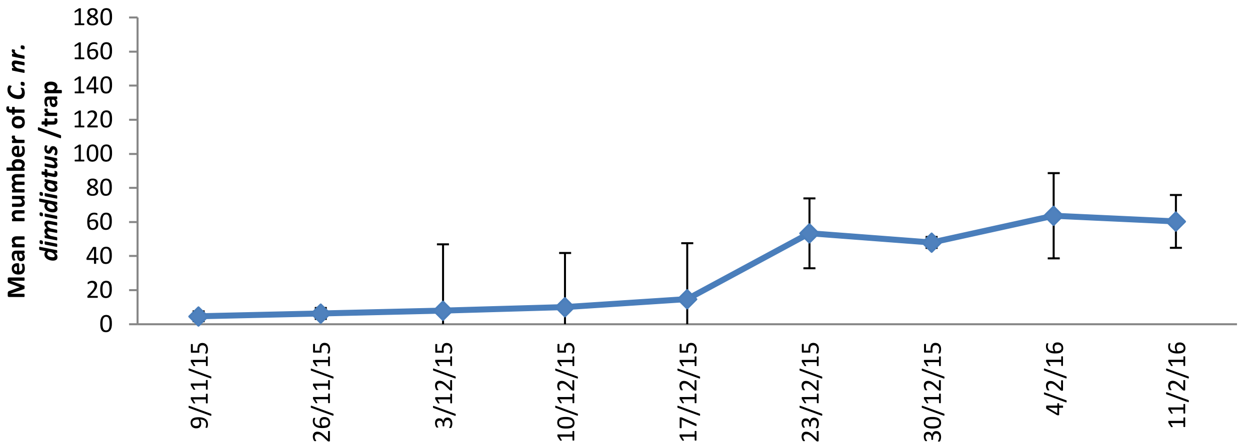

Figure 11. Mean number of *Carpophilus* nr *dimidiatus* (±SE) trapped in Riverina almond orchard No. 6 during the 2015/16 season.

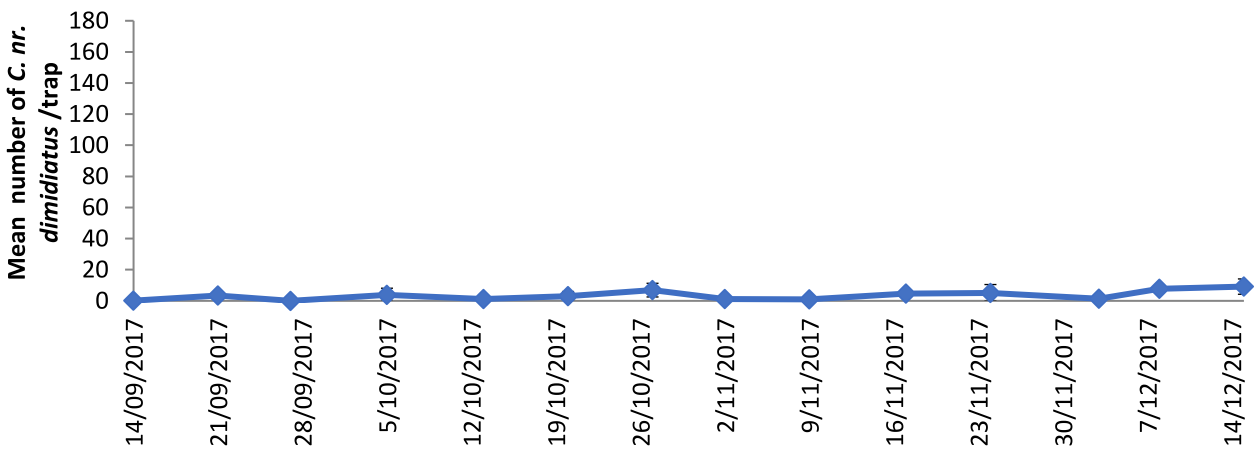

Figure 12. Mean number of *Carpophilus* nr *dimidiatus* (±SE) trapped in Sunraysia almond orchard No. 1 during the 2017/18 season.

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Project code: AL15004

Appendix 3. Diagnostic tools

Introduction

The *Carpophilus* and *Urophorus* beetles belong to the tribe Carpophilini (Nitidulidae). There are at least 200 species of *Carpophilus* known to occur worldwide (Leschen and Marris 2005) including a number of economic pests, and at least 23 species known from Australia, according to the Australian Faunal Directory (AFD – ABRS 2009).

According to the Australian Plant Pest Database (APPD) there are no specimen records of *Carpophilus* beetles collected from almonds in Australia. However in literature, *Carpophilus* species known to be associated with almonds include *Carpophilus dimidiatus,* a cosmopolitan species which is known from stored almonds and *C. gaveni* (an Australian species) known from windfall almonds (Leschen and Marris 2005).

The identification of some *Carpophilus* beetle species can be difficult because they are small and there are many species which look morphologically similar to each other externally. These require dissection, particularly of the male genitalia to tell them apart (Leschen and Marris 2005). However, even the male parameres (a characteristic part of the genitalia) can appear similar and only display subtle differences in some cases.

Literature on the identification of *Carpophilus* species known from overseas include Connell (1991) – North America; Gillogly (1962) and Brown (2009) – Pacific region; Parsons (1943) – North America and Audisio (1993) – Europe. In addition, Williams *et al*. (1983) prepared a bibliography of the genus *Carpophilus* from around the world. These works treat some of the species which have been introduced to or are endemic to Australia. Dobson (1964, 1993) described some new Australian species and Leschen and Marris (2005) provided descriptions and a key to 11 species of *Carpophilus* either known from or intercepted and of concern to New Zealand including a few Australian species. However, none of these references cover all of the described *Carpophilus* species known from Australia and there is no single reference which treats all of the species known to occur here. Many of the Australian species were described in the late 1800s and these often lack details, having no specific mention of the male genitalia features. A taxonomic revision of the *Carpophilus* species known to occur in Australia is badly needed to aid identification of species in almond orchards.

In this section, diagnostic characteristics and a brief description of the main species damaging almonds, *C*. near *dimidiatus*, are presented. Dichotomous keys and images are provided to distinguish *C.* near *dimidiatus* adults from other Nitidulidae associated with almond orchards. Descriptions, keys and images of larvae to identify them from two other commonly found Nitidulidae are also included.

Methods

Identification of trap specimens

Carpophilus beetles collected from pheromone traps and nuts in almond orchards in the South Australian Riverland, Victorian Sunraysia and New South Wales Riverina regions were used to commence the development of morphological diagnostic tools. Specimens were examined using a stereo microscope. Dichotomous keys and descriptions in Leschen and Marris (2005), descriptions in Kirejtshuk (1996) and keys in Connell (1991) and Ewing & Cline (2005) were used to aid identification. Validation of species required dissection of the male genitalia. Specimens were also compared with type and validated specimens from reference collections from around Australia. Some additional characters were recorded for adults and larvae for each of the five most common species found.

At least 10% of adult specimens already sorted to species from almonds and pheromone traps were initially re-examined to validate their identifications, particularly specimens which appeared to be unusual or different to the common species. These specimens were card mounted, labelled and retained in the Victorian Agricultural Insect Collections (VAIC) as reference specimens. Males and females can be readily separated by the presence of a round button-like tergite (last abdominal segment 10) on the male. Abdomens of male specimens of each species were dissected, with terminalia placed in genitalia capsules (with glycerol) and attached to the corresponding pinned specimens. The internal genitalia features (including the parameres) were examined and compared to illustrations in literature.

Some specimens were also tested using molecular (DNA barcoding) methods using the Cytochrome Oxidase I gene to help further confirm identification.

Lab culturing of carpophilus

To acquire egg, larval and pupal specimens of *C. davidsoni, C. hemipterus* and *C*. nr *dimidiatus*, live specimens were collected from the field and used to establish laboratory colonies at Agriculture Victoria Research (AVR) Agribio, Bundoora, Victoria. The method described by Dowd (1987) and James & Vogele (2000) was initially applied to all three species, but was not successful for *C*. nr *dimidiatus*. After some experimentation, a modified method was developed for this species. Rather than adding adult beetles to a cup containing the agar-based diet of James & Vogele (2000) and having eggs laid on the mesh cover of the cup, adults were placed in a container filled with 70% soya based Carob moth diet (Gothilf 1968) and 30% almond meal, . In this situation, the adults feed on and lay eggs into the diet, and the developing larvae also feed on the diet. When the larvae mature and enter the wandering stage, they are collected and placed onto moist vermiculite for pupation, as per Dowd (1987).

Identification of matched life stages

Adults and their matched egg, larval and pupal offspring from the laboratory colonies were used to help develop diagnostic identification tools for some of these life stages. Five specimens of each life stage (egg, larvae, pupae and adult) of *C. hemipterus, C. nr dimidiatus* and *C. davidsoni* were reared, preserved in 70% ethanol and examined under a dissecting microscope. Due to some initial issues with acquiring specimens of *C. davidsoni*, a molecular test was conducted to sequence the DNA (barcoding COI gene) for larvae from this colony to ensure a match to this species.

Adult specimens reared from egg stages were identified and determined to match the putative species. At least one specimen of each immature life stage was photographed using a Leica M205C stereo microscope to capture the diagnostic features listed below. However, the following work focused on the 5th instar larvae (the stage used in published keys and descriptions of *Carpophilus* larvae) and compared larvae with descriptions in Connell (1991) and Hayashi (1978).

Approximately 165 specimens subsampled from pheromone traps and nuts from properties in Victoria, South Australia and NSW, were card-mounted and examined. Some specimens were dissected to examine the male genitalia. These specimens are housed within the Victorian Agricultural Insect Collection and identified to genus/ morphospecies based on morphological features and compared with validated specimens and descriptions (Leschen and Marris 2005, Ewing and Cline 2005, Connell 1991).

DNA barcoding

Due to the difficulty in identifying some species of *Carpophilus* from morphological features alone, and the degree of variation within a species, a DNA barcoding test was performed on at least 85 specimens to help identify or validate species. One to two legs were removed from card mounted specimens and DNA extractions were performed using the QIAGEN Blood & Tissue DNeasy kit. Using the LCO 1490 Forward primer and HCO 2198 reverse primers, (Folmer *et. al* 1994), a partial mitochondrial COI gene fragment was acquired (~690 basepair) with PCR conditions set at 40 cycles of 94^{O} C for 30 seconds, 52 ^{O} C for 45 seconds and 72 ^{O} C for 60 seconds.

The DNA product was sent to Macrogen Inc. or Micromon (Monash University) for sequencing using Sanger DNA Sequencing technologies. Sequences were analysed using Finch TV as an initial editor and blasted against the 'Barcode of Life' Data System (Ratnasingham & Hebert 2007) or NCBI Genbank (https://www.ncbi.nlm.nih.gov/genbank/) databases for sequence matches.

Results

At least eleven Nitidulidae species were recognised from almond orchards (Table 4), mostly collected in pheromone traps, but it is likely that there are further cryptic or undetermined species amongst the pheromone trapped material. The five main species of Nitidulidae which were commonly detected through trapping in almond orchards in the Riverland, Sunraysia and Riverina, can be diagnosed using the identification keys below and include *Carpophilus* near *dimidiatus*, *C. hemipterus*, *C. davidsoni*, *Urophorus humeralis* and species of *Brachypeplus*. Further diagnostic notes on these five species are presented below. *Carpophilus* nr *dimidiatus* was the main species detected from almond nuts based on the identification of adult specimens. Larvae from the *Carpophilus davidsoni* colonies were found to match with this species when tested using DNA barcoding.

Table 4 Nitidulid species in VAIC identified from Australian (NSW, SA and VIC) almond orchards.

	Species	Morph. ID	Mol. ID	*State Collected	Comments/ Notes
1.	*Carpophilus davidsoni*	Yes	Yes	NSW, SA	Australian native species. Known to occur in Victoria on *Citrus, Cydonia, Malus, Prunus* and *Pyrus*. This species is also known to occur in NSW and Qld (according to the APPD).
2.	*Carpophilus* nr *dimidiatus*	Yes	Yes	NSW, SA, VIC	Based on DNA barcoding sequences, this is the same species as that found in Walnuts in Argentina (Reales *et al*. 2018). In this paper, the species was described as *C. dimidiatus* but based on specialist (expert) opinion and support of molecular sequences, that description is considered to be incorrect.
3.	*Carpophilus hemipterus*	Yes	No	VIC	Based on morphological features, this species is very distinctive. An introduced but cosmopolitan species known to be a pest of stone and pome fruit in Australia and known to occur in Victoria on stone and pome fruit but also on onion, citrus and species of *Plantago*. This species is known to occur widely across Australia (in NSW, NT, Qld, SA, Tas and WA).
4.	*Carpophilus* sp. 01	No	Yes	NSW, VIC	Not commonly collected in traps
5.	*Carpophilus* sp. 02	Yes	Yes	VIC	Not commonly collected in traps
6.	*Carpophilus* sp. 03	Yes	Yes	VIC	Not commonly collected in traps
7.	*Carpophilus* sp. 04	No	Yes	SA, VIC	Found in large numbers in some traps
8.	*Carpophilus* sp. 05	No	Yes	NSW, SA	Not commonly collected in traps
9.	*Carpophilus* sp. 06	No	Yes	NSW, SA	Not commonly collected in traps
10.	*Carpophilus* sp. 07	No	Yes	SA	Not commonly collected in traps
11.	*Urophorus humeralis*	Yes	No	VIC	Commonly known as the Pineapple beetle. This species is known from Victoria and is cosmopolitan - "found in decaying flowers, fruits and other organic matter and is known to attack a wide variety of fruits and grains worldwide." (PADIL 2017). This species is known from all Australian states and territories.
12.	*Brachypeplus* spp.	Yes	No	VIC	At least one *Brachypeplus* species is known to be a pest of beehives overseas (Sagili *et al*. 2016]. The presence of *Brachypeplus* (undetermined species) in almond orchards, which use bees as pollinators, could become an issue for the beehive industry. Further identification of *Brachypeplus* species in almond orchards is required.

*Note: The "State Collected" column indicates from which states the species were collected and for which there are reference specimens represented in the VAIC as collected from this project. It does not represent the complete geographic range of these species.

Key to *Carpophilus* spp. adults

1. Antennal segments without club (variously formed eg. filiform, serrate, lamellate) (Figure 14) or if clubbed at apex then abdomen without tergites visible beyond posterior margin of elytra…………………..…OTHER beetles

- Antenna with three segmented club (Figure 13); abdomen posteriorly with 2 or 3 tergites visible in dorsal view (beyond posterior margin of elytra)………………………………………………2 (*Brachypeplus*, *Carpophilus* or *Urophorus*)

2. Abdomen usually with at least three exposed tergites, (beyond posterior margin of elytra) visible from dorsal view (Figure 15) or if only two visible, then pronotal margin (in lateral view) with bead twice as thick along anterior half as along posterior half (Figure 16)……………………………………………………………………………………………….3

- Abdomen with two exposed tergites visible dorsally (Figure 17); pronotal margin in lateral view with marginal bead the same width along anterior as posterior half (Figure 18)…………………………………………………………………4

3. Body with lateral margins curved, dorso-ventrally convex; elytra with random punctures (Figure 15)….. ………*Urophorus humeralis*

- Body with lateral margins more or less parallel-sided strongly flattened dorso-ventrally; elytra with punctures in distinct rows, parallel with wing length (Figure 19)…………………………………………………………………*Brachypeplus* spp.

4. Mesosternal disc separated from sides by raised carinae with oblique lateral arms (Figure 20)………………………..5

- Elytra without distinct pattern, may be black, brown or multi-coloured but not as above; mesosternal disc without carinae…….6

5. Elytra dark brown, black without any distinctive orange colour patterns although sometimes with feint humeral angles…………………………………………………………………OTHER *Carpophilus* species (including *Carpophilus* sp. 05)

- Elytra with distinct pattern of sinuous (M-shaped) pale orange patches along posterior margin and small orange spots on anterior lateral humeral angles (Figure 5)……………………………………………………*Carpophilus hemipterus*

6. Metaventrite with large axillary space, axillary line almost straight and extending to 1/3 length along metepisternal suture (Figure 21a)…………………………… OTHER *Carpophilus* species (including *Carpophilus* sp. 02)

- Metaventrite with smaller axillary space and line is curved reaching ¼ length along metepisternal suture or axillary space not clearly present (Figure 21b)……………………………………………………………………………………………7

7. Prosternum distinctly punctate (Figure 22 a)…………………………………………………………………..8

- Prosternum weakly punctate laterally or granulate (Figure 22 b)……….…………….9

8. Hypomeron surface may be weakly punctate and male mandibles asymmetrical or if hypomeron is moderately to distinctly punctate, then specimens small with body length around 2.0 to 2.4mm; body reddish brown or light brown, sometimes with dark band along posterior margin of elytra; hind male tibia usually evenly expanding towards apex but if slightly constricted basally, then specimens usually small (<2.5 mm) and unicolourous reddish brown; male paramere usually tapering to point or rounded at apex ……………………OTHER *Carpophilus* species (including *Carpophilus* sp. 03, *C*. sp. 04, *C*. sp.06 & *C*. sp. 07)

- Hypomeron moderately punctate but margins of punctures not distinct (Figure 22a); body length 2.8 to 3.8 mm (>2.5 mm); usually dark brown/ black except for anterior lateral margin with small pale brown/ orange patch; male mandibles symmetrical; prosternum with distinct punctures all over;; male hind tibia constricted along basal 1/3 and then widens abruptly towards apex (Figure 24), male paramere apically truncate…………….…………………………………………………………………………….*Carpophilus* near *dimidiatus*

9. Body is generally unicoloured, if bicoloured then only dorsally on elytra; setae on elytra variously coloured; male mandibles may be asymmetrical or symmetrical; female 9th tergite rounded or truncate but without median tubercle or if present only very small…………………………………………………………OTHER *Carpophilus* species

- Ventrally body bicoloured, metaventrite usually darker than prosternum and abdomen but may be unicoloured; female 9th tergite truncate with median apical tubercle (Figure 26)…………….*Carpophilus davidsoni*

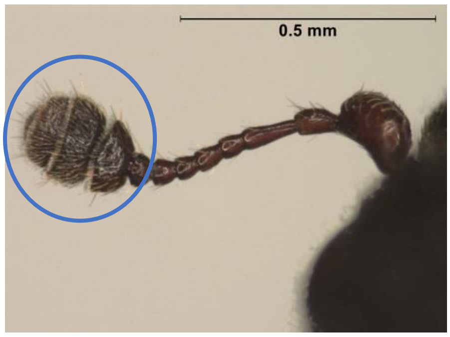


Figure 13. ***Carpophilus*** **near** ***dimidiatus*****, antenna with 3-segmented club***

Figure 14. Mycetophagidae, dorsal habitus

Figure 15. ***Urophorus humeralis*** **dorsal habitus**

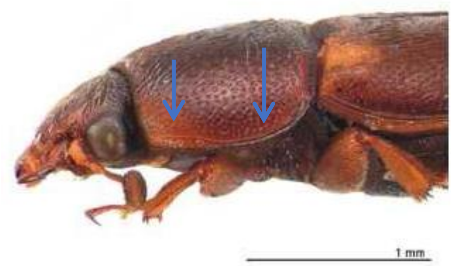


Figure 16. ***Urophorus humeralis*****, lateral head and pronotum**

Figure 17. ***Carpophilus hemipterus*** **dorsal habitus**

Figure 18. ***Carpophilus davidsoni*****, lateral head and pronotum**

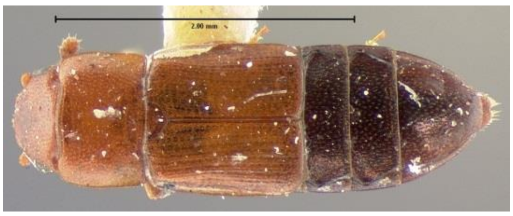


Figure 19. ***Brachypeplus*** **sp., dorsal habitus**

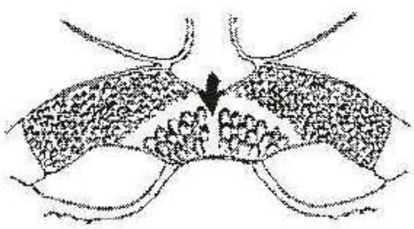
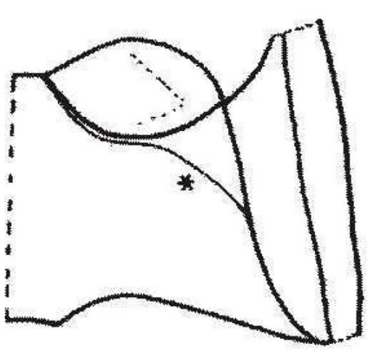


Figure 20. ***Carpophilus hemipterus*****, mesosternal disc with carinae**


a.

