Horticulture Innovation Australia

Final Report

Managing Carob Moth in almonds

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Contents

Media Summary

After three years of field and laboratory research preceded by a year of population monitoring, we have gained a good understanding of carob moth as a pest of Australia's multi-million dollar almond crops. Almond growers from Griffith to Adelaide contributed to the effort by maintaining traps for the project in their orchards. Carob moth breeds in old (mummy) nuts and lays eggs on new nuts when their hull splits, resulting in significant economic loss in some seasons from chewing damage to the kernels.

While monitoring carob moth to track its distribution and seasonal behaviour, we also investigated:

- **Options for applied controls**. We confirmed that insecticide application at hull split reduces the level of kernel damage but that this may not be cost-effective in all seasons. The optimum timing and application rates of insecticide need to be clarified as current use is based on USA recommendations for control of a related almond pest, the navel orangeworm. We also demonstrated the potential for pheromone-based mating disruption of carob moth in almonds but more work is required to clarify the efficacy and relative cost-effectiveness of this approach.
- **Associations between carob moth and mummy nuts**. We found a relationship between mummy nut populations and levels of crop damage, and continually reinforced the message that orchard sanitation is likely to always be the key to carob moth management. We believe that a serious effort needs to be made to understand the mechanism of mummy nut development and develop options to prevent or effectively remove those nuts.
- **Timing of egg laying and kernel damage**. Almonds require protection from infestation for almost the entire hull split-harvest period which is beyond the scope of single applications of the pesticides currently available for use in the crop. Wherever possible, producers and processors should consider options for improving the timeliness of their critical operations between hull split and processing, as the risk of damage by carob moth increases with every delay.
- **Female attractants**. No lure for female carob moth is currently available. We screened potential baits and found one more attractive than almond mummy nuts. This deserves more detailed investigation.

Fact sheets describing carob moth, its seasonal behaviour in almond orchards, monitoring guidelines and current management options are available from the Almond Board of Australia web site. A pocket guide to carob moth is also being produced for almond growers.

Technical Summary

Carob moth *Apomyelois* (=*Ectomyelois*) *ceratoniae* breeds in old (mummy) almond nuts and oviposits on new nuts when their hull splits. In some seasons and districts, carob moth causes significant economic loss from chewing damage to the kernels. To address the issue of carob moth, the almond industry commissioned a monitoring program in 2011/12 and a research project from 2012/13-2014/15.

Delta traps with a carob moth sex pheromone mimic lure (ISCAlure-Ceratoniae™, ISCA Technologies Inc., Riverside, California USA) were used to monitor male moth activity in orchards between the Adelaide Plains and NSW Riverina. Carob moth was recorded in most districts except the Adelaide Plains and most heavy infestations were located in large orchards in the S.A. Riverland and Sunraysia region of Victoria. Smaller orchards tended to show very little or no sign of carob moth activity, possibly because of the absence or very low numbers of mummy nuts in those orchards, due anecdotally to removal by birds. Three generations of moth activity tend to occur each season. The spring emergence typically begins in early to mid-September and lasts about three months. The second generation peaks soon after almond hull split – the point at which nuts become susceptible to oviposition by carob moth.

We used replicated field trials with 2-10 ha plots to investigate mating disruption with the sex pheromone mimic (SPLAT-EC®, ISCA Technologies Inc., Riverside, California USA), and hull split insecticide application (Altacor®, 350 g/kg chlorantraniliprole; Du Pont™) as applied controls for carob moth. We confirmed that insecticide application at hull split reduces the level of kernel damage but showed that this may not be cost-effective in all seasons. We used manual infestation of nuts to show that oviposition as late as three weeks before harvest can lead to a significant level of kernel damage. Almonds therefore require protection from infestation for almost the entire hull split-harvest period which is beyond the scope of single applications of the pesticides currently available for use in the crop. We also demonstrated the potential for pheromone-based mating disruption of carob moth in almonds but more work is required to clarify the efficacy and relative cost-effectiveness of this approach.

Through surveys of mummy nuts and nut damage assessments we found a positive relationship between mummy nut populations and levels of crop damage, and reinforced the message that orchard sanitation is likely to always be the key to carob moth management.

A lure for female carob moth is not available but would be a valuable management tool. We used choice tests with a caged carob moth populations to screen potential baits and found one that appeared more attractive than almond mummy nuts. This deserves more detailed investigation to determine the likelihood of it leading to development of a female lure.

The key drivers of mummy nut development and retention need to be determined, as do options to minimise the development and maximise the removal of those nuts. Addressing the issue of mummy nuts will have broader implications than just carob moth management, as the nuts are also utilised by carpophilus beetle (also causing almond kernel damage) and act as a source of inoculum for important almond diseases.

The timeliness of crop management operations from hull split to processing should be assessed and optimised wherever possible. The risk of significant increases in economic loss from carob moth damage grows with any delay in harvest or post-harvest disinfestation or processing.

The optimum timing and rate of chlorantraniliprole applications need to be determined. Australian usage is currently based on USA recommendations for navel orangeworm. Where chlorantraniliprole-based insecticide is used routinely, monitoring should be undertaken to detect and address any potential longer-term impacts of its use on beneficial invertebrates in the almond agro-ecosystem. The optimum method of use of SPLAT-EC® in almonds needs to be clarified. Mechanical application also needs to be assessed and demonstrated, as that will determine the economic viability of this approach. Risk assessment tools including economic thresholds need to be developed to assist commercial decision-making regarding the likely value of any applied controls in any particular season.

In the event that carob moth continues to pose a threat to the almond industry because the issue of mummy nut management cannot be adequately addressed, the sterile insect technique (SIT) could be worthy of consideration, given Australia's experience with that approach for fruit fly suppression.

Introduction

Background

Almonds are an attractive crop in Australia because the industry is considered to be profitable, stable for long term production and amenable to mechanisation to reduce management costs. The Australian almond industry has reflected this by expanding rapidly over recent years, from almost 4,600 ha in 2000 to 28,967 ha in 2014. The almond industry has become one of Australia's more significant horticultural industries and is the most valuable horticultural export industry, with export sales of $445m in 2014.

The high value of almonds relies upon high quality of the kernels and any factor that damages almond kernels directly affects the value of the crop. Insect damage in particular is considered as a serious defect, and the quality grades commonly used by industry (USDA 1997) have a low tolerance for kernels with such damage (1% for top grade whole kernels; 2% for other whole kernels).

Leading up to the 2011 harvest, carob moth *Apomyelois* (=*Ectomyelois*) *ceratoniae* which has been a long-time minor pest of almonds in Australia, was noticed as causing significant kernel quality issues for the industry. Carob moth is considered to be of Mediterranean origin but has become a pest of numerous fruit and nut crops around the world. Its larvae feed on almond hulls and kernels, reducing the kernel value to processing only, or at worst rendering the kernels unfit for human consumption. Apart from the lost value of the damaged kernels themselves, the presence of insect damaged kernels can reduce the quality grading of whole batches of nuts, resulting in greater economic loss.

Mummified nuts (mummies) that stay on trees after harvest are an important food resource for carob moth and it is possible that the growth in populations of the pest in recent years was associated with an increase in numbers of mummies in orchards. Mummies often develop as a result of hull rot, a fungal disease that develops on almonds once the hulls have split and is favoured by warm, wet conditions. Such conditions occurred across our major almond growing districts soon after hull split in 2007 and 2011. At the same time, the number of bearing trees in the industry was growing rapidly, doubling between 2004 and 2007 and more than doubling again to 2011. The exponential growth in nut production combined with favourable conditions for hull rot is likely to have resulted in very significant increases in mummy numbers and associated carob moth populations across the affected districts.

Industry response

When producers recognised carob moth as causing significant kernel damage (up to 15% in some cases), concerns were raised regarding the lack of effective management options for the pest. In anticipation of a demand for control options for the following season, the Almond Board of Australia (ABA) obtained an emergency use permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA) to allow the industry to apply insecticide against the pest. Concerns regarding the potential for significant kernel damage led major almond producers to treat large areas of orchard with insecticide to protect the 2011/12 crop. Prior to this, there had been little need for lepidopteran pesticides on almonds and none of the pesticides already approved for use on almonds were suitable for carob moth management. The only registered products at that time were a mating disruption pheromone for codling moth and oriental fruit moth and spray oil for mites. Minor use or emergency use permits were also in force for pesticides against mites, aphids and plague locusts.

In 2011, HAL project AL11009 ‘Food safety in almonds – Stage 2’ began to investigate the biology and management of fungal contamination of almonds as part of the industry’s response to a scoping study on that issue (Food Safety in Almonds Final {Interim} Report for Scoping Study, HAL Project No. AL09027). A preliminary monitoring program for carob moth was incorporated into that project because of the concerning levels of kernel damage caused by that pest and the potential role of that damage in providing sites for fungal infection. The ABA also requested that a project be developed to investigate carob moth as a pest in its own right, to generate local knowledge and begin developing management strategies for the almond industry. That project (AL12004 ‘Managing carob moth in almonds’) is the subject of this report.

Aims of the research

The aim of project Al12004 was to begin developing an effective management program for carob moth in almonds by developing a good understanding of the species as an almond pest, developing and evaluating strategies to minimise nut infestation (e.g. by suppressing carob moth’s population and/or preventing egg

laying), and informing industry of 'best bet' practices for management of the pest. These aims were embodied in the following five main components of the project:

a) Understand the seasonal phenology of carob moth on almonds in Australia
b) Evaluate mating disruption as a management tool for carob moth in almonds
c) Determine the impact of mummy nut removal on carob moth
d) Determine the optimum timing for and likely off-target impact of ovicide/larvicide applications used to prevent almond kernel infestation by carob moth
e) Inform the almond industry

As part of component a), this project continued the monitoring and grower-maintained trapping program that was initiated in 2011 under Project AL11009.

Contract variations approved during the project:

Component c) was varied to replace the mummy removal trial with a trapping trial as well as field assessments of mummy population density, to relate mummy densities to moth trap catches and kernel damage levels. The mummy removal trial originally proposed was made economically unviable when it was determined after the first season of trials that plot size would have to be increased significantly. The resultant increase in trial cost could not be accommodated within the project budget.

Component d) was varied to remove the bioassay-based assessment of the impact of insecticide on beneficial species. A significant amount of research on this subject in relation to the chemistry used against carob moth had been published since the start of this project, making this aspect of the project redundant. In place of the bioassays, a review of the relevant insecticide impact research was produced and more effort and resource was directed to additional field trials on mating disruption and moth behaviour, the need for which had already been identified.

Implications for industry & likely impacts of results

During this project we developed a good foundation of local knowledge of carob moth as an almond pest in Australia, a prerequisite to developing an effective integrated management program for the pest.

In terms of reduced kernel damage, our replicated field trials showed some benefit from one-off pesticide applications at hull split, as used by some producers, but a simple analysis suggested these sprays were not cost-effective in two of our three trial seasons. This indicates a need for the ability to forecast seasonal risks of carob moth damage, to allow producers to gauge the likely benefit of applied control in any particular season. The cost/benefit of pesticide application will very likely be different where repeat applications are used over several seasons, and this warrants some analysis. Our trials also provided 'proof of concept' and indicated the potential of mating disruption against carob moth in almonds. However, details of application methodology still need to be clarified to allow for the rigorous testing and cost/benefit analysis of this approach that industry requires to make a commercial assessment of its viability as a control option. The last season of trials was intended to achieve this but was unsuccessful due to a very low pest level and a technical failure.

Our data linking mummy population density to crop damage confirms our assumption (based on the navel orangeworm situation) and supports the industry's drive to improve orchard hygiene as a key component of carob moth management.

Preliminary screening of potential female attractants produced a promising lead which should be followed up with more detailed analysis to determine the possibility of developing a female lure for carob moth. This would be of great value for monitoring and research purposes.

Our reporting of *Trichogramma* parasitism of carob moth eggs sparked some interest amongst producers, and they would be keen to be involved in the assessment of biological control as an additional option for carob moth management.

The project also generated a greater awareness within industry of the key issues driving carob moth as an almond pest and the current 'best bet' approaches to management, especially with regards to orchard sanitation.

Literature review

Carob moth
Apomyelois (=*Ectomyelois*) *ceratoniae*
[LEPIDOPTERA: Pyralidae]

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September 2013

Introduction

Carob moth is a widespread pest of numerous commercial tree crops. It has been known as a pest in Australian almonds since at least the 1960's (Michael 1968) but seems to have become a significant concern for that industry only in more recent years (e.g. Sharp 2009). This recent rise to prominence is likely to have resulted from several factors combined. Firstly, between 2005 and 2010, the area of almond trees bearing crops in Australia grew exponentially from approximately 5,000 to 25,000 ha (ABA 2012) with over 80% located within the Sunraysia region of Victoria and Riverland region of South Australia. Secondly, higher than average rainfall in those regions after hull-split in January 2007 (over 2.3 times the long-term average) is very likely to have resulted in higher levels of hull rot, resulting in more 'mummified' nuts than usual remaining on trees after harvest. Such nuts are a major food resource for carob moth and this combination of increased prevalence of mummified nuts on a rapidly increasing number of trees would have provided the pest with an exponential growth in food resource. The problem was exacerbated by another period of heavy rain (around six times the long-term average) and high humidity around hull-split in January 2011.

To address increased industry concerns regarding carob moth, the Almond Board of Australia (ABA) commissioned a research project ('Managing carob moth on almonds', 2012/13-2014/15) to investigate options for managing the pest. This literature review is one product of that project and is intended to help inform research decisions within that project and contribute to the development of future research efforts on carob moth.

Notes:

Relatively little of the published literature on carob moth involves almonds as a host, as its pest status internationally has mostly revolved around date and pomegranate crops. Knowledge of its development and management on those crops is however still important, and can contribute to development of an effective management approach in almonds.

Some published literature proved difficult to obtain in full due to language or access issues. Where the abstracts of those papers included enough detail to be of value to this review, the references are included and noted as [Abstract only] in the bibliography.

Summaries of key details regarding the development rates of carob moth under various dietary and environmental conditions are provided in Appendix 1 for easy reference (e.g. in relation to culturing or developmental modelling).

Statements made without reference to published works are personal observations (P.O.) of the author of this review.

Background

Nomenclature

The moth *Ectomyelois ceratoniae* Zeller, in the Lepidopteran family Pyralidae, is commonly known as carob moth because of its infestation of carob pods. Other common names include date moth, knot-horn moth, blunt-winged moth and locust bean moth (Botha and Hardie 2004) and pomegranate neck worm (Mirkarimi 2002). Other notable Pyralid moths include Indian meal moth *Plodia interpunctella* and navel orange worm *Amyelois transitella*, the latter being the major pest of almonds in USA.

Ectomyelois ceratoniae appears to have been first described scientifically in 1839. Since then it has been known by several synonyms including *Spectrobates ceratoniae* [Zeller 1839] (Global Names Index 2013), *Myelois ceratoniae* [Zeller 1839], *Apomyelois ceratoniae* [Zeller 1839], *Phycis ceratoniella* [Fischer von Röslerstamm 1839], *Trachonitis pryerella* [Vaughan 1870], *Myelois tuerckheimiella* [Sorhagen 1881], *Euzophera zellerella* [Sorhagen 1881], *Phycita dentilinella* [Hampson 1896], *Hypsipyla psarella* [Hampson 1903], *Heterographis rivulalis* [Warren & N.C. Rothschild 1905], *Myelois oporedestella* [Dyar 1911], *Myelois phoenicis* [Durrant 1915] and *Laodamia durandi* [D. Lucas 1950] (ABRS 2009), as well as *Myelois pryerella* [Vaughan 1870](Aitken 1963).

In this review, 'carob moth' is generally abbreviated as 'CM'.

Description

Eggs: Soft, irregular shape but generally oval, approximately 0.7 mm long, rough textured glossy appearance, white when freshly laid turning deep pink when mature.

Larvae: Approximately 1 mm long when newly hatched, growing to 15-20 mm when mature. Colour varies between pale and dark pink, possibly depending on diet. Descriptions of microscopic diagnostic characters are available to identify CM larvae and differentiate them from similar pests such as Indian meal moth and navel orange worm (Aitken 1963; Eichlin 1983).

Table 1 lists head capsule sizes recorded for various larval instars of CM. Head capsule size is often used to determine instar, but the variability shown here within instars, due presumably to diet, suggests that before it is used for this purpose, head capsule size should be 'calibrated' to instar by close observation on the relevant diet.

Table 1. Carob moth larval head capsule width

Diet	Temp °C	RH%	Photo period	Larval instar							Reference
				1	2	3	4	5	6	7	
Sweetcorn+yeast	25	70 ±5	12:12	0.25	0.36	0.57	0.95				(Hung, Chiang et al. 2003)
Almond slices	25	70 ±5	12:12	0.25	0.32	0.44	0.58	0.78	0.95	1.05	
Date fruit				0.298	0.446	0.635	1.025	1.543			(Yaakoub 2011)

Pupae: Approximately 12 mm long, golden to dark brown. On their dorsal side, CM pupae have a dark raised 'keel' from the head to the base of the thorax, a double row of short spines along the abdomen and a pair of hooks on the end abdominal segment (Eichlin 1983). These characters separate CM pupae from those of navel orange worm and Indian meal moth.

Adults: Mottled grey in colour, approximately 12 mm long with a 20 mm wing span. A wavy line crossing the wings ⅓ the way down their length helps to differentiate carob moth from Indian meal moth with which it may be confused. Female genital morphology is apparently ambiguous but male genital morphology can be used to confirm the identity of the species (Moawad and Al-Barty 2009).

Origin & distribution

Carob moth is considered to have originated in the Middle East/Mediterranean region where it is currently a key pest of the major crops of dates, pomegranates and pistachios. It has also spread to become a pest of commercial crops in North and South America, southern Africa and Australia (Michael 1968; Eichlin 1983; Gonzalez and Cepeda 1999).

Genetic variability

As occurs with many other insect species, a high level of genetic variability has been found within CM populations and between geographically different populations. This gene pool may provide CM with opportunities to develop 'new phenotypes or behaviours' that could assist it in adapting to new situations by, for example, exploiting new hosts or adopting different overwintering sites (Mozaffarian, Mardi et al. 2008). These strategies could help CM adapt against applied control measures to some extent.

Host plants

Carob moth has been recorded to infest a wide range of host plant species including commercial crops and ornamentals as listed in Table 2 (Swezey 1923; Anon 1938; Heinrich 1956; Sonda 1963; Carrero 1966; Tokmakogiu, Soylu et al. 1967; Michael 1968; Calderon, Navarro et al. 1969; Gothilf 1970; Balachowsky 1972; Wysoki 1977; Dhouibi 1982; Eichlin 1983; Botha and Hardie 2004; International Atomic Energy Agency and Food and Agriculture Organization of the United Nations 2008; Nay and Perring 2008a; Yaakoub 2011). In some cases such as grapes, the infestation involved the dried or mummified fruit.

Table 2. Host plants recorded for carob moth.

Family	Scientific name	Common name
Anacardiaceae	*Pistacia vera*	Pistachio
Annonaceae	*Annona cherimolia*	Cherimoya
Apocynaceae	*Carissa grandiflora*	Natal plum
Arecaceae	*Livistona chinensis* *Phoenix dactylifera*	Chinese fan palm Date
Cannaceae	*Canna sp*	Canna
Fabaceae	*Acacia cavenia* *Acacia farnesiana* *Arachis hypogaea* *Butea monosperma* *Cajanas cajan* *Cassia bicapsularis* *Ceratonia siliqua* *Erythrina (Butea) monosperma* *Gleditsia macrocantha* *Gleditsia triaconthos* *Parkinsonia florida* *Robinia pseudoacacia* *Retama bovei* *Retama raetam* *Tamarindus indica*	 Peanut Flame of the forest Pigeon pea Carob Honey locust Blue palo verde Black locust Tamarind
Fagaceae	*Castanea sativa*	Chestnut
Juglandaceae	*Juglans regia*	Walnut
Malvaceae	*Sterculia acerifolia* *Sterculia diversifolia*	
Moraceae	*Ficus carica*	Fig
Myrtaceae	*Psidium guayava*	Guava
Oleaceae	*Olea europaea*	Olive
Proteaceae	*Macadamia integrifolia*	Macadamia
Punicaceae	*Punica granatum*	Pomegranate
Rosaceae	*Cydonia japonica* *Eriobotrya japonica* *Malus domestica* *Prunus amygdalus* *Prunus armeniaca*	Japanese quince Loquat Apple Almond Apricot
Rutaceae	*Citrus paradisi* *Citrus sinensis*	Grapefruit Orange
Salicaceae	*Populus japonica*	Poplar
Vitaceae	*Vitis* sp.	Grape (dried)

Carob moth was first reported in Californian dates in 1983 (Eichlin 1983) and soon became the worst date pest in that region.

It has been recorded infesting pistachios in Israel since 1984. It apparently cannot consume pistachio hull and so cannot infest pistachios with smooth intact hulls but does lay eggs on split or damaged nuts. Oviposition has for example, been observed on pistachios in the emergence holes of seed-feeding torymid wasps (Halperin 1986). If the shell of a pistachio has not actually split, CM larvae can still access the kernel through the stem end of the shell. Larvae that survive the initial processing of nuts (hulling) inside the shell, can continue their development in storage. In Iran, CM is an occasionally important pest of pistachio, but cannot survive in isolated pistachio plantations without the presence of alternative spring hosts such as pomegranate. These hosts support the pest population in the period prior to hull split in pistachio. (Mehrnejad 1993; Mehrnejad 2001). The same applies to CM in almonds (see 'Life cycle & behaviour' below).

Carob moth is considered the major pest of pomegranate in Iran where it typically damages 25-30% of this important crop (Peyrovi, Goldansaz et al. 2011). It seems that as is the case with pistachios, the infestation of pomegranates by CM may be facilitated by damage to the skin. In a survey of CM infestation of pomegranates in Iran, a significantly higher infestation rate was found in a 'Sour' cultivar, compared to

'Sweet' and 'Sour sweet' cultivars. The 'Sour' cultivar was noted to be prone to skin cracking which was considered likely to make it more susceptible to CM attack (Hashemi-fesharaki, Karimizadeh et al. 2011).

It has also been reported overseas as a significant pest of citrus (e.g. Carrero 1966) although the larvae seem very unlikely to survive in citrus because of the gumming that occurs when they burrow into the rind. However, the rind damage caused by the initial infestation has been noted to cause significant levels of fruit drop in grapefruit (Avidov and Gothilf 1960). In Italy, CM was noted to infest citrus fruit in groves already infested with citrus mealybug (*Planococcus citri*). Control of the mealybug (rather than CM directly) was suggested as the best management option (Schiliro and Bellini 1978). Field trials in Cyprus found that in different years, 10-87% of the fruit drop in grapefruit was infested with CM. Laboratory studies showed that CM only survived on, and damaged, grapefruit that were already infested with mealybug. It was suggested that mealybug honeydew could possibly be a stimulant for oviposition by CM (Serghiou 1983).

In the late 1990's CM was noted as a significant pest of walnuts in Chile where it was considered a barrier to management of other walnut pests with mating disruption. The replacement of pesticides with mating disruption specifically for codling moth for example, led to significant levels of damage by CM (Gonzalez and Cepeda 1999).

As well as being a serious field pest of orchard crops, CM is also known as a significant pest of stored product including dried figs, dates, raisins, carobs, almonds and other nuts (Heinrich 1956; citations in Warner, Barnes et al. 1990b; citations in Higbee and Siegel 2009).

In Western Australia, CM has long been known to infest a range of crops including carobs (with damaged pods), almonds, apples, citrus, figs and pomegranates (Michael 1968; Botha and Hardie 2004).

Life cycle & behaviour on almonds

Unless otherwise noted, the observations provided in this section were extracted from the only significant documentation of CM development in almond orchards - a single paper by Shmuel Gothilf reporting on field investigations on the soft-shell variety Poria 10 in Israel in the late 1970s (Gothilf 1984).

Gothilf's observations were made in the area of Heletz in western central Israel, where mean monthly minimum and maximum temperatures are on average 4° and 1°C higher respectively than those experienced in Mildura. Mean monthly maximum temperatures around Heletz are in fact very similar to those of Mildura from late winter to early autumn and 3-4°C warmer from mid-autumn to mid-winter. Mean monthly minimum temperatures are 2-7°C higher throughout the year at Heletz[1].

Survival over winter

During winter, only larvae were found in almond mummies on trees, with infestation rates up to 50%. Larvae continued to develop over winter but did not start to pupate until late winter, indicating that pupation had a higher temperature threshold than larval development.

Spring emergence

In early spring the proportion of CM in the pupal stage increased significantly. When almond mummies were stored outside under ambient conditions, moths emerged from them over a three month period from early spring to early summer (Figure 1). Seventy five percent of emergence occurred during mid-late spring. The emerging carob moths had a 1:1 male:female ratio.

[1] Comparison of long-term averages from Israel Meteorological Service and Australian Bureau of Meteorology.

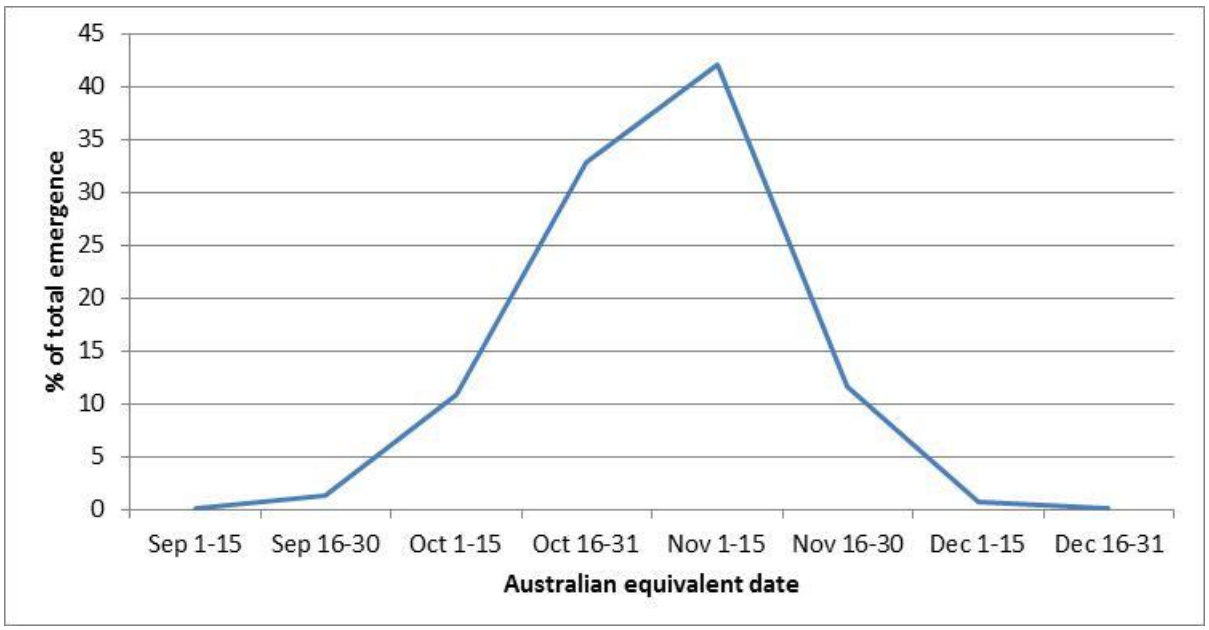

Figure 1 Carob moth emergence from overwintered generation at Heletz, Israel.

Oviposition & nut infestation

CM generally did not oviposit on or infest new almonds until hull-split. For this reason, the maintenance or growth of CM populations depended largely on the availability of food (mummified nuts or alternative host plants) between harvest and the time of hull-split in the following season.

An exception to this was that CM was found to infest unsplit almonds that were infected with anthracnose, *Colletotrichum gloeosporioides* (Table 3). This suggests that where anthracnose is absent, CM would presumably rely more on mummies for survival between spring emergence and hull split in the new season's crop. Observations in Israel did indicate that almond growing areas free of anthracnose had much lower CM infestation levels.

Table 3 Early season infestation of almonds with carob moth eggs at Heletz, Israel: % of nuts infested.

	Number of eggs per nut					
	1	2	3	4	5	Total %
New crop	0	0	0	0	0	0
New crop with Anthracnose	15.6	4.2	2.5	0.5	0.5	23.3
Mummies	13.1	4.3	2.1	0	0	19.5

Eggs were usually laid in the split of the hull. After hatch, the larvae burrowed into the hull or through the shell and into the kernel. A significant proportion of infestation occurred between the hull and shell, with the kernel left undamaged (e.g. in two samples of the soft-shell variety Poria, 25-46% of nuts were infested between the hull and shell and 18-19% inside the shell).

Typically only one larva could be found in each infested almond but multiples did occur (e.g. in one sample, a single larva was found in 89% of infested nuts, two in 8% and three in 3%).

Moth emergence from hull-split new-crop nuts began in mid-summer and continued until temperatures dropped in winter. Oviposition on new crop nuts could be assumed to follow the same pattern, suggesting that the longer harvest is delayed, the greater would be the infestation rate of new crop nuts. This was in fact observed by Gothilf whose data suggested increases of up to 73% in infestation rates for even moderate delays (11 days) to harvest.

Yearly generations
Gothilf's observations suggested three to four generations of moth emergence yearly – one from overwintered larvae, one from spring oviposition onto mummies and anthracnose-infected new crop nuts and one to two from summer oviposition onto hull-split new crop nuts.

Development rates and diet
Development rates of larvae on kernels of different almond varieties were very similar, but it was expected that infestation rates on hard-shell varieties would be lower than that on soft-shell varieties.

At around harvest time when the almond hulls were still relatively fresh, the development times of CM on a hull or kernel diet were very similar (31& 36 days respectively). Two months later when the hulls had dried further, the development time on a hull diet was over 40% longer than on a kernel diet (73 & 51 days respectively).

Infestation of almonds in storage
As mentioned earlier, CM is a known pest of a range of stored food products. In one case, approximately 5% (calculated by weight) of stored paper-shell almonds were recorded as damaged by the pest (Calderon, Navarro et al. 1969).

Interestingly, during trapping trials in stored almonds in Israel, it was found that the number of CM being caught dropped "drastically after a short period of storage" (Pisarev, Carmi et al. 1984) suggesting that at least in some situations, bulk storage conditions do not suit ongoing population development of CM.

Newly hatched and 15-day old larvae did not penetrate intact shells of the soft shell varieties 'Poira' and 'non-plus ultra'. Under typical almond storage conditions in Israel, CM may be able to double its population every three weeks (Navarro, Donahaye et al. 1986)

Life cycle & behaviour: general observations

Yearly generations
In Mediterranean citrus orchards, CM has been observed to develop 3-5 generations per year, with some of the 4th generation and all of the 5th generation overwintering as larvae (Carrero 1966; Tokmakogiu, Soylu et al. 1967). During field surveys in Turkey, CM was found overwintering as larvae in hollows in branches of carob trees (Tokmakogiu, Soylu et al. 1967).

Nay (2006) states that adult CM can be active all year in suitable environments and this would certainly seem feasible where winter temperatures remain moderate.

Diet effect on larval stages
As indicated above in Table 1, the number of larval instars for CM can be quite variable, depending partly at least on diet. In an additional example, larvae were observed to pass through five instars when feeding on pistachio kernel or pomegranate seed, while a diet of fig or date fruit resulted in seven instars developing (Norouzi, Talebi et al. 2008).

Thermal and predatory mortality
In the hot date-growing region of Coachella Valley, larvae began to evacuate fallen date fruit when the fruit temperature on the ground exceeded 46°C. On hot days fruit temperature increased very rapidly and many larvae (just under 80%) died of excess heat before they could leave the fruit. Most larvae (just over 80%) that left the fruit were predated by ants (California harvester ant *Pogonomyrmex californicus* and desert fire ant *Solenopsis aurea*) (Nay and Perring 2005; Nay 2006).

Reproductive behaviour

Calling, mating and oviposition
Adult CM generally remain still during daylight, with mating and oviposition occurring between dusk and dawn (Cox 1976). In laboratory studies, CM did not fly at temperatures of 14.9°C or below but could mate successfully at temperatures as low as 13.7°C (Nay 2006).

When studied at constant temperatures of 20, 25 and 30°C, 25°C was the most favourable for calling (releasing sex pheromone). At that temperature, calling started earlier each night and more time was spent calling. Calling also started earlier and lasted longer as the moths aged, from 1-7 days after emergence

(Soofbaf, Nouri et al. 2007). The same general behaviour was observed in the field but calling appeared to be suppressed by wind speeds exceeding 2 m/s (Sarjami, Ghanbalani et al. 2009).

In detailed laboratory studies, female CM started 'calling' and mating on the night of their emergence from pupae, with the peak rate of mating occurring during that and the following night. Calling began around the 4th hour of darkness and stopped within the first hour of light. Mating also began after several hours of darkness and peaked a couple of hours later. Oviposition began the night after mating occurred, starting during the 1st hour of darkness, and most eggs for each day were laid within the first five-six hours of darkness. About 90% of all eggs were laid in the first six days of oviposition. Adult moth emergence from pupae began two (male) and four (female) hours before darkness and peaked after one hour of darkness (Vetter, Tatevossian et al. 1997; Hung, Chiang et al. 2003).

Table 4 shows the pattern of oviposition found in several laboratory studies of CM.

Table 4. Carob moth oviposition pattern over time.

			Night (1=night of emergence)								
			1	2	3	4	5	6	7	8	
Temp	RH	Photoperiod	Mean eggs laid per female each night								Reference
25±1	75%±5	?	0	80	50	44	19	15	10	5	(Gothilf 1969a)
26	70±5	12:12?	0	49	26	15	11	7	3	2	(Navarro, Donahaye et al. 1986)
29	75 ±5	16:8	0	3	23.5	24	17	12	9	5.5	(Mehrnejad 1995)
27 ±2C	65%±10%	16:8	0	10	22	49	52	43	29	10	(Al-Izzi, Al-Maliky et al. 1987)
			% females laying fertile eggs each night								
27 ±2	65±10	16:8	0	3	34	70	65	49	30	9	(Al-Izzi, Al-Maliky et al. 1987)

Egg yield

A considerable variation in average total egg yield per female CM has been reported by various researchers, with larval diet appearing to play an important role. Examples of total egg production per female include 180 on pistachio kernel, 113 on pomegranate, 56 on fig and 48 on date fruit (Norouzi, Talebi et al. 2008); 315 on soy+sucrose diet and 201 on carob pods (Gothilf 1968); 340 on pistachio kernel (Mehrnejad 1995); 110 on wheat+date syrup diet (Alrubeai 1987); 215 on unspecified diet (Dhouibi 1982) and 113 on almond kernel (Navarro, Donahaye et al. 1986).

Interestingly, the number of sterile eggs laid per female was noted to increase with the moisture content of date fruit on which the larvae had been fed (Nay 2006).

Host selection for oviposition

Observations from several studies indicated that female CM were attracted to volatile compounds produced as a result of fungal infection of fruits. One early study found that CM larvae would feed on 'clean' carob pods but exhibited better growth on pods that were infected with fungi such as Phomopsis. It appeared that uninfected pods lacked certain nutrients that were produced or supplemented by the fungal infection (Levinson and Gothilf 1965). It may possibly be for this same reason that CM, which usually only lays eggs on almonds after hull split, will oviposit on almonds prior to hull-split if they are infected with anthracnose, as noted earlier (Table 3) (Gothilf 1984).

Also as mentioned earlier, CM larvae were found to survive on grapefruit only if the fruit were infested with mealybug (citrus mealybug; *Planococcus citri*) (Serghiou 1983). In that situation, the mealybug honeydew (or more likely the sooty mould fungus that commonly grows on honeydew?) was suggested as a stimulant for oviposition by CM on the grapefruit.

In a later study, mouldy dates were found to emit several volatile compounds attractive to mated female CM (Cosse, Endris et al. 1994). Three of the compounds, ethyl hexanoate, ethanol and acetaldehyde individually stimulated mated female CM to fly to and land on a source of the compounds in flight tunnel tests. When the three compounds were combined, their attractiveness to female CM increased to the level of mouldy dates. Ethyl hexanoate also elicited a strong response from female CM in electroantennogram tests.

It is possible, if not likely, that ethyl hexanoate is a product of microbial infection, as it did not feature in a separate study of healthy date fruit. In that study, the rates of CM infestation of three date cultivars in the orchard, and the attraction of female CM to fruit of those cultivars in wind tunnel tests appeared to be strongly associated with the presence of the volatile components acetaldehyde and 1-butanol in the fruit (Table 5) (Yaakoub 2011).