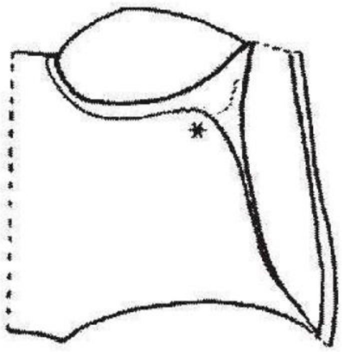
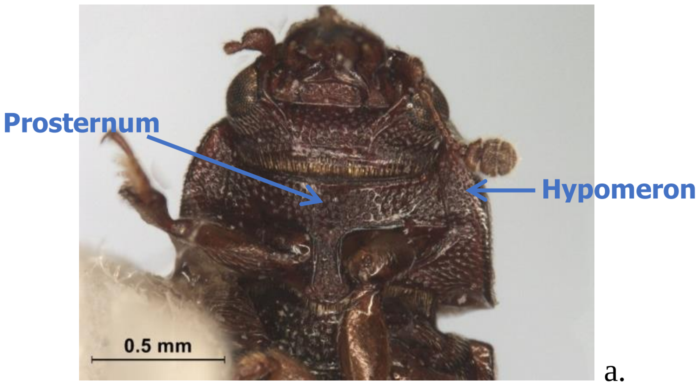

b.

Figure 21. Metaventrite with axillary space; a. *Carpophilus marginellus*, relatively large space with straight line b. *Carpophilus mutilatus*, smaller space, curved line



a.


b.

Figure 22. . Ventral prosternum and hypomeron a. *Carpophilus* near *dimidiatus*, b. *Carpophilus davidsoni*

Figure 23. *Carpophilus* near *dimidiatus* male, dorsal habitus

Figure 24. *Carpophilus* near *dimidiatus* male, hind tibia

Figure 25. *Carpophilus davidsoni*, dorsal habitus

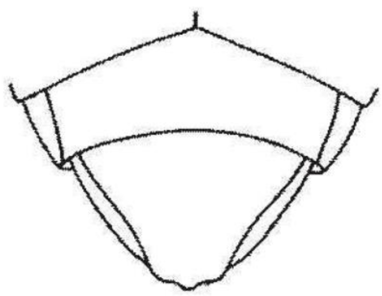


Figure 26. *Carpophilus davidsoni* female, abdomen in dorsal view apically

Diagnostic features of adults of three main species

Carpophilus near *dimidiatus*

Body length (n=4 male 2.8 – 3.8mm; n=4 female 3 – 3.6mm); black or very dark brown (sometimes lighter brown if teneral stages), unicolorous except for anterior humeral lateral angles of elytra sometimes with pale orange patches (Figure 23). Ventrally with prosternum punctate (edge of punctures distinct) and laterally (hypomeron) also punctate but margins of punctures not clearly defined. Hind tibia of male specimens narrow along basal 1/3 and then distinctly expanded to apex (Figure 24). Male paramere truncate at apex.

Carpophilus hemipterus

Body length 1.8 – 2.1 mm; dark brown with distinctive orange colouring on apical half of elytra. Mesoventrite separated from sides by raised carinae with oblique lateral arms. See Leschen and Marris (2005) page 12 for further details.

Carpophilus davidsoni

Body length 1.8 – 2.4mm; dark brown to paler brown on posterior half of elytra but this can be variable (Figure 25). Golden setae over body. Prosternum with only some weak punctures laterally or granulate, hypomeron smooth to granulate (Figure 22 b). Metaventrite often dark brown and much darker than prosternum and abdomen (but may be variable). Female 9th tergite truncate with median apical tubercle (Figure 26). See Leschen and Marris (2005) page 9 for further details.

Note: In this study it was found that *C. davidsoni* was very variable in colouration – in ventral view often with meso and metaventrite darker than prosternum and abdomen while other specimens were uniformly dark or paler brown, all over. Dorsally some specimens were uniformly very dark brown all over, while other specimens appeared to have a paler orange/brown T shaped or V shaped pattern on elytra. Based on DNA barcoding sequences, all colour forms matched with *C. davidsoni*.

Identification of Nitidulidae beetle larvae

Descriptions and diagnostic features of *Carpophilus* larvae follow Connell (1991) which covers the key features to separate Nitidulidae beetle larvae in stored products and Hayashi (1978) who described Nitidulidae beetle larvae occurring in Japan based on mature larval specimens. A few additional features were explored in this study and are highlighted with an asterisk below.

Carpophilus beetle larvae generally have white to cream coloured bodies, with a light brown sclerotised head and many with sclerotised pronotum. There are no papillae, granules or asperites on the dorsum of each abdominal segment which is generally unornamented. There is a characteristic dorsal sclerotised plate on the abdominal segment IX bearing pregomphi medially (or submedially) and urogomphi on the posterior end, which are unbranched. Note: larvae of *Urophorus humeralis* have paired sclerites with 2 to 12 asperites along each abdominal tergite segment and this can be used to help identify this species.

Features which separate the *Carpophilus* species in almond orchards relate mostly to the pregomphi and urogomphi on the dorsal sclerotized plate on segment (IX) of the abdomen and include:

1. Shape and proportion of dorsal sclerotised plate on IX segment*
2. Setal arrangement on anterior margin of dorsal sclerotised plate on segment IX*
3. Shape of pregomphi
4. Position of setae anterior to pregomphus*
5. Lateral processes present/ absent pre-apex of urogomphus
6. Space/ width between urogomphi (along mid length)
7. Shape of urogomphus
8. Presence/ absence of sclerites on thoracic segments
9. Sclerotisation around spiracle
10. Shape of body and width of abdominal segments proportionally

Further work using morphometric features and examining larvae under the Scanning Electron Microscope may reveal some further useful characters for differentiation between these three species. Inclusion of *Urophorus* *humeralis* and *Brachypeplus* larvae, the two other most commonly found Nitidulidae beetles, may also be useful in future.

Key to larvae of three main species of Nitidulidae beetle commonly found in almond orchards

1. Urogomphi absent……………………………………………………………………………………..NOT *Carpophilus* or *Urophorus*

- Urogomphi present……2

2. Abdominal tergites each with paired sclerites with 2 to 12 asperites ……………………..……….*Urophorus humeralis*

- Abdominal tergites without sclerites or if present, not bearing asperites, only setae…………………………………..3

3. Space between urogomphi narrow (midway along their length) one or less times width of urogomphus ………4

- Space between urogomphi wide, 1.5 times or more than width of urogomphus (Figure 28d-f)………….…..……5

4. Urogomphus dilated along inner margin or constricted midway beyond lateral process along outer margin, outer lateral margin with small or large process ……………………..……………………..........(OTHER *Carpophilus* species)

- Urogomphus gradually narrowing on inner and lateral margins (no outer lateral constriction) always with small outer lateral process………6

5. Distinct sclerites on meso and metathorax (Figure 27e); abdominal spiracles with sclerotized spiracular ring twice width around anterior margin compared with posterior margin; abdominal segments more or less similar in width, abdominal segment VIII only slightly wider than anterior margin of IX abdominal segment; pregomphal setae with raised bases and positioned anterior to base of pregomphiwith a distinct space (slightly less than length of pregomphus); no distinct paired medial setae along anterior margin of sclerotised plate on IX abdominal segment, or not in transverse line with bases of sublateral anterior setae (Figure 28e) ….……….*Carpophilus davidsoni*

- Sclerites absent meso and metathorax (Figure 27d); abdominal spiracles with sclerotized spiracular ring of equal width all around; abdominal segment VIII distinctly wider than anterior margin of IX abdominal segment; pregomphal setae without raised bases and positioned adjacent to pregomphal base; paired medial setae along anterior margin of sclerotised plate on IX abdominal segment in line transverse with sublateral anterior marginal setae (Figure 28d)……………………………………………………………………………………………*Carpophilus hemipterus*

6. Inner margins of urogomphi distinctly divergent…………………………………………………….(OTHER *Carpophilus* species)

- Inner margins of urogomphi parallel sided (almost rectangular in shape) with apical spine relatively short and bluntly rounded (Figure 28 c & f)………..7

7. Urogomphi separated at mid-point along length by 1 and a half times length of urogomphus, apical spine fine and tapered to a point ………………………………………………………………………………………(OTHER *Carpophilus* species)

- Urogomphi separated by at least two times length of urogomphus, apical spine stout and rounded……………………………………………………………………………..……………………*Carpophilus* near *dimidiatus*

Diagnostic features of 5th instar larvae of three main species

Carpophilus* near *dimidiatus

Diagnostic features

Weakly sclerotised lateral plates on meso and metathorax. The body is almost parallel sided with each abdominal segment being of about equal width (Figure 27 f). The abdominal dorsal sclerotized plate on segment IX, widest sub-apically when viewed in dorsal aspect (Figure 28 f); almost as long as wide, the medial seta along anterior margin distinct but slightly posterior to transverse line across bases of sublateral anterior setae (Figure 28 f); pregomphal seta distinctly removed from base of pregomphus; distance of base of pregomphi from anterior margin of plate on abdominal segment IX, at least 3 times the length of pregomphal spine; pregomphus apical spine rounded and when viewed laterally, directed posterior- dorsally (Figure 27 c, Figure 28 c). The urogomphi are short, rectangular with apical spine stout and rounded (Figure 28 c, Figure 28 f).

Differences and similarities to *C. davidsoni* and *C. hemipterus*.

The larvae are somewhat similar to *C. davidsoni* in having sclerites laterally on meso and metathorax, in general body shape with abdominal segments more or less similar in width throughout, abdominal segment VIII only slightly wider than IX. However, these species differ in the shape of the pregomphi which are rounded apically and directed posterior- dorsally in *C.* nr *dimidiatus* unlike *C. davidsoni* and *C. hemipterus* in which the pregomphi are hooked and tapered to point. The urogomphi are short and spine is rounded at apex in *C*. near *dimidiatus* but tapers to a point in both *C. davidsoni* and *C. hemipterus*. Note: Based on the key in Connell (1991) larvae of *C*. near *dimidiatus* have very similar features to those larvae of *C. nepos* with setal arrangement, shape of urogomphus (rectangular almost truncate at apex and urogomphi separated medially along their length by more than one width of the urogomphus). However, in *C*. nr *dimidiatus* urogomphi are separated by around 2 times width of urogomphus but in *C. nepos* the width is only 1.5 times length of urogomphus and width is less than one times length of urogomphi in true *C. dimidiatus* and *C. pilosellus*. Also, the shape of the urogomphal apical spine is very stout in *C*. nr *dimidiatus* but appears fine tapering to a point in *C. nepos*. This feature would need to be studied more carefully across a larger number of specimens to determine its consistency.

Carpophilus hemipterus

Diagnostic features

No distinct sclerites on dorsal abdominal segments, only on pronotum and segment IX (Figure 27 d). Abdominal spiracles with sclerotised spiracular ring of equal width all around. Abdominal segments broad and slightly flattened dorso-ventrally, the abdominal segment VIII appears distinctly wider than anterior margin of abdominal segment IX. Bases of medial setae along the anterior margin of the sclerotised plate on abdominal segment IX form a transverse line with bases of sublateral anterior marginal setae (Figure 28 d). The pregomphal setae are positioned directly anterior (adjacent) to base of pregomphi. The pregomphi are hooked and curved in a ventral direction (Figure 28 a). The urogomphi are tapered to a point (Figure 28 d).

Differences and similarities to *C. davidsoni* and *C.* near *dimidiatus*

Like *C. davidsoni* (and unlike *C*. near *dimidiatus*) the pregomphi are hooked and the urogomphi taper to a point but *C. hemipterus* differs to *C. davidsoni* species in having no sclerites laterally on the meso and meta thoraces which are distinct in *C. davidsoni* and only faint in *C*. near *dimidiatus*. It also differs to *C. davidsoni* in having distinct medial setae along anterior margin of abdominal segment IX which are not present in *C. davidsoni* and general body shape is different to both *C. davidsoni* and *C*. near *dimidiatus* with abdominal segments dorso-ventrally flattened and broader in dorsal aspect, segment VIII distinctly wider than IX.

Carpophilus davidsoni

Diagnostic features

Meso and meta thorax with distinct lateral sclerites (Figure 27 e). Abdominal spiracles with sclerotised spiracular ring twice width along anterior margin compared with posterior margin. Pregomphal setae with raised bases and positioned anterior to pregomphi with a distinct space (slightly less than length of the pregomphus) between seta and base of pregomphi. Pregomphi hooked, tapered to point at apex (Figure 28 b). The anterior margin of the sclerotised plate on segment IX has no distinct paired medial setae along anterior margin. Urogomphi with slight constriction preapically (particularly visible in lateral view along the ventral margin) and apically taper to a point (Figure 28 b & e).

Differences and similarities to *C.* near *dimidiatus* and *C. hemipterus*

The body shape is similar to that of *C*. near *dimidiatus* in having abdominal segments almost equal in width appearing almost parallel sided along abdomen and segment VIII only slightly wider compared with anterior margin of IX. It also shares with this species the presence of lateral sclerites on the meso and meta thorax (which are only weakly visible in *C*. nr *dimidiatus*). It differs to this species in the shape of the pregomphi (curved dorsoventrad and apical seta pointed) and urogomphi (apical spine pointed) and also setal pattern on the dorsal sclerotised plate of segment IX (medial setae not positioned along anterior margin – Note: small setae (less than half the length of the sublateral setae) may be present medially but are distinctly posterior to the anterior margin and the bases do not form a transverse line with the bases of the sublateral anterior marginal setae). Also, *C. davidsoni* is similar to *C. hemipterus* in the general shape of the sclerotised plate on segment IX, the pregomphi being hooked and directed ventrad and urogomphi apically tapered to a point. However, these species differ in the sclerotisation around the abdominal spiracles being twice the width around anterior margin compared with posterior margin in *C. davidsoni* while of more or less equal width in *C.* near *dimidiatus* and *C. hemipterus*, the absence of medial setae along the anterior margin of the sclerotised plate on segment IX (present in the other two species) and the node and position of the pregomphal seta in relation to the base of the pregomphus separate *C. davidsoni* and *C. hemipterus.*

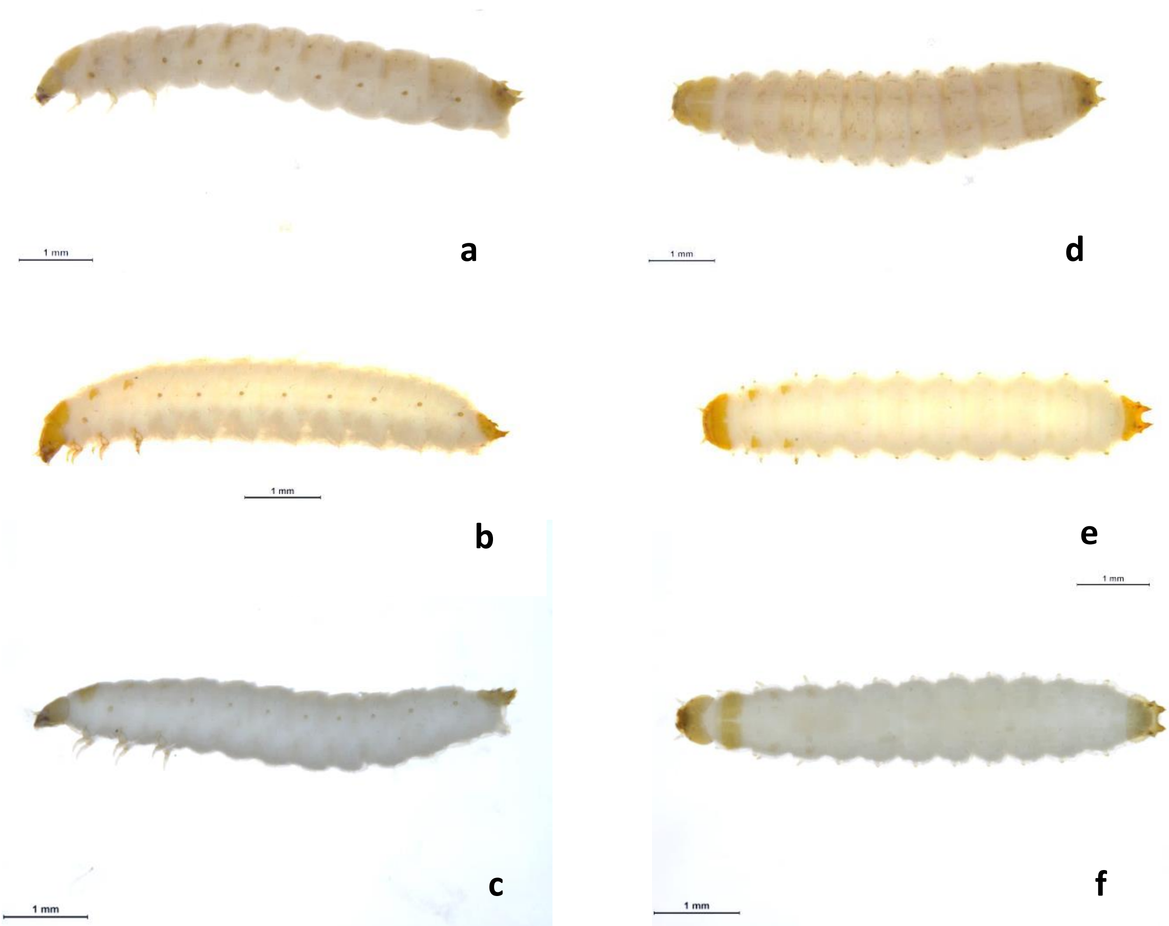


Figure 27. Lateral habitus, a. *Carpophilus hemipterus*, b. *C. davidsoni*, c. *C.* near *dimidiatus*; Dorsal habitus, d. *C. hemipterus*, e. *C. davidsoni*, f. *C.* near *dimidiatus*

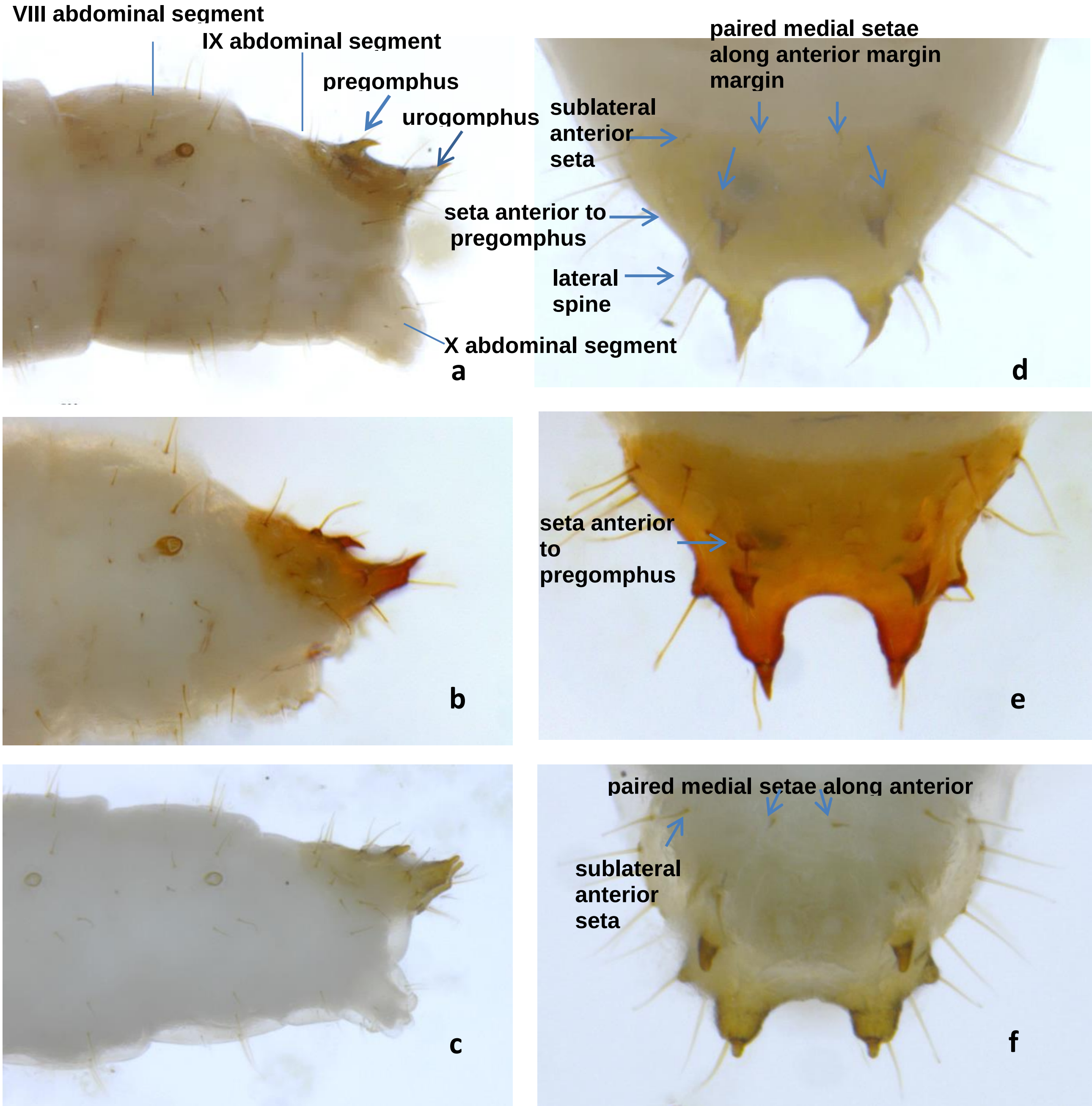


Figure 28. Lateral IX abdominal segment, a. *Carpophilus hemipterus*, b. *C. davidsoni*, c. *C.* near *dimidiatus*, Dorsal IX abdominal segment, d. *C. hemipterus*, e. *C. davidsoni*, f. *C.* near *dimidiatus*

Molecular analysis

Based on the molecular barcoding results of 56 of the 85 specimens tested, it was confirmed that 1) the five most common species identified through morphological features were correctly determined, 2) a high degree of colour and size variability is exhibited within species, particularly in relation to *Carpophilus davidsoni*, and 3) the pheromone trap samples contained at least five further *Carpophilus* species, whose identities require further work for confirmation. A DNA sequence reference library including eight Nitidulidae species has been created, and includes sequences of *Carpophilus* near *dimidiatus*, the main species found infesting almonds. It should be noted that sequences for true *C. dimidiatus*, *C. hemipterus, Urophorus humeralis* and *Brachypeplus* spp. are available on the BOLD database (Ratnasingham & Hebert 2007). In future, Nitidulidae beetles found in almond orchards may be readily matched with sequences acquired through this project. This is particularly useful for larval identification, where there are few features for diagnosis and 5th instar larva are usually needed for identification.

The DNA barcoding (partial COI gene) sequence of *C.* near *dimidiatus* was also found to be distinct from *Carpophilus dimidiatus* (GenBank accession GU217517) and was at least 12% divergent. Based on the molecular data there are no validated species in any online databases which match *C.* near *dimidiatus*, except for a specimen found in walnuts in Argentina (Reales *et al*.2018) with which it matched 100%. The Argentinian specimen was identified as *C. dimidiatus*, even though its sequence is more than 12% divergent from that species and their morphological characteristics do not match. It is believed that the naming of the Argentinian specimen is likely a misidentification of the species [personal communication with Nitidulidae specialist Dr Alexander Kiretjshuk].

Discussion and conclusion

A reference collection of at least 185 card-mounted adult specimens (labelled and databased) incorporating at least nine different Nitidulidae species from sites in the Riverland, Sunraysia and Riverina have been deposited in the VAIC, Bundoora for future examination. Five specimens of each of the five life stages (eggs, five larval instar stages, prepupal and pupal stages and adults (males and females) of reared specimens for *C.* near *dimidiatus, C. davidsoni* and *C. hemipterus* have also been retained in the collection.

Based on adult morphological features, the main pest species affecting almonds, *Carpophilus* near *dimidiatus*, can be readily distinguished from at least the four other common species, *C. davidsoni, C. hemipterus, Urophorus humeralis* and *Brachypeplus* sp., and also six other less common Nitidulidae species associated with almond orchards, using keys and images provided. Also, larvae of *C.* near *dimidiatus* were found to be distinct from larvae of *C. davidsoni* and *C. hemipterus*, and a key to separate larvae of this species from other species that could be encountered in almond orchards is provided.

Based on both morphological and molecular evidence, it is clear that the species found damaging almonds is not true *Carpophilus dimidiatus* as confirmed by at least three Nitidulidae specialists (Mr Richard Leschen, Landcare Research, New Zealand; Dr Josef Jelinek, Czech Republic; and Dr Alexander Kirejtshuk, Russia) but is likely a closely related species. Therefore this species was referred to as *Carpophilus* near *dimidiatus*. It appears to be the same as the species found recently in walnuts in Argentina (Reales *et al*. 2018) where it was referred to, apparently incorrectly, as *C. dimidiatus*. Dr Jelinek and Dr Kiretjshuk confirmed that *C.* nr *dimidiatus* belongs to the *Myothorax* subgenus (to which *C. dimidiatus* also belongs) and it is possible that it is part of the *dimidiatus* species group. According to Ewing and Cline (2005) there are at least six species currently recognised in this group. Further correspondence with Dr Kiretjshuk has assisted with the determination of this species, but more morphological and molecular investigation of specimens would help with better understanding the species identity. This requires examination of specimens from overseas collections, and clearing up previous misidentifications and incorrect synonymies found in the literature.

It is interesting to note that while *Carpophilus gaveni* has been recorded from windfall almonds (based on notes in Leschen and Marris 2005) this species was not recognised amongst the subsampled reference material. It is possible that this species is present in low numbers and was either not detected or misidentified given its very strong similarity to *C. davidsoni*. However, DNA barcoding sequences of *C. gaveni* are available on the BOLD database and none of the subsampled specimens tested appeared to match with this species.

Further study of all aspects of *C.* near *dimidiatus* (taxonomy, biology, distribution, host preferences) is clearly needed. It would also be useful in future to study specimens of *C. davidsoni* and to have a better understanding of the broad colour variations exhibited within this common species (testing further genes) and comparing with *C. gaveni* and another similar species *C. maculatus*.

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Final report: Management of carpophilus beetle in almonds
Project code: AL15004

Appendix 4. Alternative hosts and overwintering

Introduction

When carpophilus beetle was found infesting almond crops, and research on the pest commenced, almost nothing was known of the seasonal source of the species, *Carpophilus* near *dimidiatus* (*C*. nr *dimidiatus*), that was causing kernel damage. It was considered important to develop some understanding of the plant host range and overwintering sites used by carpophilus, in the hope that they may provide some practical opportunities for improved management through, for example, management of alternative host plants or destruction of overwintering beetle populations.

Five techniques were used to investigate the plant host range and overwintering sites used by carpophilus beetles. These involved extraction of beetles from fruit and nut samples collected from a wide range of exotic and native plants in different regions of Victoria, including Sunraysia; manual searches for beetles, extraction of beetles from samples of bark and leaf litter, and trapping, all in areas of native vegetation in the vicinity of almond orchards in Sunraysia; and extraction of beetles from residual almond nuts collected from the ground and trees within orchards in Sunraysia and the South Australian Riverland. The focus on native vegetation and residual nuts was informed by earlier observations of infested nuts in orchards and some evidence of greater trap catches near native vegetation.

Materials and methods

Host range

An assessment of carpophilus usage of a wide range of potential alternative hosts was achieved through collaboration with the tephritid fruit fly project, 'Risk evaluation and improvements of diagnostics of south-eastern Australian fruit flies' (CRC/NPB project 105584). Fruits from potential alternative hosts for both fruit flies and carpophilus beetles were collected in three Victorian production regions and inspected for insect larvae.

The plants chosen for this study were either known hosts of fruit fly (Hancock et al. 2000) or belong to families of plants that may be hosts of fruit fly (Blacket 2016). As the fruit host range of carpophilus overlaps with that of fruit flies, and beetles are attracted to fruits that are at similar ripening stages to those attractive to fruit fly, fruits targeted for sampling would be suitable hosts for both insects. Samples consisted of 1 to 100 fruit collected directly from the plant, including ripe fruit where possible. The sample size was restricted to the number of fruit that filled a 10 cm diameter container (500mL), which varied the total numbers of fruit collected because of variations in fruit size. Identification of host plants was conducted in the field where possible. Photographs and herbarium specimens were also collected as some species of plants such as kangaroo apples (Solanaceae) can be particularly difficult to identify. Fruit samples were maintained in individual cages at Agriculture Research Victoria (AVR), Bundoora, in a controlled environment room at 25^{o}C and 60% humidity, to allow larvae to develop and emerge as adult insects (Figure 29). All specimens that emerged from fruit were either frozen and stored for further processing (flies, wasps and beetles), or were killed with ethyl acetate prior to pinning (moths).

Out-of-orchard overwintering

In early winter 2016, native vegetation in or near almond orchards in Sunraysia was inspected for possible overwintering sites of carpophilus. The inspections were carried out by two research staff over a two day period, and involved searching for carpophilus by peeling bark from trees and by looking through debris that had accumulated under trees and bushes. During these inspections, four samples of debris (leaf litter, bark etc.) from the ground beneath native vegetation were collected and taken to the laboratory. The samples were processed through Berlese/Tullgren funnels, which utilise heat and light to drive insects out of sample material. In mid-late winter 2016, samples of paddy melon (*Citrullus lanatus*) in almond orchards were also inspected for the presence of carpophilus beetle.

In late spring and early summer 2016, six carpophilus attract and kill (A&K) traps were placed in native vegetation in the vicinity of almond orchards in Sunraysia, but at least 150 m away from the almond trees. These traps were intended to detect carpophilus beetles emerging from the areas of native vegetation. Although the attraction range of these traps is not fully known, our experience with trapping carpophilus in almond and stone fruit orchards suggested that separation of 150 m or more from the almond orchard would significantly reduce the possibility of attracting beetles from the almonds themselves. Trapping continued for five weeks.

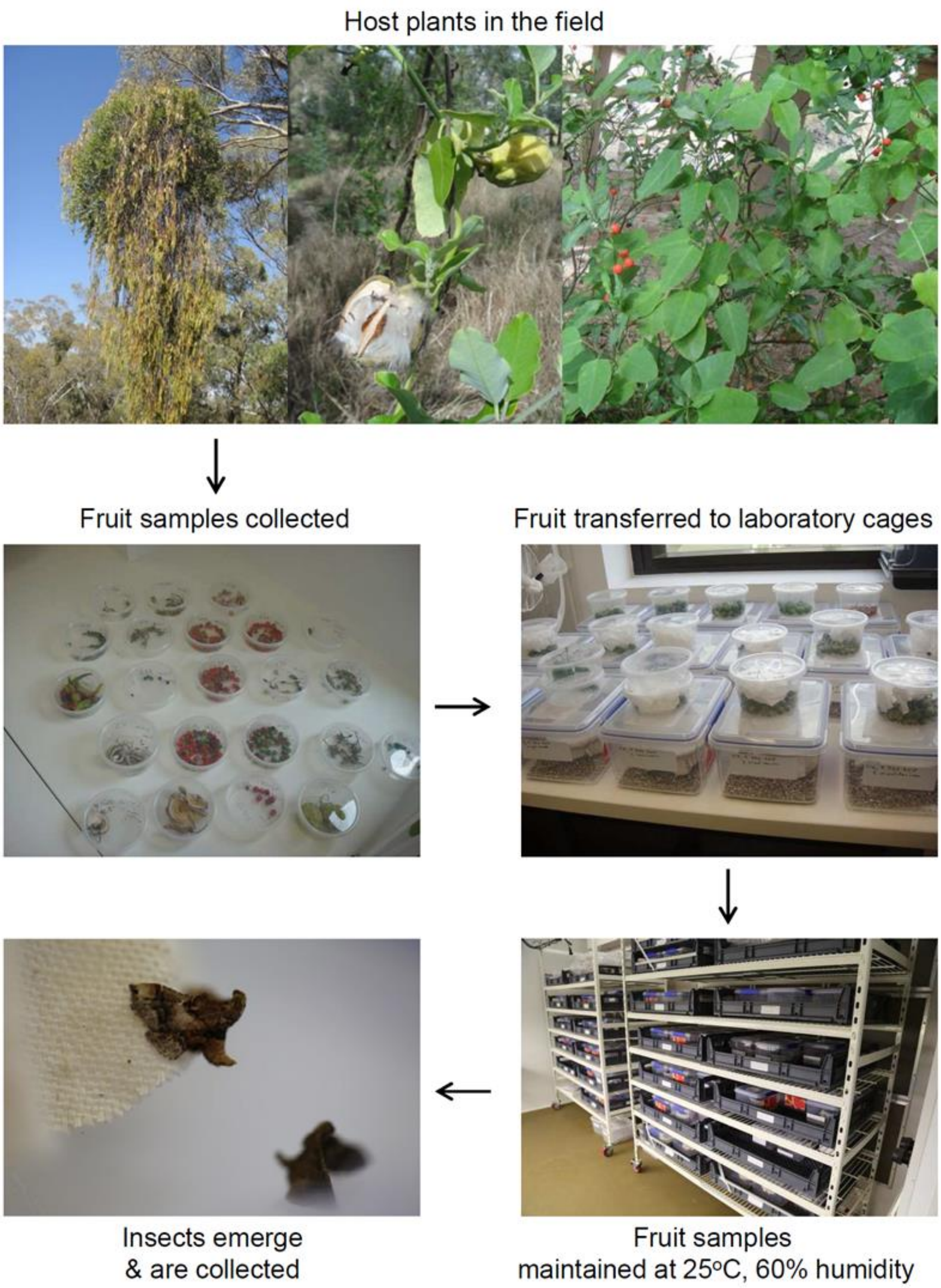

Figure 29. Field collection and host fruit incubation in the alternative host study

Within-orchard overwintering

For this study, two orchards were selected in Sunraysia and two in the Riverland. The Sunraysia orchards were selected on the basis that carpophilus was already known to be present, via trapping or nut assessments. Within those orchards, sampling was concentrated within one to two blocks that had recorded the highest levels of carpophilus damage. For the Riverland orchards where no prior data were available regarding carpophilus status, sampling was distributed across the orchards to improve the chances of detecting the pest.

To follow carpophilus development through winter, nuts were to be collected from trees and from the ground. These residual nuts, remaining in the orchard after harvest, develop into dark mummy nuts as they age. Because the almond industry had begun putting greater emphasis on orchard hygiene, including sweeping and removal of residual nuts, it was considered necessary to install secure patches of nuts within the orchards to ensure a continuity of supply of nuts for sample collection from the ground.

For each patch, Nonpareil nuts were collected from within the orchard and laid on the ground in a single layer over one square metre. For the 2016 winter sampling program, approximately 840 nuts collected from on-farm stockpiles (from the 2016 crop), were laid down in each patch. In 2017, approximately 500 nuts collected from trees (remnants of the 2017 crop) were laid in each patch. To protect the nuts from disturbance by machinery, rodents or birds, they were secured with a 1.2m x 0.85m section of 13mm bird mesh held in place by steel tent pegs. Each patch was positioned between two Nonpareil trees, such that it extended from the drip line into the tree row (Figure 30). This allowed the sampling of nuts from soil that was either dry or moist during irrigations, an important factor to consider given that some species of carpophilus have a preference for moist environments.

Figure 30. A nut patch secured under wire mesh

Six patches were installed in each orchard in late May/early June of 2016 and 2017. In each of the Sunraysia orchards, three patches were installed at each of two sites, with 160 to 260 m between sites and 60 m between patches within a site. In the Riverland, the patches were scattered across each orchard, with a maximum spread of 2.3 km within an orchard.

The periods and intervals of sample collection for the two seasons are shown in Table 5. Sampling in both regions was initially intended to cease in spring, but it was decided to maintain the sites in Sunraysia to follow carpophilus development in residual nuts through the entire season.

Table 5. Program of collection of nut samples.

District	Sampling commenced	Sampling ceased	Sampling interval
VIC Sunraysia	mid-June 2016 mid-June 2017	late-June 2017 April 2018	3 weeks 3-4 weeks
SA Riverland	mid-June 2016 mid-June 2017	Oct 2016* Nov 2017	3 weeks 3-4 weeks

* One additional late season sample was collected in late June 2017

At each sample time, four positions of nuts were collected:

- Dry – collected from the secure patches, away from the irrigation lines
- Moist – collected from the secure patches, near the dripper lines
- Tree – picked from trees near the patches
- Windfall – natural windfall nuts collected from the ground under trees near the nut patches.

Fifteen nuts in each position were collected from or around each nut patch. Where orchard hygiene practices resulted in low numbers of ‘tree’ and ‘windfall’ nuts, pooled samples of 50 nuts of each of those positions were collected from Nonpareil trees across the sample site. The nut samples were sealed in plastic zip-loc bags immediately upon collection, to minimise any loss of carpophilus beetles.

Carpophilus adults and larvae were extracted from the Sunraysia samples at AVR Irymple, using Tullgren funnels (Figure 31) in which the insects move to the bottom of the funnel and are collected in a vial of 70% ethanol. All carpophilus extracted from the samples after seven days of processing were sent to AVR Bundoora where they were identified under a stereo microscope. Samples from the Riverland orchards were processed in a similar way at the South Australian Research and Development Institute, Urrbrae, S.A.

Figure 31. Tullgren funnel processing of nut samples.

Counts (X) of carpophilus adults and larvae extracted from the nuts, were transformed to Log (X+1), to satisfy the ReML assumptions of normality and constant variance for residuals, prior to statistical analysis using a linear mixed effects model that was fitted with restricted maximum lilkelihood (ReML) in Genstat 19 software. The model included three factors - mummy position, week of the year, and property, and the two-way and three-way interactions between these three factors as fixed effects. The random effects included in the model were “site within property”, “mummy position within site within property” and “year_week within mummy position within site within property” which together defined the study design.

To quantify the effectiveness of the Tullgren process in extracting carpophilus from almonds, a test was carried out to determine the proportion of adults and larvae that were extracted. For this test, the number of adults and larvae in the collection vials of ten samples were counted after 1, 2, 4, 6 and 7 days of extraction, and the nuts were then destructively inspected to determine how many carpophilus did not exit the nuts. The percentage extracted by day was then calculated.

Results & discussion

Host range

A total of 284 containers of fruit representing 45 fruit species were collected. Table 6 indicates the timing of field collection trips to the different regions and Table 7 shows the amount of fruit from each type of host plant sampled from each region. In addition to fruits collected for this study, samples of fruit to be inspected for fruit fly were also submitted from regional AVR centres at Irymple (Sunraysia) and Tatura (Goulburn Valley).

Table 6. Field collection conducted in 2015/2016

Region	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*May*
Sunraysia								
Goulburn Valley								
Yarra Valley								
Mornington Peninsula								
Melbourne Markets								

Most of the samples did not produce any carpophilus beetles. A small number of *Carpophilus davidsoni*, *C. hemipterus*, *Urophorus humeralis*, and an unidentified species of carpophilus were obtained from pomegranate collected in Sunraysia, and *C. davidsoni* was recorded from strawberry guava from Sunraysia and kangaroo apple from the Yarra Valley (Table **8**). The species responsible for almond kernel damage, *C.* nr *dimidiatus*, was not detected in any of the samples.

These results may suggest that *C.* nr *dimidiatus* does not have a wide host range, or may have been introduced into and spread between almond orchards relatively recently, and not had the time or incentive to disperse more widely to other hosts.

Out-of-orchard overwintering

The visual inspections of native vegetation and the Tullgren funnel processing of leaf litter and bark failed to find any carpophilus beetles. The inspections of paddy melons yielded no *C.* nr *dimidiatus* or *C. davidsoni*, although large numbers of *Urophorus humeralis* and a few *C. hemipterus* were present.

During the five weeks of trapping in native vegetation, only a single specimen of *C. davidsoni* and four *C. hemipterus* were captured. *C.* nr *dimidiatus* were observed in traps placed within a nearby almond orchard during this trapping study, indicating that *C.* nr *dimidiatus* was present within the local environment, although in low numbers. These results support findings from a previous project (Hossain et al. 2005) which failed to find any carpophilus on native vegetation close to stone fruit orchards. The results suggest that native vegetation in or near almond orchards is unlikely to be a seasonal source of the *C.* nr *dimidiatus* found infesting almonds.

Table 7. Number of field *samples (i.e. containers of* fruit collected) by plant host and region

Host Plant	Sunraysia	Goulburn Valley	Yarra Valley	Mornington Peninsula	Total
Kangaroo apple			57	7	64
Hawthorn			32		32
Tobacco bush			25	3	28
Moth vine		13	3		16
Bush banana	12				12
Jerusalem cherry		6	4		10
Unknown Solanum		10			10
European black nightshade		4	3	1	8
Lillypilly		5	3		8
Tutsan			8		8
Rosehips			7		7
Boxthorn		6			6
Bridal creeper	5		1		6
Mistletoe		6			6
Pittosporum	1		4	1	6
Pomegranate	6				6
Prickly pear	5	1			6
Cotton bush		5			5
Paddy melon	4				4
Quena	2	2			4
Brush cherry		1	2		3
Bigleaf periwinkle		2			2
Japanese honeysuckle			2		2
Mango	2				2
Nitre bush	2				2
Passion fruit	2				2
Cactus	1				1
Cotoneaster			1		1
Creeper	1				1
Dodder	1				1
Dogwood			1		1
Fig			1		1
Peach	1				1
Pepper berry	1				1
Persimmon	1				1
Pigface		1			1
Pistachio	1				1
Prickly paddy melon	1				1
Pumpkin	1				1
Quandong	1				1
Quince			1		1
Red Cestrum			1		1
Rockmelon	1				1
White potato creeper			1		1
Zucchini	1				1
Total:	53	62	157	12	284

Table 8. Carpophilus species found in host plants sampled in 2015/16

Region of Sampling	Season	Host Plants	*C. nr dimidiatus*	*C. davidsoni*	*C. hemipterus*	*U. humeralis*	*Carpophilus sp.*
Sunraysia	2015/16	Pomegranate	0	2	18	5	21
Sunraysia	2016/17	Strawberry Guava	0	1	0	0	0
Yarra Valley	2015/16	Kangaroo Apple	0	7	0	0	0

Within-orchard overwintering

The ten samples used to assess the effectiveness of Tullgren funnels for extracting carpophilus from almonds contained a total of 38 carpophilus larvae and 313 adults. After two days of processing in Tullgren funnels, 100% of larvae and 95% of adults had been extracted from the nuts, indicating that this is a very efficient technique for sampling carpophilus in almonds. The remaining 5% of adults (16, all dead) were found during destructive inspection of the nuts. If those adults are assumed to have been dead at the time of sample collection, then the extraction of live adults after two days of processing would also have been 100%. To ensure maximum extraction of carpophilus from subsequent nut samples, all samples were processed for seven days.