Table 5. Volatile components of three date cultivars and their attractiveness to CM.

	Date cultivar		
Volatile component	Deglet Nour	Ghars	Degla Beidha
Acetaldehyde	✓	×	×
2-propanol	✓	✓	✓
Ethanol	✓	✓	✓
Ethyl hexanoate	×	×	×
1-propanol	✓	✓	✓
1-butanol	✓	✓	×
Female moths taking flight	50%	36%	14%
Max field infestation rate	7.75%	4.5%	1.5%

In a study of infestation rates in 13 date cultivars in Algeria, adult moth size was found to be positively associated with fruit size which suggests a benefit related either to availability or nutritional quality of the food resource. The most heavily infested cultivar however (Takermoust, 56.7% of fruit infested at late maturity), was one of the smaller fruit cultivars, and one of the least infested (Ghars, 1.65% infested at late maturity) was one of the larger fruits, suggesting that other factors such as plant volatiles play a role in determining where CM chooses to oviposit (Idder, Idder-Ighili et al. 2009).

Diapause

Diapause is a strategy to help insects survive winter conditions by halting larval or pupal development and so delaying the emergence of adults until temperatures (the following spring) are more suitable for flight, mating and egg survival.

Observations of CM behaviour in pomegranate orchards suggested that diapause was induced by day lengths less than 11 hours with temperatures below 20ºC, and subsequently broken by day lengths over 13hours with temperatures over 15ºC (Al-Izzi, Al-Maliky et al. 1985).

In line with this, under conditions of relatively low temperature (20°C) and day-length (12 hours light), (Cox 1979) found that almost all CM larvae being observed entered diapause, resulting in an average development period from egg-hatch to adult emergence, of 190 days, 111 days longer than non-diapausing larvae. Further studies found that while larval instars 1-4 developed normally under low temperature and light conditions, the developmental period for instar 5 was extended significantly, suggesting that diapause comes into play during that 5th instar (Dhouibi 1982).

Moth emergence: humidity & gender effects

Studies on CM in Mediterranean citrus suggested that relative humidity had a major influence on emergence of adults from pupae in spring (Carrero 1966). The authors concluded that a minimum RH of 70% is

necessary, combined with a minimum average temperature of 17.5°C. They also suggested that if RH exceeds 75%, higher temperatures are necessary for optimal moth emergence.

When CM was cultured on a diet of carob or acacia and kept at 25°C and 75%RH, male moths began to emerge 1-2 days earlier than females. Moth emergence peaked 5-7 days (male) and 7-8 days (female) after emergence began. Fifty percent of the moths had died by eight days after they emerged (Gothilf 1969a).

Dispersal

Over three years of study during which male CM were released into pomegranate orchards in Tunisia, 89-97% of the moths were recaptured in pheromone traps within 120 m of their release point (Mediouni and Dhouibi 2007). Although this type of study may artificially impede (and therefore underestimate) dispersal simply by having pheromone traps in place, it does provide some idea of the dispersability of CM (males at least).

Laboratory cultures

Effect of diet

Examples of the key ingredients of artificial diets used for CM are listed in Appendix A, but detailed recipes and preparation techniques are not included.

In a study using date fruit as the larval food, moisture content (MC) of the food had a significant effect on development, fitness and mortality of CM (Nay 2006). Food MC was manipulated by maintaining the cultures at particular levels of relative humidity (RH). Larval mortality and development time increased at lower food moisture content. At 32°C almost no larvae completed development at MC < 5% (RH 16%) and optimum development was at MC of 22% (RH 63%).The author of that study points out that this may explain a large part of the variability in CM development reported in lab studies (e.g. see Appendix A), especially those where relatively moist artificial diets are compared to other relatively dry foods. (Hung, Chiang et al. 2003) for example recorded larval development times of 20.2 days on an artificial diet based on sweetcorn and yeast, compared to 68 days on almond slices. This dietary effect was not restricted just to development rates, as larvae on the artificial diet gave rise to pupae that were significantly heavier than those from the almond diet. It must be noted however that because (Nay 2006) used different relative humidities to maintain different diet moisture contents, the relative humidity itself may also have influenced larval development.

Where fruits rather than artificial diets are to be used for culturing CM, the potential positive role of fungal infection in the diet should be considered. Survival of CM larvae on heat-sterilised carob pods for example, was greatly improved when the pods were inoculated with *Phomopsis sp*. (Gothilf 1969a).

When added to artificial diet, tannic acid slowed CM larval development and so extended the development period (Al-Izzi and Al-Maliky 1996).

Mating and oviposition

It is reported by some authors that more success with mating was obtained when CM were kept in a large cage under conditions that simulated the natural fluctuations in light, temperature and humidity, rather than in small containers (Gothilf 1968; Cox 1976).

(Gothilf 1968) also observed that CM appeared to lay more eggs when isolated, so he placed mated females individually under small plastic cups for oviposition. The cups were kept on rough paper (observed to stimulate oviposition) and the moths were discarded after 3-4 nights of oviposition.

In contrast, (Al-Izzi, Al-Maliky et al. 1987) obtained satisfactory results using 1 L containers for mating, with three pairs of moths per container being optimal for the production of fertile eggs. The same author reported that CM larvae are sometimes cannibalistic – a point that should be remembered if accurate measures of egg fertility (calculated by hatch rates) are required.

Constant light was found to inhibit mating in CM (Nay 2006), and contrary to the general assumption regarding egg colour, the same author noted that not all pink CM eggs are fertile.

Management strategies

A mix of practices including removal of infested fruit, mating disruption, repellents and biological and chemical pesticides has been suggested for pomegranate growers where CM causes significant crop loss (Zalom, Santibanez et al. 2012). CM is such a major pest of pomegranates in Iran that physical barriers to prevent infestation of individual fruits have been recommended to reduce damage levels (Rafiei, Farazmand et al. 2011).

Population monitoring

Field sampling

A presence/absence sampling plan has been proposed to determine CM infestation levels in Californian date plantations. The best sampling precision was obtained using 150 fruit of the type most attractive to CM, with 10 being collected from each of at least 15 palms randomly selected from the area being sampled (0.2 ha) (Park and Perring 2010). The fruit were examined externally and internally for CM to determine the percent of fruit infested as a measure of pest population density. Increasing the sample size beyond 150 slightly increased the level of error relating to the probability that 'no control would be implemented when density is just above the action threshold' (set arbitrarily at 7% of fruit infested). It should be noted that apart from their value in pest detection, sampling regimes are really only useful if meaningful action thresholds are known.

Trapping

Trap placement

In a pomegranate orchard in Iraq, using virgin female moths as the pheromone source in traps, just over 10% of the males trapped were caught at the edge of the orchard, while just under 90% were caught at the centre of the orchard (Al-Jamali 2006). In the same trials, trap height and trap density influenced the efficiency of traps as shown in the following tables.

Table 6. Distribution of male moth captures as influenced by trap height.

Trap height (m)	Percent of total male catch
2.0	19.24
1.5	78.4
1.0	2.35
0.5	0.0

Table 7. Influence of trap density on weekly catch of male CM.

Trap density (traps/ha)	Average catch (males/trap/week)
0.8	29.6
2.0	20.0

Male attractants

Using analysis of the content of female CM pheromone glands, electroantennogram studies, flight tunnel tests and field trapping tests, (Z,E)-9,ll, 13-tetradecatrienal was identified as a major component of carob moth sex pheromone (Baker, Francke et al. 1991) and an efficient procedure for its synthesis was determined (Millar 1990). The trienal component however was very unstable, even under freezer conditions, and it was

recognised that a stable form would be needed for any practical field application as a trap lure or for mating disruption.

In wind tunnel trials, a stable mimic of CM sex pheromone ((Z,E)-7,9,11-Dodecatrienyl Formate; as used in SPLAT EC) was as effective as pheromone gland extracts in stimulating male CM to fly, approach the scent source and contact the source. In field trapping trials in Californian date plantations, the formate form was more effective than the trienal form as a lure in attracting male CM, but both synthetic lures were less effective than female CM themselves (Todd, Millar et al. 1992).

Various antioxidants and UV stabilisers were tested for their value in extending the life of the pheromone mimic, and with mimic-impregnated rubber septa, 3-4 weeks of effective field life were achieved (Millar, McElfresh et al. 1997).

The stable pheromone mimic (Z,E)-7,9,11-Dodecatrienyl Formate is now commercially available, impregnated into rubber septa for use as lures in standard traps such as the sticky-based delta trap (Russell IPM 2011; ISCA Technologies 2013). These lures have a nominal field life of 4-8 weeks, depending on environmental conditions.

Female attractants & egg traps

Egg traps containing a mix of almond meal and almond oil have been used as a valuable monitoring tool for management of navel orange worm (NOW) in Californian almond orchards for many years (Van Steenwyk, Barnett et al. 1986; Kuenen, Bentley et al. 2008) and an equivalent trap for CM would be very useful.

Pomegranate fruit mixed with egg albumen, ammonium carbonate, milk or petroleum oil were tested as lures to attract female CM in pomegranate orchards in Iran. Pomegranate and egg albumen was the mix most attractive to CM (and *Carpophilus* beetles). Yellow was the most effective colour and most CM were caught in traps at ground level (Mansour 1984). Note the contrast in effect of trap height between these results and those shown for males in Table 6.

Despite these findings however, it has also been stated that after several attempts with potentially attractive substances, no suitable female-attracting bait was identified to adapt NOW egg traps for use with CM in California (Pers. Comm., Prof. Thomas Perring). Also, the need for a commercially-effective female attractant, (e.g. based on plant volatiles) has been separately noted (Nay 2006).

Degree-day modelling

The significant influence of food moisture content on CM development rates as noted earlier has implications for attempts at developing simple temperature-dependent degree-day models based on development data from laboratory cultures. At a single constant temperature of 32°C for example, generation times varied by a full month simply because of differences in relative humidity (%RH) and food moisture content (MC), from 56 days @ 63%RH, 22%MC to 88 days @ 16%RH, 5%MC. When reared on dates at 32°C and 82%RH (26% food MC), the generation times ranged from 32.8-33.9 days (636-658DD with upper and lower temperature thresholds of 38°C and 12.5°C respectively) (Nay 2006; Nay and Perring 2006; Nay and Perring 2008b). It is likely that a degree-day model for CM will need to account for the influence of food quality at different times of the season, not just simple temperature effects on developmental rates.

Table 8 lists some temperature thresholds and degree-day requirements reported for CM development by various authors. Further generational and stage-specific degree-day requirements may be determined from the data in the tables of Appendix A.

Table 8. Temperature thresholds and degree-day requirements reported for CM.

	Temperature thresholds °C			
Diet	Lower	Upper	Degree- Days per generation	Reference
Soy+sucrose	10.82		624.06	(Mart and Kilincer 1993)
Pomegranate fruit	11.76 (overall) 15.97 (eggs)	-	707.2	(Yousefi, Sendi et al. 2004)
Pistachio kernel	9.4		769	(Basirat and Mehrnejad 2005)
Date fruit (26% moisture content)	12.5	38	636-658	(Nay 2006)

Sanitation/Host destruction

In numerous crops, unharvested fruit remaining in orchards infested with CM have been found to be a major breeding resource for the pest, and orchard hygiene has been recommended as a key pest management factor.

Date

In a wet season, removal of abscised dates from bunches and from the ground in a California date orchard reduced the level of fruit damage by moths (mostly CM) at harvest by 40%. In a more typical hot, dry season, the abscised dates quickly became unsuitable for CM development, infestation rates were very low overall, and the sanitation measures did not reduce the level of damage at harvest (Warner, Barnes et al. 1990b).

Further studies confirmed that CM populations in date plantations can build up rapidly on abscised fruit that remain lodged in bunches. Removing these fruit significantly reduced the amount of CM-infested fruit within the plantation, and hence the infestation pressure on the new crop. CM did oviposit on date fruits on the ground, so sanitation practices would ideally include destruction of fallen fruit (Nay 2006; Nay, Boyd et al. 2006; Nay and Perring 2009).

The same author found that CM had a patchy distribution in the date orchard, and that the numbers of CM were spatially associated with the numbers of abscised fruit. For example, aggregations of CM were associated with aggregations of abscised fruit. If left undisturbed, the patches and gaps of CM persisted over time. After abscised fruit were removed by sanitation (and infestation rates lowered), reinfestation was directional from non-sanitised areas of the orchard (Nay 2006; Nay, Park et al. 2007), indicating that sanitation was effective when implemented on a large-enough scale.

Almond

No sanitation thresholds (density of mummies or unharvested nuts remaining in orchards) have been developed for carob moth management in almonds. A threshold of two mummies per tree has however been in place for some time in relation to management of the related pest ‘Navel orange worm’ (NOW) in Californian almond orchards (Zalom, Pickel et al. 2009). The recommended practice is for mechanical tree shaking or manual poling to be used to reduce the density of mummies on trees to below this threshold, followed by cultivation or mowing to destroy any mummies on the ground. In recent years, in response to a perceived increase in risk from NOW related to significant growth in the almond production area in California, the above threshold was reviewed, resulting in a more stringent threshold of 0.2 mummies per tree being suggested (Higbee and Siegel 2009).

General observations

In carob plantations, unharvested pods have been noted to provide breeding sites and act as a population ‘reservoir’ for CM (Gothilf 1970). Similar observations were made elsewhere, and for example, the removal and/or destruction of residual fruit infested with CM was recommended to reduce infestation levels in citrus in Turkey (Tokmakogiu, Soylu et al. 1967) and pomegranate in Iran (Kashkuli and Eghtedar 1976).

In Israel, up to 40% of split pistachio nuts remaining on trees after harvest were found to be infested with CM (Halperin 1986), suggesting that post-harvest orchard hygiene could be an important management factor for the pest in that crop. During field surveys for the pest in Iran, larvae were noted to overwinter in pistachio nuts on trees or on the ground (Mehrnejad 1993), so simply knocking unharvested nuts off the trees without destroying the nuts, may not be sufficient for adequate suppression of CM.

As one approach to orchard hygiene, a South Australian almond grower was reported as using carrion to encourage little ravens *Corvus mellori* into his orchard after harvest to clean up the remaining nuts from his trees to reduce the risk of carob moth damage and disease (Anon 2009). During the growing season a combination of scaring devices within the orchard, and carrion placed away from the orchard as a decoy, was used to protect the almond crop.

Importance of alternative hosts

The potential role of alternative host plants in supporting CM populations needs to be seriously considered in any management program. CM is the major pest of pomegranates in Iran and a considerable effort is made to control it on that crop. An analysis of CM morphology on alternative host plants (fig, pistachio, walnut) found however that plants other than pomegranate appeared to provide CM with better nutrition and hence greater opportunity for individual survival and population development (Mozaffarian, Sarafrazi et al. 2007a; Mozaffarian, Sarafrazi et al. 2007b). This had clear implications for the management of CM in that specific situation. It also suggests that the availability and role of alternative hosts should be taken into account in any crop-specific approach to CM management.

Mating disruption

The synthesis of the CM sex pheromone mimic in the 1990's raised the possibility of using mating disruption as a management tool against this pest. The approach was first used in dates in California, where (Nay 2006) identified the following challenges to be overcome:

1. Large air volume to treat, given the height of mature date palms
2. Rapid degradation of the pheromone mimic under high summer temperatures
3. Logistics of a mating disruption program (number of dispensers, dispenser renewal, monitoring)
4. High fecundity of CM and immigration from nearby non-treated orchards
5. Economic feasibility of producing the pheromone mimic
6. Length of treatment period required

To assess the ability of the CM sex pheromone mimic ((Z,E)-7,9,11-dodecatrienyl formate) to disrupt CM mating, dispensers of hollow micro-fibres loaded with the compound were used in 1.5ha, 3-20m height date orchards in California (Vetter, Millar et al. 2006). As the dispensers were effective for only a short time they had to be replaced every two weeks. Sticky traps 'baited' with five virgin female CM were used to monitor the ability of male CM to locate females under mating disruption (MD) conditions. Where date bunches were routinely dusted with malathion to protect them from CM attack, MD reduced trap catches of males, and levels of fruit damage, by over 90%. Where the dates were not treated with insecticide, MD again reduced trap catches of males, but did not reduce the level of fruit damage significantly. MD didn't appear to reduce overall population levels (after up to 18 weeks of application) as catches of males increased dramatically as soon as the dispensers were removed from the orchard at the end of the trial. In this trial, MD had been applied to protect date bunches towards the end of the season, by which time CM populations had built up over several generations. It was questioned whether early-season suppression of CM populations may yield better results.

In a further development of the MD approach, four-acre (1.62ha) plots of date plantation were treated with the CM pheromone mimic at a rate of 12.2g/ha (Park, Perring et al. 2008). The mimic was incorporated at a concentration of 2% into a waxy substrate which was applied as two 2.5g dollops per palm (121 palms/ha). A single sticky trap baited with a lure using the same pheromone mimic was placed at the centre of each treated plot. Trap catches of CM were heavily reduced for up to 12 weeks in MD plots, compared to plots that were untreated or had malathion dust applied (the standard treatment for CM in dates). Rates of fruit infestation in MD plots were the same as or lower than those in malathion-treated plots and both were significantly lower than those in untreated plots.

The product reported above is now commercially available in USA for use in CM mating disruption (ISCA Technologies 2011). This product, SPLAT-EC® uses a substrate of biodegradable food-grade ingredients including waxes to hold and slowly release the pheromone mimic over time. SPLAT products are putty-like

and are applied to trees as dollops, either manually using standard caulking guns, or through mechanised applicators that have been developed for ground or aerial use.

Sterile insect release

The mass release of sterile insects of pest species has been used to successfully suppress field populations of those pests, and some attempts have been made to apply this management approach to CM.

Irradiation of 4-9 day old CM pupae with a dose of 500-600 Gy led to reduced levels of moth emergence and high levels of moth abnormalities. At the reduced pupal irradiation dose of 400-500 Gy, the fertility of male moths and fecundity of females was very much reduced (when mated with untreated partners). Irradiation of 9-10 day old pupae with 200-300 Gy caused only slight effects on moth fertility, fecundity, morphology and egg hatch rates, and male moths from pupae treated with up to 300 Gy exhibited reduced mating competitiveness (Dhouibi and Abderahmane 2002).

Trials of mass release of irradiated sub-sterile CM indicated potential for this approach in suppressing wild populations through inherited sterility. A radiation dose of 400 Gy was found to provide the best compromise between male sterility and field competitiveness and resulted in average sterility levels of 95% for the treated insects, 97% in the next generation (F1) and 99% in the subsequent generation (F2). Genetic studies suggest that there is good potential for developing visible sex markers in CM cultures which could provide significant benefits through the easy selection, irradiation and mass release of males only. Even without a sex marker, the mass release of mixed male and female irradiated CM showed promise in suppressing wild populations (Mediouni-BenJemaa 2005).

The authors of a 2008 report on a business plan for a sterile insect production facility considered that CM either "meets biological and technological criteria for a species to be suitable for control by SIT" or that the gaps in knowledge were being actively researched at that time. Tunisia was considered to be well advanced in relation to SIT research on CM on dates, and therefore the obvious location for initial application and demonstration of SIT (International Atomic Energy Agency and Food and Agriculture Organization of the United Nations 2008).

Pesticides

Chemical pesticides

In Algeria, infestation of dates by CM was reduced from around 24% to 8% by eight applications of 100-150 g of 10% DDT dust to the centre of the palms during winter when no crop was present. The treatment was aimed at reducing CM population growth in dates that fallen and lodged around the centre shoot of the palm (Lepigre 1963).

Further trials in dates found that in dry years, CM damage was significantly reduced by treating bunches with 3-5 applications of 5% malathion dust at 2-4 week intervals, starting once the dates had reached a developmental stage susceptible to infestation. In a wet year (more favourable to CM), the pesticide applications (3-4 dustings at 2-3 week intervals) still reduced date damage significantly, but achieved better control when they began one week before the fruit became susceptible to infestation. Use of another organophosphate insecticide (4% Naled dust, 4 applications fortnightly or 8 applications weekly) also achieved significant reductions in CM damage in the wet year (Warner, Barnes et al. 1990a).

In Turkey, nine applications of Dipterex 50 WP or Lebaycid EM (both anticholinesterace insecticides) at 10-day intervals reduced citrus fruit drop associated with CM by 67.8% and 53.8% respectively. This was not considered a satisfactory level of control (Tokmakogiu, Soylu et al. 1967).

Using a different approach, trials over several years in Cyprus demonstrated significant impacts on CM infestation of grapefruit through effective insecticidal control of mealybug. Mealybug infestation had been found to be a prerequisite for CM infestation on grapefruit. The reductions in CM infestation ranged from 36-97% (Serghiou 1983).

For CM control in almonds in Australia, applications of 0.1% DDT emulsion (organochlorine), 0.05% azinphos (presumably azinphos methyl, organophosphate) or 0.15% carbaryl (carbamate) were originally recommended (Michael 1968).

When several insecticides were compared for control of CM and other pests on macadamia in South Africa, Cypermethrin (pyrethroid) applied at a rate of 20 ml/100 litres water gave the best results (Villiers and Wolmarans 1980). Applications were made in late spring and mid-summer.

Field applications of cyhalothrin EC (pyrethroid; 2.5g a.i./100l) to pomegranates resulted in approximately 70% mortality of CM (application rates and frequency were not clear). The most effective application time was just after CM egg hatch (Mart and Kilincer 1994).

Microbial/Botanical/Mineral pesticides

Field applications

In Tunisia, CM infestation of dates was significantly reduced by four applications, at 12 day intervals, of Spinosad (Tracer®). The treatment effect was inversely proportional to dose. A rate of 12.5 ml Tracer®/ha/spray was more effective in reducing CM damage than 25 ml/ha and 50 ml/ha. Over a one month period, four applications at weekly intervals, of 0.1% azadiractin sprayed at a rate of 2 l/date palm kept CM infestation levels between 1.5% and 2.7%. Over the same period, infestation rates on untreated palms increased from 2 to 16% (Khoualdia, Takrouni et al. 2002).

A combination of applications of Bactospeine® (*B. thuringiensis*) and releases of the parasitoid *Habrobracon hebetor* (no rates available) were reported to significantly reduce CM larval development in warehouse stored dates (Dhouibi and Jemmazi 1996).

Treatment of pomegranate trees with *B. thuringiensis* (no application rate data available) was reported to reduce CM infestation rates by 50% and 80% after two and four spray respectively (Alrubeai 1988).

Field applications of Thuricide® WP or Biobit® WP (both *B. thuringiensis*; 16,000 IU/mg @ 70g/100l) to pomegranates resulted in approximately 65% and 67% mortality of CM respectively (Mart and Kilincer 1994). The best results were obtained with applications just after CM egg hatch, but application rates and frequencies were not made clear.

Four whole-canopy applications of 15% kaolin particle film (Sepidan® WP) at 4-5 week intervals during late spring to early autumn reduced pomegranate infestation with CM by 74% (from 9.3% to 2.4%) in an orchard in Iran (Sheikhali, Farazmand et al. 2011).

Laboratory studies

In one study, mortality of 4th instar CM larvae reached 95% after 66 hours, and 100% after 85 hours of exposure to *Bacillus thuringiensis* (*B.t.*) (Harpaz and Wysoki 1984). The bacillus had been applied as a spray to the surface of an artificial culture medium at a rate of 48,000 IU/cm^2. When a *B.t.* rate of 24,000 IU/cm^2 was used, CM mortality was significantly lower.

In bioassays where CM larvae fed on dried grapes treated with *B.t.*, 4th instar larvae were more sensitive to *B. t.* than 2nd instars, and affected larvae seemed to turn white before dying (Elsayed and Bazaid 2011).

It was noted recently that during fermentation, *Bacillus subtilis* SPB1 produces a lipopeptide biosurfactant that exhibits significant insecticidal properties against 3rd instar CM larvae (Mnif, Elleuch et al. 2013). The author considered that the biosurfactant could be used to develop novel biopesticides against CM in storage or field situations.

Over 20 years ago, a microsporidian (PROTOZOA: Microspora), probably in the *Nosema* genus, was identified in CM larvae in Argentina (Lange 1991). This was considered to be of potential interest at the time, as several *Nosema* species had previously been developed into biological control agents for crop pests. No follow-up of this finding was apparent in the literature.

Exposure of 1st instar CM larvae to artificial diet treated with 24-384 ppm azadirachtin resulted in mortalities of 22-50% after 24 hours, and 35-75% after 120 hours. Larvae that survived the 24 to 384 ppm azadirachtin treatments gave rise to moths that laid 26% to 66% fewer eggs with 23% to 80% lower hatch rate respectively, compared to those from untreated larvae (Mehaoua, Hadjeb et al. 2013).

Fumigants

In laboratory bioassays, essential oil of *Pistacia lentiscus* was toxic to CM adults when applied as a fumigant (Bachrouch, Jemaa et al. 2010). 100% mortality of CM was achieved after 48 hours of exposure to the oil fumes. As well as causing adult mortality, the essential oil reduced the rates of mating and egg production and survival as shown in the following table.

Table 9. Reduction in CM copulation rate, fecundity and egg survival under fumigation with Pistacia lentiscus essential oil.

	Percent reduction under fumigation
Copulation rate	93.1
Fecundity	56.2
Egg hatch rate	55.4

Essential oil from Eucaluptus species appeared less toxic to CM, with *E. rudis* being the most effective of the five tested (others being *E. camaldulensis*, *E. astringens*, *E. leucoxylon* and *E. lehmanni*). *E. rudis* oil at a concentration of 13.16 µl/l air resulted in approximately 18% mortality of CM after 24 hours exposure (Mediouni Ben Jemaa, Haouel et al. 2012).

In further work by the same authors, essential oils of two of the *Eucalyptus sp*. were tested as fumigants against 5th instar CM larvae inside dates. *E. camaldulensis* and *E. leucoxylon* oil at a concentration of 131.58 µl/l air resulted in 100% mortality after three and ten days respectively, when 10% or less of the fumigated space was occupied with dates. After ten days, larval mortality for the two oils respectively was 94% and 91% when 50% of the fumigation space was occupied, and 87% and 80% when 100% of the fumigation space was occupied. Although these oils gave promising results in relation to CM mortality, their lack of persistence and high doses required may limit their practicality as replacements for conventional fumigants (Mediouni Ben Jemâa, Haouel et al. 2013).

Tests in chambers and tarp-covered stockpiles found that ProFume® (99.8% sulfuryl fluoride) was an effective and more flexible replacement for methyl bromide as a fumigant for fresh dates against carob moth eggs and larvae (Williams, Watkins et al. 2007; Williams 2009).

Repellents

In Iran, 20 vial dispensers each containing 4 ml of 1:1 v:v of *Ferula asafoetida* essential oil and ethanol (as solvent) were hung in a 1ha pomegranate orchard. A separate orchard where the vials contained only ethanol was used as a control (the trial therefore appeared to have no true replication or control treatment). The number of CM-infested fruit collected from trees and ground during the season and present on trees at the end of the season was significantly lower in *F. asafoetida* treated trees. A similar result was obtained the following season with dispensers consisting of a section of PVC tube (100mm x 100mm diam?) lined with absorbent cloth into which the oil extract or ethanol were injected. Reductions in rotten fruit on trees at the end of the season were approximately 30-60% (Peyrovi, Goldansaz et al. 2011).

Biological control

Natural enemies of carob moth

Table 10 lists parasites and predators that have been reported attacking CM (Wilkinson 1937; Biliotti and Daumal 1969; Gothilf 1969c; Kugler and Nitzan 1977; Doumandji-Mitiche 1981 ; Al-Maliky and Al-Izzi 1986; Gothilf and Mazor 1987; Zaviezo, Romero et al. 2007; International Atomic Energy Agency and Food and Agriculture Organization of the United Nations 2008; Farahani, Goldansaz et al. 2009; Idder, Bolland et al. 2009; Norouzi, Talebi et al. 2009; Farahani, Goldansaz et al. 2010b; Ksentini, Monje et al. 2010; Poorjavad, Goldansaz et al. 2011; Farahani, Goldansaz et al. 2012)

Table 10. Natural enemies associated with carob moth.

Parasitoids/Parasites	
Hymenoptera	
Braconidae	
Apanteles lacteus Nees (Microgasterinae)	*Habrobracon brevicornis* Wesm.
A. laspeyresiellus Papp	*Hypomicrogaster suffolciensis* Morly
A.myeloenta Wilkinson (77%)	*Microbracon pembertoni* Bridw
*A.*sp. group *ultor*	*Phanerotoma dentata* Panz

Ascogaster sp.	*P. flavitestacea* Fischer (Cheloninae) egg parasitoid
Bracon (Habrobracon) hebetor Say (Braconinae) (43%)	*P.ocularis* Kohl
B. lactus Wesmael (Mediouni-BenJemaa 2005) (possibly dubious reference)	*Rhogas testaceus* Reinch
B. mellitor	
Encyrtidae	
Pentalitomastix plethoricus Caltagirone (*Copidosomopsis plethorica?*)	
Pteromalidae	
Anisopteromalus mollis Ruschka	
Bethylidae	
Perisierola gallicola Kieff	*Goniozus legneri* Gordh.
P. emigrata Rohw	
Ichneumonidae	
Pristomerus vulnerator Panz	*Temelucha decorata* (Grav.) Cremastinae (22%)
Horogenes sp.	*Campoplex tumidulus Grav.* Campopleginae (up to 12% parasitism rate)
Nemeritis (*Devorgilla*) *canescens* Gravenhorst	*Venturia canescens* Gravenhorst (29%)
Gelis sp.	*Diadegma* sp
Herpestomus arridens Frav.	
Chalcididae	
Brachymeris aegyptiaca Ms. *(Brachymeria?)*	*Antrocephalus mitys* Walk.
Trichogrammatidae	
Trichogramma bourarachae Pintureau and Babault	*T. embryophagum* Hartig
T. cacoeciae Marchal	*T. evanescens* Westwood
T. cordubensis Vargas & Cabello	*T. principium* Sugonyaev & Sorokina
Eulophidae	
Pedobius sp.	

Diptera	
Tachinidae	
Clausicella suturata Rondani	*Fischeria bicolor* Robineau-Desvoidy

Acarina
Pyemotidae
Pyemotes (Pediculoides) *ventricosus* Newp.
Aceosejidae
Melichares tarsalis Berlese (oophage*)

Coleoptera
Cleridae

Hemiptera	
Anthocoridae	
Cardiasthetus nazarenus Reuter (oophage)	*Buchananiella continua* B. (oophage)
C. fasciiventris Garb. (oophage)	

* egg predator

The Hymenopteran hyperparasites *Perilampus tristis* Mayr. (**Perilampidae**) and *Pachycrepoideus vindemmiae* Rondani (**Pteromalidae**), both hyperparasites of *Apanteles* sp., have also been reported in association with CM (Farahani, Goldansaz et al. 2010a).

Field application of biological control

Dates

In Algerian dates, the parasitoids *Phanerotoma dentata*, *Bracon* (*Habrobracon*) *brevicornis* and *Nemeritis canescens* (*Venturia canescens*) were noted to be present but providing little control of CM (Lepigre 1963).

An inoculative release during summer, of 1600 *Phanerotoma flavitestacea* into a 1 ha Israeli carob plantation where it was previously absent, resulted in CM parasitism rates of 22-35%. In a 1 ha carob plantation where the parasitoid was already present, a supplementary release of 3100 *P. flavitestacea* resulted in more than a three-fold increase in parasitism rate of CM (13% to 45%) (Gothilf 1969b). The author suggests an early spring release is worth trying, to target the first generation of CM eggs.

Aerial application of *B.t.* with and without supplementary releases of the parasite *Phanerotoma flavitestacea* in a date orchard increased CM larval mortality over that occurring with natural parasitism only as shown in Table 11 (Dhouibi, Hawlitsky et al. 2000). *B.t.* was ULV formulation; Bactospeine XLV: 13,000 I.U.A.K/mg; Biobit XL: 9,000 I.U.A.K/mg; Ecotek pro: 24,000 I.U./T.n./mg.

Table 11. Impact of B.t. application and parasite release in a date orchard on CM larval mortality.

	Percent mortality of CM larvae
B.t. plus parasite release	88
B.t.	73
Natural parasitism	30
Mortality control	2

Results from five years of trials in a 100ha date plantation (Dhouibi, Hawlitsky et al. 2000) indicated that only marginal increases in rates of CM parasitism by *P. flavitestacea* were obtained by releasing the parasite:

- three times compared to once (per year or per season ?)
- at four sites per ha compared to one
- at a rate of 50 parasites per site per release compared to 25.

In Morocco, the parasitoid *Phanerotoma ocuralis* was recorded from CM in date fruit still in bunches on palms while *Bracon hebetor* was recorded from CM in fallen dates (Hassan, Mohamed et al. 2001).

Releases of *Trichogramma cordubensis* into an Algerian date orchard where it was previously absent led to parasitism of CM eggs on date fruit at rates from 47-64%. Approximately 100-150 of the parasitoids were released on each palm. Releases were made just before twilight – soon before CM oviposition would begin. No parasitised CM eggs were found on palms that didn't receive the parasitoid, even though they were close to the treated palms, indicating that at least in the short term, *T. cordubensis* did not migrate very far (Idder, Bolland et al. 2009).

Carob

Surveys in Israel found rates of natural parasitism of CM larvae and pupae in carobs reached up to 56%, with *P. flavitestacea* and *C. suturata* being the most common parasitoids (Gothilf 1969c).

Studies on carob in France found parasitism rates of CM by *Phanerotoma flavitestacea* of 2.64-13.4% (Madkouri 1978).

Pomegranate

In studies on pomegranate, the internal parasite *Apanteles* sp *group ultor* [Braconidae] preferred to infest early instar CM larvae (2-3 days old, most likely 1st and 2nd instar, and some 3rd instar) (Al-Maliky and Al-Izzi 1986). CM larvae older than 7 days were mostly rejected by the parasite. The great majority of mature wasp larvae emerged from 2nd and 3rd instar CM larvae and very few emerged from 1st and 4th instar larvae. *A.* sp. *group ultor* parasitised CM during the entire pomegranate season. Parasitism rates ranged from 10% in mid spring to 35% in mid-autumn to over 50% in mid-winter (as parasite larvae/pupae overwintered). Mated female *A.* sp. *group ultor* lived an average of five days (range 3-8) and on average parasitised 58 CM larvae (range 37-82). The 2nd, 3rd and 4th days of oviposition accounted for 58% of the total production of parasite progeny. These studies were carried out at 27°C, 55% RH and 16 hour day length. In further studies by the same author, total development time for *Apanteles* sp *group ultor* decreased with increasing temperature, ranging from 59 days at 16°C to 14 days at 29°C (Al-Maliky, Al-Izzi et al. 1988). A lower developmental threshold of approximately 10°C was estimated. Photoperiod did not have any marked effect on oviposition by adult parasites or development of the parasite larvae. This gives the parasite an advantage over CM whose development is slowed by shorter photoperiods (see Appendix).

Mass releases of *Trichogramma embryophagum* into a 0.5 ha pomegranate orchard in Iran were considered to have increased CM egg parasitism by 200% (to 53%) and reduced CM trap catches by 50% and fruit damage at harvest by 66% (to 24%). A total of two million *T. embryophagum* were released in the form of parasitised *Ephestia kuehniella* eggs on small cards. Ten releases were made, at ten-day intervals during late spring to mid-summer (Mirkarimi 2000). Note: the treatment was not replicated, just compared to a non-treated orchard.

Field releases of 15,000 *Trichogramma cocaeciae* per tree in a Tunisian pomegranate orchard reduced the average rate of fruit infestation with CM from 13.2% to 2.4% (Lebdi-Grissa and Ayed 2008).

Infestation of pomegranates in Iran was reduced by 65% (from 30% to 10.5%) by releasing *Trichogramma embryophagum* eggs into the orchard (Karami, Mirabzadeh et al. 2011). No data on release rates was given and the treatments were not replicated.

Parasitism rates were greater in unharvested pomegranates still in the trees while CM infestation rates were greater in fruit on the ground (Farahani, Goldansaz et al. 2012). Collection and destruction of unharvested fruit from the ground and trees as a sanitation for CM control resulted in reduced parasite populations. The authors recommended destroying leftover fruit from the ground to remove CM but leaving fruit in the trees to maintain parasite populations.

Almond

Release of an estimated 950,000 *Pentalitomastix plethoricus* (CM egg parasitoid) across 2ha of an almond orchard led to its establishment in that orchard and resulted in a parasitism rate of 12% of CM larvae the following season (Gothilf 1978).

Natural levels of parasitism (P. flavitestacea) in almonds collected during summer ranged between 0.5-5.0% (Gothilf 1984).