It should be noted that the Tullgren extraction process is more likely to extract adults than larvae. Regarding larvae, the system will be more likely to extract more mature larvae than early stages such as 1st and 2nd instars. Early instar larvae are generally very delicate and sluggish, and are therefore more likely to desiccate or become stuck in the sample or on the wall of the funnel compared to late instar larvae, so are likely to be under-represented in the following data.

'Windfall' and 'Tree' nuts were available in sufficient numbers for collection throughout the entire sampling period. These nuts provide the most reliable picture of the development of carpophilus in the orchard blocks being sampled, as they represent a natural scenario, in comparison with the patches of nuts laid down under mesh. When considering the various factors associated with sample collection, significant interactions were found between factors, with mummy position (on <u>trees</u>, natural <u>windfalls</u>, or laid under mesh on <u>moist</u> or <u>dry</u> ground) involved with all significant interactions (except for mummy position and year_week in Sunraysia) (Table 9). Overall, nuts collected from the ground contained more beetles (both adults and larvae) than those collected from trees. This contributed to the significant interactions involving mummy position.

The numbers of adults extracted from windfall nuts in Sunraysia in 2016/17 were not significantly different to those extracted from moist nuts (Table 10), although there were significant differences on some dates (Figure 32). Dry nuts had significantly more adults than did nuts on the trees but had significantly fewer adults than in either moist or windfall nuts (Table 10). Nuts on the trees had significantly lower numbers of larvae (Figure 33) than in the other categories and moist nuts had significantly more larvae than in the other categories. There were no significant differences between numbers of larvae in dry and windfall nuts (Table 10).

Table 9. Statistical significance of sample factors, based on ReML analysis - Log(Count + 1).

	F Prob			
	Sunraysia		Riverland	
Factor(s)	**Adults**	**Larvae**	**Adults**	**Larvae**
MummyPosition	<0.001	<0.001	<0.001	<0.001
Year_Week	<0.001	<0.001	<0.001	<0.001
Property	0.007	0.952	0.431	0.699
MummyPosition x Year_Week	0.094	0.194	0.001	<0.001
MummyPosition x Property	<0.001	0.036	<0.001	0.019
Year_Week x Property	<0.001	<0.001	0.040	<0.001
MummyPosition x Year_Week x Property	<0.001	<0.001	0.022	<0.001

In the Riverland, there were significantly fewer adults and larvae in nuts on trees than in the other positions (Table 10, Figure 34, Figure 35). There were no significant differences in numbers of adults in dry, moist or windfall nuts. In contrast to Sunraysia, windfall nuts had significantly fewer larvae than in dry nuts, and there were no significant differences in larval numbers between moist and windfall nuts, or moist and dry nuts (Table 10).

These results demonstrate the importance of mummy position, especially regarding the higher infestation levels in windfall nuts compared to nuts on trees, and suggest that considerable improvements in beetle control might be achieved by concentrating efforts into removing residual nuts from the ground before the onset of spring. Nuts on the ground would be easier to remove than nuts still on the trees but would need to be destroyed or disposed of at considerable distances from almond orchards to prevent re-infestation.

Table 10. Effect of mummy position on adult and larval numbers in mummies. Two farms sampled in each state and in each farm three sites were selected for sampling.

	Mummy position				F Prob	LSD 5%
	Dry	**Moist**	**Tree**	**Windfall**		
Sunraysia-Mean log adult count	**1.303 b**	**1.501 c**	0.592 a	1.509 c	<0.001	0.0675
Sunraysia-Mean log larval count	0.943 b	1.114 c	0.349 a	0.778 b	<0.001	0.170
Riverland- Mean log adult count	0.3342 b	0.3354 b	0.0543 a	0.3299 b	<0.001	0.0957
Riverland- Mean log larval count	0.4528 c	0.3792 bc	0.0563 a	0.2991 b	<0.001	0.126
All data are means of log(X+1) transformed counts. Numbers followed by the same letters within rows are not significantly different.						

Carpophilus beetles were found infesting residual nuts in almond orchards year-round, and the very great majority were identified as *C.* nr *dimidiatus*, the species causing damage to almond crops. Adults and larvae survived in the nuts in low numbers over winter in Sunraysia (Figure 32 & Figure 33) and the Riverland (Figure 34 & Figure 35). In Sunraysia, the numbers of adults and larvae began to increase in mid spring and late spring respectively. This is likely due to mature larvae pupating to adults and egg laying commencing, as daily temperatures started to increase in spring. Average numbers of adults and larvae per nut peaked (3.4 and 8.25 respectively) around the time of hull split in early January, then declined. The decline in numbers of larvae was likely due to them maturing to adults, while the decline in numbers of adults is possibly due to the new crop nuts becoming very attractive to adults after hull split, as has been observed in the field.

These results highlight the potential for residual nuts within almond orchards to maintain high carpophilus populations. A planting density of 255 trees/ha together with the peak infestation level shown in Figure 33 (8.25 larvae/nut) and conservative figures of five windfall nuts per tree and 500 eggs produced per female, could yield over 2.6 million eggs/ha, one egg for every nut in a 3 t/ha crop.

In Sunraysia, from mid-spring to mid-autumn, drip irrigation was applied for eight hours per day, every day, so nuts in the 'moist' zone were exposed to wet or damp soil almost constantly. During that period, as can be seen in Figure 32 and Figure 33, numbers of carpophilus adults and larvae tended to be greater in 'moist' nuts compared to 'dry' nuts, suggesting that nuts, and in particular kernels, with a higher moisture content are more favoured by *C.* nr *dimidiatus*.

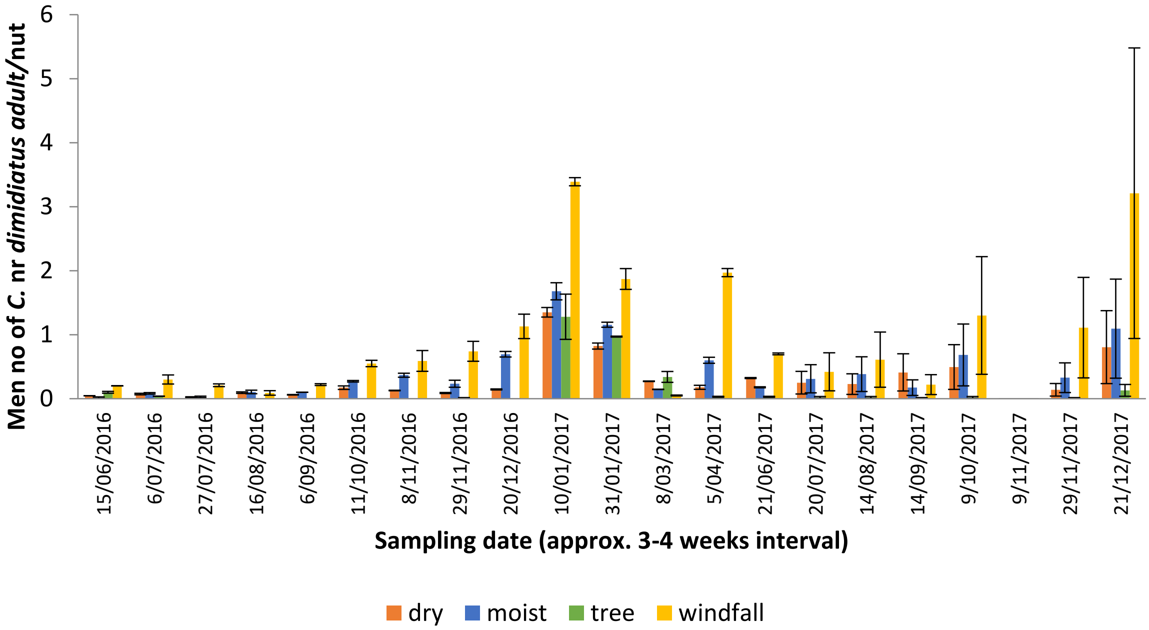

Figure 32. Mean number (±SE) of *C.* nr *dimidiatus* adults extracted from residual nuts collected from an almond orchard in Sunraysia. Dry, collected away from irrigation dripper lines; Moist, collected from near irrigation dripper lines; tree, picked from trees; windfall, collected from the ground under trees.

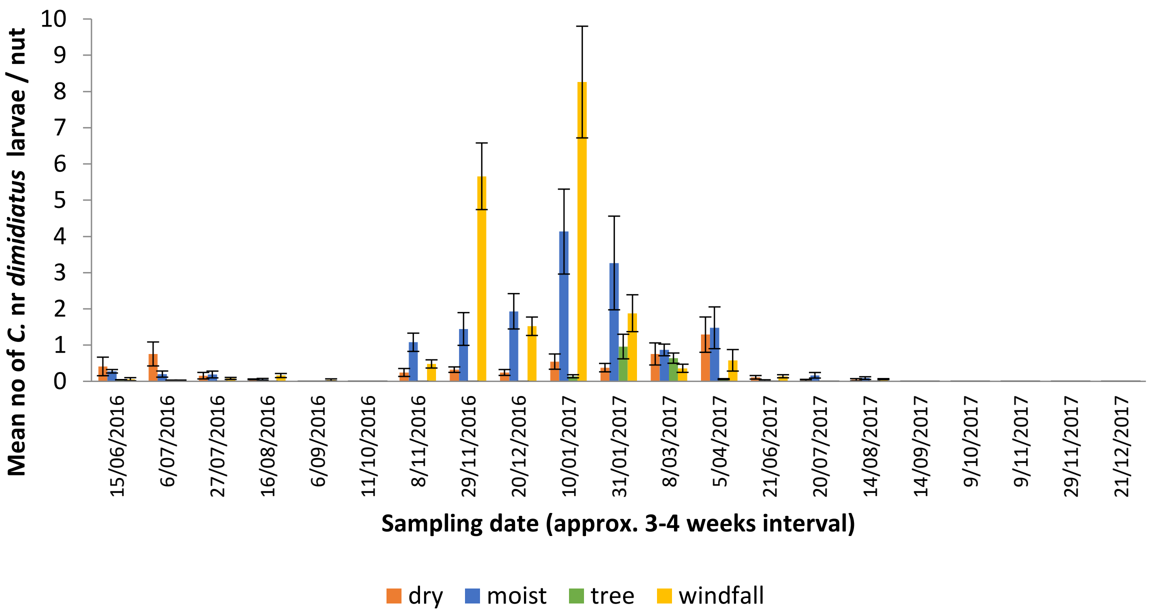

Figure 33. Mean number (±SE) of *C.* nr *dimidiatus* larvae extracted from residual nuts collected from an almond orchard in Sunraysia. Dry, collected away from irrigation dripper lines; Moist, collected from near irrigation dripper lines; tree, picked from trees; windfall, collected from the ground under trees

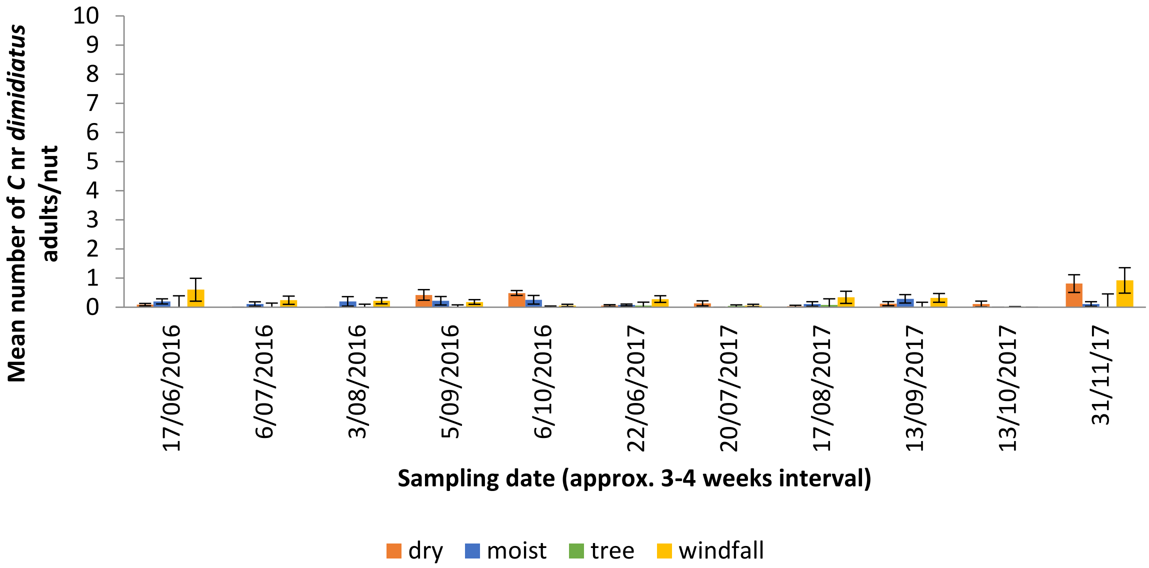

Figure 34. Mean number (±SE) of *C.* nr *dimidiatus* adults extracted from residual nuts collected from an almond orchard in the Riverland. Dry, collected away from irrigation dripper lines; Moist, collected from near irrigation dripper lines; tree, picked from trees; windfall, collected from the ground under trees.

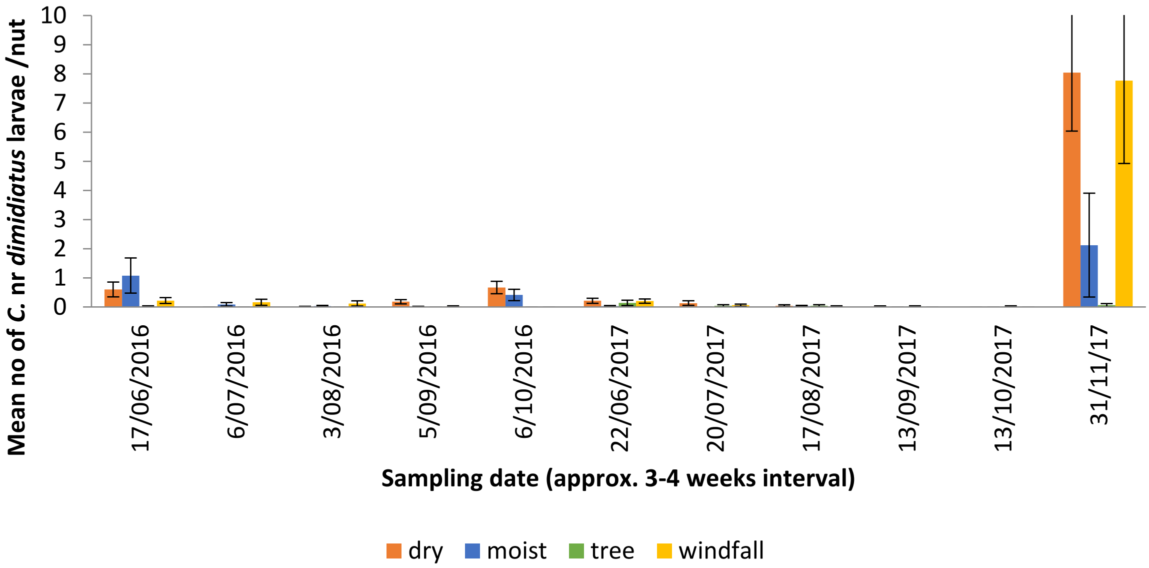

Figure 35. Mean number (±SE) of *C.* nr *dimidiatus* larvae extracted from residual nuts collected from an almond orchard in the Riverland. Dry, collected away from irrigation dripper lines; Moist, collected from near irrigation dripper lines; tree, picked from trees; windfall, collected from the ground under trees.

Conclusions

The lack of detection of *C.* nr *dimidiatus* in a wide variety of potential host fruits could suggest that it may not have a wide host range, or possibly is a relatively recent introduction and has not yet dispersed widely. The results of the host survey should however be interpreted cautiously, as so few carpophilus of any species were detected, even though the survey included known hosts of carpophilus.

The apparent absence of *C*. nr *dimidiatus* in native vegetation near infested almond orchards suggests that such areas may not act as a significant source of this pest infesting almonds.

The levels of infestation of residual and mummy nuts with *C*. nr *dimidiatus* indicate that regardless of whether *this species* does make use of alternative hosts or habitats, its potential for massive population development in those nuts within orchards should be the major concern for producers. It seems likely that regardless of any other management technique used, preventing or destroying residual/mummy nuts or rendering them unsuitable for infestation in some way, will be a critical factor for success in managing this pest in almonds.

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Project code: AL15004

Appendix 5. Efficacy of the current A&K system

Introduction

It became clear during the 2014 harvest that carpophilus beetle was causing serious levels of kernel damage in almonds. After consultation with Agriculture Victoria Research (AVR), producers in areas affected by this pest decided to implement a program of 'attract and kill' (A&K) trapping in an effort to protect their crops in the following season. Although the A&K system developed by AVR was used successfully to protect fresh stone fruit crops from carpophilus, the effectiveness of this approach in almonds was not known. At the time, however, A&K was the only available option that had potential to control carpophilus beetles, other than broad-spectrum pesticides.

Management of carpophilus with A&K in stone fruit orchards involves high-density trapping, with one to three traps per hectare. However, whilst stone fruit orchards are of tens of hectares, almond orchards are often hundreds to thousands of hectares: consequently, this density of trapping was not going to be feasible. Almond producers therefore compromised with trapping densities that were much lower, but economically and logistically manageable.

Questions that required answering regarding A&K in almonds included, (i) Does the stone fruit A&K system attract all the species of carpophilus present in almonds, and with equal effectiveness? (ii) At the trapping densities used in almonds, does the A&K system control the damaging species sufficiently to reduce crop damage? When it became apparent that the stone fruit A&K system was not protecting almond crops sufficiently, a further question arose: How does the damaging species respond to the food attractant currently used in the traps?

To address these questions, the project team collaborated with almond producers to a) ensure that the A&K system operated as effectively as possible, b) share trap catch and crop damage data, and c) collect samples of carpophilus from traps for analysis. The project also commenced bioassays of the stone fruit A&K co-attractants against the key species of carpophilus in almonds and stone fruit, in response to a Project Steering Committee recommendation to place more research focus on improving A&K attractants. These bioassays were to improve our understanding of the field response of damaging carpophilus species to A&K in almond orchards, and inform the next step in improving that A&K system.

Method

Field assessment of industry implementation of A&K

Details of carpophilus A&K traps, their field installation and maintenance, collection and identification of trap samples, and the geographic distribution of A&K implementation in almonds during this project are presented in Appendix 2.

Attraction of carpophilus species: The ability of A&K traps to attract the species of carpophilus found in almonds was assessed by analysing trap catches and comparing those with the range of species found through all sampling techniques used in and near almond orchards. Those techniques included:

- destructive inspection of new crop nuts at hull split (Appendix 2)
- destructive inspection of new crop nuts at harvest (see below).
- extraction of beetles from residual nuts within almond orchards (Appendix 4)
- manual searches of native vegetation and extraction of beetles from bark and leaf litter samples from native vegetation (Appendix 4)

Impact on kernel damage levels: The effect of broad-scale implementation of A&K on levels of carpophilus damage to almonds was determined through damage assessments of Nonpareil almond kernels collected at the time of commercial harvest in 2017 and 2018. For these assessments, in-hull samples of Nonpareil were collected from three almond orchards in the Robinvale district of Victoria's Sunraysia region in 2017, and four orchards in 2018. All orchards had maintained an A&K program for carpophilus management from 2014/15 to 2016/17. Two orchards continued their A&K program through 2017/18 while the other two maintained only isolated traps for beetle sample collection that season. Where an A&K program was in place, the traps were arranged in a near regular grid across the orchard.

Samples of approximately 1000 nuts were collected in pairs, one within 2-3 metres of an A&K trap ('near'), and the other in the same area of the orchard but as far as possible from the nearest 3-4 A&K traps in the trapping grid ('far'). Some characteristics of the samples are shown in Table 11. Samples were collected as soon as possible after the trees had been shaken for harvest (early-mid March 2017; mid-February to early March 2018). Each sample was collected from the ground under two adjacent trees, and placed in plastic mesh onion bags. The bagged samples were cool-stored at approximately 7°C until a subsample was hand-shelled and inspected for kernel damage. Care was taken during the inspections to differentiate between carpophilus and carob moth damage, based on the characteristic feeding pattern of the two pests, and the presence/absence of frass, webbing, and the insects themselves.

Table 11. Almond harvest sampling protocols across three orchards in the 2017 & 2018 seasons.

Harvest	Orchard	Trapping density Ha/trap	Distance of 'far' sample from nearest trap (metres)	No. of pairs of near-far samples
2017	1	5	119	8
	2	5	138	5
	3	15	159	4
2018	1	*	150	6
	2	*	150	3
	3	15	150	4

* Isolated traps for sample collection only.

Bioassays of current A&K attractants against key carpophilus species

Behavioural bioassays were conducted to assess the response of *C.* nr *dimidiatus* (the species damaging almonds) to the food co-attractant currently used in A&K traps, in comparison with that of *C. davidsoni*, one of the species specifically targeted by the co-attractant. The co-attractant used in the assays is a liquid component of the 'Carpophilus Catcha® Trapping System' (Insect Management Services P/L, Baccus Marsh, Victoria), and is a proprietary aqueous blend of 3-methyl-1-butanol, 2-methyl-1-butanol, ethyl acetate, acetaldehyde, sec butanol and ethanol.

Cultures of *C. davidsoni* and *C.* nr *dimidiatus* were established at AVR AgriBio, Bundoora, using adults collected from stone fruit and almond orchards respectively. These species were selected for bioassays as they are the major carpophilus pests for their respective crops (see also Appendix 2). Culturing methods are described in Appendix 3.

The bioassays were performed in a wind tunnel, 162 cm long and 67x67 cm in cross section. The walls and top were transparent plastic, but the floor had a rougher surface so that beetles falling on their backs could turn over easily. An air flow rate of 0.4 m s^{-1} and a temperature of approximately 27°C were maintained during the assays.

The evening before assays were run, approximately 400 adults of the test species (of mixed sex and age) were transferred from their culture into a glass jar. A crumpled paper towel was placed in each jar as a walking substrate. No food or water was provided as it is known that the beetles respond better to food odours after being deprived of food. The following morning, beetles were transferred to the wind tunnel and the laminar flow air stream was switched on. Assays began when 5-10 beetles were consistently in flight.

For each assay, the co-attractant (test odour) or clean air (control odour) was delivered into the wind tunnel from glass flasks via plastic tubing, and emitted at a height of 0.4 m above the floor of the wind tunnel. A circular piece of filter paper was attached around the delivery end of the tube to facilitate beetles landing. Carpophilus landing on the filter paper during a two minute period were counted, after which the odour delivery was switched off. Carpophilus remaining on the filter paper were dislodged by gentle tapping. After 5-8 minutes the odour delivery was switched back on for the next assay using the same odour. After one odour had been assayed 3-4 times, the odour delivery was switched to the alternative source (test or control odour) and the same procedure followed until the second odour had been assayed 3-4 times. This whole process was then repeated over a period of six to eight hours until each odour had been assayed 20 times.

Carpophilus were removed from the wind tunnel at the end of the experiment by allowing them to enter cups of diet set on the floor, after which the wind tunnel was cleaned thoroughly using a vacuum cleaner. The two test species of carpophilus were assayed on different days to avoid cross contamination of species within the wind tunnel.

Our previous experience with wind-tunnel assays of *C. davidsoni* responses to the stone fruit co-attractant (Bartelt & Hossain 2006) served as a means of verifying whether the assay equipment and beetles behaved as expected in the current tests.

Results & Discussion

Evaluation of industry implemented A&K

Attraction of carpophilus species

Analysis of samples from A&K traps deployed in almond orchards in SA, VIC and NSW found that the traps attracted all carpophilus beetle and related Nitidulid species that were found in those orchards through destructive sampling of nuts, extraction from nuts and leaf litter, and manual searches. Trap catches included *Carpophilus davidsoni*, *C.* nr *dimidiatus*, *C. hemipterus*, *Urophorus humeralis*, *Brachypeplus spp*. and unconfirmed *Carpophilus* species that could represent up to six or more species. The damaging species, *C.* nr *dimidiatus*, was trapped in relatively low numbers in comparison to the stone fruit pest *C. hemipterus*. Refer to Appendices 2, 3 & 4 for details of species composition and seasonal population dynamics of carpophilus beetle as indicated by A&K trap catches.

Impact on kernel damage levels

At the 2017 harvest, mean kernel damage across the three orchards ranged from 2.5% to 6.4%. Although there appeared to be a slight trend towards less damage near A&K traps, the difference was significant only in one orchard (Figure 36). The difference in damage levels between 'near' and 'far' samples ranged from 21% to 62%, but the lowest damage level of 2.5% was still unacceptably high from a commercial perspective.

At the 2018 harvest, mean kernel damage levels in our samples were considerably more variable, ranging from 1.8% to 27.5% across the same three orchards, and were again unacceptably high by industry standards (Figure 37). These levels closely reflect the within-orchard variation of 0-25% damage with a mean of 8-9%, reported by industry (confidential, pers. comm.). Although Figure 37 suggests a very slight trend of lower levels of kernel damage near A&K traps, there was no significant difference in damage levels between 'near' and 'far' samples on any of the orchards sampled.

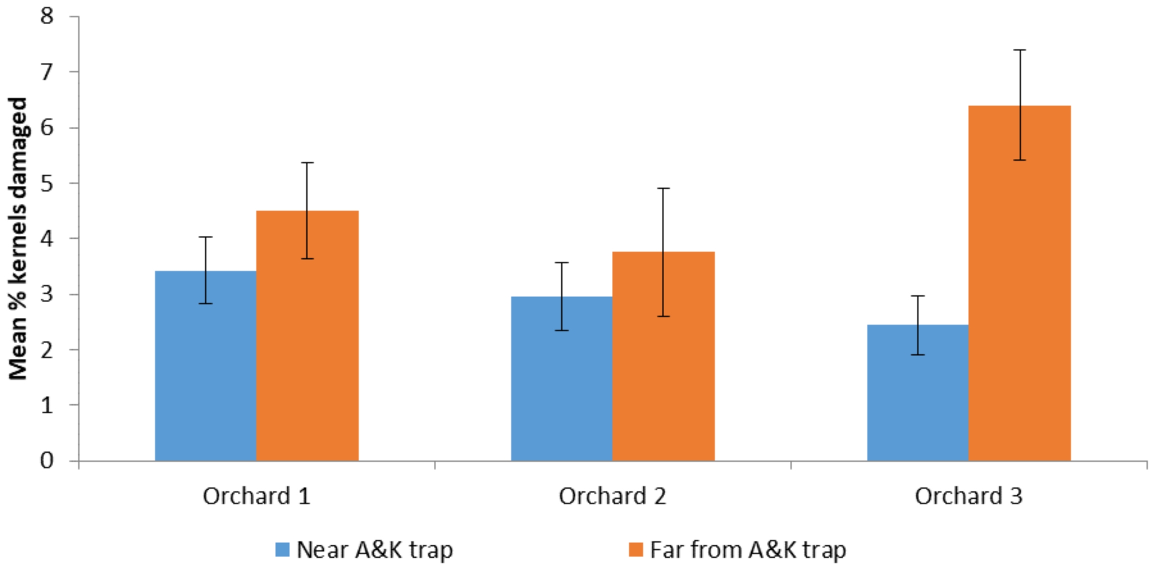

Figure 36. Mean % kernel damage in Nonpareil at harvest (±SEM), in orchards in Sunraysia, 2017. A significant difference was observed only on orchard 3.

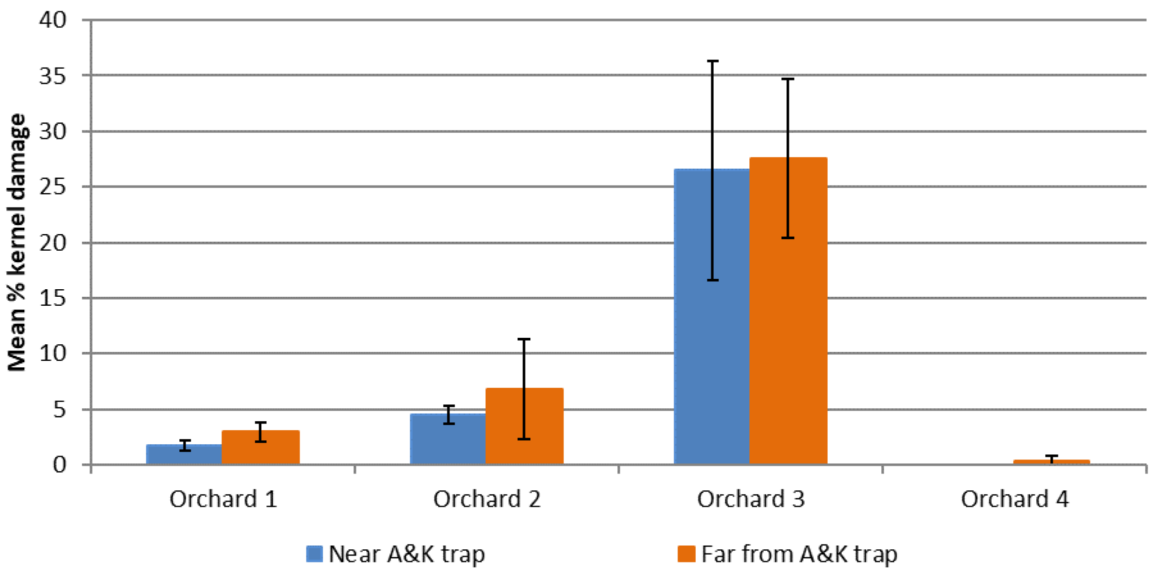

Figure 37. Mean % kernel damage in Nonpareil at harvest (±SEM), in orchards in Sunraysia, 2018. No significant differences were observed.

Between-orchard and between-year differences in damage levels seen here may relate to differences in population levels of the damaging species of carpophilus (*C.* nr *dimidiatus*), possibly in response to differences in prevalence of mummy nuts resulting from the level of hygiene achieved within those different orchards and years. Data collected during this project has demonstrated the extent to which *C.* nr *dimidiatus* utilises mummy nuts in orchards, with those nuts supporting high population levels of the pest (Appendix 4). Clarifying the association between mummy nut prevalence, *C.* nr *dimidiatus* activity and kernel damage is one objective of new project AL16009. Unfortunately, the A&K trap data for 2018 was not sufficient to provide any comparison of adult beetle population levels between the two years.

The instance on Orchard 3, of lower levels of kernel damage in samples from near A&K traps in 2017, and the apparent trends in the 2017 and 2018 harvests were encouraging. However, the unacceptably high damage levels observed in both years indicate clearly that the current A&K system, as implemented in almonds to date, is not sufficient to protect the almond crop.

It is likely that the low level of kernel damage observed in Orchard 4 in 2018 was related to very low mummy nut populations resulting from a high level of orchard hygiene and bird activity., but that association is yet to be confirmed.

Bioassays of current A&K attractants against key carpophilus species

These bioassays were conducted to determine whether differences in response of the two species of carpophilus beetle to the A&K stone fruit co-attractant, could help to explain the apparent lack of efficacy of the current A&K system against the species damaging almonds. The response of *C. davidsoni* to the co-attractant in these assays was in line with our previous work conducting similar assays (Bartelt & Hossain 2006). Beetles began to fly one to two hours after release into the wind tunnel, and when the co-attractant odour was delivered into the wind tunnel, a characteristic response usually began within 20 to 30 seconds.

The response of *C.* nr *dimidiatus* towards the stone fruit co-attractant was significantly lower than that observed for *C. davidsoni* (Figure 38). The mean number of landings per two minute period for *C.* nr *dimidiatus* (1.05±0.18) was less than half of that for *C. davidsoni* (2.55±0.26)(χ^2 = 12.5, P < 0.001).

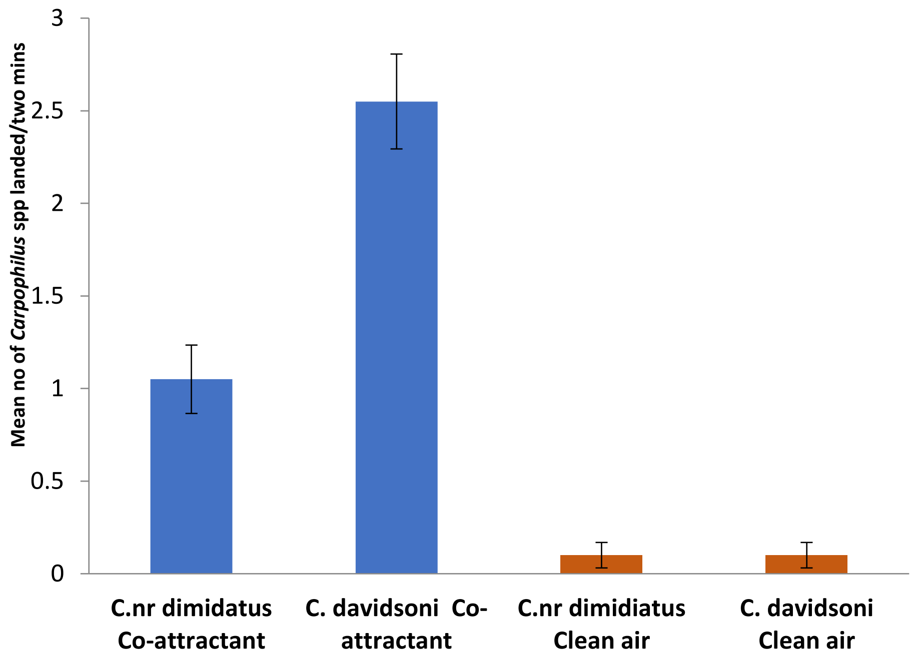

Figure 38. Mean number of *Carpophilus* spp(±SE) landing per two minute period in a wind tunnel experiment using stone fruit co-attractant.

The attractant in the carpophilus A&K trap is a combination of two odour components – an aggregation pheromone produced by the beetles and a “co-attractant” made from fermenting host plant odours (volatiles). These two components work together synergistically to attract male and female beetles. The stone fruit co-attractant currently used in the Carpophilus Catcha® Trapping System was designed to attract *C. davidsoni*, the major carpophilus pest of fresh stone fruit, and is based on odours of fermenting stone fruit juice. The results of these latest wind tunnel assays show that although *C.* nr *dimidiatus* does respond to the stone fruit co-attractant, the blend is not as attractive to this species compared to *C. davidsoni*. These results support the research direction of the new project AL16009 (An Integrated Pest Management program for the Australian almond industry), which focuses on designing an effective co-attractant for *C.* nr *dimidiatus*.

Another possible explanation for the lack of efficacy of the current A&K system in protecting almonds from *C.* nr *dimidiatus* relates to the ecology of the species. Effective A&K requires a high proportion of the pest population to be in a dispersal phase (actively searching for food or other suitable resources), within dispersal distance from the trap, have suitable weather conditions for flight, be receptive to the attractants in the trap (food odour and pheromone), and enter the trap once they find it. Carpophilus beetles are unlikely to be dispersive until their food resources become limiting, and are known to be unreceptive to aggregation pheromone unless they are hungry.

Observations of their feeding behaviour and anecdotal evidence that they prefer to remain in nuts until the food resource is exhausted, suggest that a major factor in performance of the traps may be the proportion of individuals receptive to the attractant. Hossain et al. (2006) demonstrated that the response by *C. davidsoni* to aggregation pheromone combined with co-attractant is more effective when flight activity is high but food availability is low. The number of mummified nuts typically observed on almond trees over winter and through spring and early summer provides an abundant source of food that may reduce receptiveness of *C.* nr *dimidiatus* to the attractant in the current A&K traps. A study of the response of starved *C.* nr *dimidiatus* to the pheromone plus co-attractant would be in order to test this hypothesis. If receptiveness is improved by starvation, then that lends greater importance to the removal of mummified nuts over winter to improve response of the pest to the traps. Our assessment of the numbers of *C.* nr *dimidiatus* infesting different categories of nuts in orchards over winter indicated that nuts left on the ground, including windfall nuts, have significantly higher numbers of both adult beetles and larvae compared to nuts left on the tree (Appendix 4, this report). Mechanical removal of nuts on the ground before the onset of spring would probably be less expensive and logistically more feasible than hand labour to remove mummies from trees. This might reduce the available food supply to such a level that residual beetle populations will be sufficiently starved for high proportions of those populations to be receptive to the aggregation pheromone and co-attractant emitted by the traps, before the new season nuts become attractive at hull split.

Conclusions

The stone fruit A&K traps currently in use, do attract the carpophilus and related Nitidulid species that have been found in almond orchards to date, including *C.* nr *dimidiatus*, the species causing damage to almonds.

When used in almonds, the A&K system did reduce kernel damage levels in one instance, but overall did not provide sufficient protection for the crop. This may indicate shortcomings in the strength of attraction of the trap lure (pheromone plus food co-attractant). The stone fruit co-attractant currently used in the carpophilus A&K system attracts the species responsible for almond kernel damage, but far less effectively than it attracts species causing damage to fresh stone fruit, for which it was designed. A co-attractant that effectively targets *C.* nr *dimidiatus* is required, and this is the subject of considerable research effort as a major component of the new Project AL16009. Also, the mode of action of the carpophilus A&K system, together with the behaviour of *C.* nr *dimidiatus*, indicate that the management of mummy nuts in orchards, by removal, destruction or other treatment, is likely to be crucial for successful management of the pest through A&K. The cost-effective application of A&K for carpophilus management in almonds is likely to require a combination of high lure attractiveness to the damaging species, low deployment cost, and good orchard hygiene.

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Appendix 6. Estimating trapping space for Carpophilus species captured in Attract and Kill traps placed in an almond orchard near Robinvale, Victoria during summer 2016-17.

Introduction

The capture of insects in traps depends on a number of factors working in concert. Most traps are stationary, requiring their targets to either approach the trap by chance or in response to chance encounters with an attractive stimulus emitted from the trap (Miller et al., 2015). Active traps are those that use some form of attraction to draw insects towards the trap. Colour, UV light, smell, contrast or silhouette, or a combination of two or more of these agents can be sources of attraction in active traps. Examples of active traps are pheromone traps, UV light traps, food traps, and coloured traps that mimic fruit. Passive traps are those that have no intrinsic attraction but rely on interception of a moving insect. Examples of passive traps are pitfall traps and clear acetate sheets coated with adhesive, which intercept meandering walking or flying insects respectively.

Active traps such as pheromone traps are commonly used in pest management but, although a lot of early research attempted to assign threshold values to the numbers of insects caught in a trap, the answer to the fundamental question of how to interpret capture of an insect in a trap has remained complex. The magnitude of the catch depends on the size of the target pest population, the proportion of individuals that are receptive to the attractant, the dispersal activity and propensity for long distance movement, suitability of weather conditions for dispersal, the spatial arrangement of the pest population in the environment relative to the location of the trap in that environment, the willingness and ability of the insects to enter the trap, and the ability of the trap to retain an insect once it has entered the trap (Miller et al., 2015).

Pest insect populations are not usually uniformly distributed throughout the environment nor even in smaller subunits such as orchard blocks, but exist in clumped distributions or aggregations often referred to as hot spots (Sciarretta and Trematerra, 2006). Flight capacity should not be confused with dispersal distance. Flight capacity refers to the total length of track flown by an insect. Most flying insects use a meandering flight path until they encounter an attractive stimulus and therefore the length of flown track is usually considerably longer than the observed dispersal distance. Dispersal distance is the distance between start and finish points (i.e. a straight line between the points) of the dispersal phase. The probability of capture of a moving insect declines as the starting distance from a trap increases (Turchin and Odendaal, 1996).

Mark-release-recapture studies have been used to estimate dispersal distance by analysing capture in a grid of traps radiating out from a central release point. Such studies are spatially and temporally restricted but can provide data on within-population dispersal (Peacock and Ray, 2001), although the frequency of dispersal distances recorded is usually skewed towards short distances due to the higher probability of an insect intersecting traps closer to the release point.

Geostatistical techniques can provide improved understanding of spatial phenomena in agricultural ecosystems (Trematerra et al., 2004). Spatial dependence of observations such as trap data can be examined by calculating semivariograms (Sciarretta and Trematerra, 2006). A semivariogram is a plot of semivariance as a function of distance between samples. Spatial analysis software groups possible sample pairs into user-specified classes, called lags, of approximately equal distance ranges. Models fitted to semivariogram data generate lines that reach a plateau, or sill, that indicates the distance at which data values are no longer autocorrelated (Johnston, 1999).

Here we report on use of variograms to estimate the trapping radius of carpophilus attract and kill (A&K) traps in an almond orchard.

Materials and Methods

An area of approximately 414 ha of almond orchard within the property known as Carina, located near Robinvale in NW Victoria was used as an experimental site to investigate interaction between A&K traps targeting species of carpophilus beetles found in almond orchards. A total of 104 traps were placed in the experimental area as indicated in Figure 39.

Figure 39. Trap layout in the experimental area.