A total of 31,900 *Goniozus legneri* and 5.4 million *Copidosomopsis (Pentalitomastix) plethorica* and 1,505 *Diadegma* sp. were released over eight, seven and four almond orchards respectively, in Israel. Follow-up sampling 1-5 years later found *G. legneri* present at seven of the release sites with up to 30% of CM larvae parasitised by this species. *C. plethorica* was found at three sites in up to 9% of CM larvae, and *Diadegma*

was recovered from only one site in very low numbers. *G. legneri* appeared to work most efficiently under hot summer conditions and established well in California where it was introduced for NOW control. The failure of *Diadegma* sp. to establish may reflect the relatively very low numbers released. This species is known to attack CM on almonds in Australia (Gothilf and Mazor 1987).

As indicated by the preceding reports, levels of parasitism found in CM in almonds have been generally low. Nevertheless, this biological control could provide a useful contribution to an overall system of integrated pest management for CM in almonds.

Conclusions

Carob moth has been a pest of almonds in Australia for many years, but rose to prominence as a major pest only in relatively recent years. This was most likely due to the rapid increase in numbers of bearing trees, combined with wetter-than-average summers that resulted in greater retention of nuts (mummies) on trees after harvest. Whether carob moth declines in importance with a return to more 'normal' drier conditions remains to be seen, but international experience suggests that it is here to stay and will require active management to minimise crop loss.

The wide host range of this pest and its importance in a number of key crops overseas indicates that its contribution to damage or loss in other commercial crops in Australia (e.g. pistachio, citrus, walnut, pomegranate, pome fruit, carob) would be worth assessing. It is possible that these industries, if affected by carob moth, may contribute to the current or future research efforts.

Effective management of carob moth by the almond industry is likely to require one or more of:

- Prevention of population build-up in the orchard (e.g. remove the pest's food source – mummies – from the orchard, or suppress oviposition by the generation of moths emerging in spring).

  The nature of Australian almond production suggests that carob moth depends almost entirely on mummies as a food resource in Australian almond orchards, so good sanitation practices that achieve very low densities of mummies in orchards would be an ideal approach to CM management. However, experience to date suggests that this is unlikely to be achieved economically in the short-medium term.

  Because the spring moth emergence is over an extended period (three months), it is possible, if not likely, that the multiple pesticide applications required to target the bulk of that emergence would be uneconomic. A longer-acting treatment such as mating disruption may be economically feasible at that stage of the season.

- Protection of the new crop from damage (e.g. prevent infestation of new crop nuts and avoid any delays to harvest).
  The application of ovicidal or larvicidal pesticides from hull split onwards, or mating disruption from at least two weeks prior to hull split are strategies that may minimise or prevent the infestation of new crop almonds. A key issue for industry is the length of time between hull split and the completion of harvest, as new crop nuts are prone to infestation during that entire period.

- Protection of the harvested crop from damage (i.e. protect shaken and stockpiled nuts from further oviposition and remove existing infestations from harvested nuts).

  Overseas experience with CM in dates and navel orange worm in almonds is that the pests will oviposit on fruits/nuts on the ground, so shaken and stockpiled almonds should be considered at risk of oviposition by CM.

  Overseas experience and local observation also indicates that CM development in almonds continues during storage (field stockpile and warehouse), so removal of existing infestation with prompt processing, fumigation or other means should be a priority to protect the harvested crop.

Research on the sterile insect technique to combat CM continues overseas. This approach could be worthy of consideration in the future, given Australia's experience with SIT for fruit fly suppression.

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Appendix A: Carob moth developmental periods

A1. Oviposition period at constant temperatures

Diet (base ingredients)	Temp °C	% Relative Humidity	Photoperiod (light:dark)	Oviposition period Days (& average)	Reference
soy, casein, cellulose, dextrose	27 ±2	75±10	16:8	2-8	(Al-Izzi, Al-Maliky et al. 1987)
Soy+ molasses+ pomegranate or fresh pomegranate	27 ±1	75±10	16:8	5-5.9	(Ghavami 2006)
Soy+sucrose	25 ±1	70±5	14:10	1.3-4 (3.3)	(Mart and Kilincer 1993)
	30 ±1	70±5	14:10	2.6-5 (3.7)	
Pistachio kernel	29	75 ±5	16:8	13 (95% by day 8)	(Mehrnejad 1995)
Sweetcorn+yeast	25	70 ±5	12:12	4.9-13.5 (9.2)	(Hung, Chiang et al. 2003)
Almond slices	25	70 ±5	12:12	1.6-7.4 (4.5)	

A2. Egg incubation periods at ambient temperatures

Season	Mean daily temperature °C	Incubation period (days) on unspecified artificial diet	Reference
Late spring-mid summer	22-34	14.3 ±2.6	(Al-Izzi, Al-Maliky et al. 1985)
Summer	30-33	7.6 ±0.7	
Late summer-mid autumn	28-31	5.4 ±1.2	
Mid autumn-late spring	26-5.5-22	19.6 ±1.4	

A3. Egg incubation periods at constant temperatures

Diet (base ingredients)	Temp °C	% Relative Humidity	Photoperiod (light:dark)	Incubation period (days)	Survival %	Reference
soy, casein, cellulose, dextrose	27 ±2	65 ±10	16:8	4-6		(Al-Izzi, Al-Maliky et al. 1987)
Wheat, date syrup	30 ±1	65 ±5	16:8	3.5		(Alrubeai 1987)
Soy, sucrose	15, 20	70 ±5	16:8	-	0	(Cox 1976)
	25	70 ±5	16:8	4-5		
	30, 35	30-90 ±5	16:8	3-4		
Soy+ molasses+ pomegranate or	27 ±1	75±10	16:8	4-4.9		(Ghavami 2006)

fresh pomegranate						
Soy+sucrose	15	80		-	0	(Gothilf 1969a)
	20	80		8-9	80.5	
	25	80		4	88.1	
	27	80		3.5	90.5	
	30	80		3	95.5	
	34	80		3-3.5	85.3	
	<34	20			80-90	
	20-30	10			50	
	34	10			24	
	45	any			0	
Soy+sucrose	25	70 ±5	14:10	3-4 (3.63)	84.4	(Mart and Kilincer 1993)
	30	70 ±5	14:10	2.6-4 (3.22)	85.1	
Pistachio kernel	29	75 ±5	16:8	2.4-3.1 (2.7)		(Mehrnejad 1995)
Sweetcorn+yeast	25	70 ±5	12:12	3.9	91.5	(Hung, Chiang et al. 2003)
Almond slices	25	70 ±5	12:12	4	72.7	
Pomegranate	30	70 ±5	16:8	3		(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	3.06		
Fig	30	70 ±5	16:8	3.06		
Date	30	70 ±5	16:8	3.05		

A4. Larval development periods at ambient temperatures

Season	Mean daily temp °C	Developmental period (days) on unspecified artificial diet						Reference
		Larval instar					total	
		1	2	3	4	5		
Late spring-mid summer	22-34	5.1	3.4	4.7	4.3	3.8	21.2 ±2.6	(Al-Izzi, Al-Maliky et al. 1985)
Summer	30-33	4.8	4.1	3.9	4.0	6.7	23.3 ±3.5	
Late summer-mid autumn	28-31	5.1	4.6	4.0	3.3	3.6	21.1 ±2.5	
Mid autumn-late spring	26-5.5-22						204.5 ±3.2	

A5. Larval development periods at constant temperatures

Diet (base ingredients)	Temp °C	% Relative Humidity	Photoperiod (light:dark)	Larval period, Days & (average)	Survival %	Reference
soy, casein, cellulose, dextrose	27 ±2C	65%±10	16:8	9-29		(Al-Izzi, Al-Maliky et al. 1987)
Wheat, date syrup	30 ±1	65 ±5	16:8	15-18 (16.4)		(Alrubeai 1987)
Soy, sucrose	20	70 ±5	16:8	29-41 (35)	10	(Cox 1976)
	25	70 ±5	16:8	19-22 (21)	57	
	30	70 ±5	16:8	16-18 (17)	73	
	30	30 ±5	16:8	25-36 (30)	40	
Soy+ molasses+ pomegranate or fresh pomegranate	27±1	75±10	16:8	18-19		(Ghavami 2006)
Soy+sucrose	24±1	75±5		28		(Gothilf 1969a)
Soy+sucrose	25	70 ±5	14:10	22-44 (29.7)		(Mart and Kilincer 1993)
	30	70 ±5	14:10	16-29 (21.2)		
Pistachio kernel	29	75 ±5	16:8	21-30 (22.9)		(Mehrnejad 1995)
Sweetcorn+yeast	25	70 ±5	12:12	20.2	86.2	(Hung, Chiang et al. 2003)
Almond slices	25	70 ±5	12:12	68.0	54.4	
Pomegranate	30	70 ±5	16:8	24.9		(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	29		
Fig	30	70 ±5	16:8	41.3		
Date	30	70 ±5	16:8	72.9		

A6. Photoperiod effects on larval development

	Larval development period (days) on unspecified artificial diet at 27 ±2°C, 55±10% RH			
Photoperiod L:D by larval instar	Max	Min	Mean	Reference
12:12 instars 1-5	65	28	47.3	(Al-Izzi, Al-Maliky et al. 1985)
12:12 instar 1-4 then 16:8	52	26	36.6	
12:12 instar 1-3 then 16:8	30	16	22.1	
16:8 instars 1-5	20	9	13.5	

A7. Pre-pupal development periods at constant temperatures

	Temp	RH	Photoperiod	Pre-pupa period (days)	Reference
soy, casein, cellulose, dextrose	27 ±2C	65%±10%	16:8	1	(Al-Izzi, Al-Maliky et al. 1987)
Soy+ molasses+ pomegranate or fresh pomegranate	27 ±1°C	75±10%	16:8	1.4-3.3	(Ghavami 2006)
Soy+Sucrose	25	70±5	14:10	1-2	(Mart and Kilincer 1993)
	30	70±5	14:10	0.6-2	

A8. Pupal development periods at ambient temperatures

Season	Avg daily temperature	Pupal period (days) on unspecified artificial diet	Reference
Late spring-mid summer	22-34	9.2 ±1.9	(Al-Izzi, Al-Maliky et al. 1985)
Summer	30-33	10.7 ±1.5	
Late summer-mid autumn	28-31	26.1 ±5.7	
Mid autumn-late spring	26-5.5-22	9.9 ±3.3	

A9. Pupal development periods at constant temperatures

Larval diet	Temp °C	% RH	Photoperiod	Pupal period (days)	Reference
soy, casein, cellulose, dextrose	27 ±2	65±10	16:8	4-13	(Al-Izzi, Al-Maliky et al. 1987)
Wheat, date syrup	30 ±1	65 ±5	16:8	6-9 (7.2)	(Alrubeai 1987)
Soy, sucrose	20	70 ±5	16:8	13-16 (15)	(Cox 1976)
	25	70 ±5	16:8	7-9 (9)	
	30	70 ±5	16:8	5-8 (6)	
	30	30 ±5	16:8	5-7 (6)	
Soy+ molasses+ pomegranate or fresh pomegranate	27 ±1	75±10	16:8	5.4-6.4	(Ghavami 2006)
Almond kernel chopped	26	70 ±5	12:12?	7-11 (8)	(Navarro, Donahaye et al. 1986)
Soy+sucrose	25	70±5	14:10	7-9	(Mart and Kilincer
	30	70±5	14:10	5-7	

					1993)
Pistachio kernel	29	75 ±5	16:8	6-8 (7.2)	(Mehrnejad 1995)
Sweetcorn+yeast	25	70 ±5	12:12	7.9	(Hung, Chiang et al. 2003)
Almond slices	25	70 ±5	12:12	9.5	
Pomegranate	30	70 ±5	16:8	7.1	(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	7.1	
Fig	30	70 ±5	16:8	7.2	
Date	30	70 ±5	16:8	7.2	

A10. Total development period from oviposition to adult emergence

Larval diet	Temp °C	% RH	Photoperiod	Total period (days)	% survival	Reference
Wheat, date syrup	30 ±1	65 ±5	16:8	26.2		(Alrubeai 1987)
				30-43		(Carrero 1966)
Soy+ molasses+ pomegranate or fresh pomegranate	27 ±1°C	75±10%	16:8	31.3-32.2		(Ghavami 2006)
Almond in cracked shell	26	70 ±5	12:12?	47-82 (62)	14	(Navarro, Donahaye et al. 1986)
Almond kernel	26	70 ±5	12:12?	42-73 (55)	20	
In-shell almond chopped	26	70 ±5	12:12?	37-58 (45)	54	
Soy+sucrose	26	70 ±5	12:12?	36-46 (39)	62	
Soy+sucrose	25	70 ±5	14:10	37-51 (44)		(Mart and Kilincer 1993)
	30	70 ±5	14:10	27-39 (32.5)		
Pomegranate	30	70 ±5	16:8	35		(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	39.1		
Fig	30	70 ±5	16:8	51.5		
Date	30	70 ±5	16:8	83.2		
Pomegranate	30	70 ±5	16:8	34		(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	38		

Fig	30	70 ±5	16:8	48		
Date	30	70 ±5	16:8	72		
Pomegranate	20	65 ±5	14:10	98		(Yousefi, Sendi et al. 2004)
	25	65 ±5	14:10	53		
	30	65 ±5	14:10	36		
	35	65 ±5	14:10	28.5		

A11. Total development period from egg hatch to adult emergence

Larval diet	Temp °C	% RH	Photoperiod	Total days Range & (mean)	% survival	Reference
Soy, sucrose	20	70 ±5	16:8	43-72 (48)		(Cox 1976)
	25	70 ±5	16:8	27-39 (30)		
	30	70 ±5	16:8	21-25 (23)		
	30	30 ±5	16:8	31-44 (37)		
Soy+sucrose	25-28	75±5		26-36 (29.3)	95	(Gothilf 1968)
Soy, sucrose	20	70 ±5	16:8	(49)	67	(Cox 1979)
	20	70 ±5	12:12	(79)	3	
	30	70 ±5	24:0	(23)	100	
	30	70 ±5	20:4	(25)	77	
	30	70 ±5	16:8	(29)	67	
	30	70 ±5	13:11	(27)	47	
	30	70 ±5	12:12	(26)	37	
	30	70 ±5	0:24	<50	83	
Dry carob	25			97-127 (114)	10	(Gothilf 1969a)
Green carob	25			29-43 (33)	63	
Grapefruit	25			46-105 (66)	29	
Almond kernel	25			41-55 (49)	50	
Almond hull-fresh	21-32			31		(Gothilf 1984)
Almond kernel-fresh	21-32			36		
Almond hull-dry	26			73		
Almond kernel-dry	26			51		
Almond kernel (diff varieties, 10	25-26			72-80	59-88	

months post-harvest)						
In-shell almond chopped	26	70 ±5	12:12?	37-71 (54)	80	(Navarro, Donahaye et al. 1986)
Almond kernel	26	70 ±5	12:12?	41-60 (49)	15	
Almond in cracked shell	26	70 ±5	12:12?	45-114 (76)	40	
Almond in intact shell	26	70 ±5	12:12?	-	0	
Soy+sucrose	32	82	16:8	32		(Nay 2006)
Dates	32	82	16:8	38-41		
Pistachio kernel	29	75 ±5	16:8	26-37 (30)		(Mehrnejad 1995)

A12. Adult longevity

Larval diet	Temp °C	% RH	Photoperiod	Adult longevity (days)	Reference
soy, casein, cellulose, dextrose	27 ±2C	65%±10%	16:8	2-10	(Al-Izzi, Al-Maliky et al. 1987)
Wheat, date syrup	30 ±1	65 ±5	16:8	♀ 8.44	(Alrubeai 1987)
				8-9	(Dhouibi 1982)
Soy, sucrose	Some indication that adult longevity increases with decreasing temperature and increasing humidity				(Cox 1976)
Soy+ molasses+ pomegranate or fresh pomegranate	27 ±1°C	75±10	16:8	♀ 4.3-6	(Ghavami 2006)
Carob pods	25	75±5		9	(Gothilf 1969a)
Almond kernel	26	70±5	12:12?	♀2-10 (5.7) ♂1-9 (4.6)	(Navarro, Donahaye et al. 1986)
Dates	32	82	16:8	♀7.2-9.1 ♂8.1-11	(Nay 2006; Nay and Perring 2008b)
Soy+sucrose	32	82	16:8	♀(7.9) ♂(6.8)	
Sweetcorn+yeast	25	70 ±5	12:12	♀(16.5)	(Hung, Chiang

				♂(9.6)	et al. 2003)
Almond slices	25	70 ±5	12:12	♀(10.6) ♂(11.4)	
Pomegranate	30	70 ±5	16:8	7.4	(Norouzi, Talebi et al. 2008)
Pistachio kernel	30	70 ±5	16:8	6.2	
Fig	30	70 ±5	16:8	6.2	
Date	30	70 ±5	16:8	6	

Materials & Methods

To address the main aims of this project, several separate laboratory and field-based research activities were carried out. Each of these components of the research work, including their specific materials, methods, discussion and conclusions, is reported in the following chapters of this report.

Seasonal phenology and distribution of carob moth in almonds.

Aim

To understand the seasonal developmental cycle and behaviour of carob moth in almonds to inform the development of management options.

Introduction

Carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae* is an economically significant pest of a wide range of tree crops globally. It has been a minor or sporadic pest of almonds in Australia for many years, but became a significant kernel quality issue for the industry after the unusually wet summers of 2009/10 and 2010/11. It is possible that the growth in populations of the pest during those wet seasons was associated with an increase in numbers of 'mummy' nuts (nuts remaining on trees after harvest). Mummies are an important food resource for carob moth, and often arise from nuts that are affected by hull rot, a fungal disease that develops during wet summer conditions.

After observing high infestation levels during the 2011 harvest, the Australian almond industry commissioned research into carob moth with a view to development of cost-effective management options. In 2011, HAL project AL11009 'Food safety in almonds – Stage 2' began to investigate the biology and management of fungal contamination of almonds. A preliminary monitoring program for carob moth was incorporated into that project because of the concerning levels of kernel damage caused by the pest and the potential role of that damage in providing sites for fungal infection. Project AL12004 'Managing carob moth in almonds' was subsequently developed to investigate carob moth as a pest in its own right.

This aspect of Project AL12004 sought to acquire local knowledge on the distribution and seasonal development of carob moth in Australian almond orchards. This was achieved through a range of activities including moth trapping programs, spot surveys for the presence of nut infestation, spatial distribution surveys of moth activity and nut infestation, and construction and assessment of a degree-day model for carob moth.

Materials and methods

Carob moth identification

Early in the first season of the project (October 2011) we preserved specimens of what we believed to be carob moth, that we had reared from almonds, and provided them to the DEDJTR Biosciences taxonomy group for confirmation of their identity.

Carob moth is generally described as being 12mm in length, a measurement that matches most observations made during this project. However, occasionally during our trapping exercises we found moths that had the colour and patterning typical of carob moth but were considerably smaller, approximately eight mm in length. We had also received queries from industry participants in the trapping program regarding small moths.

To clarify the identity of these small moths, we sent specimens of what appeared to be small and large carob moths to the DEDJTR Biosciences taxonomy group for DNA sequencing and analysis. The moths had been caught during trapping for our field trials.

DNA was extracted from the heads of three small and three typical-sized moths. One section (containing more than 500 base pairs) of the mitochondrial COI (Cytochrome Oxidase I) gene was then sequenced to examine genetic variation between the specimens. The DNA sequence was then compared to records in the GenBank and BOLD DNA sequence databases (Benson et al. 2011, Ratnasingham et al. 2007) to confirm the identity of the specimens.

Natural enemies of carob moth

A wide variety of natural enemies have been recorded to attack carob moth (refer to the literature review below). During assessments of almond samples for carob moth infestation and damage throughout the project, we found a range of parasitic and predatory invertebrates that may play a role in natural control of the pest. Specimens of these species were preserved, and where necessary sent for identification by the DEDJTR Biosciences taxonomy group.

Seasonal trapping of male carob moth

To gather data on the geographical distribution and seasonal activity of carob moth in almond orchards, a trapping program was established early in the project. The program was initiated in August 2011 as a component of Project AL11009 "Food safety in almonds: Stage 2", when nine orchards between Adelaide and Griffith were each provided with five white plastic delta traps, male carob moth lures (ISCAlure-Ceratoniae™, ISCA Technologies Inc., Riverside, California USA), record sheets and an information sheet on carob moth identification and trap maintenance (see attachments). Orchard managers at each site agreed to maintain the traps and send weekly moth counts to the project officers for collation.

It was also intended to monitor carob moth oviposition using navel orangeworm (NOW) traps baited with an almond meal and oil mix used for NOW(). However, a discussion with a USA researcher with experience of carob moth management in dates (Perring[2], pers. comm. 2011) revealed that attempts to use the traps with carob moth had been unsuccessful as a suitable attractant for female carob moth was lacking. Subsequently, for this project, carob moth oviposition was monitored through inspection of mummy and new season nuts for fresh eggs. In addition, some preliminary screening was carried out in an effort to identify potential female attractants for use in moth or egg traps. That work is reported in the chapter "Investigating potential female attractants".

Because carob moth is a significant pest of pistachio nuts in some countries (Halperin 1986, Mehrnejad 1993), a Sunraysia pistachio orchard was included in the trapping program. The program was expanded to 15 orchards for the 2012/13 season then rationalised to 13 sites for 2013/14 and 2014/15.

Construction of a degree-day model

Degree-day models can be useful tools in insect pest management by providing some capacity to predict key life cycle events such as moth emergence or oviposition. These predictions can then be used to refine the timing of pest monitoring activities or applied control measures such as mating disruption. To assess the potential value of a predictive model for carob moth in almonds, a spreadsheet-based degree-day model to predict the timing of moth emergence and egg hatch was constructed. The model uses the double-sine method for calculating degree days (Zalom et al. 1983) and published data on the development of carob moth under varying conditions of temperature and diet (Cox 1976, Hung et al. 2003, Alrubeai 1987, Norouzi et al. 2008, Nay & Perring 2008, Yousefi et al. 2004, Cox 1979, Gothilf 1984, Nay & Perring 2006). Rather than base the model solely on temperature, larval diet was taken into account because of the impact that diet can have on developmental rates of the species (Nay & Perring 2008, Norouzi et al. 2008). For this purpose, particular attention was paid to carob moth developmental rates on larval diets of fresh and old almond kernel and hull.

Spot surveys of overwintering mummy nuts for carob moth infestation

In August of 2011, 2012 and 2014, samples of mummy nuts were collected from Nonpareil trees in almond orchards in the Victorian Sunraysia and South Australian Riverland regions to assess the levels of carob moth infestation that were being carried into the new season. The sampled orchards were located along a 1,100 km section of the Murray River, from approximately Swan Reach at the west of the South Australian Riverland to Swan Hill at the centre of the Victorian Mallee. This area accounts for over 85% of Australia's almond plantings.

At each sample site and time, 100 mummy nuts were collected from within an orchard block, from randomly selected trees scattered throughout the block. If no mummies could be found after 30 minutes of searching, the search at that site was terminated. The collected nuts were returned to the laboratory and examined under a dissecting microscope for the various life stages of carob moth (eggs, larvae, prepupae and pupae).

These spot surveys together with moth trap data were used to determine the potential levels of pressure from this pest in different orchards. Altogether, samples were collected from twelve different orchards. Not all the same blocks were sampled in every year, as some were removed for replanting while others were added to the program.

Surveys of field-stockpile nut infestation

In collaboration with Project AL11009 'Food safety in almonds – Stage 2', this project undertook to follow the carob moth infestation of almonds from the AL11009 field trial site, through the stockpiling process. This was to gain information on the fate of field infestations and on the timing and site of additional

[2] Thomas Perring, Professor of Entomology, University of California, Riverside.

infestation, should it occur during the stockpiling process. The field site and stockpile were to be monitored by Project Al11009 for the development of mould infection in almonds.

The field site was a 3.9 ha orchard block in the Robinvale district of Sunraysia. Within the block, 14 plots of five Nonpareil trees were delineated, with two plots in each of seven rows. The plots were four rows apart, and within rows were separated by buffers of five trees.

Prior to hull split (6 Dec 2011) and when hull split was well advanced (1 Feb 2011), two current season nuts were collected from each of the five trees in each plot. The ten nuts per plot were pooled together and all the nut samples were returned to the laboratory where they were inspected for carob moth infestation and damage, using a dissecting microscope.

The crop from the trial site itself could not be followed through the stockpile process because of commercial considerations. Subsequently, a separate Nonpareil stockpile was made available and environmental monitoring equipment installed to collect data for project Al11009.

On 4 May 2012, nut samples were collected from four positions along the stockpile, 20-40 m apart, and at four depths (0.5-5 m) for assessment of mould infection. From each of those samples, subsamples of 15 nuts were taken and inspected under a dissecting microscope for carob moth infestation and damage.

On 29 June 2012, nut samples were collected as above, and also from the outer lower edge of the stockpile. These samples were assessed for mould infection and any that showed signs of insect damage were provided to this project for assessment of carob moth infestation.

The stockpile trial was terminated in July 2012, as a commercial decision was made to process the nuts.

Sequential surveys of carob moth development in nuts

Spring 2011 – autumn 2012

To determine the pattern of carob moth development in almond nuts through a production season, a sequence of repeat surveys was conducted at one orchard site in Sunraysia. The surveys were carried out across a 2.7 ha section of a 20 ha block, which itself was part of approximately 9,000 ha of almond plantings. The trees were 30 years old and were spaced 7.2 m between rows and 5.5 m between trees. The area used for the surveys also contained carob moth traps as part of the trapping program described above.

Eight surveys were conducted at 2-4 week intervals from 13 Oct 2011 (new crop nuts) and 20 Oct 2011 (mummy nuts) until 15 Mar 2012. A follow-up survey was conducted on 8 May 2012. At each sample time, one mummy nut and one new crop nut were collected from each of 100 Nonpareil trees distributed throughout the survey area. One mummy nut was also collected from the ground under the same trees during the first seven surveys (until 17 Feb 2012) after which time the trees were shaken and the ground swept for harvest.

All nut samples were returned to the laboratory and examined under a dissecting microscope for insect damage and for the various life stages of carob moth and any other invertebrates appearing to cause damage to the nuts.

Summer 2014/15

In 2014/15 a shorter set of surveys was used to follow the development of carob moth infestation of almonds during the critical period of hull split to harvest. The surveys were carried out across a 5.6 ha section of an 11 ha block which was located in the same district as that used for the 2011/12 surveys. The trees were eight years old and were spaced 7.2 m between rows and 4.6 m between trees.

Five surveys were conducted at 11 to 18 day intervals from 29 Dec 2014 (prior to hull split) to 26 Feb 2015 (just after tree shaking for harvest). For each survey, one mummy nut and one new crop nut were collected from each of 100 Nonpareil trees distributed in a 10x10 grid pattern such that every fifth tree (23 m apart) in every fourth row (29 m apart) was sampled. Nut samples collected during these surveys were assessed for infestation and damage as described above.

Assessment of kernel damage from deficit irrigation trial

Between 2009/10 and 2013/14, DEDJTR operated a field trial to investigate the impact of deficit irrigation on almond trees and yields. The trial was located on a commercial orchard in Sunraysia, using trees that were six years old at the start of the trial. During the assessments of nut samples for the trial the presence of insect damage including carob moth was recorded, and that data is presented in this report.

Table 12 lists the irrigation treatments applied to trees during the trial. Apart from these treatments, the trees were managed as per standard farm practice.

Table 12. Irrigation treatments applied in the deficit irrigation trial.

Irrigation treatment	% of recommended irrigation
C (Control)	100%
W (Wet)	120%
RDI85	85% (50% mid Jan-mid Feb; 100% all other times)
RDI70	70% (50% mid Nov-mid Feb; 100% all other times)
RDI55	55% (50% mid Sep-mid Feb; 100% all other times)
SDI85	85% all season
SDI70	70% all season
SDI55	55% all season

Spatial distribution survey of mummy nut infestation

On 25 Oct 2012, a one-off survey of Nonpareil mummy nuts was conducted to gain some insight into the distribution of carob moth infestations within an orchard block. The survey took place across a 20 ha block of 30 year old trees in an orchard in Sunraysia. The block had a row spacing of 7.2 m and tree spacing of 5.5 m. For the survey, twenty mummy nuts were collected from every eighth tree in every sixth row, resulting in a 43 m by 44 m sampling grid. In total 120 trees were sampled, with ten samples being collected in each of 12 rows. Where the intended sample trees carried too few mummy nuts, additional nuts were collected from trees within the same row, up to three trees away from the nominal sample tree.

The nut samples were assessed for carob moth infestation as described above.

Results & discussion

Carob moth identification

Specimens of moths reared from almonds collected in the Sunraysia district were confirmed in November 2011 to be carob moth, *Ectomyelois ceratoniae*. Regarding its scientific name, carob moth has numerous synonyms of which *Ectomyelois ceratoniae* and *Apomyelois ceratoniae* are two that were in use at the time. The former synonym was selected for use during the project because it appeared to be the accepted name and was used in the majority of scientific papers being published at the time. Currently the latter synonym appears to be the accepted version, so all reporting for this project now uses the scientific nomenclature *Apomyelois* (=*Ectomyelois*) *ceratoniae*.

DNA analysis confirmed that small moths that are occasionally caught in carob moth pheromone traps, and that have the colour and patterning typical of carob moth, were indeed carob moth. All six moths analysed had the same mitochondrial COI DNA sequence as each other, indicating that they were all the same species, and the DNA sequence had a 100% match with carob moth (under the synonym *Ectomyelois ceratoniae*) in the GenBank and BOLD DNA sequence databases.

Variations in adult size of carob moth have been attributed to larval diet. For example, Idder et al. (2009) found moth length to range from 7.2 mm to 12.2 mm depending on the date variety that the larvae had fed upon, and Nay (2006) found the weight (and presumably size) of newly emerged moths to vary by up to four times depending on the development stage of their host date fruit. It is likely that the moth size variation observed in almonds similarly relates to the variation in larval diets, which range from fresh moist hull or new kernel to old dry hull and mouldy kernel.

This information on moth size variability has been fed back to growers and used to update the project's first fact sheet on carob moth and has been incorporated into a second fact sheet and a carob moth ID card.

Natural enemies of carob moth

Two species of parasitoid wasps including *Trichogramma carverae* (HYMENOPTERA: Trichogrammatidae) were reared from carob moth eggs collected from almond orchards in the Sunraysia/Riverland region. *T. carverae* is produced commercially in Australia for mass release against a range of lepidopteron crop pests, and in the past has been released in Australian almond orchards to assist with management of carob moth (James Altmann, pers. Comm.). The fact that this species is commercially available makes it an ideal choice for investigations into the potential of biological control of carob moth.

Three larval/pupal parasites including *Goniozus jacintae* (HYMENOPTERA: Bethylidae) were also recorded from lepidopteron pupae in almonds during the project. Given that almost all larvae and pupae encountered in almonds during the project were of carob moth, it is most likely that these wasps had parasitised carob moth. To confirm this however will require DNA analysis of the remains of pupae from which the wasps emerged.

The predatory bug *Orius sp*. near *chadwicki* (HEMIPTERA: Anthocoridae) was also found in almonds from field samples. This bug is common in Victoria and elsewhere in Australia and feeds on a range of small soft insects and eggs, including carob moth eggs as confirmed in a simple laboratory test during the project. A second predatory Anthocorid bug *Lasiellidea sp.* may also have been present in almond samples but that is yet to be confirmed.

European earwigs *Forficula auricularia* (DERMAPTERA: Forficulidae) are known to be general predators and have been very numerous in some almond orchards, to the extent that orchard managers have expressed concern regarding the levels of damage they have seen these earwigs causing to almond foliage. Predation of carob moth eggs and small to medium-sized larvae by European earwigs was confirmed in a simple laboratory test during the project. Any program to target European earwig as a pest of almonds should take into account the potential benefits they provide ('ecosystem services') in the way of pest suppression.

Two other general predators that are common in horticultural areas and were found in almond samples from our field trials were the green lacewing *Mallada signata* (NEUROPTERA: Chrysopidae) and the 'red and blue beetle' *Dicranolaius bellulus* (COLEOPTERA: Melyridae). Both species are known to consume insect eggs and soft-bodied insects and are very likely to prey on carob moth eggs and small larvae that are accessible on or in almond nuts.

The natural enemies of carob moth discussed above are only those encountered during the assessment of almond samples from project field work. A broader range of parasites and predators is most likely associated with carob moth across Australia's almond growing districts. The contribution of these species to natural suppression of carob moth populations has not been quantified but should be considered as a potentially useful component of any IPM approach to carob moth management in almonds.

Seasonal trapping of male carob moth

Initially five traps were provided to each orchard participating in the trapping program. At the end of the first trapping season, a simple comparison was made of how accurately the fluctuations in moth activity would have been detected using three instead of five traps. Figure 2 shows the average weekly moth catch per trap from sites with low, moderate and high carob moth populations, using all five traps (12345) or all possible combinations of three out of the five traps (123, 124…345). Based on this simple comparison, it was decided that three traps would provide data of satisfactory accuracy for the monitoring of carob moth population trends, so from 2012/13 onwards, only three traps were used at each trapping site. This helped to reduce the workload for orchard managers who maintained the traps and also the running costs of the program. Any sites that recorded zero moth catches in a season, maintained only a single 'sentinel' trap from the following season onwards.

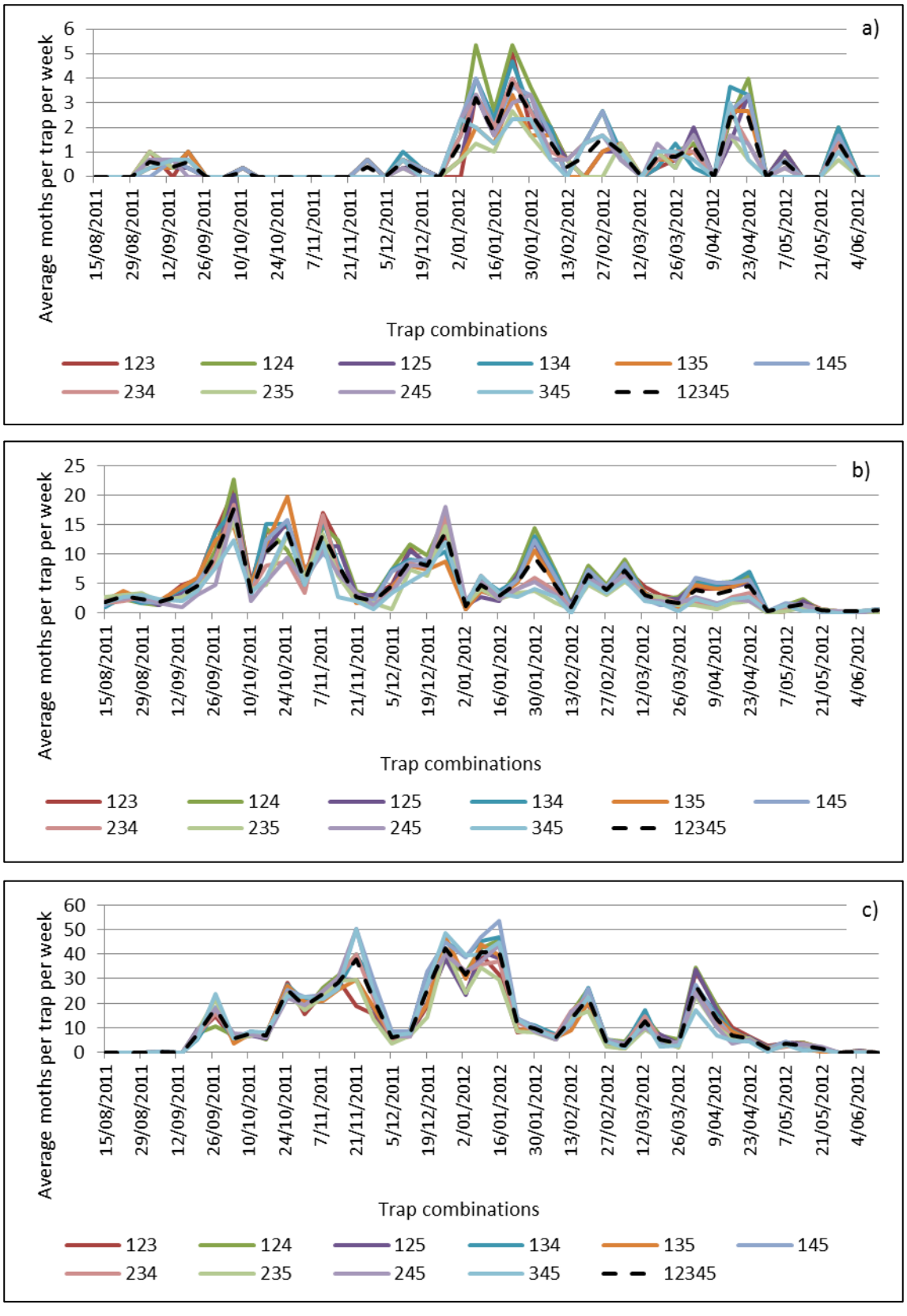

Figure 2. Average weekly trap catch over five and three traps in orchards with low (a), moderate (b) and high (c) carob moth populations.