The traps comprised of a five litre black plastic funnel trap, a three-species pheromone lure containing aggregation pheromones of *Carpophilus davidsoni*, *C. hemipterus* and *C. mutilatus*, a synthetic food co-attractant, and an insecticidal pest strip to kill beetles that enter the trap (For details of trap components and maintenance, see attachment 'AL15004 Factsheet-Managing Carpophilus beetle in almonds 2016-17.pdf'). Trapping commenced in early November 2016 and continued until mid-February 2017. The traps were serviced every week for population monitoring purposes, however for this experiment only insects in the traps when they were serviced on 5 December 2016, 28 December 2016, and 23 January 2017 were collected. The location of each trap was registered via GPS so that the co-ordinates could be used in spatial analysis software to examine interactions between traps in order to estimate separation distances at which trapping spaces started to overlap. The number of beetles captured in each trap was estimated using the volumetric methods developed by Hossain et al. (2009a) and then the entire catch from each trap was individually packaged and transported to the DEDJTR entomology laboratory at AgriBio, Bundoora where the packaged dry samples were stored under refrigeration (2°C) until the contents were sorted according to species. For samples containing less than 300 beetles, the entire catch was sorted into species. For packages containing more than 300 beetles, a subsample of up to 300 randomly selected beetles was drawn from the bulk sample and sorted into species. Species identification was based on the keys described by Dobson (1954, 1964), for more details see Appendix 2. Due to the amount of time and resources required to process the samples for species identification, only the insects collected on 5 December 2016 were identified to species level. The insects collected on 28 December 2016 and 23 January 2017 were used to assess spatial distribution of the combined species catch data.

The spatial analysis software VESPER 1.6 was used to fit a variogram model to the data using the weighted non-linear least squares method (Jian et al, 1996). Goodness of fit was assessed by AIC (Akaike Information Criterion) where the lowest AIC indicates the best model (Webster and McBratney, 1989). To interpret the variogram the point of inflection in the fitted line corresponds to the point where the horizontal sill occurs. The horizontal (X) axis indicates distance and the vertical (Y) axis is semivariance. Dots represent the calculated values and their colour, ranging from pink (smallest number) to blue (highest number), indicates the number of pairs for variance estimation at each lag. The distance increments represented by each lag are determined by dividing the maximum interpolation distance by the number of lags. The initial variogram calculation used 30 lags. Thirty lags over a maximum distance of 2500m gives lags of 83m. Where no sill was evident with variograms calculated on 30 lags, the variogram was recalculated using 80 lags. The blue line is the current estimate of the model. The value of the x-coordinate corresponding to the point of inflection is the separation distance between traps at which autocorrelation (indicating overlapping of trapping space) ceases. Assuming that the trapping space can be represented by a circle centred on the trap then this point is the distance between traps at which the circumferences of the trapping spaces just touch. The radius of the trapping area is therefore half the distance of separation.

Results

The most prevalent species in the traps cleared on 5 December 2016 was *C. hemipterus*, accounting for approximately 56% of the catch. The next most prevalent beetles were *Brachypeplus* spp., accounting for about 23% of the catch. *C.* nr *dimidiatus* accounted for only about 3% of the catch (Figure 40) and had a calculated trapping radius of 319m compared to *C. hemipterus* at 548m (Table 12). The variogram calculated with 30 lags for *C. davidsoni* had a good fit (AIC = 260) to the observed data but did not demonstrate a sill (Figure 41c), suggesting a trapping radius >1250m. Recalculation using 80 lags generated a sill at 678m, indicating a trapping radius of 339m, but the fit was poor (AIC=901) (Figure 41d).

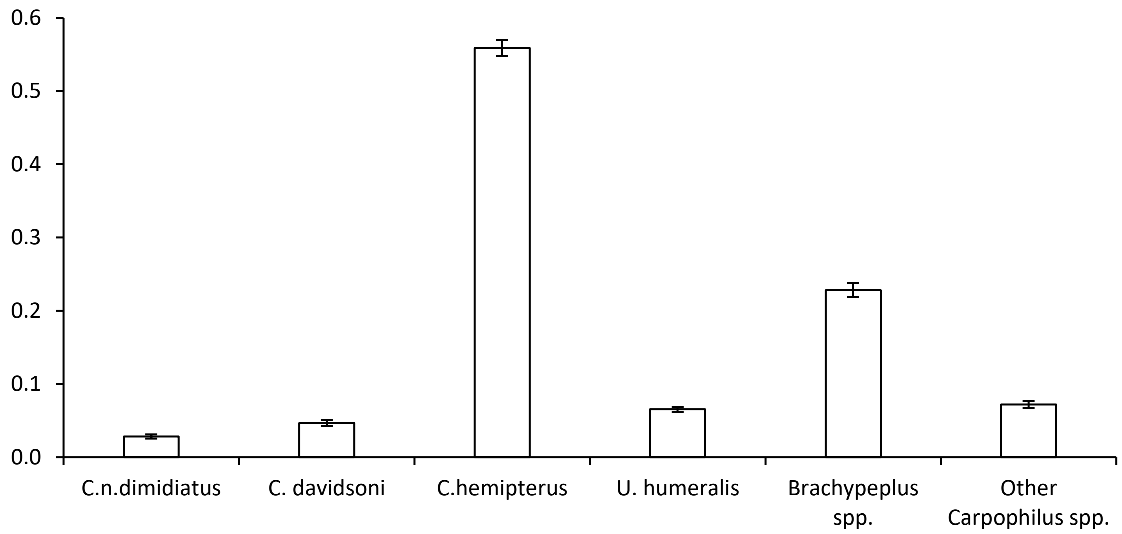

Figure 40. Mean proportion (±SE) of each species out of the total numbers of beetles in subsamples from traps (N=104). Collection date 5 December 2016.

The trapping radii of 545m and 447m derived from the variograms for total catch on 5 December 2016 and 28 December 2016 were similar, but the results for 23 January 2017 suggest a much larger trapping radius of 807m (Table 14).

Table 12. Trapping radius for each species, calculated from variograms presented in Figures 3-13.

Date	Description	No. Lags	Sill	AIC	Radius (m)
5 Dec 2016	Total	30	1090	556	545
	C. nr *dimidiatus*	30	638	119	319
	C. davidsoni	30	No sill observed	260	>1250
		80	678	901	339
	C. hemipterus	30	1096	525	548
	U. humeralis	30	1090	273	545
	Brachypeplus spp.	30	1280	470	640
	Other *Carpophilus spp*.	30	No sill observed	360	>1250
		80	1556	1032	778
28 Dec 2016	Total	30	894	653	447
23 Jan 2017	Total	30	1614	574	807

Discussion

Attraction of carpophilus beetles to ripening fruit is generally due to emission of odours consisting of blends of alcohols, aldehydes and esters (Smilanick et al., 1978). These volatiles also act synergistically with the male-produced aggregation pheromone (Bartelt and James, 1994) and have been used effectively in attract-and-kill stations to control *C. davidsoni* in stone stone fruit orchards (Hossain et al., 2006). Presence of food volatiles stimulates carpophilus beetles to land on, and enter traps (Hossain et al., 2012). Carpophilus spp. are strong fliers, with flights of 4 km recorded in mark-release-recapture studies (Barnes and Lindgren, 1940) and greater than 1 km in flight mill studies (Wu and Laughlin, 1994), allowing them to migrate into ripening crops from neighbouring areas (Hossain et al., 2009b).

The high proportions of *C. hemipterus* and *Brachypeplus* spp. captured in the traps were unexpected because neither have been found infesting nuts in the field. The results for *C. davidsoni* indicating a trapping radius greater than 1250m suggest that it may be able to detect lower concentrations of pheromone than can the other species. The high AIC values associated with the 80-lag variograms for *C. davidsoni* and “other *Carpophilus* spp.” indicate that the trapping radius calculated from those variograms is not reliable and that a radius of greater than 1250m is more likely.

The variogram for *C.* nr *dimidiatus* indicates a trapping radius of about 319m. Given that this species is the most prevalent species in almond nuts it is unlikely that the size of the target pest population had a negative impact on magnitude of the catch. The proportion of individuals that are receptive to the attractant, the dispersal activity and their propensity for long distance movement, and suitability of weather conditions for dispersal are more likely factors influencing the magnitude of the catch for this species. A trapping radius of 319m suggests that they are capable of reasonably long flight distances but this could be explored further in a flight mill. Also, nothing is known about their weather preferences for flight or their behaviour around traps, and these points warrant investigation.

A trapping radius of 319m equates to a trapping area of approximately 32 ha but this does not mean that one trap per 32 ha would be an effective trapping density for A&K. Effective A&K requires a high proportion of the pest population, within dispersal distance from the trap, to be receptive to the attractant. It is therefore imperative to further explore the factors influencing receptiveness, be they nutritional status of the pest or composition of the attractant (pheromone, co-attractant, or a combination).

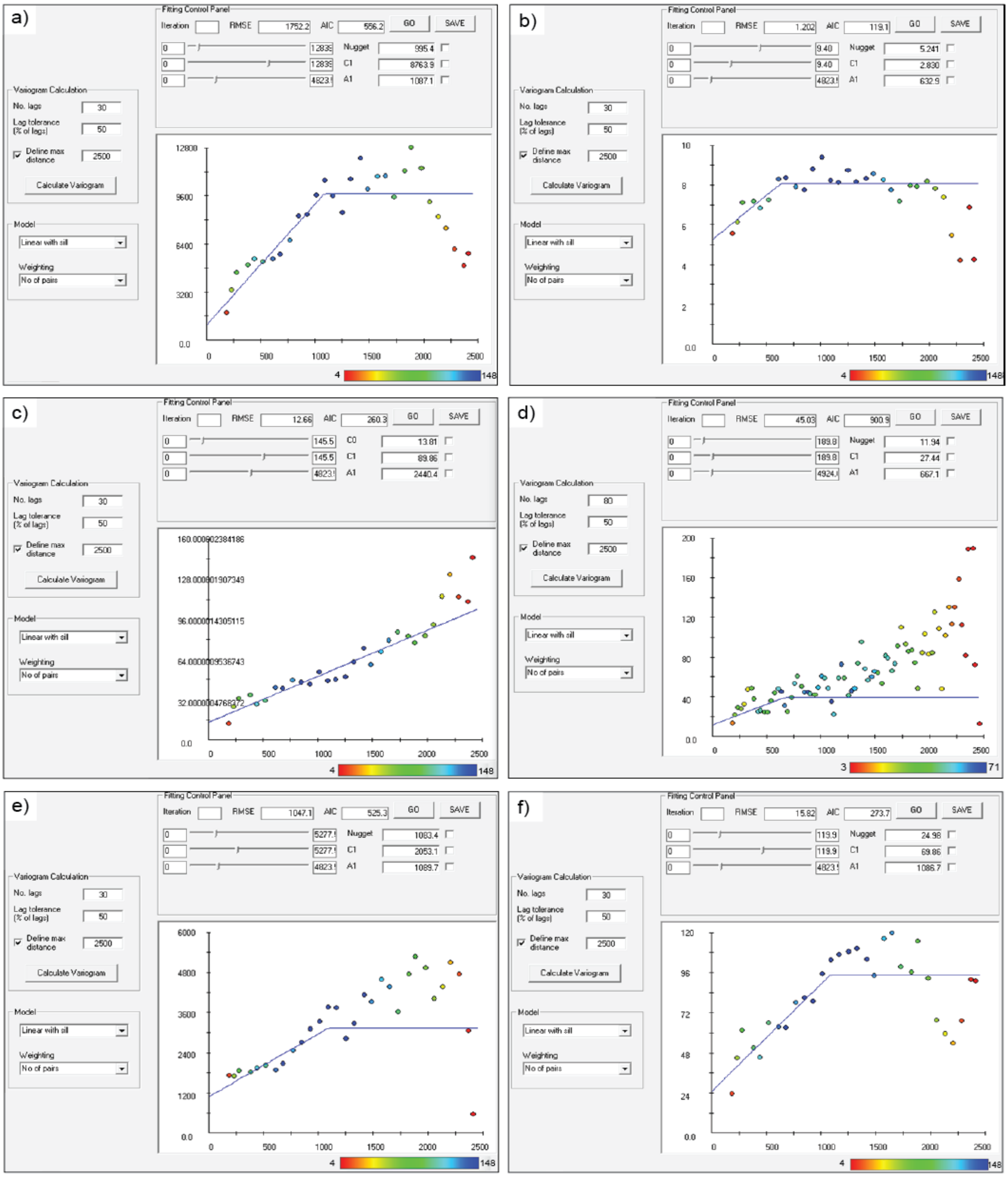

Figure 41. Variograms calculated for *Carpophilus* captures (a-f). a) Total capture, 5th December, b) *C. nr dimidiatus*, 5th December, c) *C. davidsoni*, 5 December (30 lags), d) *C. davidsoni*, 5 December (80 lags), e) *C. hemipterus*, 5 December (30 lags), f) *U. humeralis*, 5 December (30 lags).The horizontal (X) axis indicates distance and the vertical (Y) axis is semivariance. Dots represent the calculated values and their colour, ranging from pink (smallest number) to blue (highest number), indicates the number of pairs for variance estimation at each lag.

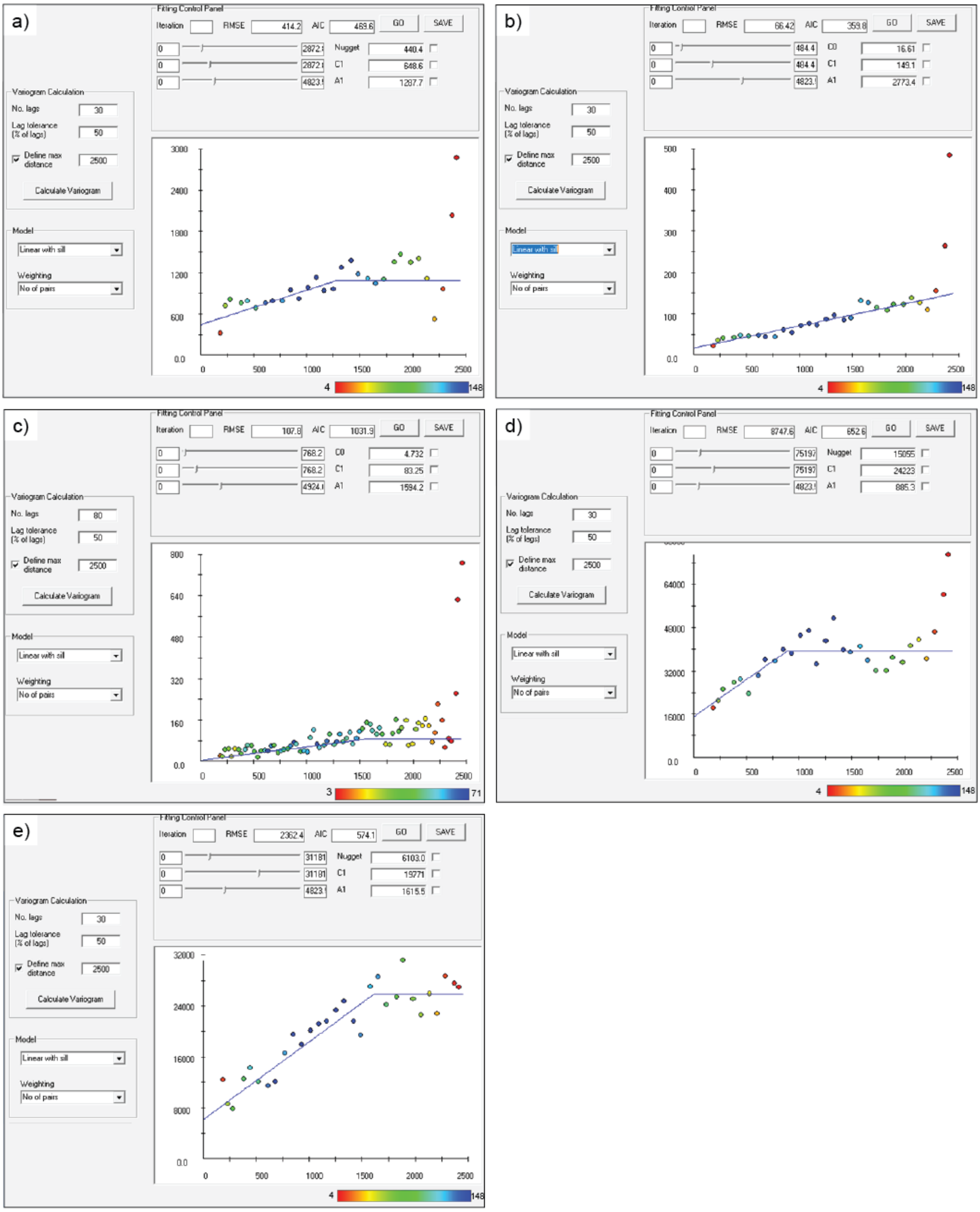

Figure 42. Variograms calculated for *Carpophilus* captures (a-e). a) *Brachypeplus* spp, (30 lags), b) Other *Carpophilus* spp. (30 lags), c) Other *Carpophilus* spp. (80 lags), d) Total capture, 28 December (30 lags), e) Total Capture, 23 December (30 lags). The horizontal (X) axis indicates distance and the vertical (Y) axis is semivariance. Dots represent the calculated values and their colour, ranging from pink (smallest number) to blue (highest number), indicates the number of pairs for variance estimation at each lag.

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Appendix 7. Pesticide screening

Introduction

In an effort to protect almond crops from damage by carpophilus beetle, some producers have trialled applications of pesticides based on the synthetic pyrethroid Bifenthrin. Bifenthrin is the only pesticide active ingredient registered in Australia for use against carpophilus beetle in any crop. Being a broad-spectrum contact insecticide, bifenthrin poses a considerable risk to IPM in almonds because of its toxicity to natural enemies of other pests such as mites, and 'flare-ups' in populations of pest mites were in fact observed in almond orchards treated with this chemical.

The 'pesticide screening' component of this project was intended to assess the efficacy and cost-effectiveness of potential IPM-friendly pesticides against carpophilus, to provide producers with additional options for management of this pest without the associated risk of secondary pest outbreaks. During the initial stages of this component, the Project Steering Committee (February 2017) recommended that lab-based insecticide trials stop, and that the focus be moved to co-attractant research to improve the efficacy of A&K traps. That recommendation was implemented through a contract variation, subsequently approved by Hort Innovation. The pesticide screening research completed up to that point is reported below. The project also continued to support the evaluation of pesticide options for the industry by supplying 15,000 carpophilus beetles to Bayer for use in field testing of new pesticides. The project team also assisted with the design of Bayer's field trails to improve data quality.

Literature review

A review of the scientific and 'grey' literature on chemical management of carpophilus beetles (family Nitidulidae), found no references to chemical control in almonds. Carpophilus is a relatively very new pest in almond orchards and consequently the information reviewed below relates to chemical control of this beetle in other crops such as stone fruit, figs and dates.

Most literature suggested early harvest and orchard sanitation can help reduce the damage potential of these pests, as can the use of less susceptible varieties. Since carpophilus beetles do not appear until fruit crops are mature, and the observation of pesticide witholding periods is considered critical, insecticide use on crops is discouraged. However, some degree of control was obtained in severe infestations by using carbaryl or bifenthrin. Although these insecticides may kill beetles present in the crop at the time of application, as long as fruit/vegetables are present, they cannot prevent additional beetles from reinfesting crops.

In Australia, control of carpophilus beetle using bifenthrin and fipronil was trialled in stone fruit, but results were inconclusive. It was recommended that bifenthrin should be applied only when necessary, as the use of this ingredient can often lead to high populations of two spotted mites late in the season (Thwaite 2001). Carpophilus larvae are more difficult to manage, so repeated applications may be necessary to break the beetle life cycle. Insecticides recommended in Florida to control Nitidulid beetles in strawberries include Brigade® (bifenthrin), Diazinon® (organophosphate), and Pyrenone® (pyrethrin). Some formulations of Malathion® (organophosphate) and Sevin® (carbaryl) are registered in USA for control of these beetles on other crops and are allowed for use on strawberry. Although insecticide use on strawberry crops is limited by frequent harvests, frequent and thorough applications are recommended during the early period of beetle activity (Rondon et al 2017).

Blumberg (2008) reported that Nitidulid beetle control in Israeli date plantations is based mainly on applications of insecticide. He also reported that a single application of an appropriate insecticide in midsummer and a second one 3-4 weeks before harvest were used as routine measures for control of these beetles in Israel; similarly to the measures against all moth species that attack the ripening date fruit. Since the 1960s, Israeli date growers have used various organophosphates for controlling fruit pests, and malathion has provided the most satisfactory control of Nitidulids (Kehat et al. 1985, Blumberg et al 2001).

Blumberg (2008) also reported that in the early 1980s, malathion was replaced with azinphosmethyl (organophosphate), that was more effective against Nitidulids. In addition, selective and supposedly more environmentally friendly insecticides of various groups were tested. The synthetic pyrethroids cypermethrin and cyhalothrin, and the Insect Growth Regulators (IGRs) diflubenzuron, hexaflumuron and teflubenzuron were found effective, and these three IGRs were recommended for commercial use (Bitton et al 2007, Kehat et al 1985). Although the IGRs do not affect adult beetles, they cause sterilisation of their eggs and high mortality of the beetle larvae (Ascher et al 1986, Blumberg et al 1985). Additional trials, carried out during 2003–2006, indicated that the pyrethroids lambda-cyhalothrin and bifenthrin, the neonicotinoid imidacloprid, and the chloronicotinoid thiacloprid were highly effective in controlling sap beetles (Blumberg 2008).