Figure 3 shows the distribution of carob moth across the major almond production districts according to this project's moth trapping program and nut surveys. Since the trapping program was initiated in 2011/12, it has continued to confirm that areas of the Victorian Sunraysia and South Australian Riverland regions are the hotbeds of carob moth activity, with other districts showing relatively little or no sign of the pest. This is no surprise, given that the Riverland/ Sunraysia regions hold over 85% of Australia's almond plantings (Anon

2014), mostly in large and almost contiguous orchards that provide suitable conditions for the build-up of pests like carob moth. The orchards with moderate to very high trap catches were of several hundred hectares or more in size.

Carob moth is also known to be present in Western Australia but the project could not obtain any reliable data from almond orchards in that region.

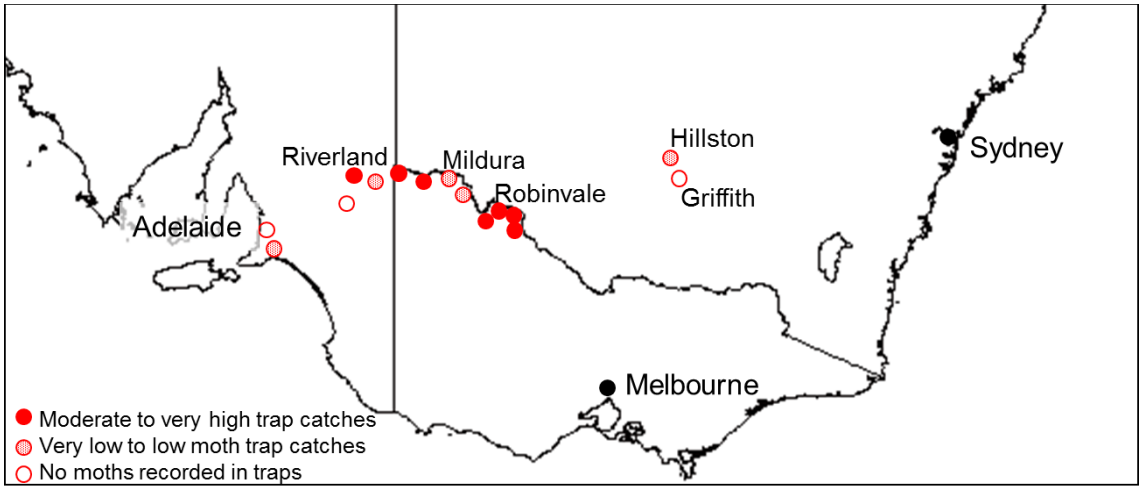

Figure 3. Approximate locations of carob moth trapping sites and their indicative population levels.

The following charts (Figure 4 - Figure 7) show examples of the variation in moth activity within and between seasons in almond blocks of relatively low, moderate and high carob moth population levels.

Generally, each orchard in the trapping program has tended to maintain similar levels of moth activity from season to season, apart from the occasional unusually high single count. Even within this consistency though, it can be seen from the trap data that activity levels of carob moth were somewhat higher during 2013/14 than in the other seasons of trapping.

Data from a number of the trapping sites suggest that up to three full generations of moth emergence occur each season. In some seasons and sites, there are indications of the start of a fourth generation in late autumn before temperatures become too cold for moth emergence and flight. Overseas experience is that under field conditions, carob moth typically develop three to four generations yearly (Gothilf 1984, Lebdi-Grissa 2005).

An important point in relation to the protection of new season almonds, is that the peak in moth activity relating to the second generation of moth emergence coincides with almond hull split (early January) – the point at which the new crop becomes susceptible to infestation by the pest.

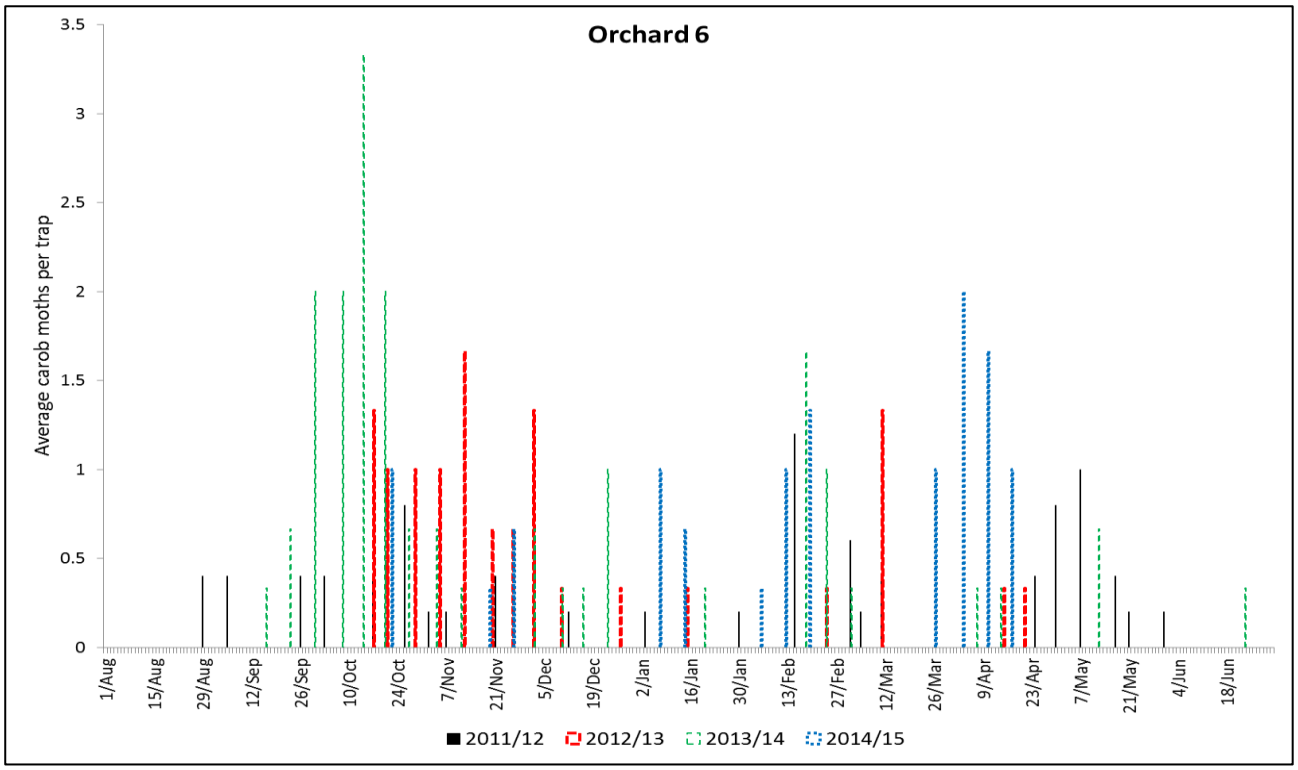

Figure 4. Average weekly trap catch of male carob moth, trap site 6, 2011-2015.

Orchard 6 is a small property (< 20 ha) where mummy nuts are very scarce, and its moth trap counts have consistently been very low. Anecdotal evidence suggests that efficient bird predation of mummy nuts on small properties like this is a key reason that they remain relatively free of carob moth.

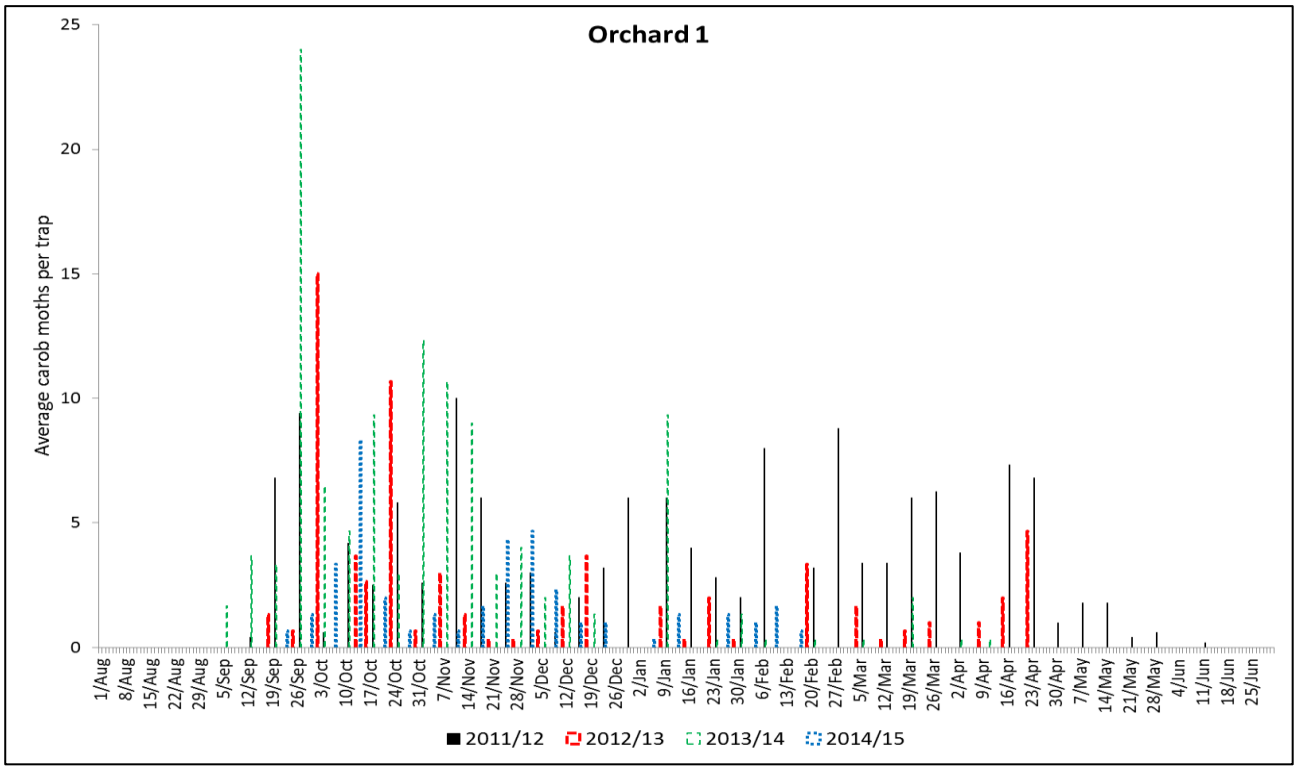

Figure 5. Average weekly trap catch of male carob moth, trap site 1, 2011-2015.

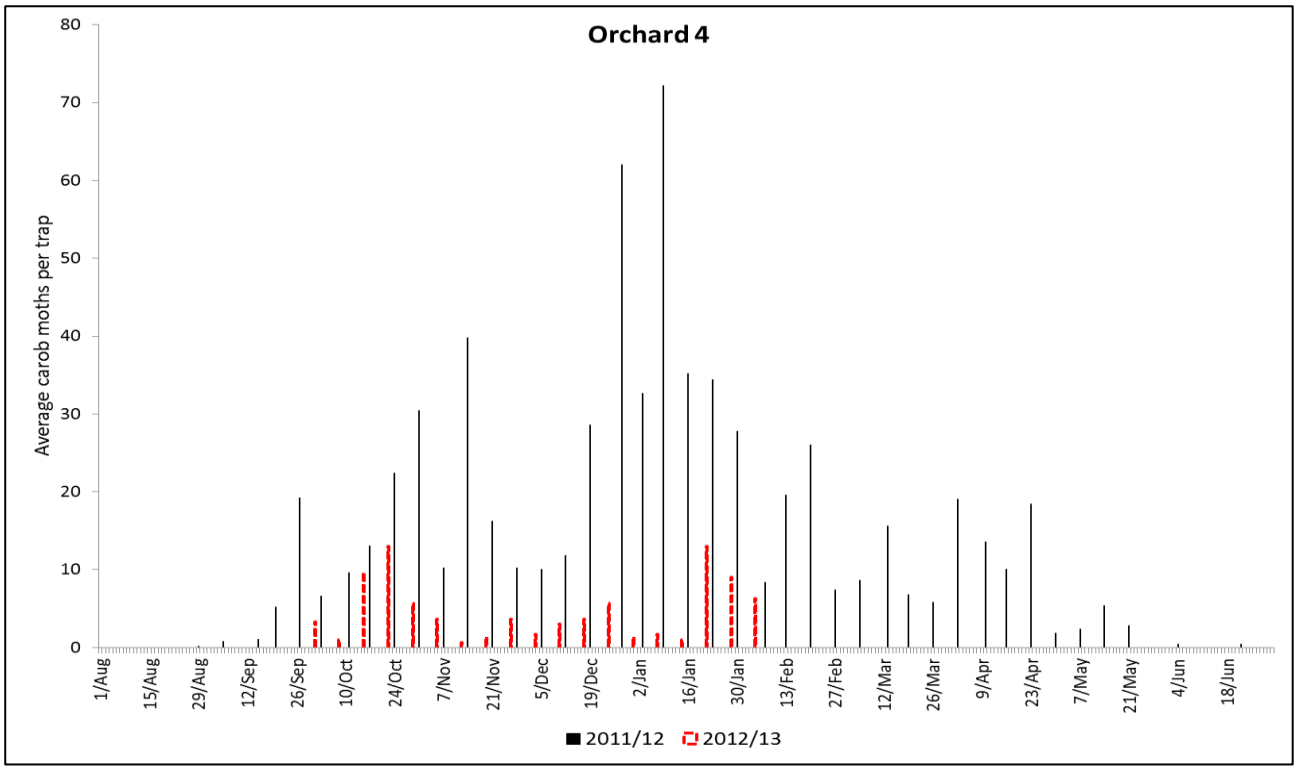

Figure 6. Average weekly trap catch of male carob moth, trap site 4, 2011-2013 (insecticide applied during 2012 hull split).

One exception to the season-to-season consistency in moth activity is orchard 4 (Figure 6). The insecticide chlorantraniliprole was applied to this orchard at hull split in 2012, which may have a bearing on the lower levels of moth activity in the following season.

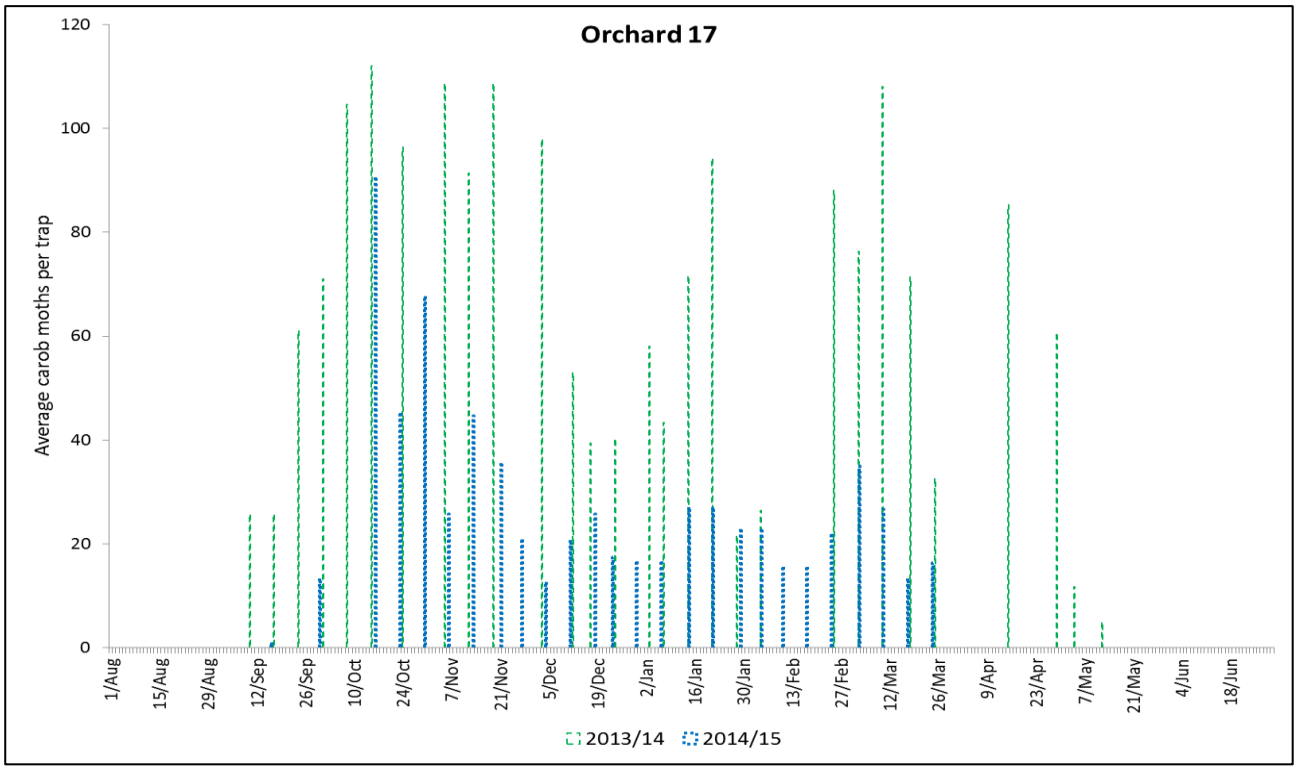

Figure 7. Average weekly trap catch of male carob moth, trap site 17, 2013-2015.

Construction of a degree-day model

A prototype degree-day model for carob moth has been compiled and two examples of its output using different orchards and seasons are shown below (Figure 8, Figure 9). Given a 'biofix' date at the start of the season, in this case the date of first moth catch, the model calculates the expected start dates and duration of

subsequent generational flights. Egg development and hatch times are incorporated in the calculations. If enough reliable data from carob moth trapping becomes available, analysis of that together with weather data should allow the prediction of the biofix date to be added to the model.

The curves on the charts are purely arbitrary normal distributions fitted between the expected start and end dates of moth emergence to provide a guide as to the expected timing and idealised pattern (not magnitude) of moth activity. The duration of the first flight (moths that developed from overwintering larvae) is based on local and overseas observations and trap data. The date for 1% hull split is predicted by a degree-day model developed in California (Tombesi et al. 2010) that has been incorporated into the carob moth model spreadsheet.

Output from the model confirms the capacity of carob moth to develop three full generations each season, with a possible start to a fourth in late autumn/early winter. It has also been useful in the interpretation of trap data where generation peaks in moth activity are not always well defined.

The carob moth model may not be necessary for timing of applied treatments for carob moth as these are currently based on emergence of the first generation of moths (detected by trapping) for spring insecticide applications, and hull split (determined through field monitoring of nut development) for insecticide applications at 2-5% hull split. Mating disruption which also has potential as a management tool must be applied at least two weeks prior to hull split, so requires a hull split predictor such as the Californian model.

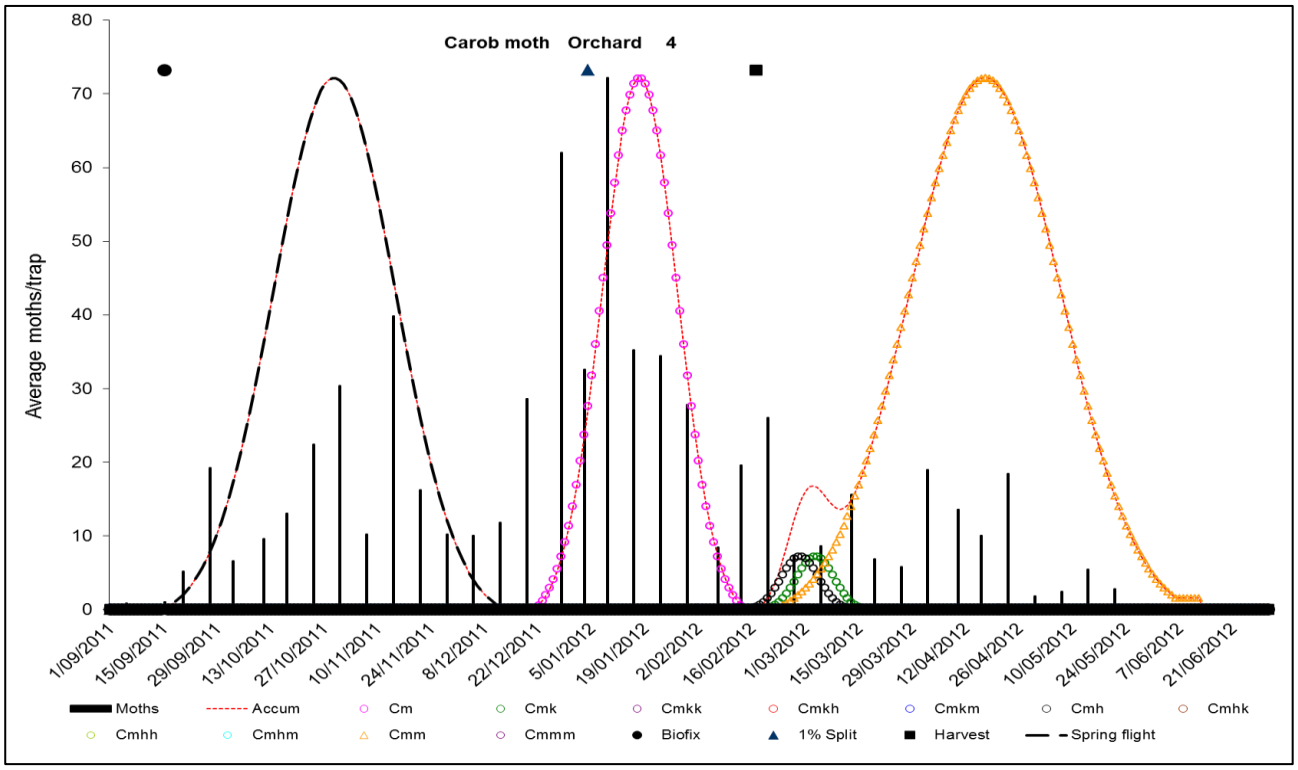

Figure 8. Degree-day model screen, orchard 4, 2011/12.

The model may however have value in forecasting events such as flight/oviposition periods for mass release of egg parasitoids or the start of the autumn generation if a post-harvest insecticide was to be used to minimise development of an overwintering population. With further development, the model could also contribute to our understanding of season-to-season variations in carob moth activity, such as the higher population levels observed during 2013/14 as mentioned above.

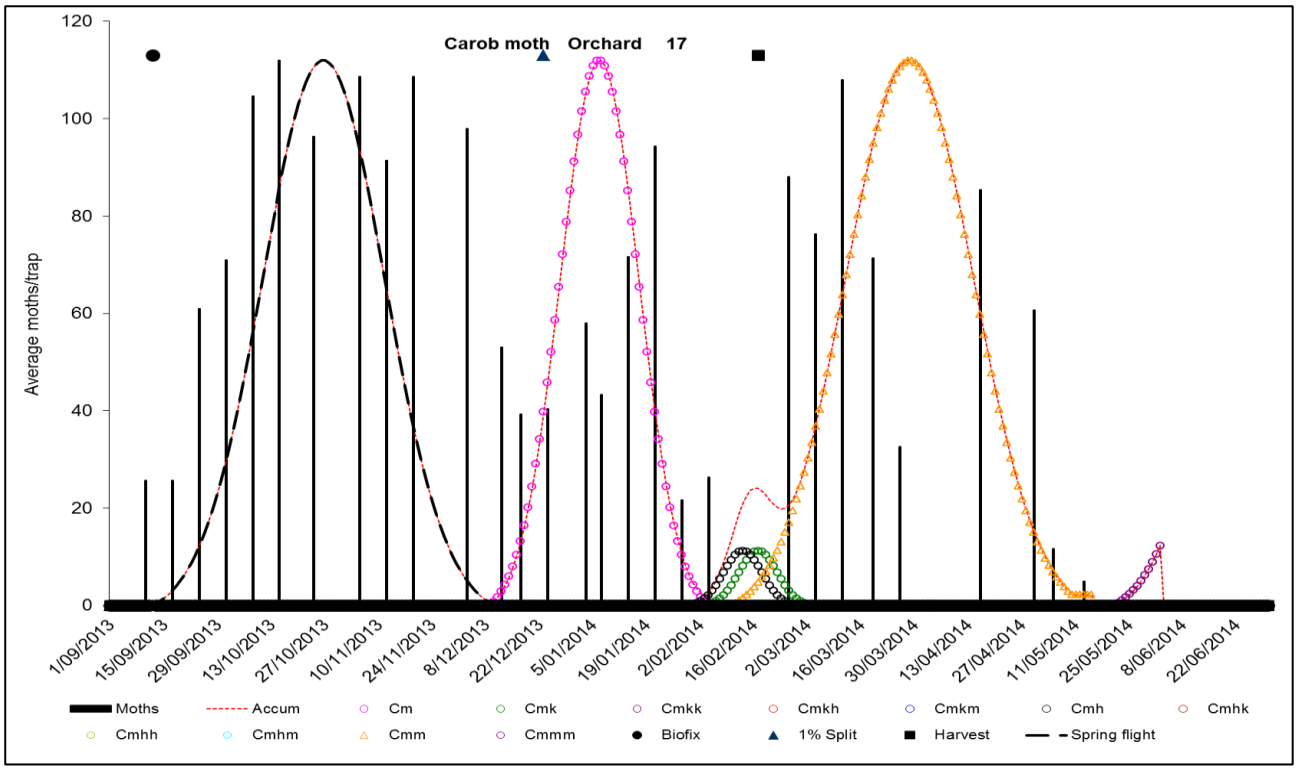

Figure 9. Degree-day model screen, orchard 17, 2013/14.

One limitation that has been encountered during validation of the model is the narrow climatic range occupied by Australia's almond production districts. Validation of insect developmental models based on temperature and other environmental parameters requires field data that relate to developmental stages of the insect species (e.g. moth trap counts) together with relevant weather data. To test models effectively, these data should be obtained from different locations that provide some variation in relation to the relevant parameters (e.g. temperature). However, most of the data available on carob moth in Australia is from traps in the Sunraysia/Riverland regions – an area fairly homogenous in relation to climate. In this situation, model validation can still be performed but will not be as robust as if more varied data was used.

Spot surveys of overwintering mummies

Table 13 lists the infestation rate of mummy nuts in late winter across the surveyed orchard blocks. As can be seen, the infestation levels within the samples varied significantly from year to year and between orchards.

All samples with higher infestation rates (20% or higher) were from blocks closely associated with large plantings of almonds, of 500 to several thousand hectares. Mummy nuts were generally plentiful in late winter in these blocks.

In all cases where blocks were small (less than 30 ha of almonds), it was difficult if not impossible to find enough mummy nuts for the samples. On these properties there was evidence of nuts hanging on the trees after harvest, but few, if any, contained a kernel. Anecdotal evidence from orchard managers indicates that the predation of nuts by various bird species is largely responsible for the lack of mummies on their smaller properties as mentioned earlier, while the impact of birds on very large properties appears more limited. It was also noted during discussions with orchard managers, that as could be expected, critical orchard operations such as spraying for hull rot, harvest shaking and winter shaking for mummy removal could all be performed in a timely manner on small orchards, whereas this is generally difficult to achieve uniformly across large properties. There was also some suggestion that attention to detail regarding these practices could be afforded more on smaller properties. The economies of scale that benefit crop production unfortunately do not appear to be well suited to optimum management of mummies.

Table 13. Carob moth infestation levels in almond mummy nuts in late winter.

	% of Nonpareil mummies infested in late winter		
Orchard site	2011	2012	2014
Riverland 1	-	-	-
Riverland 2			4.0%
Riverland 3		0.0%	11.0%
Riverland 4	47.0%	20.0%	33.0%
Sunraysia 1		15.0%	
Sunraysia 2	2.0%	* 14.8%	2.0%
Sunraysia 3	0.0%	0.0%	0.0%
Sunraysia 4		0.0%	11.0%
Sunraysia 5	55.1%	19.0%	
Sunraysia 6	31.4%	3.0%	
Sunraysia 7		8.9%	48.0%
Sunraysia 8	c 7.0%	0.0%	
Sunraysia 9		1.5%	9.0%
-	No mummy nuts found		
Blank	Orchard not surveyed that year		
*	Old nuts from crotch of trees. None found hanging on trees		
c	Carmel variety		

The importance of mummy nut management as a pest risk reduction strategy can be seen from a simple calculation. If a 10 ha almond block carries 20 mummy nuts per tree into spring, with a 40% carob moth infestation rate, then 1-2 million carob moth eggs could be produced by the spring generation of moths emerging within that block. The much higher mummy population densities present in some orchards increases the risk even further.

Surveys of field-stockpile nut infestation

No sign of carob moth infestation or damage was detected in current season nuts from the pre or post-hull split samples from the Project AL11009 trial site.

Of the 180 nuts from the 4 May 2012 stockpile samples inspected for insect damage, none contained any live carob moth, 4.4% showed signs of carob moth chewing damage and 2.8% had suffered kernel damage.

Of the 979 nuts from the 29 June 2012 stockpile samples assessed for mould, none contained any live carob moth and 54 (5.5%) showed signs of carob moth damage.

No further samples from this stockpile were assessed as the nuts were processed in late July 2012. Because the timing of processing of any particular stockpile was driven by commercial forces, i.e. the demand for a certain grade of nut, or for the crop from a particular farm, the availability of a stockpile for longer-term monitoring and sampling could not be guaranteed by the processor (as was learnt in this situation), unless a stockpile or portion thereof was purchased for the purpose. That option was beyond the scope of both projects. This and the added complication of fumigation as a standard practice led us to discontinue stockpile sampling for carob moth and focus on field populations of the pest.

Targeted research to determine specifically the efficacy of current fumigation practices against all almond nut pests, and options for improvement, are currently (June 2015) being flagged as a worthwhile area of investment for the almond industry.

Sequential surveys of nut infestation

Spring 2011 – autumn 2012

The rate of infestation of mummy nuts with carob moth detected in surveys from mid spring 2011 to late autumn 2012 is shown in Figure 10. The fact that most early season infestation is restricted to the hull and shell (as seen from the relatively low proportions of nuts with kernel damage), is simply because at that time carob moth is present mostly as eggs (Figure 11), which are usually laid on the hull and sometimes the shell. The high infestation rate of mummy nuts at this site allowed for a considerable carob moth population to

develop, prompting the producer to apply an insecticide during hull split (20-21 Jan 2012) in an attempt to limit damage to the new season crop. As Figure 11 and Figure 12 show however, despite the insecticide treatment the mummy nuts continued to carry a significant level of infestation.

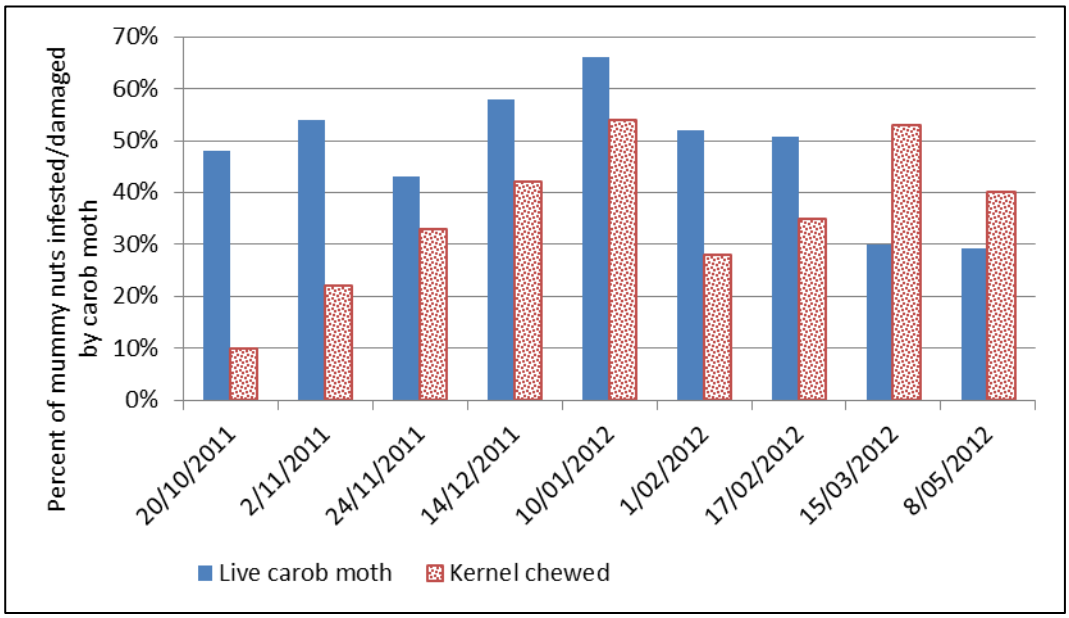

Figure 10. Rates of infestation and kernel damage by carob moth in mummy nuts on trees, spring-autumn 2011/12.

As would be expected from the pattern of moth activity recorded at this site (trap site 4, Figure 6), the peak in appearance of fresh eggs (mid Dec to mid Jan) coincides with the almond hull split period. It is also interesting to note that egg-laying continued into early May.

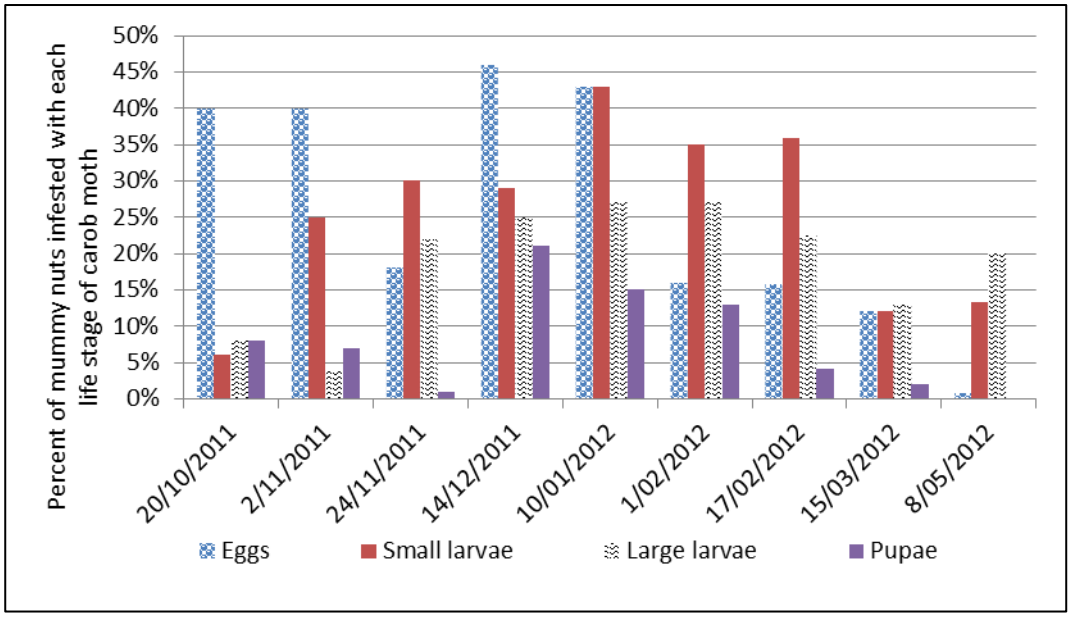

Figure 11. Presence of carob moth life stages in mummy nuts on trees, spring-autumn 2011/12.

The levels of live infestation of mummy nuts collected from the ground (Figure 12) were considerably lower than those recorded in mummies still hanging on trees. It is likely that carob moth larvae in nuts on the ground are subject to higher levels of predation, by ants for example, as has been found in date plantations (Nay & Perring 2005).

Out of 1,030 nuts collected from the orchard floor (790 mummies, 240 hull split new crop), only two carried fresh carob moth eggs. Both were mummy nuts that are very likely to have had the eggs laid on them while still on the tree.

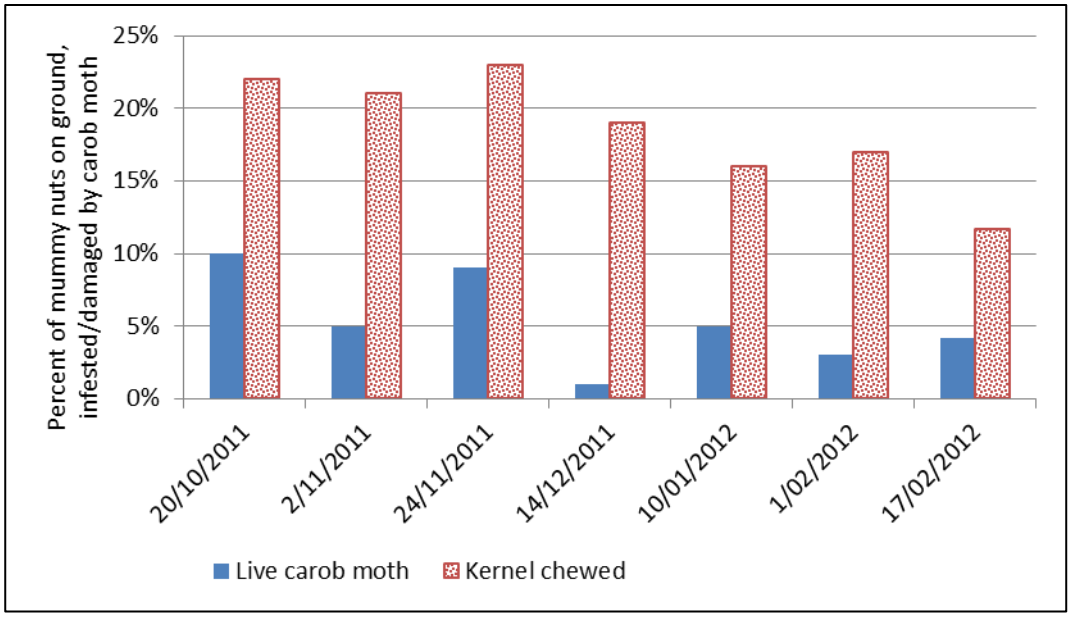

Figure 12. Rates of infestation and kernel damage by carob moth in mummy nuts on the ground, spring-autumn 2011/12.

As was expected, no sign of carob moth infestation was found in new season nuts until soon after the start of hull split, when 4% of nuts carried fresh eggs (Figure 13 & Figure 14). Kernel damage then occurred within the following three weeks. From hull split until harvest, the maximum infestation rate in the new crop reached only 9% compared to 66% in mummy nuts, and most of that infestation was limited to between the hull and shell, as indicated by the relatively low level of kernel damage compared to overall infestation.

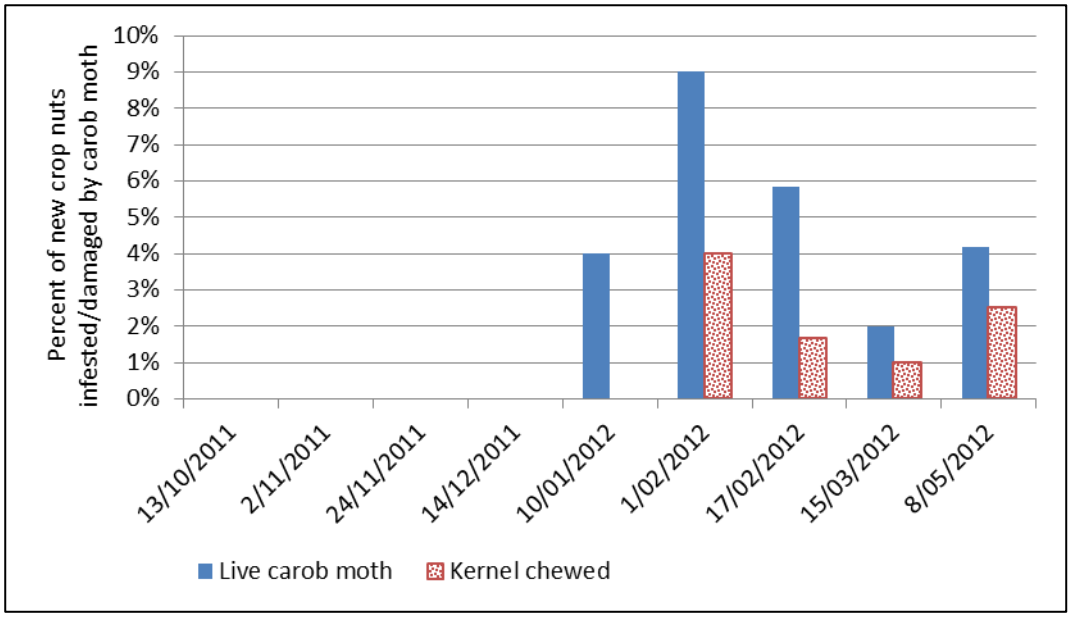

Figure 13. Rates of infestation and kernel damage by carob moth in new crop nuts, spring-autumn 2011/12.

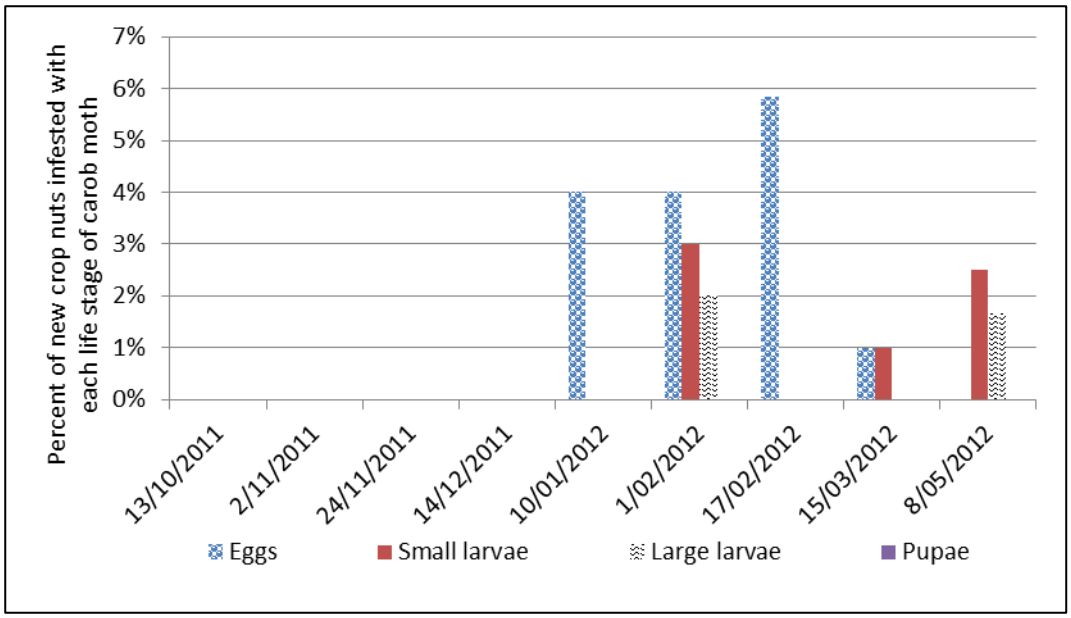

Figure 14. Presence of carob moth life stages in new crop nuts, spring-autumn 2011/12.

Carob moth's preference for mummy nuts over new season nuts as egg laying sites can be seen in Figure 15.

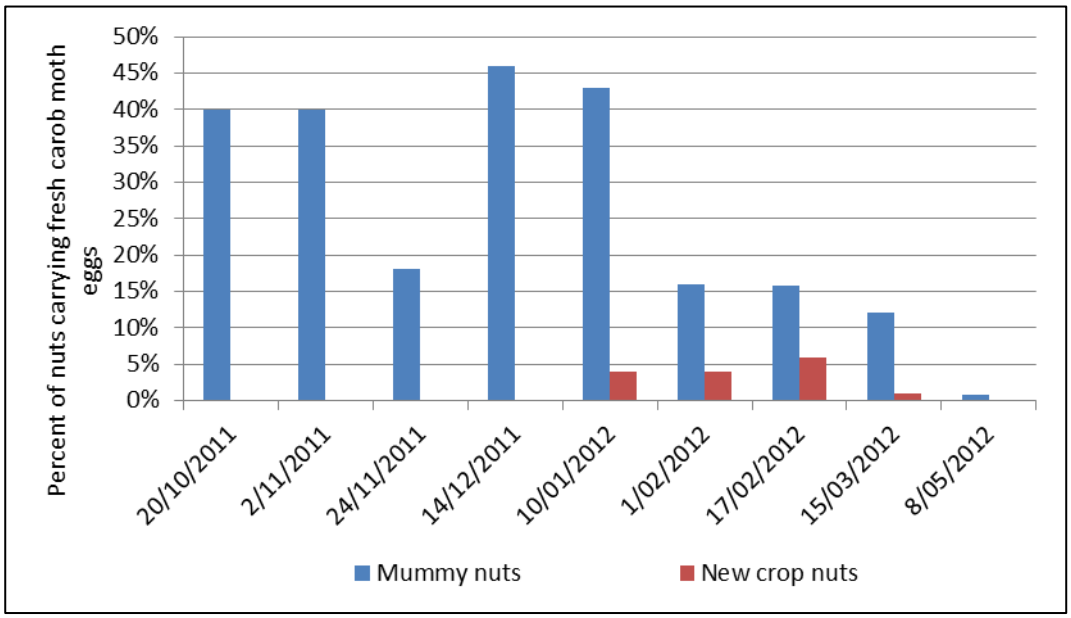

Figure 15. Presence of fresh carob moth eggs on mummy and new crop nuts, spring-autumn 2011/12.

During these surveys, up to 16% of current season nuts and 61% of mummy nuts on trees were found to carry old carob moth egg cases (up to 20 per nut) but have undamaged kernels. This may indicate significant levels of mortality from such factors as heat, desiccation, predation and parasitism, amongst young larvae before they can become established in the nuts.

Summer 2014/15

During the summer 2014/15 surveys, mummy nuts on trees maintained a fairly constant level of carob moth infestation (Figure 16). The levels of kernel damage exceeded those of live infestation simply because some mummy nuts that had been used and vacated by carob moth had not yet been reinfested by fresh oviposition.

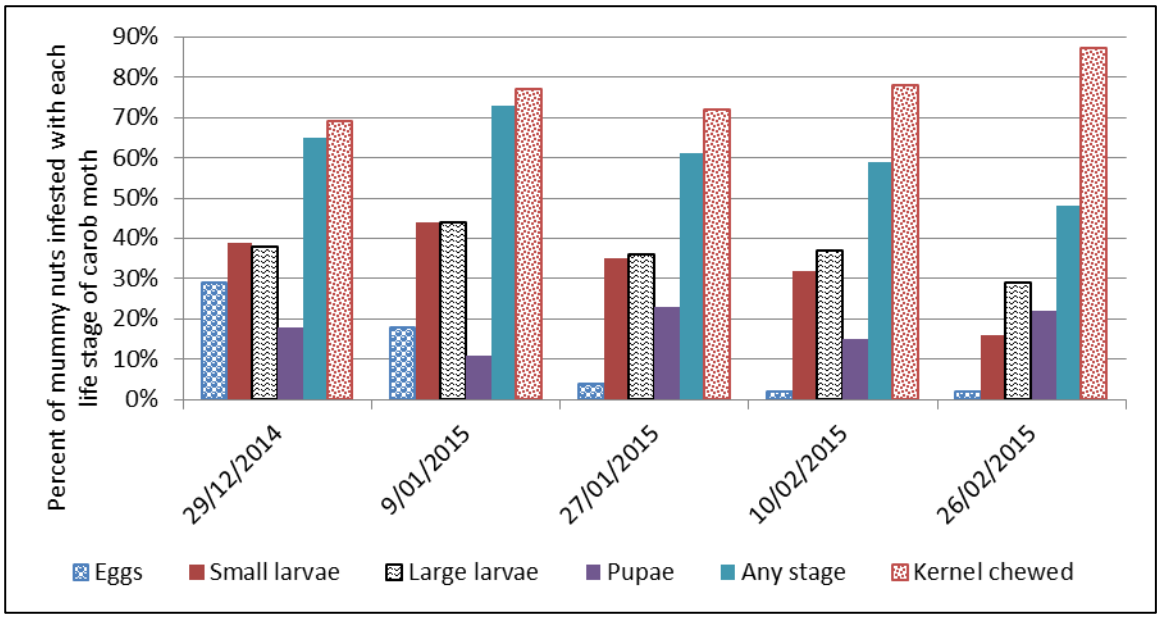

Figure 16. Presence of carob moth life stages in mummy nuts on trees, summer 2014/15.

As found in the earlier surveys, new season nuts became infested with carob moth eggs soon after the start of hull split, with kernel damage ensuing within the next three weeks (Figure 17). By the time of harvest, an average of 7% of new kernels within the survey block had been chewed by carob moth.

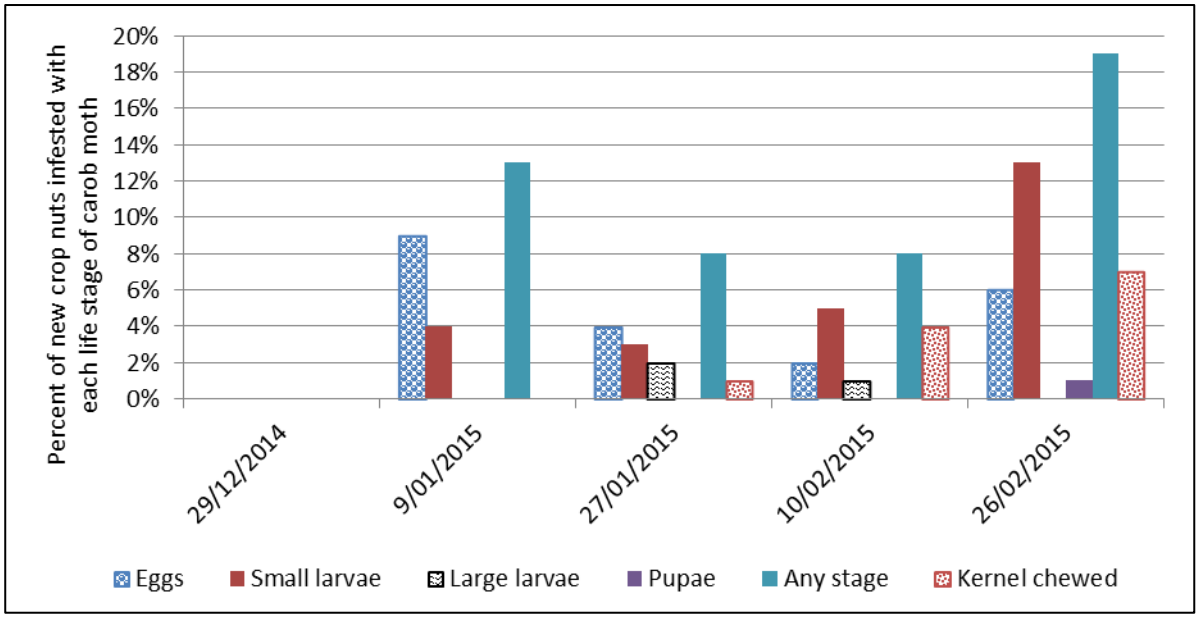

Figure 17. Presence of carob moth life stages in new crop nuts, summer 2014/15.

Deficit irrigation trial

Data relating to the kernel damage noted during assessments of nut samples from the deficit irrigation trial are shown in Figure 18. Higher levels of damage were associated mostly with the driest treatments, RDI55 and SDI55. The trees in those treatments were under considerably higher levels of water stress, resulting in earlier onset of hull split. Because trees in all six irrigation treatments were harvested at the same time, the relatively higher levels of kernel damage seen in the dry treatments are most likely due simply to those nuts being susceptible to oviposition by carob moth for a longer period due to earlier splitting. In a commercial situation, deficit irrigation would be applied to entire blocks or orchards, in which case the entire blocks or orchards would be expected to experience earlier split and therefore be suitable for earlier harvest. This would avoid the increased risk of damage observed in the trial under the dry treatments.

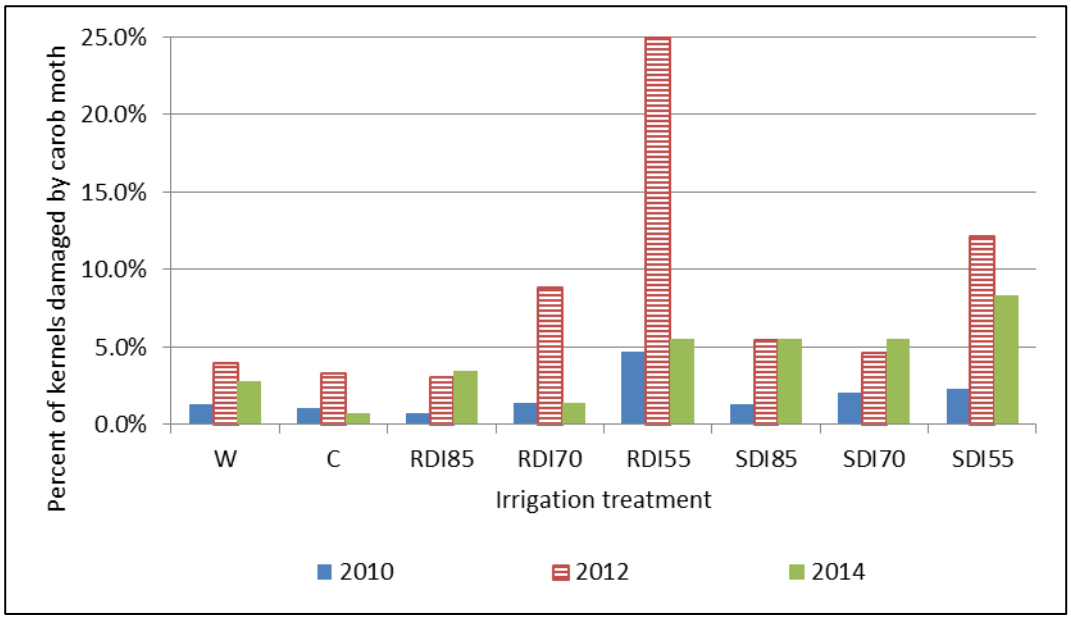

Figure 18. Deficit irrigation treatments and rates of kernel damage by carob moth in new crop nuts at harvest, 2010-2014.

Spatial distribution mummy survey

The spatial survey of mummy nuts in spring found the distribution of carob moth infestation to be very patchy across the 19 ha block (Figure 19). A total of 2,019 mummy nuts were examined during the survey, but as the figure shows, no intact mummy nuts were found in the north-west corner of the block. This corner and the whole western edge of the block were bordered by scrub that linked directly to larger areas of native vegetation including river habitat. The lack of mummies in this area of the block was very likely due to bird activity, a subject that has been the focus of recent research (Luck 2013, Luck et al. 2014).

Because of the patchy distribution of carob moth infestation in the nuts, small isolated samples could easily provide a misleading picture of the infestation status of a particular block. More detailed analysis of results from structured sampling such as this will be required to determine the optimum sampling regime to detect and quantify carob moth infestation across almond blocks. Until this is achieved, sampling of nuts for carob moth infestation should involve the collection of nuts from as many trees as is practical, scattered throughout a block.

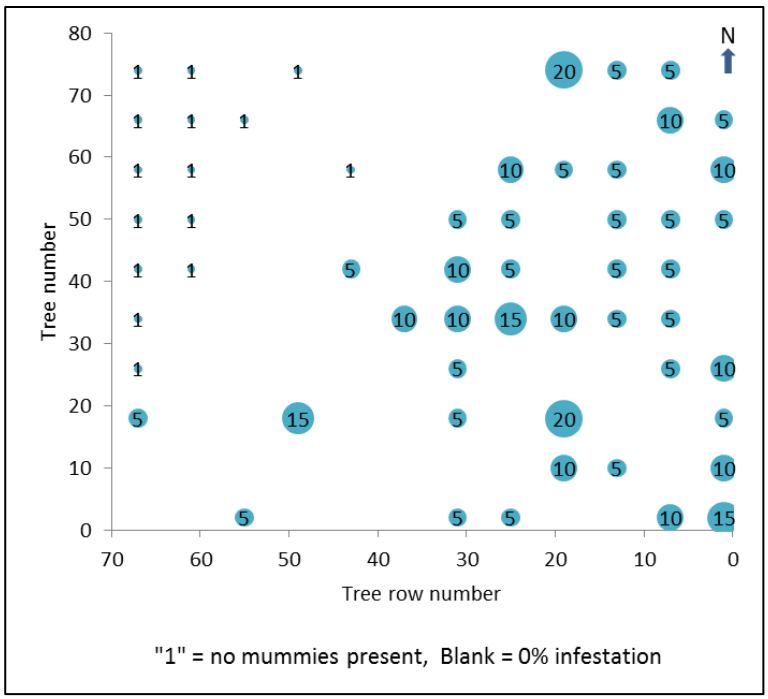

Figure 19. Percent infestation of mummy nuts with carob moth across a 19 ha (396 m x 473 m) orchard block.

Conclusions

Carob moth was confirmed to be present throughout Australia's almond growing zone, from Adelaide to Griffith, with orchards of high populations of the pest located in the Sunraysia/Riverland region. Heavily infested orchards were at least several hundred hectares in size and tended to carry significant loads of mummy nuts. In contrast, smaller orchards tended to carry few if any mummy nuts and maintained low counts of carob moths in traps. High rates of infestation of overwintering mummy nuts with carob moth highlight the importance of good orchard sanitation in management of this pest. Removal of mummy nuts by birds is likely to play an important role in sanitation of small orchards, as might the timeliness of orchard management operations.

Trap data and output from a degree-day model confirmed that carob moth develops three full generations each year in almonds in Australia, starting in the first to third week of September with emergence of moths that developed from overwintering larvae. Based on trap catches, in the absence of widespread management action against the pest, orchards tend to maintain similar infestation levels from season to season, in that heavily infested orchards remain heavily infested, and similarly, light to moderately infested orchards continue to trap light to moderate numbers of moths.

Sequential surveys of nuts from trees confirmed a heavy season-long use of mummy nuts by carob moth and no infestation of new nuts until hull split. Infestation of new season nuts begins almost immediately after hull split. Kernel damage occurs within three weeks and increases significantly over the following few weeks, which highlights the importance of avoiding delays in harvest as much as possible.

The patchy distribution of infested mummies within an orchard block indicates that small numbers of isolated samples have a high chance of missing infestation hotspots and that nut sampling to detect the presence and level of infestation needs to be distributed across a block.

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Investigating applied control of carob moth in almonds.

Aim

Evaluate mating disruption and insecticide application as potential management tools for carob moth in almonds.

Introduction

Carob moth *Apomyelois* (=*Ectomyelois*) *ceratoniae* is an economically significant pest of a wide range of tree crops globally. It has been a minor or sporadic pest of almonds in Australia for many years, but became a significant kernel quality issue for the industry over the past four to five years, after unusually wet periods during the harvest seasons of 2007 and 2011. It is possible that the growth in populations of the pest has been associated with an increase in numbers of 'mummy' nuts (nuts remaining on trees after harvest). Mummies often arise from nuts that are affected by hull rot, a fungal disease that develops during wet summer conditions. Mummy nuts are an important food resource for carob moth as it does not infest new almond nuts until hull split (typically early January). Because of this, carob moth relies on mummies for the period between harvest in one season and hull split in the next.

After observing high infestation levels during the 2011 harvest, the industry decided to take management action to protect future crops. The only response available to the industry for the 2012 harvest was a pesticide application at hull split. This option was made possible by an emergency use permit from the Australian Pesticides and Veterinary Medicines Authority (APVMA) arranged by the Almond Board of Australia (ABA).

In California, a commercial product (SPLAT-EC™) containing a mimic of the carob moth sex pheromone has been developed and used to protect date crops from carob moth by disrupting the pest's mating behaviour (Todd et al. 1992; ISCA Technologies 2011). Such a product has the potential to protect almond crops from damage while avoiding the disadvantages of broad scale pesticide use. Mating disruption (MD) of a pest is based on the fact that the target pest species uses a sex pheromone to allow males to locate females for mating. This approach involves releasing a synthetic version of the sex pheromone throughout a crop area such as an almond orchard. This prevents males from following the pheromone trail released by females and so prevents or delays mating and further infestation of the crop. Some mating usually still occurs due to chance encounters between the sexes. The trials reported here were established to evaluate MD of carob moth in almonds under Australian conditions, in comparison with the insecticide treatment that the industry had begun to use.

Materials and methods

Trial site

All trials reported here were located in a commercial almond orchard in the Sunraysia region of Victoria, Australia. Drip irrigation and fertigation were used throughout the orchard and the orchard floor was generally maintained free of vegetation. The major almond variety (every second row) was Nonpareil, while Price, Monterey and Carmel were used as pollinators in the alternate rows. The suitability of the orchard for carob moth trials was confirmed by an initial assessment of the population density and distribution of 'mummy' nuts and carob moth throughout the area. This assessment was performed on Nonpareil trees at and around the centre of what would become 15 experimental treatment plots for the 2012/13 trials.

2012/13 Trials

Mating disruption (full-rate) & insecticide

Trial design

The area used for the trial was approximately 59 ha in area, measuring 605 m by 974 m. It contained 83 rows (oriented north-south) spaced at 7.2 m and 178 trees per row spaced at 5.5 m giving a total of 14,774 trees. The trees were five years old and were a mix of three varieties in a repeating row pattern of Nonpareil (NP)-Price-NP-Carmel-NP-Carmel. Further blocks of almonds were adjacent to the north and west sides of the trial area, while the south and east sides faced onto dry grassland. The trial area was divided into 'sub-blocks' by two north-south headlands and two east-west headlands approximately 14 m wide. Fifteen

treatment plots of 2 ha each were arranged within the trial area, in a rectangle of three columns by five rows of plots. Each plot measured 25 trees by 20 rows. A 2 ha plot size was chosen as it was considered manageable while also being 20% larger than the plots used in successful Californian trials of SPLAT-EC™ against carob moth in dates(Park & Perring 2008). The plots were positioned so that they were separated from adjacent plots or headlands by buffers of at least three trees.

The trial site was selected at short notice, as the originally proposed site was found during pre-trial assessments to have very low mummy infestation levels. As a result, there was not sufficient time to carry out pre-trial moth trapping to inform the allocation of treatments to plots. Instead, the three experimental treatments were allocated randomly to the three plots in each row of the trial.

Treatments

Mating disruption

The product used to disrupt mating of carob moth was SPLAT-EC™ (ISCA Technologies Inc., Riverside, California USA). This putty-like product is a proprietary blend of various waxes and other compounds and contains 2% of the active ingredient (Z,E)-7,9,11-Dodecatrienyl formate, a synthetic mimic of the female carob moth sex pheromone. SPLAT-EC™ is applied using a caulking gun calibrated to deliver a standard sized dollop. On 21 Dec 2012 one dollop of approximately 2.5 g of SPLAT-EC™ was applied to the trunk of each of the 494 trees within each of the MD plots, providing an application rate of approximately 617 g/ha as recommended by the manufacturer. The dollops were applied to the trunk or a main scaffold branch of each tree, between 1.5 and two metres above the ground. They were positioned, as far as was practical, on the south side of the trunk or branch to minimise excessive exposure to direct sunlight.

Laboratory studies have found that under favourable temperature and diet conditions, adult female carob moths typically live for a maximum of 11 days, and lay 90% or more of their eggs within their first 9 days (e.g. Hung et al. 2003; Navarro et al. 1986). A two week lead time for the MD treatment prior to hull split was chosen to minimise the risk that females who mated prior to the treatment application would still be able to lay eggs in the hull split nuts. This timing also coincided with the start of the second generation of carob moth emergence.

Insecticide

On 4 Jan 2013 (1-5% hull split) the insecticide plots were treated with Altacor® (350 g/kg chlorantraniliprole; Du Pont™) using standard practice for the orchard. Chlorantraniliprole is an ovicide/larvicide and its application at hull split is intended to protect almonds from infestation by carob moth eggs that are laid from hull split onwards. The product was applied at a rate of 280 g/ha in 1,500 l water/ha using an airblast sprayer travelling at 6 km/hr. As per label instructions, a non-ionic wetter (Deluge Low Foam™; Victorian Chemical Co. P/L) was included at the rate of 15 g active ingredient/100 l water. When spraying the outer rows of the insecticide plots, the spray nozzles facing towards a headland or non-insecticide trial plot were turned off.

Control

Control plots were left untreated.

Mating disruption (half-rate)

To assess the possibility of using reduced rates of the MD treatment, a small pilot trial was established in a separate area of the same property.

Trial design

Eight treatment plots of 0.5 ha each were arranged within a 20 ha trial area. Each plot measured 13 trees by 10 rows. The plots were positioned so that they were separated from adjacent plots by at least 50 m. The potential movement of mated females from untreated areas into the small plots was not a concern, as the success or otherwise of the half-rate treatment was to be gauged by trap catch levels only. The following treatments were allocated randomly to the eight plots.

Treatments

Mating disruption

Mating disruption plots were treated with SPLAT-EC™ at approximately half the recommended rate, i.e. 332 g/ha. The same application procedure was used as for the full-rate trial except that the 2.5 g dollops were only applied to every second tree.

Control
Control plots were left untreated.

Apart from the different treatments described above, all trial plots were subject to the same farm management practices.

Data collection & analysis

Pest population assessment

To assess the level and distribution of carob moth infestation of mummy nuts within the full-rate trial area, 25 mummies in total were collected from Nonpareil trees around the centre of each trial plot on 14 Dec 2012. This gave a total of 375 mummies which were returned to the laboratory and examined for carob moth eggs, larvae and pupae.

The distribution of mummy nuts was gauged more formally on 3 Jan 2013, by a visual assessment of trees, using a score of 0 (no mummies), 1 (one to five mummies per tree) and 2 (six or more mummies per tree). Within each plot, the 25 trees in the centre Nonpareil row were assessed.

Male moth trapping

One measure of the efficacy of SPLAT-EC™ in disrupting mating of carob moth is the degree to which pheromone trap catches of male carob moth are suppressed, as an indication of the ability of SPLAT-EC™ to prevent males from locating point sources of the sex pheromone (i.e. traps or female moths).

The ability of male carob moths to locate a point source of sex pheromone under each treatment regime was measured using pheromone-mimic traps. One white plastic delta trap containing a sticky base and baited with a lure containing the carob moth sex pheromone mimic (ISCAlure-Ceratoniae™, ISCA Technologies Inc., Riverside, California USA) was placed 1.5-2 m above ground in a tree at the centre of each trial plot. The traps were installed on 14 Dec 2012 in the full-rate trial and 8 Jan 2013 in the half-rate trial.

Counts of trapped male carob moths were made weekly during the trials and the moths were removed after each count. The pheromone-mimic lures were replaced after six weeks. The new replacement lures had been 'aged' by storing them outdoors in a shady position for one week. This was done to avoid the slight repellent effect reported with new lures.

Nut infestation

The main measure of the efficacy of SPLAT-EC™ in disrupting carob moth mating is the reduction in levels of nut infestation and kernel damage, as an indication of reduced mating and fertile oviposition onto the crop.

Just prior to commercial harvest, five samples of 100 new crop Nonpareil nuts each were collected from each of the full-rate trial plots. One sample was collected from the centre tree in each plot. The other samples were collected from trees half way between the centre tree and each of the four corners of the plot. The nuts were returned to the laboratory where their hull, shell and kernel were inspected under a dissector microscope and assessed for infestation and damage by carob moth. In the results reported below, 'Live' carob moth includes live eggs, larvae or pupae, and 'Any sign' of carob moth includes old pupal cases, webbing or chewing damage to the hull, shell or kernel typical of carob moth, regardless of whether any live insect is present. Samples were collected on 19 Feb 2013.

Kernel damage

After the trees had been shaken for commercial harvest, but before the nuts were swept into windrows (22 Feb -1 Mar 2013), five 'bulk' samples, each of approximately 1.8 kg equivalent kernel weight of new crop Nonpareil nuts were collected from each of the 15 plots of the full-rate MD trial. As per the pre-shake sampling, one sample was collected from the centre tree in each plot. The other samples were collected from trees half way between the centre tree and each of the four corners of the plot. The nuts were collected into woven onion bags to ensure that they were well ventilated. The nuts were stored in a glasshouse at ambient temperature until the commercial crop from the trial-site farm was processed. This was to allow any carob moth in the samples to develop for the same time as those in the commercial crop, assuming a 'worst case' situation of no fumigation. At that time (15-19 Apr 2013) the samples were hulled and shelled using a small-scale almond huller/sheller (Jessee Equipment Manufacturing, Chico, California). One thousand kernels from each of the five sample points per plot (75,000 kernels in total) were then inspected under a dissector microscope and assessed for damage by carob moth. Considering the pre- and post-shake samples, a total of 82,500 kernels were assessed for damage in the full-rate MD trial.

Data analysis

For all three seasons of trials, data comparing the effects of treatments on amount of nut infestation and kernel damage were analysed using ANOVA, assuming a completely randomised design and using protected LSD (at 5% significance level) to compare the treatment means. Residual diagnostics were performed to check for the validity of ANOVA assumptions and a permutation test was also performed to confirm the ANOVA results for each analysis conducted.

Checks for relationships between mummy population density and levels of nut infestation or kernel damage were performed using scatterplot matrix and correlation analyses with a two-tailed test to determine whether the correlations (relationship between variables) were significant or not as a precursor to more sophisticated regression analyses. The results showed that there were no significant correlation in most cases, therefore regression analyses were not performed.

2013/14 Trials

Mating disruption (half-rate) & insecticide

Trial design

Because it was considered possible that the 2012/13 treatment effects were compromised by the immigration of mated female moths from untreated areas into the 2 ha treatment plots, it was decided to increase plot size for the 2013/14 trials in an effort to minimise any such edge effect.

Very limited information is available on dispersal of carob moth. In a single trapping trial overseas, the majority of male moths released from a single point were recaptured within 120 m of the release point (Mediouni & Dhouibi 2007). If the dispersal of mated females was assumed to be similar, then having trees for nut sampling at least 150 m from the nearest untreated trees should significantly reduce the chance of fertile oviposition on those sample trees by mated females from outside the treated area. This would require square treatment plots to be at least 300 m wide, i.e. at least 9 ha in area.

To satisfy this requirement, four replicates of three 10 ha plots were established over an area of the orchard that was not used in the 2012/13 trials. Each plot was separated from the next by a 5 m headland along their northern and southern borders, and at least ten rows of trees (>70 m) along their eastern and western borders. Each plot had an 11 m headland running north-south through the approximate centre of the plot.

Treatments

Mating disruption

Based on results from the 2012/13 trials, the MD plots were treated with SPLAT-EC™ at approximately half rate (332 g/ha as 2.5 g dollops applied to every second tree). Apart from this rate difference, the treatment was applied as in 2012/13. The application was made over 3-5 Dec 2013.

Insecticide

The insecticide plots were treated with Altacor® (Du Pont) between 23-27 Dec 2013 using standard practice for the orchard as described for 2012/13.

Control

Control plots were left untreated.

Mating disruption (quarter-rate)

A separate small pilot trial was also established to assess the effectiveness of SPLAT-EC™ at one quarter of the recommended rate.

Trial design

Three treatment plots of 0.5 ha were arranged within each of the five untreated control plots of the 2012/13 trial site. Each plot measured 13 trees by 10 rows. The following three treatments were allocated randomly within each group of three plots and the SPLAT-EC™ was applied on 5 Dec 2013.

Treatments

Mating disruption (half-rate)

SPLAT-EC™ was applied at half rate (332 g/ha as 2.5 g dollops applied to every second tree).

Mating disruption (quarter-rate)
SPLAT-EC™ was applied at approximately quarter of the recommended rate (178 g/ha as 1.25 g dollops applied to every second tree).

Control
Control plots were left untreated.

Data collection & analysis

Male moth behaviour

For the half- and quarter-rate trials, the effect of MD and insecticide treatments on male carob moth behaviour was assessed with pheromone mimic traps checked weekly as per 2012/13. In 2013/14 however, five traps were installed in each half-rate treatment plot (60 traps in total). One trap was placed at the centre of the plot, and one halfway between the centre trap and each corner of the plot. A single trap was placed at the centre of each quarter-rate trial plot.

For the trap placement trial, the traps were inspected and replenished with fresh virgin female moths every 2-3 days.