James and Curtis (2013) reported that Rimon® (novaluron) is a benzoylurea inhibitor of chitin biosynthesis (an insect growth regulator type of insecticide) that very effectively reduced the presence of larvae of the Nititulid *Haptoncus luteolus* generated from adults that enter strawberry fields (Price and Nagle 2010). The adulticides Assail® (acetamiprid) and Brigade ® contributed to a comprehensive program of beetle control that included removal of all ripe fruit from the field and use of Rimon ® (novaluron) larvicide (James and Curtis 2013).

In Australia, the only insecticides registered for use against carpophilus in any crop are based on the active ingredient bifenthrin. In almonds, bifenthrin is not registered for any purpose, but its use against carpophilus is currently allowed under the APVMA Minor Use Permit 82138 (expires 31/Mar/2019). However, as mentioned earlier, use of bifenthrin has generally led to high populations of mites and thus increased the need for miticide applications.

Laboratory bioassays

Materials and methods

Adults of *Carpophilus hemipterus* and *C*. nr *dimidiatus* were sourced from laboratory colonies at DEDJTR, Agribio. Because of colony limitations, the adults of *C*. nr *dimidiatus* were of mixed age.

A commercial formulation of a pesticide was provided as 'commercial in confidence' for use in this laboratory bioassay. The pesticide was mixed with water at the label rate (70ml product/100L) and applied to plastic petri dishes using a Potter tower. The spray residue was then left to dry. For the untreated control, a spray of distilled water was applied to petri dishes and also left to dry. This bioassay work was funded by the company involved and no funding was used from the levy fund.

To test the contact toxicity of the pesticide, 20 adults of *C. hemipterus* were placed in one of the petri dishes containing dry residue of the pesticide (Treated), and another 20 adults placed in a petri dish that had received a water spray (Control). Mortality of the beetles was recorded at 12, 24, 36 and 48 hours after they were placed in the treated and control dishes. Abbott's formula (Abbott 1925) was used to adjust treatment mortality figures to account for mortality in the untreated controls. The same procedure was followed with *C*. nr *dimidiatus*.

This initial bioassay was not replicated because the carpophilus populations in the laboratory colonies had only just started to increase in number and it was considered important to allow the colonies to become more established to allow for more extensive testing in future.

Preliminary results

Clear differences were observed between the two species, in control and treatment mortality (Table 13). *C. hemipterus* showed no control mortality throughout the 48 hour period, and only 10% mortality after 24 to 48 hours of exposure to the pesticide residue. In contrast, *C*. nr *dimidiatus* suffered a control mortality of 30% after 12 hrs and 50% after 24 hrs. Also, mortality of *C*. nr *dimidiatus* was obvious after 12 hours of exposure to the pesticide residue and the uncorrected mortality reached 80% after 36 hrs of exposure.

Adjusting for control mortality suggests that pesticide-attributed mortality in *C*. nr *dimidiatus* was approximately 60%, but the general rule in pesticide testing is to reject results when control mortality exceeds 20%, as it did with that species.

Table 13. Percentage mortality of *Carpophilus* nr *dimidiatus* and *C. hemipterus* after exposure to a field dose of insecticide under laboratory conditions.

Species	Treatment	12 hours	24 hours	36 hours	48 hours
C. nr *dimidiatus*	Control	30%	50%	50%	50%
C. nr *dimidiatus*	Treated	70%	70%	80%	80%
C. hemipterus	Control	0%	0%	0%	0%
C. hemipterus	Treated	0%	10%	10%	10%

Discussion

Given that this initial bioassay was not replicated due to colony limitations, and that the *C.* nr *dimidiatus* adults used were of mixed age, further bioassays with sufficient replication and standardised beetle age would be required to provide a robust assessment of the pesticide's efficacy against carpophilus and to allow dose response curves to be determined. As mentioned above, these assessments did not proceed because of a contract variation that halted the pesticide screening in favour of other components of the project. The preliminary screening of potential pesticides for management of carpophilus beetle in almonds is one component of new project AL16009 (An Integrated Pest Management program for the Australian almond industry).

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Appendix 8. Outputs

[Publications listed in the body of the report have been submitted separately in PDF format]

Formal and informal presentations by project officers

Formal presentations to industry forums, conferences and workshops

12/6/2013	McCormick Centre for the Environment, Renmark. Almond R&D Forum. Presentation on Carob moth to industry audience, including a 'heads up' on Carpophilus infestation and damage noted in the 2013 harvest. [75]*
18/6/2014	McCormick Centre for the Environment, Renmark. Almond R&D Forum. Presentation on Carpophilus to industry audience. [92]
28-30/10/2014	16th Australian Almond Industry Conference, Glenelg. Presentation on Carpophilus beetle and its management. [373]
28/4/2015	Olam research day, Euston Club, Euston. Presentation of current knowledge on carob moth and carpophilus beetle behaviour, monitoring and management in almonds to Olam orchard managers.
1/9/2015	ABA Office, Berri. Presented update on Carpophilus in almonds to Riverland growers. [2]
28/10/2015	McCormick Centre for the Environment, Renmark. Almond R&D Forum. Presentation on Carpophilus to industry audience. [117]
3-4/5/2016	AVR Irymple. Select Harvests insect management workshop. Six presentations on IPM, CM, CB, orchard hygiene.
27/7/2016	Loxton Research Centre, Loxton. Almond grower workshop on Carpophilus. Presentation on background to Carpophilus work, and current research. [42]
5/5/2017	Presented results of the 2016/17 season's research to the ABA Production Committee.
17-20/9/2017	Terrigal, NSW. Australian Entomological Society, 48th AGM and Scientific Conference. Presented paper on use of A&K against carpophilus in almonds.
5-9/11/2017	Adelaide. VII International symposium on almonds and pistachios. Presented poster on CB A&K in almonds
* Values in brackets indicate number of attendees at the event	

Training sessions/discussions on A&K trap maintenance and sample handling

6/10/2015	Almas Orchards, Bannerton. Project officers discussed Almas' trapping program, trap maintenance and sample handling procedures for sample collection for the CB project.
6/10/2015	Select Harvests, Carina. Project officers and field/technical industry staff discussed Select's trapping program, trap maintenance and sample handling procedures for sample collection for the CB project.
7/10/2015	Olam, Nenandie. Project officers met with Olam trap maintenance staff to discuss Olam's trapping program, trap maintenance and sample handling procedures for sample collection for the CB project.
7/10/2015	Select Harvests, Lake Powell. Project officers and field/technical industry staff discussed Select's trapping program, trap maintenance and sample handling procedures for sample collection for the CB project.
28/7/2016	Euston Club, Euston. Olam workshop for pest scouts. Presentations on carob moth and Carpophilus. [4]
15/9/2016	Olam, Wandown farm office. Project officer presented a training session on Carpophilus trap maintenance and sample management for five pest scouts.
16/9/2016	Select Harvests, Carina. Project officer presented a training session on Carpophilus trap maintenance and sample management for technical and field staff.
13/10/2016	Select Harvests, Amaroo. Project officer presented a training session on Carpophilus trap maintenance and sample management farm staff..

Informal presentations/discussions by project officers at industry meetings

4/4/2014	Olam office, Carwarp. Ag Vic researchers and industry reps discussed CB damage levels, spray trial, CB species, biology & A&K as possible management option.
4/9/2014	DEPI Mildura. Ag Vic researchers and industry reps discussed options for Carpophilus monitoring/trapping in the 2014/15 season. Field visit to Lake Powell – collected C. species from mummies along drip line and stockpile.
14-17/10/2014	Orchards at Griffith [9], Robinvale [16], Mildura [7], Riverland [14] & Adelaide Plains [11]. ABA Orchard walk program. Discussions on carob moth and Carpophilus monitoring and management with orchard managers – included 13 orchard walks.
4/3/2015	AVR Mildura. Ag Vic researchers and industry reps discussed progress re: Carpophilus trapping and specimen identification.
6/5/2015	Select Harvests, Carina. Industry update meeting on Carpophilus with Ag Vic researchers and industry reps.
4/8/2015	AVR, Irymple. Pre-season Carpophilus update meeting. Project officers and industry reps discussed results of 2014/15 trapping and industry plans for 2015/16 season. Project officers then discussed details of Almas' trapping plans, data collection and sample handling (for use by CB project) with Almas Almonds.
5/8/2015	AVR Irymple. Project officers discussed details of Select Harvest, Bright Lights and Olam trapping plans, data collection and sample handling (for use by CB project) in separate meetings with industry reps.
9/9/2015	Select Harvests, Carina. Regular industry update meeting on Carpophilus, with Ag Vic researchers and industry reps.
7/10/2015	Olam, Nenandie. Project officers met with Olam technical staff, Mike Strmska & Kevin Brooks (USA consultants) to discuss CB and approaches to management.
18/11/2015	Select Harvests, Carina. Regular industry update meeting on Carpophilus with Ag Vic researchers and industry reps.
18-22/1/2016	Hillston district. Project officers visited local orchards to collect CB samples and discussed the CB project and management of the pest during an open field session for local almond growers.
26/7/2016	Select Harvests, Carina. Regular industry update meeting on Carpophilus with Ag Vic researchers and industry reps.
31/8/2016	Select Harvests, Carina Farm office. Project officers and field/technical industry staff discussed a Carpophilus trapping density trial planned for Carina in Nov 2016.
28/6/2017	Select Harvests, Carina. Regular industry update meeting on Carpophilus and other pest issues with Ag Vic researchers and industry reps.
29/6/2017	AVR Irymple. Project officers discussed 2016/17 trial results and plans for 2017/18 season with representatives of Olam and Almas separately.
5/3/2018	Select Harvests, Lake Powell. Project officer discussed Select's trap data and the project's nut sampling program.

Guidelines for A&K trap maintenance and sample management.

Tips for maintenance of traps and management of beetle samples for the Carpophilus project.
Mofakhar Hossain & David Madge, Dept. of Economic Development, Jobs, Transport and Resources, Vic.
Please feel free to contact Mofakhar (0417 500734) or David (0427 233692) with any queries on Carpophilus trapping.

Traps

Storing pheromone buttons and co-attractant. It is best to store new pheromone buttons in a freezer, or at least refrigerated to maximise shelf life. Co-attractant solution must be refrigerated (about 4°C) or it quickly loses its effectiveness.

Inspection routine. As far as possible, maintain a strict routine of trap inspections so that any particular trap is always checked on the same day of the week. This helps to maximise the efficacy of the traps as well as maintain the reliability of the beetle count data as an indicator of beetle activity through the season.

Trap marker. Traps will be easier to find if they are marked with some brightly coloured 'flagging' tape. Traps that are being used for sample collection for the Carpophilus project could be marked in a different colour as a reminder that the beetles in that trap need to be collected, not thrown out.

Food attractant. It is important to tip out any old food attractant before adding fresh liquid. The old liquid will have lost many of the compounds attractive to Carpophilus beetles, and would dilute the fresh liquid, making it less effective. The old liquid should not be tipped out in the orchard, but should be put into a container, and disposed of away from the orchard. Refill the tub weekly with 200-250 ml of new attractant. Ensure the new liquid is thoroughly mixed before use, by shaking the bottle well.

Pheromone lures/plugs. Replace pheromone lures fortnightly. Old lures can maintain some attractiveness to Carpophilus for a long period, so they should be left in the trap when new lures are added. Use extra wires or longer wires to hold the lures.

Insecticide strip. Store new insecticide strip in a cool, dry place, but not in a fridge used for food or drinks. Use gloves or forceps to handle new and old insecticide strip. Replace the strip every month and remove the old strips from the orchard.

Cleaning traps. It is desirable to keep traps clean by removing excess dust, spider webs etc with a brush or rag as necessary. Spider webs in particular can make traps less effective or ineffective by preventing the entry of beetles.

Beetle samples

Wind. The beetles are very light and can be lost in gusts of wind when a trap is open, and especially when you are pouring them out of the trap, so take care when it is windy. A funnel may help to protect the beetles from wind when you pour them from the trap into a measuring cylinder.

Other insects in traps. To improve the accuracy of beetle counts, large insects such as moths should be removed from the trap before the catch is poured into the measuring cylinder. This can be done easily with a pair of tweezers/forceps.

Record sheets. Traps that are to be used for sample collection should be highlighted on the record sheets as a reminder that the beetles from those traps should be saved for the Carpophilus project.

Beetle counts. Once a beetle catch has been measured, the number of beetles should be recorded before the beetles are discarded. This gives you a chance to double-check whether the beetles should be saved.

Labels. Each beetle sample needs to be labelled clearly with the Producer (company) name, farm name, block name/number, trap number and date. Pre-printed sticky labels can be provided to make this easier. Please make sure that you write all the necessary information on the label.

Zero counts. If a trap contains no Carpophilus beetles, write 'zero' on the sample label and include the label with the other samples. This is important to allow us to differentiate between zero counts and missed counts.

Esky. In warm weather, a cold esky will help to keep beetle samples in good condition.

Sample storage. At the end of the day, the labelled beetle samples should be stored in a coolroom or fridge, not a freezer. Frozen samples break down more rapidly when they thaw and become more difficult to handle and identify.

Steering Committee: Terms of reference

The Project

Purpose

The Project 'Management of Carpophilus beetle in almonds' aims to provide the Australian almond industry with improved knowledge and expertise in management of Carpophilus beetle, and with a cost-effective alternative to pesticides for management of this pest.

Background

Carpophilus beetle was discovered damaging new season almond kernels in Victoria in 2013 and in 2014 caused damage serious enough that growers, with guidance from the Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Victoria, began using an attract and kill (A&K) system developed for Carpophilus management in stone fruit by DEDJTR in the early 2000's. The almond industry and DEDJTR collaborated in preliminary trapping studies and nut assessments in 2014/15 that confirmed the presence of Carpophilus beetle across almost 70% of Australia's almond plantings.

Preliminary studies conducted in 2014/15 suggest that at least four species of Carpophilus are associated with almonds in Australia. The stone fruit A&K system caters for three of these. The fourth species, the one most closely associated with almond kernel damage so far, has not yet been fully identified. The effectiveness of the current A&K system against the fourth species is not known.

The Project will assess the efficacy of the current A&K traps for reducing the Carpophilus population in almonds to levels that provide cost-effective control. If the 4th species proves to be a significant pest not controlled by the current A&K traps, a contract variation will be sought to develop improved traps. The Project will also perform preliminary screening of potential pesticide options for management of Carpophilus beetle.

The Steering Committee

Purpose

The Steering Committee will provide input and guidance to the Project on the behalf of key stakeholders, to assist the Project in meeting its stated objectives.

The Committee may also make recommendations to the Project and stakeholders regarding variations to the original Project contract.

Objectives

The objectives of the Committee are to:

1. Review progress of the Project's activities and outputs against contracted milestones.
2. Raise relevant issues on behalf of the Project's stakeholders more broadly.
3. Assess these and other issues as they arise within industry or the Project and consider how the Project may be affected by or take advantage of those issues.
4. Consider the Project activities as originally contracted, and potential new areas of research on Carpophilus beetle, and propose contract variations if they are deemed necessary to maintain the Project's relevance to the almond industry.
5. Assist with the dissemination of information from the Project to the broader almond industry.

Membership

The Committee shall comprise a minimum of:

- One representative from the Almond Board of Australia
- Three representatives from the project research team
- Three representatives from the almond production/processing industry
- One representative from HIA

- Current membership is given in Appendix 1

Chair

The Committee shall appoint a Chairperson whose role will be to manage the Committee meetings, facilitate discussion and ensure Committee decisions are enacted.

The Chairperson may delegate Committee roles and responsibilities as required.

Meetings

Steering Committee meetings shall be held at least two times per year, but may be held more often if that is considered necessary or desirable. Meeting may be either face to face or via electronic media such as teleconferencing or skype. An update report covering project progress will be issued to all members prior to the meeting.

Records

At each meeting, a DEDJTR member of the Committee will be appointed to record the minutes of the meeting and to forward the minutes, within two weeks after the meeting, to Committee members for revision and approval.

Appendix 1

Composition of the Committee as of 23/02/16

Name	Organisation/Company	Position
Mofakhar Hossain	Dept. of Economic Development, Jobs, Transport and Resources Victoria	Chair
David Madge	Dept. of Economic Development, Jobs, Transport and Resources Victoria	Member
Greg Baker	South Australian Research and Development Institute	Member
Ross Skinner	Almond Board of Australia	Member
Ben Brown	Select Harvests	Member
Robert Wheatley	Olam Orchards	Member
Troy Richman	Almas Almonds	Member

Steering Committee: Minutes of meetings.

Management of Carpophilus beetle in almonds - HIA project AL15004

Record of proceedings - Steering Committee meeting: April 20, 2016

Meeting commenced 1:23pm at DEDJTR's, Irymple office.

Present

Mofakhar Hossain(DEDJTR, Chair), David Williams (DEDJTR), David Madge (DEDJTR, Note taker), Ross Skinner (Almond Board of Australia), Troy Richman (Almas Almonds), Ben Brown (Select Harvests).

Apologies

Robert Wheatley (Olam Orchards), Greg Baker (SARDI), Brenda Kranz (HIA).

Proceedings

MH provided a summary of project progress to date, following the information in the printed report that had been provided to committee members.

Extensive discussion took place during and after the presentation, the key points, comments and questions from which are summarised below.

The industry members present were satisfied with the project's progress to date and emphasised that they would be happy to provide the project with any assistance necessary to help generate useful data.

The formal meeting closed at 3:00pm and was followed by informal discussions on Carpophilus beetle and carob moth until 4:40pm.

Actions arising from the meeting and discussions

1. Costings of insect damage.

Industry representatives to develop costings of the value of Carpophilus and carob moth damage to their companies (including costs of pest management and losses from kernel damage, resorting etc).

2. Monitoring/trapping video

RS to contact MH/DM by 31/5/16 to organise production of ABA video(s) demonstrating current best practice for monitoring/trapping.

3. Monitoring/trapping guidelines

MH/DM to review and update guidelines on Carpophilus monitoring and A&K by 31/8/16.

4. Next meeting.

MH to check project milestones and circulate a proposed date (early December if possible?) to fit in with milestone reporting.

Summary of key discussion points and questions

(Italics indicate project staff comments and responses to questions)

a) In a simple field test by a commercial orchard, more Carpophilus beetle (CB) were caught in traps high in the tree canopy where the crop load is heavier, compared to lower traps. How do CB population levels and crop damage levels relate to crop load?

b) Were CB larvae found during the processing of nut samples? Yes, and the larvae were found to feed on almond hulls. Would this have negative implications regarding the return of hulls to the orchard floor during or after harvest? Unlikely as those hulls would be finely chipped.

c) Nut sampling has focussed on Nonpareil but many CB have been found in Carmel. Will other varieties be checked? The project found large numbers of CB between the hull and shell of hull-split Carmel in spot-checks during and after the Nonpareil harvest, but very little kernel damage was observed in Carmel. Monitoring of CB in varieties other than Nonpareil will be considered during project planning for the 2016/17 season.

d) To account for possible impacts of environmental factors on CB behaviour and life cycle, the collection of trap and nut sample data as performed this past season needs to be repeated over several years as proposed. As an example, rainfall data shows that the 2015/16 season was significantly drier than the previous four seasons, and this may have influenced the response of CB to traps, or the mix of species active in orchards, in that season.

e) Carob moth (CM) was considered by some to be worse than CB this past season. Was this likely due to environmental factors or Attract & Kill trapping reducing CB levels in comparison to CB? A&K would be expected to take several years to bring the CB population levels down.

f) It was commented that CB issues in almonds seem to be less under sprinkler irrigation compared to drip irrigation. This may be due to sprinkler irrigated surface soil drying out between irrigations, while drip irrigation tends to keep soil (and nuts on the soil surface) continually moist (and attractive to CB?). This indicates that the combination of drip irrigation and windfall nuts would support the build-up of CB populations in the months before harvest, and would warrant sweeping and destruction of those windfalls to reduce the risk infestation of the new crop.

g) Taxonomic work to identify the Carpophilus species that is mostly associated with kernel damage is ongoing, and it is becoming clearer that it is unlikely to be C. dimidiatus. Where does the damaging species come from? - almond machinery is imported from many places and the industry does not want to risk importing more of the same or new pests. Is the species an issue in USA for example? The available information suggests that the suspect species is not known elsewhere as a crop pest. An additional species, C. maculatus is being found in trap samples from S.A., but its distribution in other districts is not yet known (samples not processed yet).

h) The cost of CB damage to the almond industry will be important information to gather, especially for use in funding applications. [see Action 1]

i) Thresholds and timing of hygiene for CB and CM would be useful to help plan orchard work programs. E.g. nut fall continues after winter hygiene shaking/sweeping. When should new windfalls be swept/destroyed to minimise their contribution to infestation of the new crop? CM develop within nuts, so nut removal and destruction before moth emergence in Spring would be useful. CB develop largely within nuts but it is assumed they exit the nut to pupate in the soil. Optimal timing for nut removal for CB management is not yet clear.

j) A rain event in January 2016 triggered hull rot, CB trap catches and nut damage, with higher damage levels in wetter areas. It is known that moisture and humidity is important for CB development, so this may help to explain that observation.

k) We need to be mindful of the source and quality of nut infestation/damage data. E.g. heavily damaged nuts (lighter, more brittle) can be 'lost' from the product stream during handling and processing, resulting in overall damage levels being underestimated, the later the data is collected along the production/processing chain. Some producers are collecting more data, starting at the orchard block level, to get better estimates of damage levels. It would be useful to do some structured intensive sampling (50/Ha?) to determine the minimum sampling density required for reliable data.

l) Where are the 'updated guidelines' mentioned in the project's Monitoring & Evaluation Plan? This refers to future training events and publications. The guidelines on CB monitoring and trapping available on the ABA website are current at this time. These guidelines and other relevant project outputs will be updated progressively as new information is developed, rather than waiting until the end of the project. Could the project contribute to ABA videos on 'current best practice' CB monitoring and trapping? Yes – ABA is to initiate contact on this. [see Actions 2 & 3]

m) Regarding testing of attractiveness of traps to CB, can this be done in the lab or just in the field? Initially wind tunnel trials would be used. Also, it will be important to know just what species 'near dimidiatus' really is, if work on pheromones is to progress.

n) Can we look at disinfestation of stockpiles? This is not within the scope of the current project but could be possible by working with processors and their own stockpiles. HIA has been approached to fund disinfestation of table grapes and summer fruit – almonds could be an addition to that work. Pest resistance to fumigants (a major issue in grains) needs to be addressed. Disinfestation needs controlled environment storage, not stockpiles which currently involve a mix of sealed bunkers, unsealed dirt etc. It is also important to know the source of infestations and timing of damage in crops, stockpiles, transport etc to save wasting time in disinfestation.

o) Concern was raised regarding the impact of Talstar® (Bifenthrin) on mites in almonds. The product is registered for CB management in almonds and the ABA has received feedback regarding significant issues with pest mites following its use.

p) The Steering Committee is required to meet twice each year. Early December was suggested for the next meeting and a tentative date will be proposed, timed to fit with reporting for project milestones. [see Action 4]

Management of Carpophilus beetle in almonds - HIA project AL15004

Record of proceedings - Steering Committee meeting: Feb 15, 2017

Meeting commenced 1:35pm at DEDJTR's, Irymple office.