Nut infestation & kernel damage

The collection of samples and assessment of nut infestation and kernel damage followed the 2012/13 procedure except that the bulk kernel assessments were increased to 1,200 kernels from each of the five sample points per plot in the half-rate trial (72,000 kernels in total). Pre-shake samples were collected from the half-rate trial from 10-17 Feb 2014 and post-shake samples between 25 Feb and 3 Mar 2014. Considering the pre- and post-shake samples, a total of 78,000 kernels were assessed for damage. Nut and kernel assessments were not performed for the quarter-rate trial.

Mummy population assessment

Prior to harvest we assessed the mummy population density by counting mummies in 25 trees (5 trees x 5 rows) centred around each of the 60 trap trees in the half-rate trial (1500 counts in total).

2014/15 Trials

Based on results from the earlier trials, the trial protocol for 2014/15 was revised to include: selection of a trial site with a lower and spatially less variable mummy population and so hopefully a lower and more evenly spread moth population; and smaller plot sizes to allow for greater treatment replication.

The trial was located in a block of six-year-old trees that had not been used in the previous trials. This block had a row spacing of 7.2 m and tree spacing of 5 m.

Although the quarter-rate trial of 2013/14 gave promising results, it was decided to maintain the application rate of SPLAT-EC™ at half-rate until the issues of dollop placement and kernel damage were resolved.

An additional trial investigating the placement of pheromone traps and SPLAT-EC™ dollops was also established but is not reported here for reasons of commercial confidentiality. For the same reasons, some specific details of the experimental methods used in 2014/15 have been omitted from the following section of this report.

Mating disruption (half-rate) & insecticide

Trial design

For this trial, an area of orchard was selected on the basis that it had a lower and much more evenly distributed population of mummies in comparison with the previous season. The area's mummy status was determined by a pre-trial assessment (described below). Eighteen treatment plots of 3.8-4.8 ha were established in a row-column design, as six replicates of three-plot rows with each row containing all three treatments. Mummy population assessments and early-season moth trap data were used as covariates to determine the most appropriate allocation of treatments to treatment plots.

Treatments

Mating disruption
Plots were treated from 12-14 Dec 2014 with SPLAT-EC® as 2.5 g dollops applied to every second tree. Because of the closer tree spacing compared to the previous trials, the effective application rate SPLAT-EC™ was 345 g/ha. The dollops were applied to plastic bag closures as described above, and allowed to set

for one to two days. The tags were then clipped to small branches in the trees, using purpose-made applicators.

Insecticide
The pesticide plots were treated with Altacor® (Du Pont) between 8-15 Jan 2015 using standard practice for the orchard as in previous seasons. The spread in application dates was due to unfavourable weather at the time. The pesticide was applied at a rate of 280 g/ha in 1500 l water/ha using an airblast sprayer travelling at 6 km/hr. As per label instructions, a non-ionic wetter (Horti-Wet 370, SST Australia) was included at the rate of 15 g active ingredient/100 l water.

Control
Control plots were left untreated.

Data collection & analysis

Pre-trial mummy assessments

Each trial plot was divided into 25 subplots of approximately 85-106 trees, depending on plot size. On 14-15 Aug 2014, the numbers of mummies on one Nonpareil and one pollinator tree at the centre of each subplot were recorded, giving a total of 900 counts of mummies across the trial site.

Male moth trapping

On 21 Aug 2014 a single pheromone trap was placed at the centre of each plot at a height of approximately 1.5-2 m as per the 2012/13 procedure. On 25 Sep 2014, another four traps were placed in each plot, one halfway between the centre trap and each corner of the plot. Once in place, the traps were monitored weekly for male carob moths.

Nut infestation & kernel damage

All samples for whole-nut assessments and bulk kernel assessments were collected between 6-9 Feb 2015, after the trees were shaken. Samples were collected from the centre trap tree in each plot and from trees 30 m north, south, east and west of the centre tree. From under each sample tree, enough nuts were collected to yield approximately 2 kg of kernel for damage assessments, and a separate sample of 100 nuts was collected for whole nut assessments. The bulk samples for kernel damage assessments were stored at ambient temperature for three weeks before being hulled and shelled. They were then stored sealed in plastic bags at approximately 7°C until inspected for damage. 1,500 kernels by weight, per plot, were assessed for insect damage, giving a total of 144,000 kernels assessed, when combined with the 100-nut samples.

Observations from a commercial spray application

One component of the overall research project on carob moth was to determine the pattern of carob moth development in almond nuts through a production season, using a sequence of repeat surveys. Eight surveys were conducted at 2-4 week intervals from 13 Oct 2011 to 8 May 2012. The surveys took place at one orchard site in Sunraysia, across a 2.7 ha section of a 20 ha block, which itself was part of approximately 9,000 ha of almond plantings.

At each sample time, one mummy nut and one new crop nut were collected from each of 100 Nonpareil trees distributed throughout the survey area. The nut samples were returned to the laboratory and examined under a dissecting microscope for insect damage and for the various life stages of carob moth. Results from those surveys are reported in the chapter 'Seasonal phenology and distribution of carob moth in almonds', apart from one aspect regarding the impact of an application of Altacor® (chlorantraniliprole) on carob moth in mummy nuts. Data relating to that aspect is included in the results presented below.

The orchard containing the survey site was treated on 21 Jan 2012 with Altacor® at label rates (280 g/ha) in response to producer concerns regarding potential levels of crop damage by carob moth. One of the routine surveys was conducted two weeks later. During inspection of the survey nut samples, carob moth larvae were classified as:

- 'Healthy' if they responded actively as they usually do, to being poked with a probe.
- 'Morbid' if they were alive and able to move but did not respond as above.
- 'Dead' if they were obviously dead (e.g. starting to shrivel) or appeared normal but showed no signs of life when probed.

The broad nature of the spray usage in the area at that time meant that no comparable unsprayed block was available for sampling, to allow a spray vs no spray comparison.

Results & discussion

2012/13

Pest population assessment

The rate of infestation of nut mummies with carob moth varied from 4-36% across the 15 trial plots, with an average of 16.3%.

The mummies themselves were distributed relatively evenly between plots, which had an average score of 1.484 (min 1.04, max 1.88). All plots contained trees with 1-5 mummies and six or more mummies (25 trees assessed per plot).

Mating disruption (full-rate) & insecticide

Male moth trapping

Figure 20 shows average weekly male moth counts per treatment for the duration of the trial. Trap catches were generally heavily suppressed under MD, from after the application of SPLAT-EC™ (21 Dec 2012) until after harvest (15 Feb – 19 Apr 2013). This result indicated that in the almond orchard, SPLAT-EC™ significantly reduced the ability of male carob moths to locate a point source of sex pheromone (a trap), and so could also be expected to reduce their ability to locate females for mating.

Suppression rates of 90% or more were observed for 14 weeks, followed by eight weeks of generally 70% or greater suppression. As would be required for commercial success of MD, this period extended from before hull split to the end of harvest.

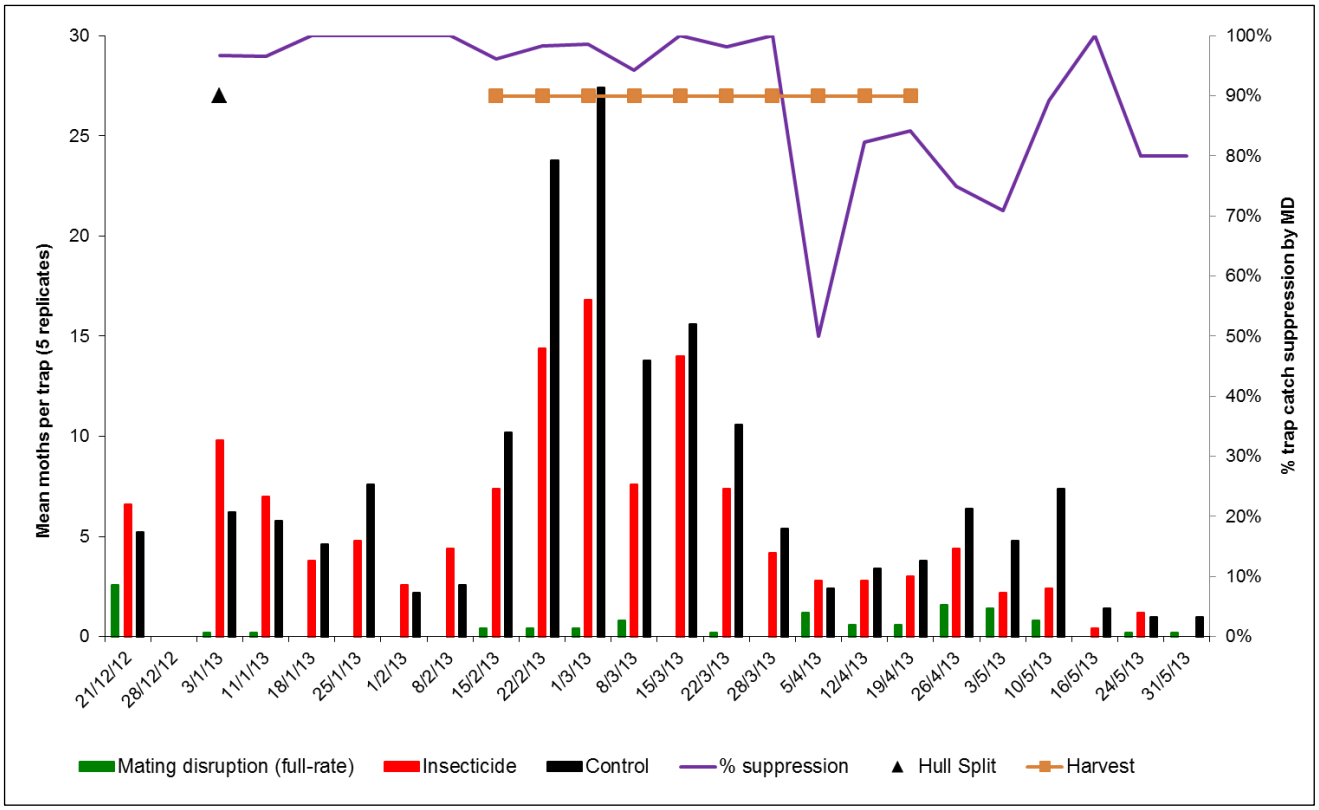

Figure 20. Male carob moth trap catches, full-rate mating disruption, 2012/13.

The application of Altacor® in early January appears to have resulted in reduced levels of moth activity in the following generation (late February-early March), as would be expected from a treatment that targets eggs and young larvae.

Nut infestation (pre-shake samples)

As Figure 21 shows, nut infestation levels just prior to harvest were low across all treatments. Although a trend is apparent, the MD treatment did not significantly reduce the percent of nuts with kernel damage, live carob moth or signs of infestation, in relation to control trees. The insecticide treatment did reduce the percent of nuts with any sign of carob moth infestation and with live carob moth significantly compared to the control treatment, but was not different from mating disruption in those parameters. Kernel damage levels in the whole nut samples averaged 0.12-0.16% across the treatments and no treatment effects were detected.

Kernel damage (post-storage samples)

After storage, the levels of kernel damage in bulk nut samples from the insecticide plots were significantly lower ($P<0.01$) than those from untreated control or MD plots (Figure 22). The levels of kernel damage in nuts from the control and MD plots had increased significantly during storage, indicating the necessity for fumigation if harvested nuts cannot be processed rapidly.

The most likely explanation for the lack of improvement in kernel quality under MD was considered to be the immigration of mated females into MD plots from the surrounding untreated areas. The same immigration could have occurred into insecticide plots, but egg and larval mortality from the insecticide treatment would be expected to result in reduced infestation levels, as were observed.

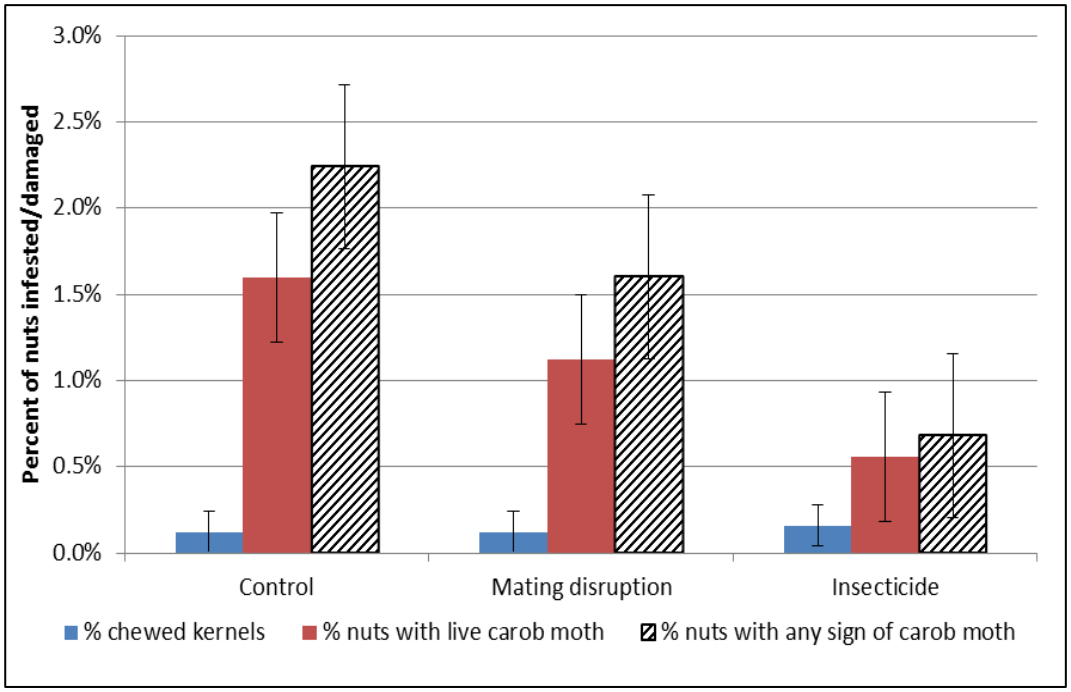

Figure 21. Infestation of whole nuts with carob moth at harvest, full-rate mating disruption, 2013 (P=0.05).

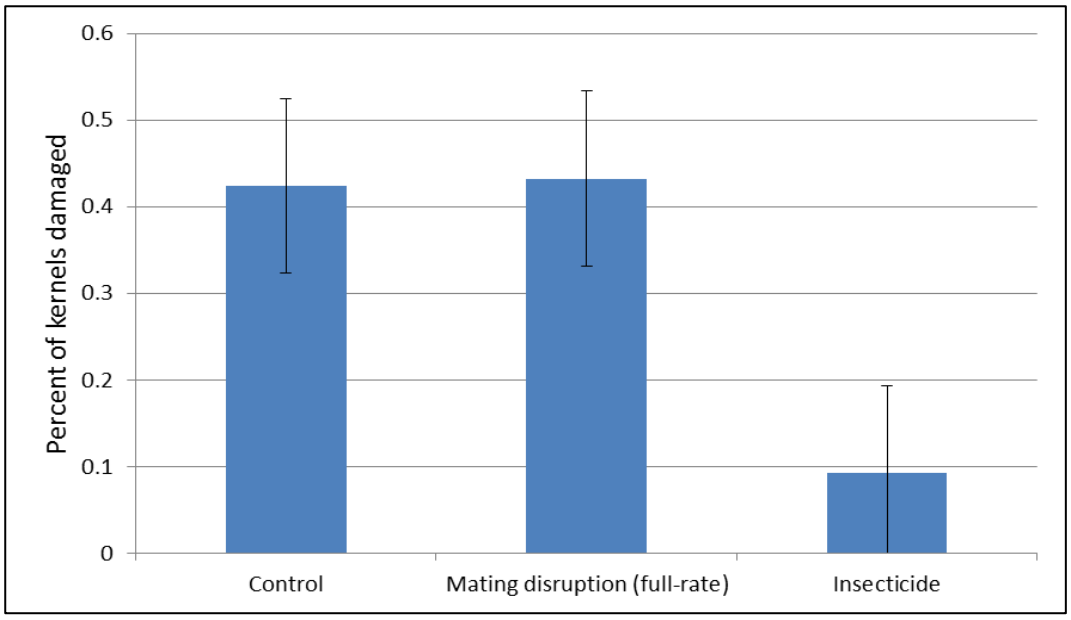

Figure 22. Kernel damage in bulk samples after storage, full-rate mating disruption, 2013 (P=0.05).

Mating disruption (half-rate)

Male trapping

Application of MD at half the recommended rate suppressed moth trap catches to the same degree as the full rate treatment (Figure 23). This suggested that the cost of implementing MD may be able to be significantly reduced by using lower rates.

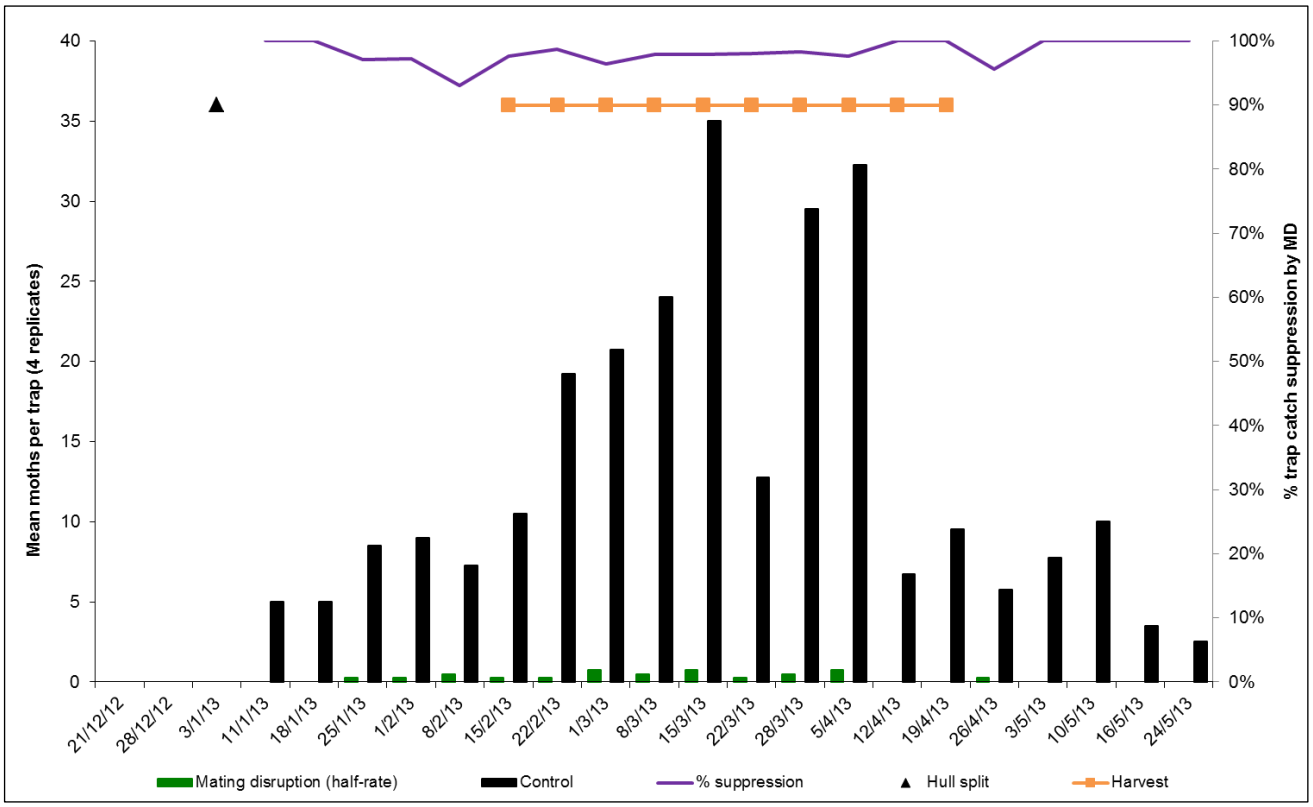

Figure 23. Male carob moth trap catches, half-rate mating disruption, 2012/13.

2013/14

Mating disruption (half-rate) & insecticide

Male moth trapping

Figure 24 shows pheromone trap data for the 2013/14 MD trial (half-rate). Levels of moth activity were higher than during the 2012/13 trial. Trap suppression was generally maintained at over 80% from the time of MD application to the start of harvest, but then this effect tended to break down. As was seen in the previous season, reduced moth activity in the February-March generation appears to have resulted from the Altacor® application in early January.

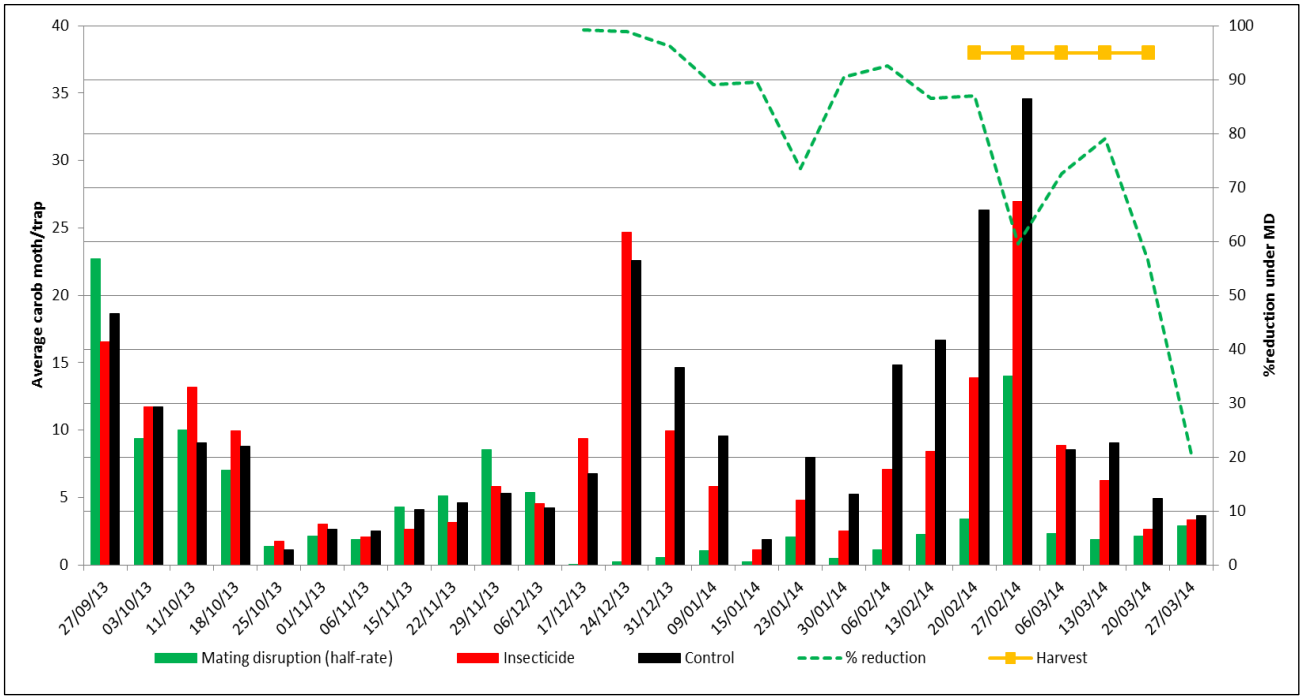

Figure 24. Male carob moth trap catches, half-rate mating disruption, 2013/14.

Nut infestation (pre-shake samples)

The pre-harvest nut infestation levels observed in 2014 (Figure 25) were significantly greater than those seen in 2013. The insecticide treatment resulted in a significantly lower percentage of nuts with kernel damage or any sign of infestation compared to the MD treatment, but neither the insecticide or MD treatment were different to the control. There were no significant differences in kernel damage between the three treatments. The result seen for the MD treatment was largely due to unusually high levels of infestation in two of the four treatment plots. A review of the orchard plots and practices failed to find any explanation for this result.

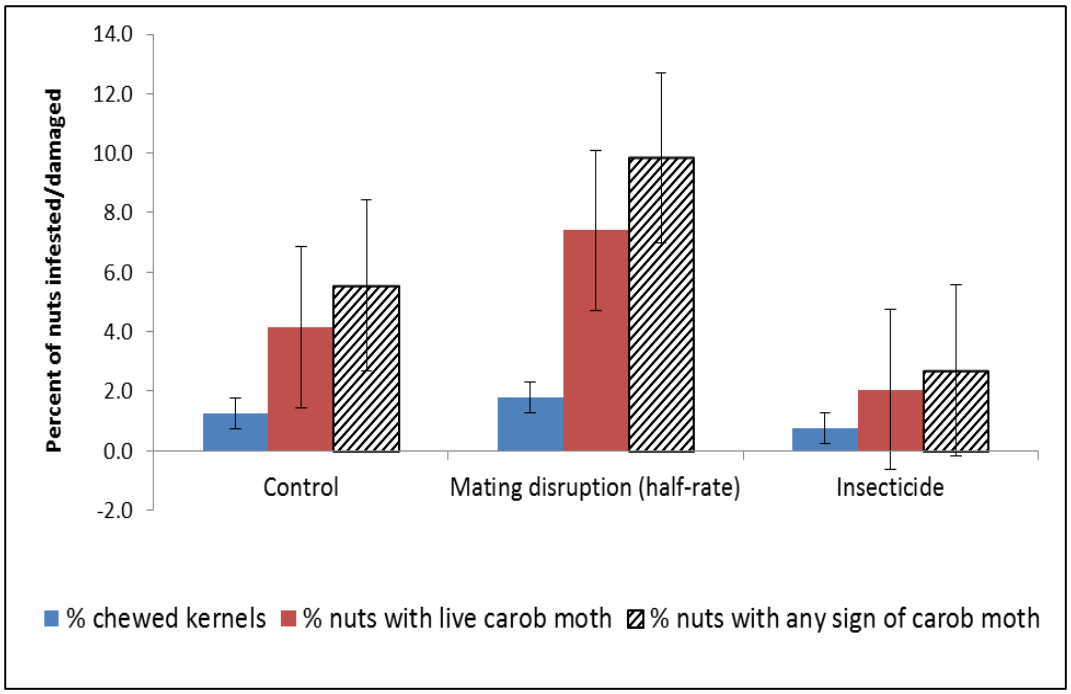

Figure 25. Infestation of whole nuts with carob moth at harvest, 2014 (P=0.05).

Kernel damage (post-storage samples)

During storage without fumigation, kernel damage levels across all three treatments increased more than two-fold when compared to pre-harvest levels (Figure 26), again emphasising the need for rapid processing or treatment of infested crops after harvest. As in 2012/13, the levels of kernel damage in bulk nut samples from the insecticide plots were significantly lower than those from untreated control or MD plots.

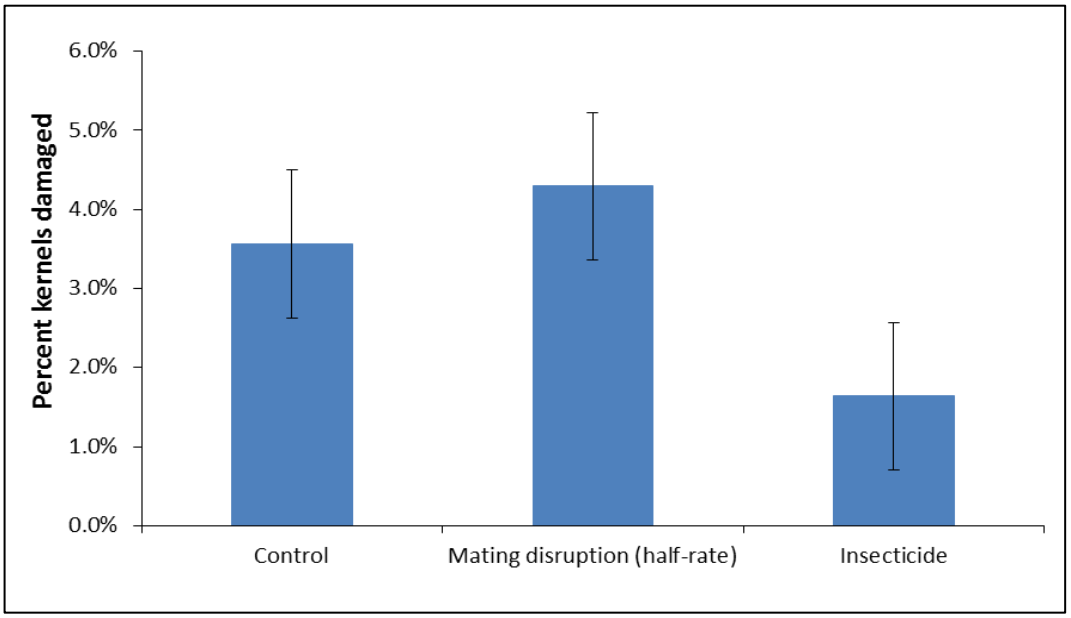

Figure 26. Kernel damage in bulk samples after storage, half-rate mating disruption, 2014 (P=0.05).

Mummy population density

The mummy population density across the trial site was highly variable within plots (0-320 mummies per tree; average 24) and between plots (average 2.2-42.9 mummies per tree). Such a high level of variability in the key food resource for carob moth would have added to the difficulty in obtaining clear treatment differences. Also, the high populations of carob moth supported by these high populations of mummies are likely to have contributed to greater than desired levels of mating under MD, simply due to chance encounters between male and female moths.

Mating disruption (quarter-rate)

Male trapping

Trap suppression under quarter-rate SPLAT-EC™ followed generally the same pattern as that for half-rate as can be seen in Figure 27. This is an interesting result as it was thought that the rate of pheromone release from the 1.25 g dollops used in the quarter rate trial may drop significantly sooner than that from the 2.5 g dollops used in half and full-rate applications.

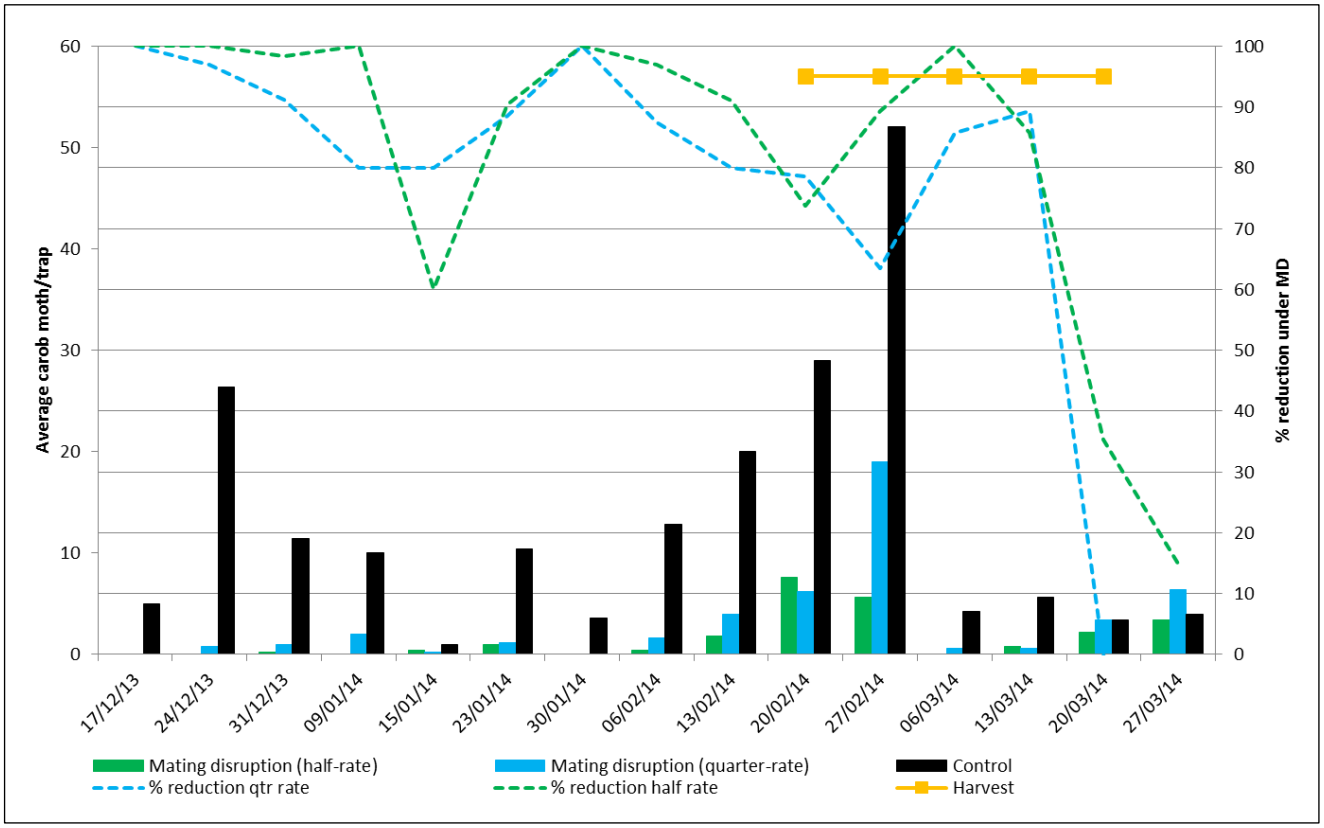

Figure 27. Male carob moth trap catches, half & quarter-rate mating disruption, 2013/14.

2014/15

Mating disruption (half-rate) & insecticide

Pre-trial mummy assessments

The number of mummies per tree across the trial site ranged from 0 to approximately 200, compared to the 0-320 range within the 2013/14 trial. The between-plot variation in average mummies per tree of 1.9-21.7 was significantly lower than that of the 2013/14 site (2-43/tree). The between-replicate variation in average mummies per tree (4.2-17.9) was taken into account in the trial design and was potentially useful in determining the influence of mummy population density on moth activity and nut infestation.

Male moth trapping

The spring generation of moths occurred as expected (Figure 28), and as hoped, the numbers were lower than in the previous seasons' trials and so were at more reasonable levels for successful application of MD. However, as was observed in the SPLAT placement trial, the level of moth activity across the trial site was very low from November onwards. Across the entire trial, the average moth catch after the spring peak was just under 0.2 moths per trap per week. This makes it difficult to draw any inferences regarding treatment effects although the apparent trends generally mirror those of the previous seasons' trials. It did seem however that the percent reduction in trap catch under MD dropped more rapidly than in the previous trials.

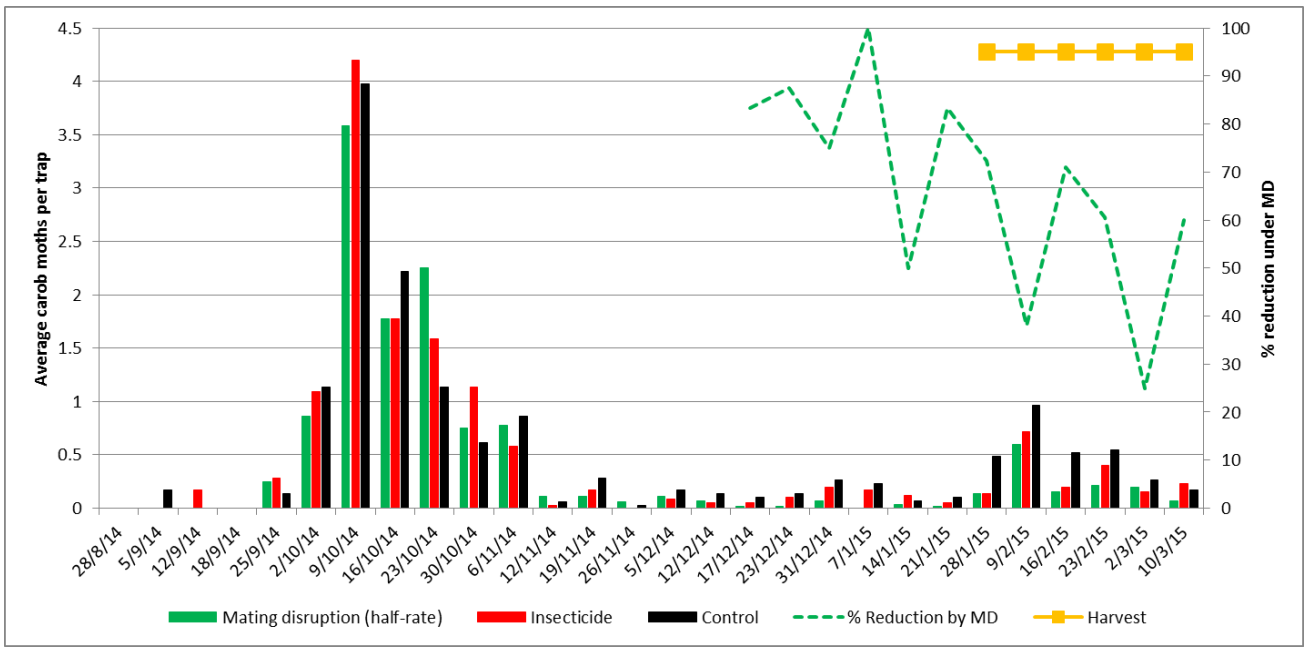

Figure 28. Male carob moth trap catches, half-rate mating disruption, 2014/15.

During trap inspections late in the season, it was noticed that the SPLAT-EC™ dollops were missing from some of the plastic tags. A more thorough check found that only 20% of the dollops were still in place. Those dollops were evidently stuck to the tags quite securely, as evidenced by the fact that the retention rate of dollops was the same on trees that had and had not just been shaken for harvest. A subsequent test found that SPLAT-EC™ dollops applied to the same plastic tags were quite securely attached to the tags after seven days of curing. It seems likely that the 1-2 days of curing used during the preparation of SPLAT-EC™ for the trial was not sufficient. Although the tags could be successfully applied to the trees with the SPLAT-EC™ dollops intact, it is probable that the dollops were, for several days at least, prone to being dislodged if they were shaken strongly. This is likely to have happened during windy conditions that occurred within 2-4 days of the tags being applied. During that period, maximum wind speeds reached 54 km/h (Beaufort scale 7, 'near gale'). The loss of a significant proportion of SPLAT-EC™ dollops early in the trial would help to explain the more rapid drop in level of trap suppression compared to previous seasons and would have obvious implications for nut infestation and kernel damage levels in the MD plots.

Nut infestation & kernel damage

As could be expected under the conditions of very low pest pressure in 2014/15, nut infestation levels were also low. No treatment differences were detected in levels of nut infestation or kernel damage in the small whole-nut samples (Figure 29), but in the bulk kernel samples the insecticide treatment significantly reduced the level of kernel damage compared to the control treatment only (Figure 30). The levels of kernel damage found in this trial reflect those reported by industry for the 2015 harvest (0.1% with insecticide use; <1% without insecticide, personal communications).

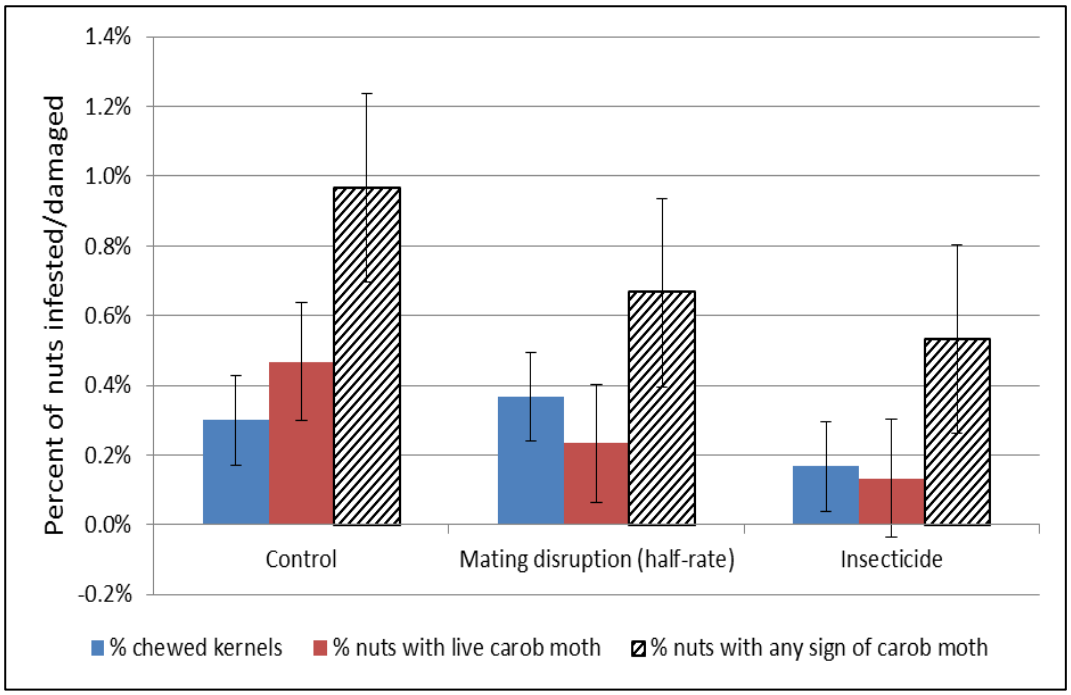

Figure 29. Infestation of whole nuts with carob moth at harvest, 2015 (P=0.05).

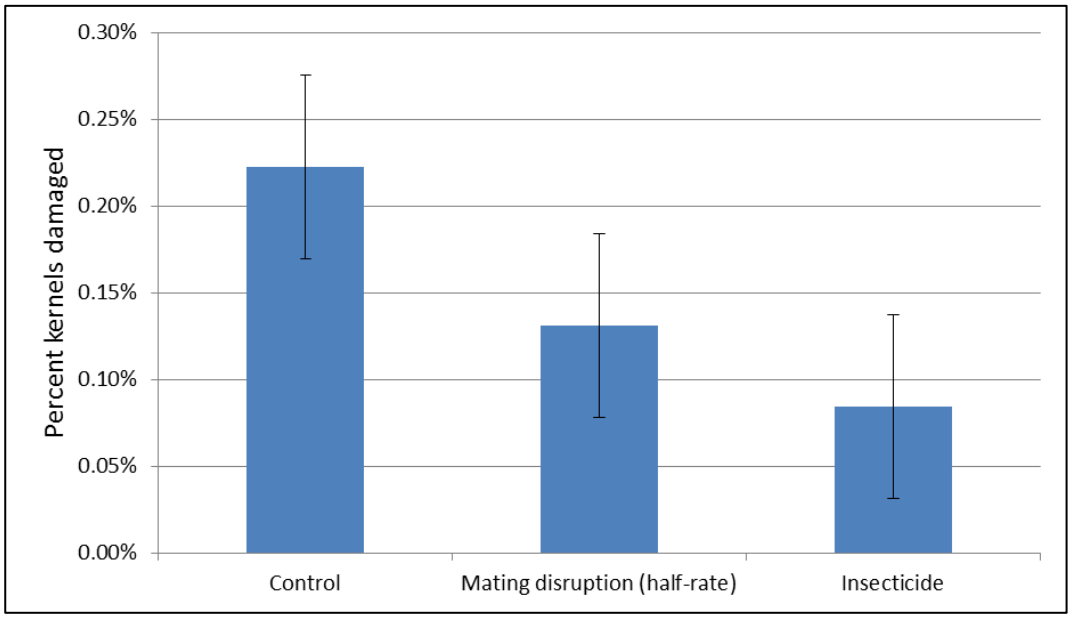

Figure 30. Kernel damage in bulk samples after minimal storage, 2015 (P=0.05).

Observations from a commercial spray application

Figure 31 shows the occurrence of mortality and morbidity observed in the 100 carob moth larvae that were found in mummy nuts collected from a commercial orchard two weeks after an application of Altacor® (chlorantraniliprole). Of those larvae, 47% appeared healthy, 37% were dead and 16% showed signs of morbidity. This contrasts with the previous five surveys in the series, where dead or unresponsive larvae were very rarely encountered, suggesting that the mortality and morbidity observed were very likely a result of the insecticide application. It should be noted that assuming the larval mortality and morbidity were due to the insecticide, the treatment appeared to leave almost half of the population untouched. In this orchard, that meant that a very significant carob moth population remained. This may be one indicator of the difficulty in achieving thorough spray coverage in almonds generally, and penetration of insecticide into mummy kernels in particular.

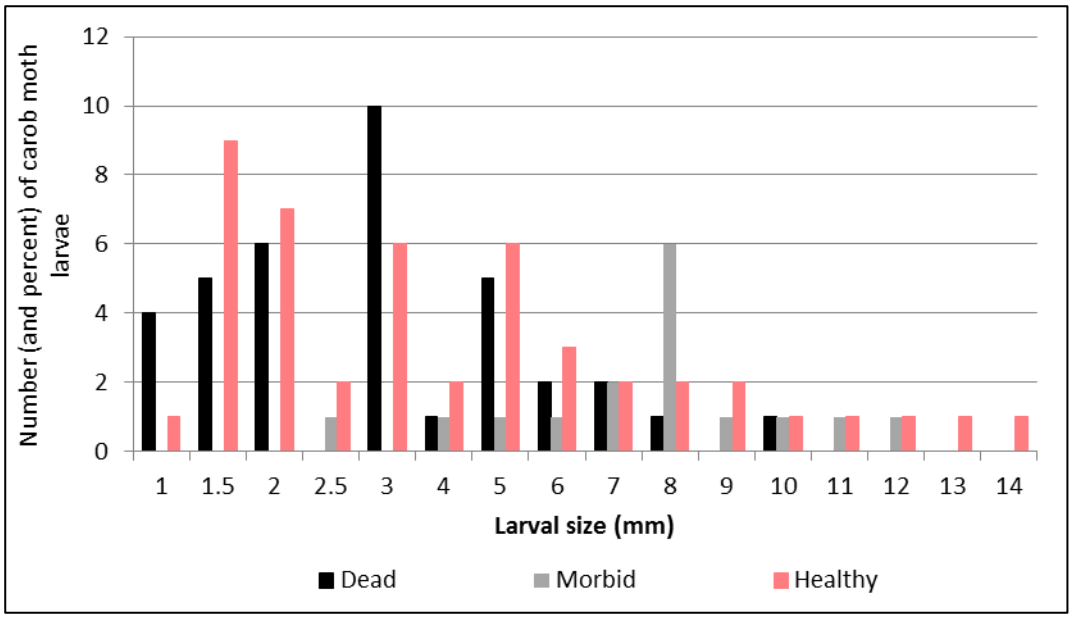

Figure 31. Mortality and morbidity amongst carob moth larvae in mummy nuts, two weeks after an application of chlorantraniliprole.

Cost/benefit estimate of insecticide treatment

In Table 14 an attempt has been made to provide a simple cost/benefit analysis of the one-off applications of insecticide used in the trials reported above, compared to the Control treatment, for each of the three years of trials. Mating disruption is not included in the analysis as it is not yet an effective option for producers and costs have not been firmly established. The analysis uses industry figures for the value of top grade kernel in all years, second grade kernel in 2015, and spray application costs. Second grade kernel values for 2013 and 2014 are estimates, calculated using the same ratio of top grade:second grade as applied in 2015. An average yield of 3.2 t/ha has been assumed. Levels of kernel damage for each year and treatment are from the trials reported above, and for the purpose of the analysis it is assumed that carob moth damage is the only defect affecting kernel quality.

Table 14. Simple cost-benefit analysis of insecticide application for carob moth.

Harvest year	Kernel value (top grade)	Kernel value (2nd grade)	Treatment	Kernel damage	Potential change in value due to lower grade (prior to sorting)		Value of damaged kernel (removed by sorting)		Benefit from insecticide compared to control	Cost of insecticide & application	Net gain/loss
	$/t	$/t		Avg %	$/t	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha
2013	$5,580	$4,799	Control	0.424%	-$781	$0	0.014	$76		$0	-$76
2014	$8,500	$7,310	Control	3.570%	-$1,190	-$3,808	0.114	$971		$0	-$971
2015	$11,500	$9,500	Control	0.222%	-$2,000	$0	0.007	$82		$0	-$82
2013	$5,580	$4,799	Insecticide	0.092%	-$781	$0	0.003	$16	$59	$200	-$141
2014	$8,500	$7,310	Insecticide	1.640%	-$1,190	-$3,808	0.052	$446	$525	$232	$293
2015	$11,500	$9,500	Insecticide	0.084%	-$2,000	$0	0.003	$31	$51	$265	-$214

The potential change in value is a result of downgrading of the crop from top to second grade due entirely to excessive insect damage, using the USDA 'Standards for grades of shelled almonds' (USDA 1997). This standard allows a maximum of 1% 'serious damage' which includes insect damage. The 'potential change' value is included to illustrate the order of magnitude of the potential loss in value due to carob moth in a high pest pressure season (2014). In reality an attempt would be made to sort the crop to reduce kernel damage levels to below the 1% threshold.

The 'value of damaged kernel' is simply the value of kernels damaged by carob moth and ideally rejected during processing of the crop. It does not account for any value regained from the further processing of damaged kernel or extra value lost due to accidental rejection of good kernel with chewed kernel during the sorting process.

The ‘benefit from insecticide’ relates to how much the insecticide treatment reduced kernel damage compared to the untreated control. As can be seen in terms of ‘net gain/loss’, the insecticide treatment was economically beneficial only in 2014 – the season of high pest pressure. In the other two seasons the cost of insecticide application exceeded the benefits gained, resulting in a net loss compared to no treatment at all.

To balance out the per hectare spray costs in the three years would have required increases of 36, 27 and 23 kg/ha of undamaged kernel respectively in those years, equivalent to 1.12%, 0.853% and 0.72% of average yield. Linking these requirements to thresholds for the drivers of kernel damage, such as moth activity or mummy population density would give producers the tools to make informed decisions seasonally regarding the value or otherwise of insecticide applications.

It must be noted that this analysis involves only one-off insecticide applications at hull split. Different cost/benefit outcomes are likely to be obtained with applications that are timed differently (e.g. spring) or repeated over a number of seasons.

Overall discussion

Mating disruption

The fact that SPLAT-EC™ at full, half and even quarter rates (617 g/ha, 332 g/ha & 178 g/ha respectively) resulted in high levels of carob moth trap suppression for at least ten weeks, and more moderate suppression for a further 3-8 weeks indicates that it has potential for effective MD of the pest.

Although reduced application rates are desirable for lowering the cost of treatment, it should be noted that the full-rate application provided a slightly longer period of high-level trap suppression (14 vs 11 weeks) and a significantly longer period of more moderate suppression (8 vs 3 weeks) compared to the half-rate treatment. We have found in other research that nuts infested with carob moth eggs as late as three weeks before harvest can suffer significant kernel damage. Effectively, this means that the crop requires protection from infestation for the entire harvest period. This may be compromised by lower application rates if they provide protection for a shorter period. The option of extending the effective field life of SPLAT-EC™ dollops should also be pursued with the manufacturer, as should the relative merits of different dollop sizes in relation to the rate and period of release of the pheromone mimic.

The MD treatment failed to reduce levels of nut infestation or kernel damage significantly in any of the three seasons compared to the control treatment, and was not significantly different from the insecticide treatment in two of the seasons. This clearly indicates that while pheromone communications for carob moth were inhibited in some areas, the effect was not distributed broadly enough to achieve disruption of mating on a whole-plot basis.

It seems most likely that the distribution of SPLAT-EC™ dollops within trees is a critical factor in determining the success of this approach to MD. The 2014/15 trial that was established to investigate this produced no result due to very low moth numbers overall and a partial failure of the treatment application. A trial such as this is still needed to clarify the issue of trap and dollop placement, to allow the application of SPLAT-EC™ to be optimised for a fair assessment of its potential.

Also, there are several possible modes of action involved in mating disruption. These include but are not limited to sensory overload, false trail following and competitive attraction. The distribution of numerous point sources of pheromone (e.g. the spatial density of dispensers) is important for competitive attraction, whereas a small number of high-volume pheromone emitters may be more appropriate to achieve sensory overload. The mode of action varies between insect species and is important for determination of appropriate methods of achieving mating disruption. The results obtained overseas with mating disruption of carob moth in dates and pomegranate suggest a competitive attraction mode of action, but this should be confirmed with more detailed trapping experiments.

To achieve accuracy in the application rates of SPLAT-EC™ used during these trials, all the dollops were placed in trees manually. To be economically viable however will require mechanical application using applicators mounted on aircraft or ground vehicles. This approach will need to address the challenge of placing appropriately sized dollops in almond trees that often have lightly-foliaged open structures that would provide flimsy targets for airborne dollops. Adaptation and field assessment of existing mechanical SPLAT-EC™ applicators used in USA would be the first step in this process.

Insecticide

In all three seasons, when compared to the untreated control, single applications of Altacor® at early hull split appeared to reduce moth activity in the following generation. In the first season only, the insecticide treatment resulted in a significantly lower level of infestation of nuts by carob moth compared to the control treatment, as measured in 100-nut samples soon after harvest. In the second season only, the insecticide treatment reduced the levels of kernel damage and any signs of infestation in the 100-nut samples compared to the MD treatment only. No effect of the insecticide treatment was detected on kernel damage levels in the 100-nut samples in any season, but it did result in significantly lower levels of kernel damage compared to the control treatment in all seasons when measured in bulk samples after 3-8 weeks storage. Results from other trial work during this project indicate that a single application of Altacor® at the start of hull split would leave the new crop of nuts exposed to infestation by carob moth for a significant period of time (refer to 'Kernel damage and the timing of carob moth oviposition in almonds'). It is possible that this accounts for the relatively low proportion of treatment comparisons that showed significantly different results.

Industry assessments of spring vs hull split applications of Altacor® and repeat applications of this insecticide over several seasons have also yielded potentially interesting results which should be assessed in detail and investigated more thoroughly if warranted. This could include the impact of insecticide application on infestation levels of mummy nuts, given that the apparent impact of a single Altacor® spray on carob moth larvae in mummy nuts suggests difficulties in targeting that population of the pest with insecticide.

One of the issues regarding insecticide use in almonds is the difficulty often encountered in achieving good spray coverage throughout the trees (Rosenzweig and Furness 2014). Thorough coverage is critical for an insecticide that is intended to target carob moth eggs and newly hatched larvae, given that the eggs are typically laid inside or very close to, the split in the hull.

The results of the very simplified cost/benefit analysis of one-off insecticide applications clearly indicate the need for economic thresholds and risk assessment/predictive tools to determine in advance the potential crop loss from carob moth damage and the likely benefit of an application of insecticide or alternative treatment.

Conclusions

In the trials reported above, the sex pheromone mimic released by SPLAT-EC™ as a mating disruption treatment successfully interfered with pheromone communication in carob moth in almonds, as measured by its suppression of catches of male carob moth in traps baited with the same pheromone mimic or with unmated female carob moths.

The treatment did not however reduce levels of kernel damage during the trials, for reasons that we now believe we understand. The 2014/15 trial that was designed to address this should be rerun using a site and season of sufficient carob moth population level and allowing for a longer dollop curing period.

Further discussions should be held with the manufacturers of SPLAT-EC™ to identify opportunities to improve field performance of the product, such as changes to formulation or dollop size to extend the release period of the pheromone mimic. Mechanical application of the product in almonds also needs to be assessed.

Single applications of Altacor® at hull split reduced levels of kernel damage, but on simple analysis, were not cost-effective in all seasons. Industry data derived from on-farm trials and routine usage of Altacor® needs to be evaluated to determine whether more rigorous assessments of insecticide options such as spring applications are warranted.

Given the variable benefits gained from one-off insecticide applications, economic thresholds and risk assessment tools should be developed to assist decision-making regarding the potential value of insecticide use.

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DRAFT PAPER FOR PUBLICATION

Relationships between almond mummies and carob moth development and activity.

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Abstract

The relationship between carob moth activity, kernel damage and the amount of mummified nuts in almond orchards in the Sunraysia area of Australia was examined over three growing seasons. Capture of male carob moths in pheromone traps was not correlated with population densities of infested mummies. A strong relationship existed between kernel damage at harvest and average number of mummies per tree at a plot level in 2013/14 but was not evident in other growing seasons. Relative susceptibility of common varieties to mummy development was Price> Nonpareil > Monterey > Carmel.

Introduction

The carob moth *Apomyelois* (=*Ectomyelois*) *ceratoniae* has become a significant kernel quality issue for the Australian almond industry over the past four to five years, after unusually wet periods during harvest (Sharp 2009). Mummified nuts (mummies) hanging on trees are an important food resource for carob moth (Gothilf 1984) and it is possible that the growth in populations of the pest has been associated with an increase in mummy numbers resulting from increased levels of hull rot disease. Hull rot is favoured by warm, wet conditions and often results in nuts adhering to the tree and not dropping during harvest.

In almond orchards, carob moths survive over winter as larvae, that only begin to pupate in late winter, in mummy nuts (Gothilf 1984). In early spring they complete their development and emerge as adult moths. They do not infest new almond nuts until hull split (typically early January), so need an alternative food source over the intervening period. In most almond orchards, the only apparent food source available is mummy nuts. Given the importance of mummies to carob moth, it is considered likely that in any particular orchard, the development of carob moth populations and the level of crop damage they cause would be related to the mummy population levels within the orchard. This was found to be the situation within the Californian almond industry, where their major pest navel orangeworm (*Amyelois transitella*), a close relative of carob moth, also utilises mummy nuts and can be managed to a large degree by good orchard hygiene involving the removal and destruction of mummies (Higbee & Siegel 2009; Zalom et al. 2009).

This paper explores possible relationships between mummies, carob moth activity and crop damage, as a first step towards development of mummy population thresholds for use by orchard managers who are considering orchard hygiene as a management tool against the pest.

Materials and methods

During the 2012/13 to 2014/15 seasons, trials were established to investigate mating disruption and insecticide application for the management of carob moth in almonds. The same trial sites were also used to gather data on the population levels and distribution of mummy nuts. At the completion of the trials, the mummy population density and infestation rate data were combined with moth trap counts and kernel damage data to investigate relationships between these factors.

The management trials reported here were located in a commercial almond orchard in the Sunraysia region of Victoria, Australia. Drip irrigation and fertigation were used throughout the orchard and the orchard floor was generally maintained free of vegetation. The major almond variety (every second row) was Nonpareil, while Price, Monterey and Carmel were used as pollinators in the alternate rows.

In addition to the structured trials mentioned above, spot surveys of mummies for overwintering carob moth that were conducted over several years, were also assessed for insights into the relationship between mummy populations and levels of activity of the pest.

2012/13 Management trials

Trial layout
The 2012/13 management trial used 15 square 2 ha treatment plots to provide five replicates of three treatments. Each plot contained 20 rows of 25 trees.

Male moth trapping
One white plastic delta trap containing a sticky base and baited with a lure containing the carob moth sex pheromone mimic (ISCAlure-Ceratoniae™, ISCA Technologies Inc., Riverside, California USA) was placed 1.5-2 m above ground in a tree at the centre of each trial plot. The traps were installed on 14 Dec 2012 and were monitored weekly.

Nut infestation
Just prior to commercial harvest (19 Feb 2013), five samples each of 100 new crop Nonpareil nuts were collected from each of the 15 trial plots, one from the centre tree in each plot and the other four from trees half way between the centre tree and each of the four corners of the plot. The nuts were assessed for infestation and damage by carob moth.

Kernel damage
After the trees had been shaken for commercial harvest, a 'bulk' sample of new crop Nonpareil nuts was collected from each of the 75 sample positions used for the nut infestation samples. After being hulled and shelled, 1,000 kernels from each bulk sample were assessed for damage by carob moth.

Mummy assessments
The mummy population in each trial plot was assessed on 3 Jan 2013 by a simple visual assessment of trees, using a score of 0 (no mummies per tree), 1 (one to five mummies per tree) and 2 (six or more mummies per tree). Within each plot, the 25 trees in the centre Nonpareil row were assessed.

2013/14 Management trials

Trial layout
The 2013/14 management trial used 12 square treatment plots of 10 ha each for four replicates of three treatments. Each plot contained 44 rows of 58-60 trees.

Male moth trapping
Five white plastic delta traps as described above were placed 1.5-2 m above ground in each plot, one in the centre tree in each plot and the other four in trees half way between the centre tree and each of the four corners of the plot. The traps were installed on 20 Sep 2013 and were monitored weekly.

Nut infestation & kernel damage
Sample collection and assessment of nut infestation and kernel damage followed the same procedure as in 2012/13 except that the bulk samples were increased to provide 1,200 kernels for damage assessment. The pre-harvest samples were collected between 10-18 Feb 2014 and the bulk samples were collected between 25 Feb and 3 Mar 2014.

Mummy assessments
From 10-13 Feb 2014, the numbers of mummy nuts in each of 25 trees (5 rows x 5 trees) around each trap were counted, giving a total of 125 counts per plot and 1500 overall. Because the orchard blocks were comprised of 50% Nonpareil and 50% pollinators (three varieties), in alternate rows, the mummy counts included all four varieties, Nonpareil, Price, Carmel and Monterey. Samples of mummies were also collected and assessed for infestation with carob moth. Those samples were taken from Nonpareil trees just outside the treatment plots to avoid the management trial being compromised by mummy removal. Mummy infestation levels were used to generate estimates of the total number of infested mummies around the five traps per plot (infestation rate x mummies/125 trees = infested mummies/125 trees).

2014/15 Management trials

Trial layout
For the 2014/15 mating disruption trial, 18 approximately square treatment plots of 3.8-4.8 ha were established to provide six replicates of three treatment plots. Each plot contained 28-32 rows of 37-42 trees.

Mummy assessments
Each of the 18 treatment plots was divided into 25 subplots of approximately 85-106 trees, depending on plot size. The numbers of mummies on one nonpareil and one pollinator tree at the centre of each subplot were recorded on 14-15 Aug 2014, giving a total of 50 trees assessed for mummies per plot and 900 across the trial site.

Male moth trapping
A pheromone trap was installed at a height of 1.5-2 m at the centre of each plot on 21 Aug 2014. By 12 Dec 2014 four additional traps were installed in each plot at the same height, one halfway between the centre trap and each corner of the plot. Once installed, the traps were monitored weekly.

Nut infestation & kernel damage
Samples for whole-nut assessments and bulk kernel assessments were collected between 6-9 Feb 2015, after the trees were shaken. Samples were collected from under the centre trap tree in each plot and from trees 30 m north, south, east and west of the centre tree. Enough nuts were collected from under each sample tree to yield 1,500 kernels for damage assessments, and a separate sample of 100 nuts was collected for whole nut assessments. The bulk samples for kernel damage assessments were stored at ambient temperature for three weeks before being hulled and shelled. They were then stored sealed in plastic bags at approximately 7°C before being inspected for damage.

Winter mummy surveys
In August 2011, 2012 and 2014, samples of mummies were collected from almond orchards in the Victorian Sunraysia and South Australian Riverland regions and assessed for carob moth infestation. The samples of 100 mummy nuts were collected from orchard blocks that contained carob moth traps being maintained for this project.

These spot surveys were intended to gather data on the geographic spread of the pest and potential levels of pest pressure in different orchards. Altogether, samples were collected from twelve different orchards, but not all orchards were sampled in every year.

The prevalence of mummies at each sample site was rated arbitrarily as ‘none’, ‘few’, ‘moderate’ and ‘numerous’ and each rating was allocated a numeric log-based score. A ‘mummy prevalence x infestation’ score was calculated by multiplying the prevalence score by the percentage of mummies that were infested with live carob moth at the time of sampling.

Results and Discussion

2012/13
No clear relationships were apparent between mummy population densities measured using the simple mummy scoring procedure, and total number of moths captured over the season by traps in each plot (Figure 32), whole nut infestation levels (Figure 33) or kernel damage levels (Figure 34). Since the scoring system used to assess mummy populations apparently lacked the precision required to detect relationships involving mummy population density, a more detailed procedure for assessments of mummy populations was used for subsequent years.

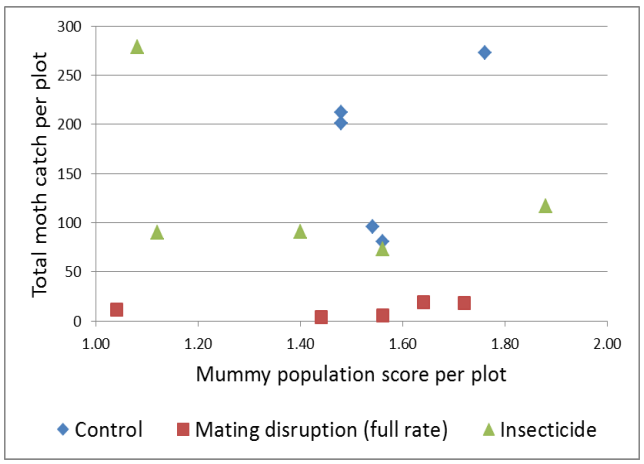

Figure 32. Total carob moth catch and mummy population score per plot in the 2012/13 management trial.

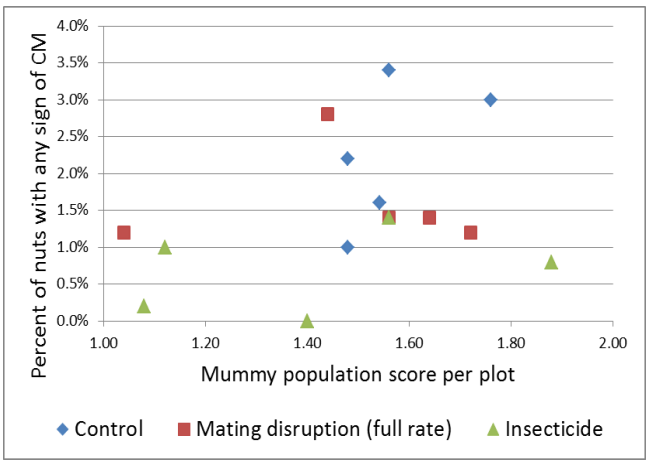

Figure 33. Percent of whole nuts with signs of carob moth infestation and mummy population score per plot in the 2012/13 management trial.

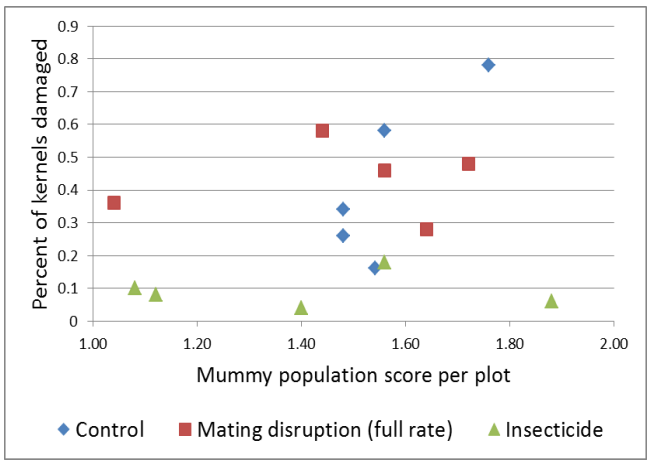

Figure 34. Kernel damage and mummy population score per plot in the 2012/13 management trial.

2013/14

Mummy populations and moth activity levels

Over the period between the start of trapping (27 Sep 2013) and the application of mating disruption (3-5 Dec 2013), there did not appear to be any clear association between mummy population densities and total moth trap catches at a trap level in the untreated or treated plots (Figure 35).

There also did not appear to be any clear association at a plot level between population densities of infested mummies and total moth trap catches (Figure 37) or maximum spring catch per trap (Figure 36).

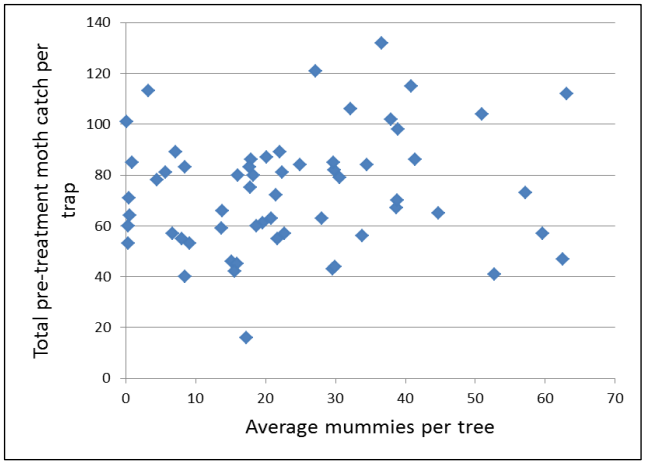

Figure 35. Total moth catch per trap prior to treatment applications, and mummy population density in 25 trees around each trap, 2013/14.

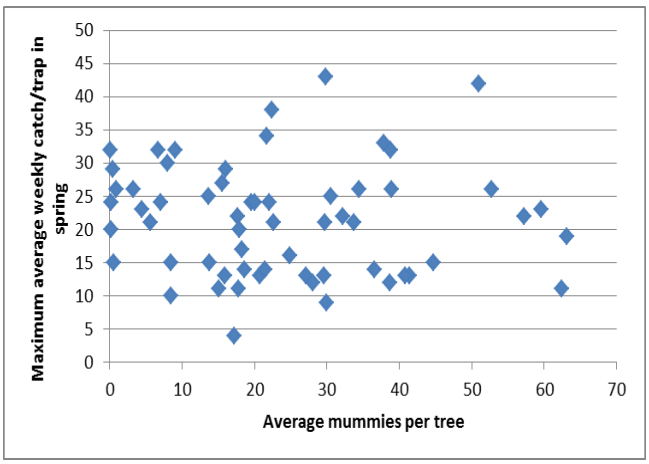

Figure 36. Maximum average weekly catch per trap in spring, and mummy population density in 25 trees around each trap, 2013/14.

It was assumed that a 1:1 sex ratio existed amongst emerging carob moth adults (Gothilf 1984, Mediouni and Dhouibi 2007) and therefore capture of male moths in pheromone traps, prior to deployment of any treatments that may differentially affect one sex, would reflect the overall population trends. However, male moths generally are more active dispersers than female moths, which tend to stay closer to the trees in which they emerged. Capture of female moths would therefore possibly be better correlated to mummy density but in the absence of a female attractant the next best option is to investigate the relationship between mummy density and nut damage, since it is the females that lay the eggs that produce larvae that in turn feed on the nuts.

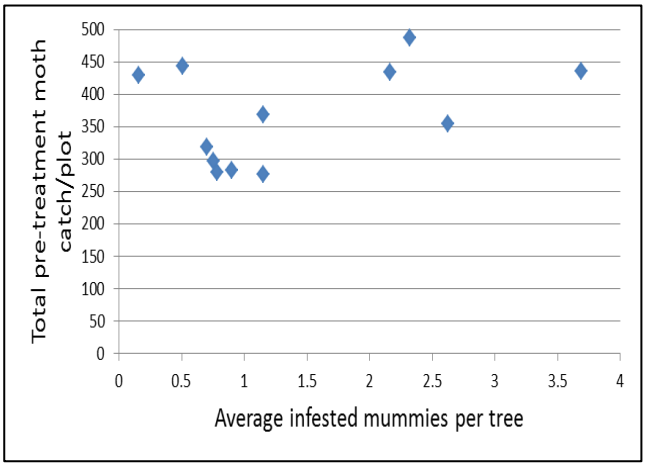

Figure 37. Total moth catch per plot prior to treatment applications, and average number of infested mummies per tree in 25 trees around each trap, 2013/14.

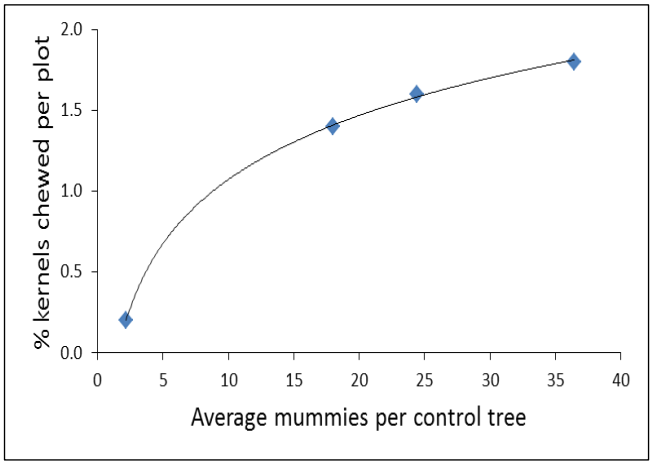

Figure 38. Relationship between kernel damage at harvest and average mummies per tree (Control plots only), 2014 (Y=0.572Ln(X)–0.246; R^2=0.9997).

Mummy populations and kernel damage levels

Given that both mating disruption and insecticide application are designed to control the pest and therefore very low levels of damage should occur in treated plots, only the untreated control plots were used to test the influence of mummy density on nut damage. A good association was found between kernel damage levels at harvest and average numbers of mummies per tree at a plot level (Figure 38). Interestingly, the decline in kernel damage with lower mummy population densities trends towards a threshold of one to two mummies per tree for negligible damage. This is the threshold originally determined for use by the Californian almond industry when using tree hygiene to manage navel orange worm, a close relative of carob moth (Engle and Barnes 1983).