Present

Mofakhar Hossain(DEDJTR, Chair), David Madge (DEDJTR, Note taker), Brenda Kranz (HIA via telephone), Greg Baker (SARDI), Zubair Shahzad (Olam Orchards); Troy Richman (Almas Almonds), Ben Brown (Select Harvests).

Observers: Tim Kennedy (Select Harvests), Abby White (Almas Almonds), Geoff Furness (SARDI contractor), Brett Rosenzweig (Almond Board of Australia),.

Apologies

Ross Skinner

Proceedings

Introductions and previous minutes

Everyone present briefly introduced themselves and their role in the project, after which the minutes of the previous meeting were reviewed and accepted by the committee.

Costings of insect damage. Some costings of insect damage were developed with industry data for the 2015/16 season. Better estimates of overall losses in crop value, increased management costs and potential savings from improvements in pest management would be valuable to inform industry and support future research proposals. DM is to approach industry for more detailed damage/management/processing cost data for the 2017 harvest.

Monitoring/trapping video. This production is running behind time but should be completed by the end of March 2017.

Monitoring/trapping guidelines. The guidelines were reviewed, and a revised draft produced by early August 2016, but further amendments and delays in the approval process meant that they were not finalised for publication until November 2016. The revised guidelines were posted to the Almond Board website on 1/3/2017.

Next meeting. To permit more effective reporting of seasonal results, the proposed timing for this meeting (early December 2016) was altered to February 2017 with approval from Committee members.

Report on project progress to date

MH provided a summary of project progress to date, following the update report that had been emailed earlier to committee members. The following points were raised directly in relation to the progress report:

- GB-There were some distinct peaks in trap catches of Carpophilus beetle (CB) during the season – do these peaks indicate a temperature effect? MH-That is possible as CB activity is very dependent on environmental conditions such as wind and temperature.
- GB-It is surprising that very few mummy nuts on trees were found infested with CB over winter.
- TR-Was any sampling done of pollinator varieties to see if they are infested by CB at the same split stage as Nonpareil? DM-Not to date, because of the workload involved with Nonpareil sampling and processing. We will attempt to sample pollinators if time permits.

Discussion (summary of key points and questions)

(Italics indicate project staff comments and responses to questions)

a) ZS-The A&K system currently in use was designed for other CB species. Can work be done on C. near dimidiatus (C.nr d; the species actually causing kernel damage in almonds). BB-Can any desktop or lab studies be done to fast-track potential attractants for C.nr d? Would this work be within the scope of the current project? MH-We haven't had an in-house chemist until now. We did collect almond volatiles for this purpose last season but couldn't have the analysed. We are running lab cultures of C.nr d for pesticide testing, and these could be used for attractant testing. We have a wind tunnel at Tatura and will soon have a new facility at Bundoora, however testing of potential new attractants is not covered by the current Carpophilus project budget.

b) BB-Where would the project want to go next? MH-Continue with the current season's work and meet later (June?) to consider changing direction. TR-delaying a possible change in research direction until June would not allow time for potential new attractants to be available for field testing early next season.

c) MH-We have a commitment to complete the pesticide assessments first. BK-The project should make sure that any chemistries being assessed are the most appropriate for potential registration in almonds to avoid wasting any effort.

d) DM-Would the industry put highest priority on assessing pesticides or investigating potential better coattractants? ZS,TR,AW,BB-coattractant is most important. BK-If the industry priority is coattractant, this needs to be communicated to her.

e) MH-The new wind tunnel at Bundoora is not yet available (maybe late April). The DEDJTR chemistry group will meet next week and can discuss the wind tunnel, CB attractants and other work relevant to the CB project. GB-SARDI has a wind tunnel that could be used if needed to speed up testing of attractants.

f) GF-What is the position regarding CB pheromones? MH-The Tri-lure currently used in A&K traps attracts several species including C.nr d. At this stage it is probably better value to look at improving the coattractant rather than investigating a new pheromone. Also, DEDJTR has a PhD student investigating the impacts of fungi on CB, looking at potential attractants. That project could possibly include C.nr d (It currently does not because the PhD project requires a target species that is relatively well known, and very little is known about C.nr d).

g) BK-The project should determine what it can do (re: attractant chemistry) in the short-medium term; get a proposal to industry for comment, and then to BK for comment; then renegotiate the contract if necessary.

h) DM-For your info, the DEDJTR chemistry group is working on lures for carob moth, CB, fruit fly and codling moth, with the aim of collecting preliminary data to support a cross-industry project proposal on lures and A&K systems.

i) DM-Given the industry's priority for work on CB attractants, we should probably collect almond volatiles for analysis very soon while unsplit nuts are still available, otherwise we'll have to wait another year for the opportunity.

Actions arising

a) DM is to approach industry for more detailed damage/management/processing cost data for the 2017 harvest.

b) BR to complete the monitoring/trapping video by 31/3/2017

c) MH to discuss the wind tunnel and possibility of short-term work on coattractants at the next DEDJTR chemistry group meeting and coordinate a proposal for industry to consider regarding a contract variation (if necessary) to shift the focus from pesticide assessments to work on attractants.

d) DM to organise collection of almond volatiles or samples for volatiles extraction asap.

Next meeting

MH proposed that the next meeting be held at the DEDJTR AgriBio complex in Bundoora, Melbourne in June/July 2017. All except one Committee member would be able to attend and that member would send a representative. Tentative dates for this meeting will be sent to the Committee later.

Close

The meeting closed at 3:06 pm.

Management of Carpophilus beetle in almonds - HI project AL15004

Record of proceedings - Steering Committee meeting: Aug 2, 2017

Meeting commenced 11:33am at DEDJTR, Agribio, Bundoora.

Present

Mofakhar Hossain (DEDJTR, Chair), David Madge (DEDJTR, Note taker), Ross Skinner (ABA CEO), Zubair Shahzad (Olam Orchards); Abby White (Almas Almonds, for Troy Richman)

Observers: Paul Cunningham (DEDJTR), David Williams (DEDJTR), Daniel Lai (DEDJTR), Maisy Bennett (student), Solange Camilleri (student).

Apologies

Brenda Kranz (HI), Greg Baker (SARDI), Ben Brown (Select Harvests).

Proceedings

Introductions and previous minutes

Everyone present briefly introduced themselves and their role. The minutes of the previous meeting were then reviewed and accepted by the committee, after discussion on the following 'actions arising':

DM is to approach industry for more detailed damage/management/processing cost data for the 2017 harvest. Some of this data is already in hand. Access to further data is being organised (pending a 'commercial-in-confidence' agreement).

BR to complete the monitoring/trapping video by 31/3/2017. Done.

MH to discuss the wind tunnel and possibility of short-term work on co-attractants at the next DEDJTR chemistry group meeting and coordinate a proposal for industry to consider regarding a contract variation (if necessary) to shift the focus from pesticide assessments to work on attractants. A variation was agreed to, and work on co-attractants, including use of the new wind tunnel facility, will begin shortly.

DM to organise collection of almond volatiles or samples for volatiles extraction asap. Samples of split and unsplit nuts were collected the week following the meeting, and were used for preliminary volatile extractions at AgriBio, Bundoora.

Report on project progress to date

MH provided a summary of project progress to date, following the update report that had been emailed earlier to committee members.

Discussion (summary of key points and questions)

(Italics indicate project staff comments and responses to questions)

a) ZS-If the damaging species of carpophilus only increases in number in traps in Dec/Jan is there any value in trapping from as early as August? MH-We have only one season of good data to see this trend. It may be risky to shorten the trapping period without knowing if the trend is consistent from year to year, although it would allow for concentration of the trapping effort around the critical period leading up to hull split. We cannot make any recommendation on a single year's results. Seeing the same result over 2-3 years would give us confidence to recommend a shorter, more concentrated trapping effort.

b) DW-Does anything stop A&K being applied from early until after harvest, apart from cost? AW/ZS-That is what industry is currently doing. DW-The experience with A&K in stone fruit was that it took about four years to reduce beetle populations to acceptable levels.

c) RS-Given the big focus on hygiene, is flail mowing the most effective way to destroy nuts on the ground? DM-we plan to assess this within the next few weeks. We also hope the IDM project delivers more effective mummy prevention through improved disease management. AW-How does the rotary hoe compare with flail mower or slasher for nut destruction? We do not know yet. How deep would nuts have to be buried to prevent adult survival/emergence? Experience in stone fruit is that deep burial (1m?) would be required.

d) AW-Regarding the data on kernel damage from samples 'near' and 'far' from A&K traps, how long were the nuts on the ground before collection? She has noticed rapid changes in damage levels if nuts are left lying on the ground for even short periods (hours?). We tried to harvest asap after tree shake and had to wait for shakers in some instances. [extra note: we will keep this in mind for the next harvest and will aim to collect sequential samples from the same area to quantify changes in damage levels over time before pick-up]

MH also outlined the future research plans being built into a project proposal for Almond IPM, including:

- Improved orchard hygiene

- Biology, phenology and chemical ecology of the damaging species C. nr dimidiatus
- Identification of better A&K co-attractants
- Opportunities to adjust components of the current food attractant
- Identification of the pheromone for C. nr dimidiatus
- Better formulations of lures (encapsulated, bubble lure etc.) to extend field life and reduce maintenance costs

Actions arising

No specific actions for follow-up were flagged at the meeting.

Next meeting

A meeting date was not set. Tentative dates will be sent to the Committee for consideration.

Close

The meeting closed at 12:50 pm.

Final report: Management of carpophilus beetle in almonds
Project code: AL15004

Appendix 9. Monitoring and Evaluation

Monitoring & Evaluation Plan **HIA Project AL15004** **2015/16 – 2017/18** **Management of Carpophilus beetles in almonds**

	Measures	Evaluation method	Reporting
Longer-term outcomes (timing dependent upon medium-term outcomes)			
"Less than 10% of orchard blocks will have crop quality downgraded due to Carpophilus damage"	% of blocks downgraded due to Carpophilus damage[1]	• Quality assessment data from processors	
Medium-term outcomes (up to 5 years post-project)			
"Industry will have access to a cost-effective alternative to pesticides for Carpophilus management"	Access to the effective and improved management practices for use of A&K system	• Interview with commercial agents/suppliers	
	% of producers aware of the above system % of producers using the alternative to pesticides	• Industry survey • Interviews with producers	
Short-term outcomes (by project completion)			
"Industry will have improved knowledge and expertise in management of Carpophilus beetle"	% of producers aware of Carpophilus as an almond pest % of producers aware of the existence of multiple Carpophilus species which may pose different levels of risk to almonds % of producers aware of updated guidelines for management of Carpophilus	• Industry survey • Interviews with producers	HIA Final Report
Activities and Outputs (during project)			
Prepare diagnostic tools	No. of species included in descriptive tools	• Preparation of draft publication • Data and project documentation • Technical papers	HIA Milestone and Final Reports
Determine the species complex, distribution and behaviour	No. of orchards represented by trap samples No. of trap samples analysed for species composition No. of weekly nut samples collected and assessed		
Determine attraction of current A&K system	No. of traps established for field trials No. of trap samples analysed for species composition		
Investigate the optimal spatial distribution of A&K traps	No. of orchards and traps represented in spatial analysis of trap data		
Investigate alternative hosts for Carpophilus	No. of orchards/potential hosts sampled		
Assess pesticide options	No. of pesticide options tested in laboratory		
Inform industry	No. of awareness/training sessions on monitoring protocols with industry staff No. of fact sheet publications/revisions No. of presentations to industry meetings/conferences No. of technical articles drafted		

[1] Baseline data relating to % damage levels in orchard blocks and % blocks affected by CB will be collected during the project, using industry quality assessment reports.

Management of carpophilus beetles in almonds: HI Project AL15004 Monitoring & Evaluation producer interview

Date:________________ Interviewee:____

1. What area (ha) of almonds do you manage in relation to pest control?	
2. As far as you know, what are the serious pests of almonds in Australia? (may or may not be a pest in your area/orchard)	CB CM Mites Aphids Birds
3. If CB not mentioned…Have you heard of CB in almonds? (memory trigger)	No ➔ Q7
4. When did you first become aware of CB being a pest in almonds?	June/13 R&D Forum Apr/14 Crisis meeting Carwarp June/14 R&D Forum Oct/14 Orchard walks Oct/14 ABA Conf Glenelg Oct/15 R&D Forum
5. Briefly, what do you know about management of CB?	Traps: monitor/A&K Hygiene Spray Fumigate
6. Without trying to remember any names, what do you understand about there being different species of CB and their association with damage to almonds?	Several spp in orchards One spp causing damage
7. Can you tell me where you got your information on CB and its management?	ABA staff ABA website/factsheets R&D Forum Conf Orchard walks AgVic project direct Internet Consultant
8. Where would you go for the latest information on pest/CB management? (Most recent guidelines?...)	ABA staff ABA website/factsheets AgVic project direct Internet Consultant

Table 2. Producer interviewees

Ben Brown	GM Horticulture, Select Harvests, SA, VIC, NSW
Zubair Shahzad	Manager, Technical Services, Olam Orchards Australia
Mary Cannard	Agronomist, Olam Orchards Australia, VIC
Abby White	Technical Manager, Almas Almonds, VIC
Peter Ryan	Agronomist/Farm Manager, Brownport Almonds, VIC
Scott McKenzie	Technical/Operations Manager, Century Orchards, SA
Robert Cox	Orchard Manager, Amoretto Foods, VIC
Narender Pathania	Rmonpro Developments,VIC
John Kennedy	Agronomist, Lacton Pty Ltd, SA
Brian Slater	Orchard Manager, Aroona Farms, VIC
Kelvin Trezise	Orchard Manager, Aroona Farms, SA
Tim Preusker	CMV Farms, SA
Peter Freeman	Producer, SA
Neale Bennett	Producer, VIC
Philip Costa	Producer, SA
Dean Dinicola	Producer, NSW
Paul Rossetto	Producer, NSW
Jim Belehris	Producer, SA
Peter Cavallaro	Producer, SA

Table 3. Awareness of management options amongst interviewed producers

	% of producers	% of producers in high risk CB areas	% of total bearing area
Monitoring traps	42%	56%	85%
A&K traps	63%	67%	92%
Orchard hygiene	84%	100%	98%
Pesticide	53%	67%	89%
Earlier harvest	11%	22%	26%
Minimise hull rot	11%	22%	29%

Table 4. Sources of producer technical knowledge on carpophilus

	% of producers	% of producers in high risk CB areas	% of total bearing area
Ag Vic project direct	42%	67%	86%
ABA website/factsheets	32%	22%	52%
Industry peers	16%	22%	11%
ABA R&D Forum	42%	22%	9%
Stonefruit growers	5%	11%	6%
ABA staff	32%	11%	5%
ABA Almond Conferences	11%	11%	2%
Almondco agronomists	11%	0%	2%
ABA orchard walks	11%	11%	1%
Internet	5%	11%	1%
Corporate seminars	5%	11%	2%
Consultants/agronomists	5%	0%	0%
Nutgrower Magazine	5%	0%	0%

Activities and Outputs

The data in the following numbered sections, relating to project activities and outputs, align with the Project's seven major research components as detailed in the project proposal.

1) Prepare diagnostic tools
    a) No. of species included in descriptive tools
        i) Four species are included in the adult and larval identification keys. The species were selected based on their prevalence in almond orchards, and to date are the species most likely to be encountered by almond growers and their agronomists/advisors.
2) Determine the species complex, distribution and behaviour
    a) No. of orchards represented by trap samples
        i) The regions (SA Riverland, VIC Sunraysia, NSW Riverina) and numbers of orchards represented in trap samples analysed during the project were 2015/16: SA 3, VIC 7, NSW 1; 2016/17: SA 3, VIC 8, NSW 1;

2017/18: SA 1, VIC 8, NSW 1. This geographical spread of orchards allowed the project to gather valuable information on the distribution and species mix of carpophilus between regions while focussing most effort on the district suffering the greatest impact from the pest (Sunraysia).

b) No. of trap samples analysed for species composition

i) The numbers of traps sampled for beetle analysis during the project were 2015/16: SA 15, VIC 51, NSW 3; 2016/17: SA 15, VIC 51, NSW 6; 2017/18: SA 3, VIC 51, NSW 0.

c) No. of weekly nut samples collected and assessed

i) 2,700 new crop samples over 16 weeks (Dec 15-Mar 16, pre-hull split to harvest); 704 mummy nut samples (512 VIC, 192 SA, Jun 16-May 17); 246 new crop samples (Dec 16-Mar 17, pre-hull split to harvest), 576 mummy samples (384 Vic, 192 SA, Jun 17-Apr 18).

3) Determine attraction of current A&K system

a) No. of traps established for field trials

i) For the three years of field work, 133, 139 and 87 industry traps were identified for sample collection and analysis. Those traps were serviced, and samples collected, by orchard field staff. The lower number in the final year reflects a commercial decision by one producer to minimise input into A&K and instead focus resources on orchard hygiene.

b) No. of trap samples analysed for species composition

i) The numbers of trap samples used for beetle analysis during the project were 2015/16: SA 15, VIC 51, NSW 3; 2016/17: SA 15, VIC 51, NSW 6; 2017/18: SA 3, VIC 51, NSW 0.

4) Investigate the optimal spatial distribution of A&K traps

a) No. of orchards and traps represented in spatial analysis of trap data

i) 106 A&K traps (54 project traps supplementing 52 industry traps) were established in one contiguous area of a single orchard in 2016/17, to allow for spatial distribution analysis of trap catches.

5) Investigate alternative hosts for Carpophilus

a) No. of orchards sampled

i) A detailed manual inspection of vegetation and leaf litter for carpophilus beetles was performed in one orchard.

b) No. of potential hosts sampled

i) 45 species of fruit including Australian natives were collected and assessed for the presence of carpophilus as part of a survey of potential fruit fly hosts.

6) Assess pesticide options

a) No. of pesticide options tested in laboratory

i) Initial screening of one pesticide commenced in 2016/17, but was halted due to a contract variation.

7) Inform industry

a) No. of awareness/training sessions on monitoring protocols with industry staff

i) Four training sessions for industry trap maintenance staff were held at the start of the 2015/16 season (7 orchard field staff), and three for the 2016/17 season (9 staff). These staff were responsible for servicing traps across the majority of orchard area in the Victorian Sunraysia district. Training for the 2017/18 season was managed by producers in-house.

b) No. of fact sheet publications/revisions

i) One fact sheet 'Carpophilus beetle: Preliminary guidelines – Monitoring and Attract and Kill' was produced by the project team nine months prior to the start of Project AL15004, to assist producers who were keen to commence a trapping program. Those guidelines, published as an ABA 'All about almonds' fact sheet, were subsequently updated for the 2015/16 and 2016/17 seasons.

c) No. of presentations to industry meetings/conferences

   i) Information on current research and management options for carpophilus as an almond pest was presented formally to three ABA R&D Forums, one ABA almond industry conference, one international symposium on almonds and pistachios, three corporate producer pest management workshops, two general grower information sessions, a series of orchard walks and one ABA Production Committee meeting. Informal presentations and discussions were also held at over 20 additional meetings with producers and producer groups (Appendix 8).

d) No. of technical articles drafted

   i) None to date.