Varietal susceptibility to mummy formation

A greater than three-fold difference in the population density of mummies was evident between varieties, with the pollinators Carmel and Monterey carrying the least mummies and Price carrying the highest whereas Nonpareil was intermediate (Table 15). This difference is likely to be due to a number of factors including varietal susceptibility to hull rot and the timeliness of harvest for each variety. Holtz and Tetviotidale (2008) have in fact rated the susceptibility of almond varieties to hull rot based on the number of 'strikes' of the disease found on trees, and these ratings generally match the mummy densities we found.

Table 15. Average number of mummies per tree by almond variety, 2014.

Variety	Number of trees assessed	Average mummies per tree	Susceptibility to hull rot (Holtz & Tetviotidale 2008)
Price	120	43.8	High
Nonpareil	855	27.6	Very high
Monterey	150	15.4	Low
Carmel	375	12.1	Low

2014/15

Mummy assessments

The distribution of mummies within and between the 18 management trial plots (subdivided into 25 subplots for mummy counts) indicates considerable spatial variability (Figure 8). The numbers in each cell are the number of mummies per tree averaged over the two trees at the centre of each subplot. The average number of mummies per tree at the plot level (Figure 9) demonstrates the gradient in mummy population density from row one to six that was incorporated into the design of the management trial.

	Plot Column															
	1					2					3					
Row	85	79	73	67		53	47	43	37	33	25	19	15	9	5	
Tree																
5	4.5	1	3	4	2	1.5	0	2	0	1.5	0	0	5	5	5.5	
13	5	1	20.5	4	0	1	0	3	0	0.5	0.5	7.5	1.5	3.5	2.5	
21	16	1.5	23.5	2.5	4.5	3	16.5	2	3	5	4.5	6	29	11	5	1
29	9	8	31.5	4	44.5	10	18	14	7	16	7	4.5	11	10	0.5	
37	43	12.5	24	8	31	10	11	33	51.5	14.5	28	9	12	11.5	4	
46	51	10	25.5	7	13	20.5	30.5	28	32.5	23.5	19.5	30	14	53.5	16.5	
54	20.5	9.5	26.5	3.5	3	24	11.5	14	63	7.5	20.5	9	20	6	18.5	
62	1	3.5	36.5	7	14	15	13.5	7.5	11	19.5	11.5	8.5	15.5	5	3	2
70	10.5	6.5	48.5	11	13	12	8.5	18.5	27	16	11.5	7	24.5	21.5	28.5	
78	21.5	11.5	26.5	4	19.5	15.5	29.5	23.5	37.5	33.5	16	5.5	25	4.5	2.5	
4	26	4.5	52	6.5	33	7	38	9	12.5	3.5	17	4.5	30.5	11	7	
11	4.5	7.5	53	2	9	13	10	20.5	21	21.5	8.5	8.5	13	10.5	18.5	
18	26	3.5	1.5	6	3	10.5	14	11	28.5	23	12.5	4	10	10.5	3.5	3
25	1	4.5	15	4.5	5.5	10.5	9.5	6.5	4.5	12.5	9.5	10	9.5	2	10	
32	7.5	7	16	6	9	14	15	12.5	9.5	22	7.5	6	15.5	12.5	8	
41	25.5	6	30.5	6.5	10.5	5.5	13.5	10	14.5	7	13.5	4	15.5	9.5	3.5	
48	27	2	23.5	3	8	8	15	17.5	24.5	15	13	2.5	13.5	4.5	15.5	
55	21	4.5	17.5	2	3.5	15.5	10.5	6.5	7.5	22.5	12	11	24.5	13	9	4
62	22	17.5	25.5	3	8.5	6	6.5	6	6	7.5	10	13	4.5	5	8	
69	26	9	11	9.5	4.5	7	6	6	8	5.5	4	2.5	18	4.5	4	
4	16.5	2	0.5	1.5	6	10.5	9	14	8	2.5	8	5.5	12.5	6.5	3.5	
11	13	2.5	2	5.5	14	6	13	4	0.5	5	5.5	4	10	6	17	
18	0	3	11	8	6	8.5	11.5	5	12	17.5	6	12.5	6	5.5	9.5	5
25	14.5	3.5	14	3.5	5.5	6.5	22	5	8	15	11.5	5	7	7.5	2	
32	6.5	1	10	4.5	5.5	4.5	6	8.5	7.5	3.5	23.5	9.5	11.5	8	8.5	
41	4	1	8	2	4	13.5	10	7	7	13	6	8.5	15.5	9	4.5	
48	0.5	4.5	4	2	2	6	5.5	3.5	7	8.5	3	4	16.5	7	1	
55	0.5	0	5.5	0	3.5	1.5	4.5	0	7.5	4	1	3	11	3.5	4	6
62	1.5	0.5	0	0.5	0.5	0	6.5	3	1	8.5	0.5	8	3.5	2.5	7.5	
69	1.5	1.5	0	1	0	0	12	3.5	4	1.5	1	0	1	2	5.5	

Plot Row

Figure 39. Average number of mummies per tree on two trees at the centre of each subplot, 2014/15.

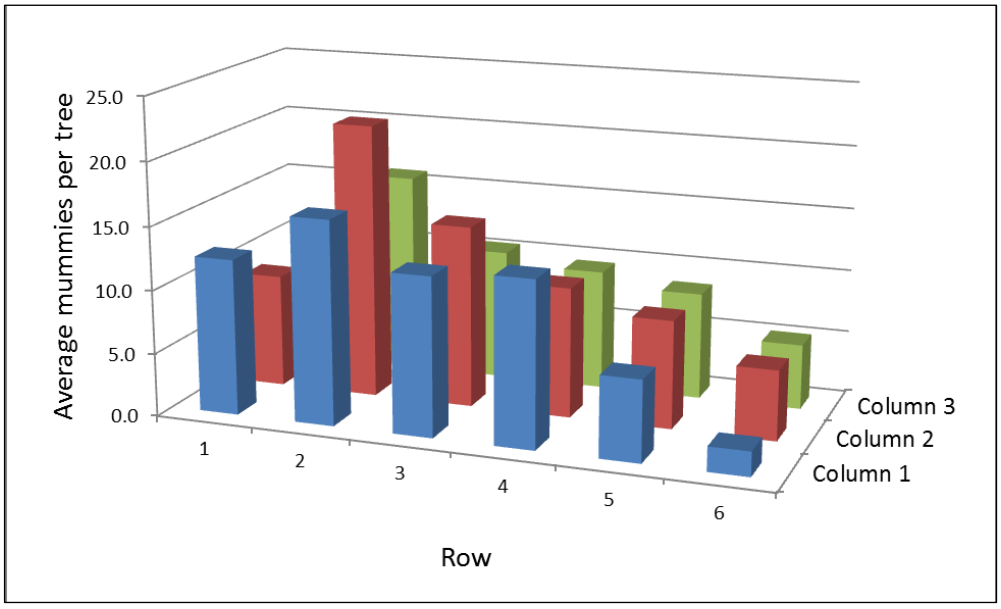

Figure 40. Average mummies per tree for each trial plot, 2014/15

Mummy populations and moth activity levels

Even though a widespread survey of mummies was performed across the trial site and the carob moth population was monitored with 180 traps, no obvious association was found between mummy population density and levels of moth activity, either at plot level (Figure 41 & Figure 42) or trap level (Figure 43).

Mummy populations and kernel damage levels

Similarly, no clear association was found between mummy population density and levels of kernel damage in untreated trees (Figure 44).

These results may be due in part to the very low population levels recorded for carob moth throughout the trial site over the season.

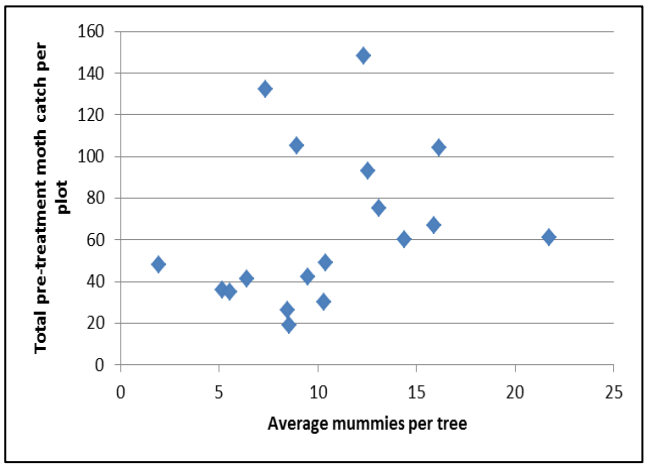

Figure 41. Total moth catch per plot prior to treatment applications and average number of mummies per tree within plots, 2014/2015.

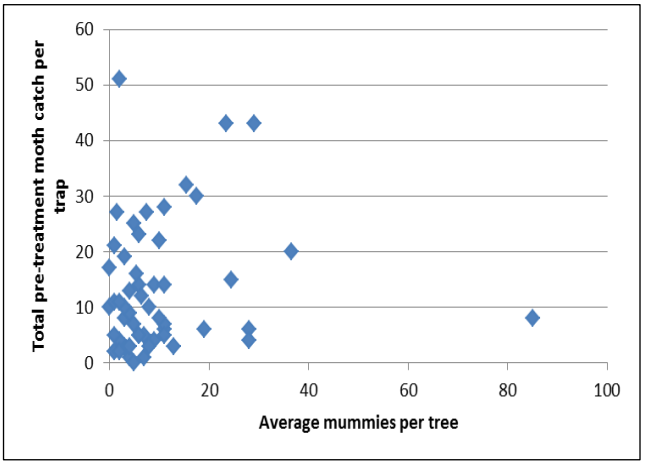

Figure 43. Total pre-treatment moth catch per trap and average number of mummies per tree in trees adjacent to traps, 2014.

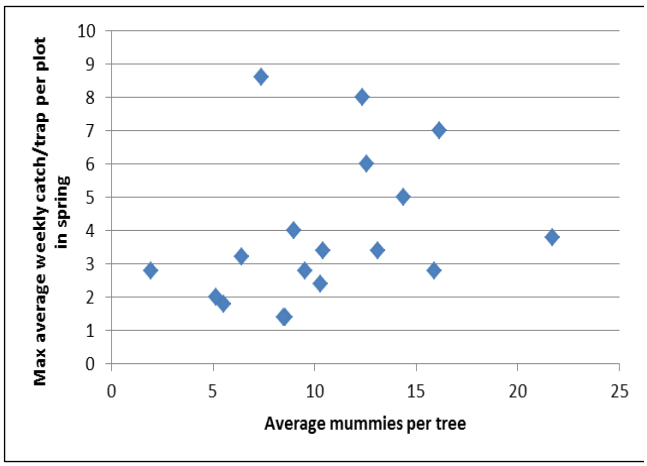

Figure 42. Maximum average weekly catch per trap per plot in spring and mummy population density in 25 trees around each trap, 2014.

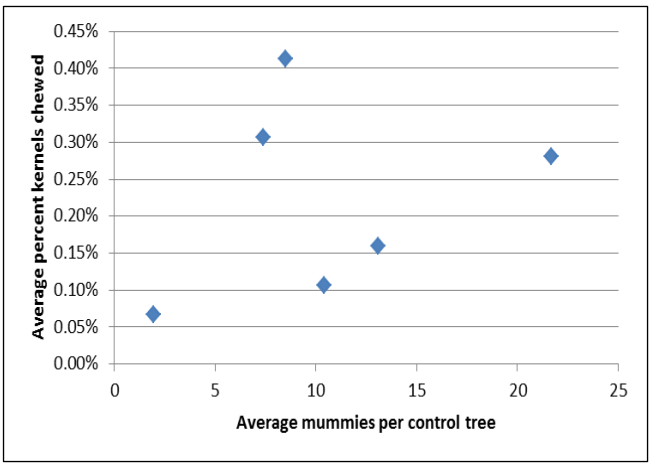

Figure 44. Kernel damage and mummy population density (control plots only) 2014.

Winter mummy surveys

Figure 45 shows how higher levels of carob moth activity in spring can be expected in orchards that carry greater loads of infested mummies in late winter. This was not unexpected.

The same data is presented in Figure 46 but without rates of mummy infestation being taken into account. Again, this result is not surprising but it is interesting that it was not obvious in the data from the 2013/14 and 2014/15 management trials. The apparent association between mummy prevalence alone (ignoring infestation levels) and spring moth activity suggests that, as indicated by Figure 38, the extra work in assessing mummies for accurate carob moth infestation levels may not be necessary for the development of thresholds for mummy population levels.

Of potentially greater value is the pattern shown in Figure 47 which indicates that the level of carob moth activity during the critical post-hull split period may still be fairly closely related to the mummy load in late winter. If this link can be confirmed and strengthened, it may be useful in the development of economic thresholds for mummy population density or moth activity, if combined with the kernel damage data discussed earlier.

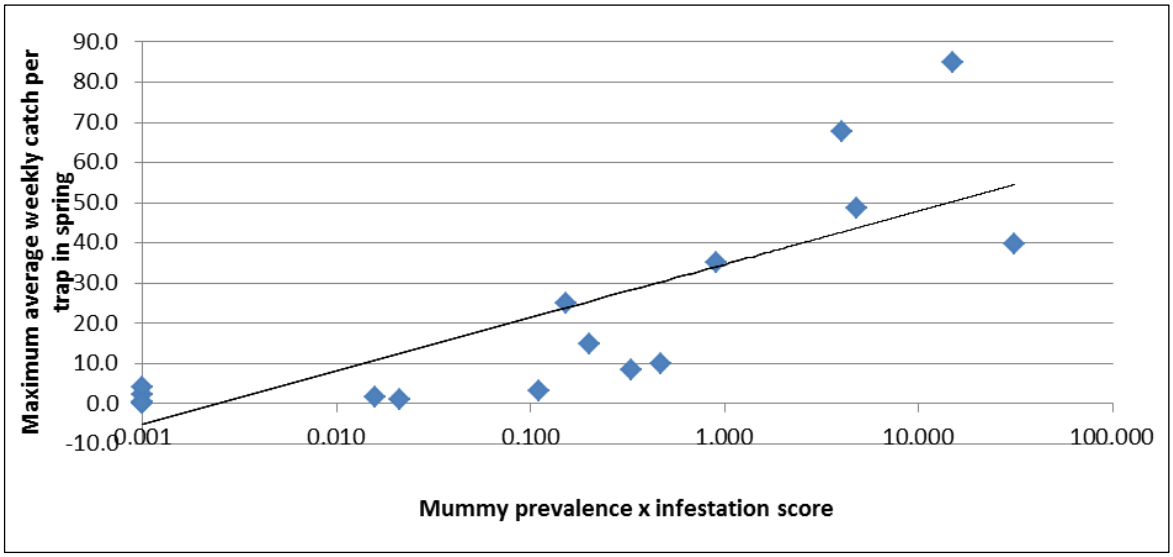

Figure 45. Maximum average weekly catch per trap of carob moth over the spring generation as influenced by winter mummy population and infestation levels (y = 5.7586ln(x) + 34.687; R^2 = 0.6634)

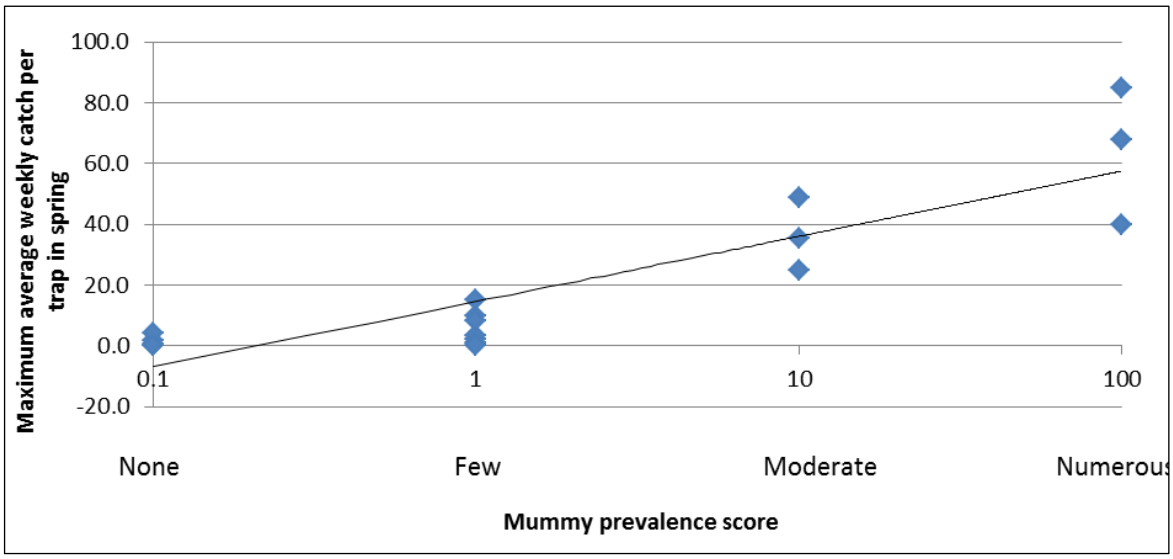

Figure 46. Maximum average weekly catch per trap of carob moth over the spring generation in orchards of varying mummy loads in late winter (y = 9.3255ln(x) + 14.562; R^2 = 0.7772).

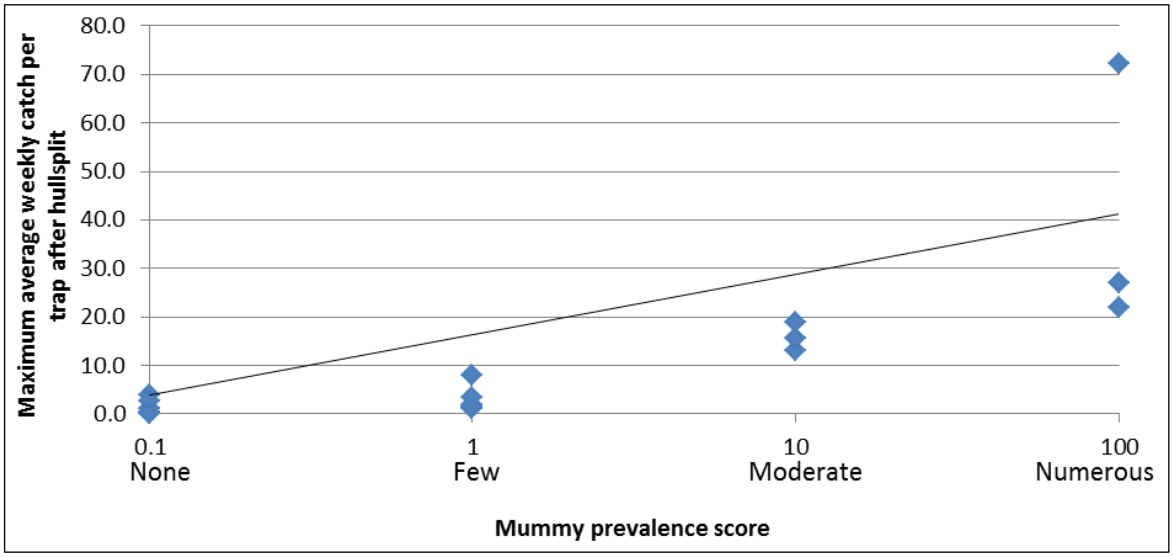

Figure 47. Maximum average catch per trap of carob moth after hull split, in orchards of varying mummy loads in late winter ($y = 0.3744x + 3.7649$; $R^2 = 0.6446$).

Carob moth activity at the management trial site remained at very low levels during the 2014/15 season, making it difficult to determine any associations between mummy population levels and moth activity or kernel damage in that season.

The association between mummy population density and kernel damage levels found in 2013/14 was logical and interesting but needs to be confirmed, especially at the lower values of each factor, before it could be considered as a general guide for the management of orchard hygiene.

The above association used data from a narrowly targeted, intensive mummy survey (25 trees around each nut sample site) compared to the broader and lightly scattered survey of 2014/15 (50 trees distributed evenly across each plot). Even though carob moth is a mobile pest, it is possible that the influence of mummy infestations on damage to new nuts is a localised effect. This needs to be clarified, possibly through a broadly-applied intensive survey. Data from such a survey could also be subsampled to examine how the survey's precision is influenced by varying intensities and spatial range of the mummy counts. This would assist in the development of effective sampling regimes and economic thresholds for producers.

The fact that no clear association was found between mummy population density and moth trap catches under any of the sampling regimes used during the management trials could suggest several scenarios, including:

- The mobility and dispersal behaviour of male moths within orchards may overshadow any localised influence of mummy populations.
- The pheromone traps may have relatively low attractiveness to males compared to female carob moths (the project found some evidence of this). If so, the traps are likely to suffer more from this competition under conditions of higher female population density. If female population density is related to that of mummies, then trap estimates of male moth activity could become less reliable as mummy (and female) densities increased. This could mask any association between population levels of mummies and male moths.

Both scenarios point to the need for an effective trap to monitor female carob moths – the real drivers of kernel damage in almonds. This would help to clarify the mummy-moth relationship and assist the development of economic thresholds for moth activity.

The apparent and logical link between winter mummy population density determined from simple mummy surveys, and moth activity levels in spring and following hull split, if confirmed, may also help contribute to the development of economic thresholds for both mummies and moth activity.

Conclusions

There is some evidence for a good association between local mummy population density and levels of kernel damage at harvest, but this needs to be confirmed.

A broadly-applied survey of intensive localised mummy counts could help to confirm the above association and clarify the optimum survey approach.

The lack of association found between mummy population density and male moth trap catches could possibly be due to moth behaviour or be an artifact of trap efficacy. Development of a female trap would help to clarify any relationship between mummy population density and moth activity.

The apparent association between late winter mummy loads and moth activity in spring and after hull split should be examined in more detail with more rigorous mummy population estimates. This should be combined with kernel damage assessments with a view to developing economic thresholds for moth activity and mummy loads.

Acknowledgements

This project was funded by Horticulture Innovation Australia using the almond growers levy, voluntary contributions from industry and matched funds from the Australian Government. Funding was also provided by the State of Victoria 'Growing Food and Fibre' initiative via the Department of Economic Development, Jobs, Transport and Resources.

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Kernel damage and the timing of carob moth oviposition in almonds.

Aim

Investigate the effect that timing of carob moth oviposition (egg laying) has on subsequent levels of kernel damage in current season almonds.

Introduction

Carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae* is a widespread pest of numerous fruit and nut crops globally and has caused significant levels of kernel damage in some of Australia's major almond producing districts since the 2011 harvest. In almond orchards, carob moths survive over winter mostly as slowly developing larvae in mummy nuts (nuts remaining on trees after harvest). In early spring they complete their development, pupate and start to emerge as adult moths. From that time until hull split in the current season crop, oviposition, larval and pupal development in carob moth is restricted to mummy nuts.

From the start of hull split onwards (typically early January), oviposition and subsequent development of carob moth occurs in both mummies and current season nuts. Kernel damage in the new crop occurs when carob moth larvae burrow into and feed on the kernel. Infestation of current season nuts does not always lead to kernel damage as carob moth activity is often limited to the zone between the hull and shell.

This aspect of project AL12004 sought to determine the relationship between the timing of oviposition on current season nuts and the resulting levels of kernel damage. The intention of this work was to determine whether the new crop required protection from infestation by carob moth for the entire period between hull split and harvest, or whether there was a particular window of time when protection was critical. The rationale behind this research was that if oviposition occurs later in the season, there is less time for larvae to damage the kernel, and potentially less need for protection of the crop. This information is required to fine-tune the timing and so optimise the effectiveness of mating disruption and hull split insecticide applications against carob moth. Both management approaches were the subject of research during project AL12004 and the latter is currently used by a large proportion of the Australian almond industry.

The link between kernel damage and timing of oviposition was investigated in small field trials between 2013 and 2015.

Materials and methods

The trials reported here were located in two commercial almond orchards in the Sunraysia region of Victoria, Australia. The major almond variety in both orchards was Nonpareil (50%), with Price, Monterey and Carmel used as pollinators.

For each trial, twigs carrying current season Nonpareil nuts were enclosed in gauze sleeve cages to protect them from natural oviposition and the nuts were progressively 'seeded' at different times with fresh carob moth eggs. The 2013 trial was used to test the methodology for trials in subsequent seasons.

Trial site

The 2013 and 2014 trials were located in an almond orchard known to be infested with carob moth. An infested orchard was chosen to avoid the chance of accidental introduction of carob moth into an otherwise clean orchard. In 2013 and 2014 respectively, four and three Nonpareil trees were used, all positioned within a single row within the orchard.

The 2015 oviposition trial was located in an orchard that appeared to have a naturally very low carob moth population. This was to minimise the likelihood of oviposition into the cages by the local population, which appeared to be an issue in the 2014 trial. An alternative approach would have been to use a tightly-knit sleeve cage material to provide more of a physical barrier to oviposition. Such a material however, could also be expected to alter the environment of the enclosed twigs and nuts considerably, in relation to air movement, humidity, temperature and or light intensity, with potential effects on development of the carob moth that were to be introduced into the cages as eggs.

For the 2015 trial, twenty Nonpareil trees in two rows were used.

Sleeve cages

Sleeve cages used for these trials were 30-50 cm long, 20 cm in diameter and were made of fine nylon gauze. The cages were installed by pulling them over twigs and tying the ends with tie wire. This effectively prevented carob moths from accessing nuts on the enclosed twigs for oviposition. Any nuts that showed signs of splitting at the time were removed before the twigs were caged.

For the 2013 trial, 18 cages enclosing a total of 457 nuts were installed on 8 Jan 2013, just at the start of hull split. Twigs were selected to each be carrying 20-40 unsplit nuts.

In 2014, twigs were selected to each be carrying at least four unsplit nuts. Fifty-one cages enclosing a total of 404 nuts were installed on 10 Dec 2013, prior to the start of hull split.

In 2015, 48 sleeve cages were used, each placed around a twig carrying 3-7 unsplit nuts. The cages were installed on 12 Jan 2015. Before being enclosed in the cages, any obviously split nuts on the twigs were removed and the suture area of the remaining nuts was inspected with a 10x hand lens to confirm that the nuts had not started to split and that no carob moth eggs from early natural oviposition were present.

Manual seeding of nuts with eggs

For the trials in all three years, fresh carob moth eggs were obtained from a laboratory culture maintained by the project. The eggs had been laid onto sheets of thin card which were then cut into small pieces ('egg cards') 1-2 cm long, each carrying several eggs.

In 2013, each week for three weeks starting on 11 Jan, split nuts in 14 'treatment' cages were manually seeded with several fresh carob moth eggs by placing an egg card into the split in the hull using fine forceps, taking care not to damage the eggs. Each nut that was seeded with eggs was marked to indicate the date of seeding. At the end of the trial, some cages contained nuts seeded at different times, and all cages contained nuts that had not been manually seeded. The remaining four cages containing a total of 98 nuts, were treated as 'controls' and were not seeded with eggs at any stage.

The cages and twigs that they enclosed were collected from the orchard on 1 Mar 2013 after the trees had been shaken for commercial harvest, and were stored at ambient temperatures until they were assessed for infestation and damage by carob moth on 10-13 May 2013. This storage period simulated a 'worst-case' scenario in relation to the delay between harvest and processing in a non-fumigated stockpile situation.

In 2014, each week for six weeks starting on 2 Jan, split nuts in cages were manually seeded with several fresh carob moth eggs. The same methods were used as in 2013 except that any particular cage only received eggs on a single date. This was to avoid the potential for cross-infestation between nuts seeded on different dates, that may arise from wandering larvae or second generation oviposition as may have been the case in 2013. At the time of seeding caged nuts with eggs, most of the cages contained some unsplit nuts. Those nuts were left in place and were not manually seeded with eggs at any time. Seven cages containing 67 nuts were left unseeded as controls.

The cages and nuts were collected from the field on 27 Feb 2014 and stored at ambient temperatures until the nuts were assessed on 31 Mar 2014. As in the previous season's trial, this storage period simulated the delay between harvest and processing in a non-fumigated stockpile situation.

In 2015, each week from 12 Jan -23 Feb 2015, all the nuts in six sleeve cages were manually seeded with two to four fresh carob moth eggs as described earlier. Six cages containing 30 nuts in total were left unseeded and marked as controls. All cages and enclosed nuts were collected from the field on 3 Mar 2015, several days after commercial harvest, and were stored at approximately 6°C to halt further carob moth activity until the nuts were assessed one week later. The delay between harvest and assessment was minimised in an attempt to avoid the 'secondary' infestation of nuts by 2nd generation moths during storage, as may have occurred in the previous trials. The 2015 trial therefore represented a commercial situation where processing of the crop or stockpile fumigation occurred promptly after harvest.

Data collection

Each year, all nuts from the sleeve cages were inspected using a dissecting microscope and were assessed for the presence of different life stages of carob moth and carob moth damage to the hull, shell and kernel.

In the reporting of results:

- 'Any sign of CM' includes nuts that showed any sign of carob moth damage such as chewing damage to the hull, regardless of whether or not live carob moth was actually present at the time of assessment.
- 'CM present' refers to nuts that contained any live life stage of carob moth.
- '2nd generation' refers to nuts that contained moths or old pupal cases, indicating that eggs placed into the nuts had developed through a full generation to pupation and moth emergence, and potentially mating and oviposition.

Results & discussion

The following table summarises the numbers of sleeve cages and nuts used in the oviposition trials over 2013-2015.

Table 16. Number of sleeve cages, seeded nuts and nuts in the 2013-2015 trials.

Year	No. of seeded cages	No. of seeded nuts in seeded cages	Total No. of nuts in seeded cages	No. of control cages	Total No. of nuts in control cages	Total No. of nuts caged
2013	14	43	359	4	98	457
2014	44	210	337	7	67	404
2015	42	212	212	6	30	212

Table 2 lists the dates that nuts were seeded with eggs in 2013. Only three nuts were seeded in the first week as hull split had not progressed far and only those three nuts had split.

Table 2. Date and number of nuts seeded with carob moth eggs, 2013.

Date of seeding	11/01/2013	18/01/2013	25/01/2013	Total
Nuts seeded	3	26	14	43

As indicated by the 0% infestation of nuts in 'Cages not seeded' (Figure 48), the sleeve cages appear to have functioned as intended in preventing natural oviposition on caged nuts, and the level of infestation of seeded nuts confirms that the seeding technique was a satisfactory way to infest nuts with carob moth.

At the time of assessment, 13 of the 14 treatment cages contained signs of development of a 2nd generation (i.e. pupation and moth emergence). Oviposition by that generation of moths on nuts that were not manually seeded with eggs would explain the almost 16% of such nuts that became infested (Figure 48, 'Nuts not seeded'). An alternative explanation could be that some larvae from the manually placed eggs moved to nuts that had not been seeded.

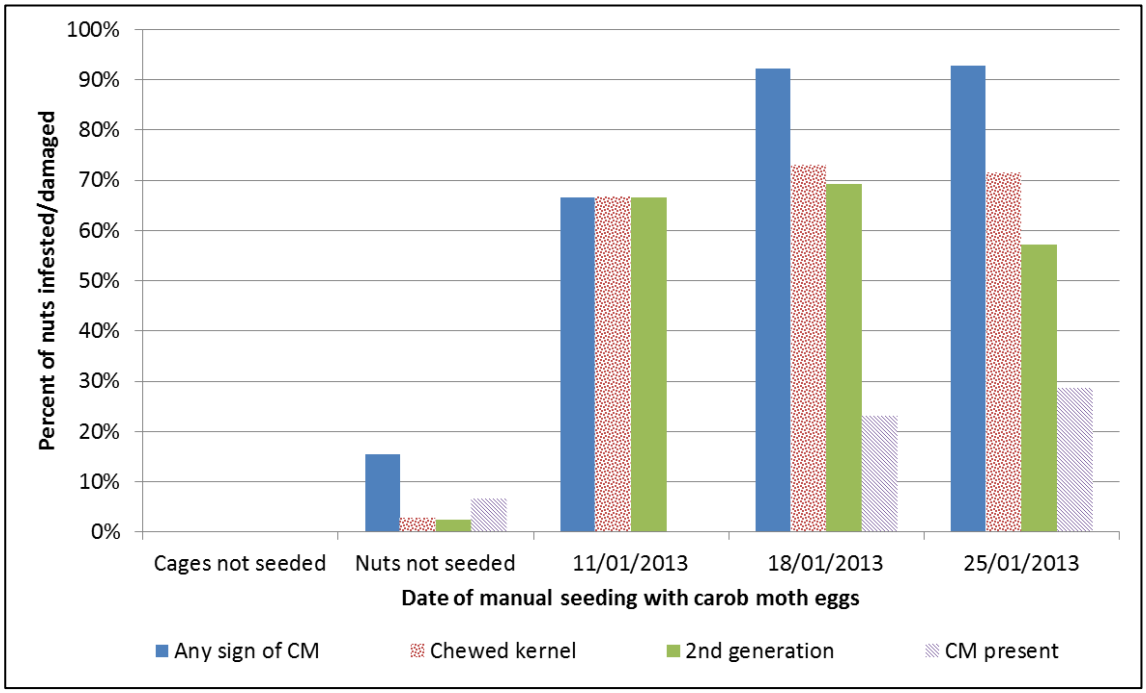

Figure 48. Infestation and kernel damage levels resulting from manual seeding of almonds with carob moth eggs, 2013. Note: Only three nuts seeded on 11/1/2013.

The dates of seeding of nuts in 2014 are listed in Table 3.

Table 3. Date and number of nuts seeded with carob moth eggs, 2014.

Date of seeding	2/01/2014	9/01/2014	16/01/2014	23/01/2014	30/01/2014	6/02/2014	Total
Nuts seeded	15	14	28	49	40	64	210

In contrast with the results from 2013, high levels of infestation were observed in nuts that were not directly seeded with eggs (Figure 50) and in nuts in cages that received no eggs at all in 2014 (Figure 49Figure 51, 'Nil').

These results suggest a failure of some aspect of the experimental technique. Two of the 51 sleeve cages were found to be torn slightly at the end of the trial but this obviously does not explain the overall result. One possible explanation could be that the relatively high level of carob moth activity recorded in the orchard during the 2013/14 season led to a significant amount of oviposition onto caged nuts through the gauze sleeve, but this cannot be confirmed. Another possibility could be that the delay between harvest and assessment allowed for the majority of nuts in all seeded cages to become infested through oviposition by moths of the 2nd generation, although this would not explain the infestation of nuts in unseeded cages.

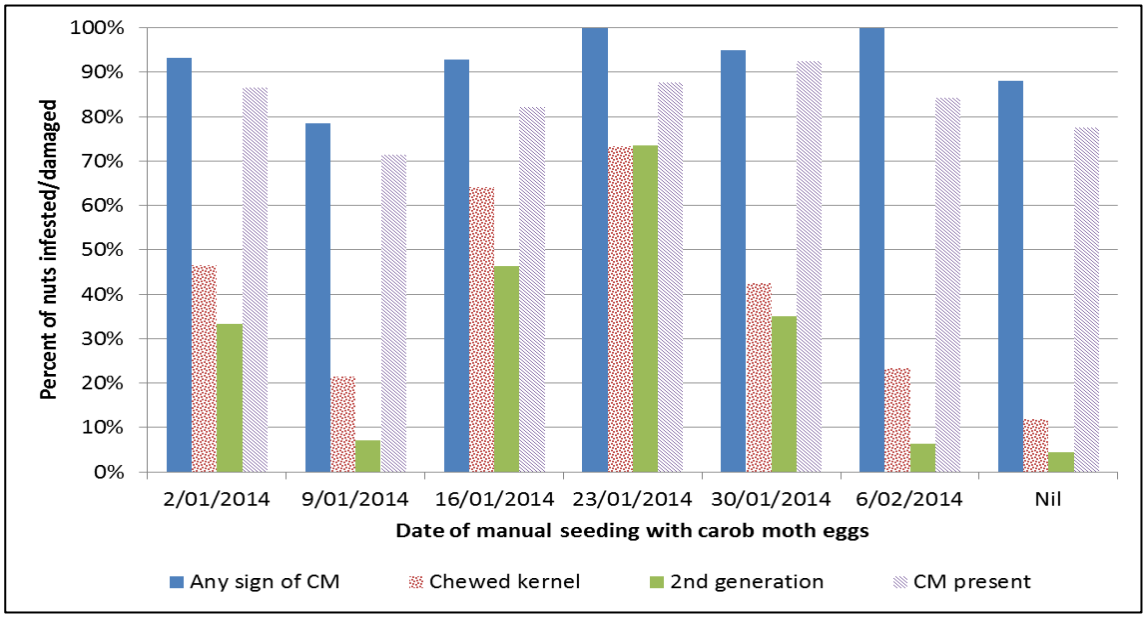

Figure 49. Infestation and kernel damage levels of almonds manually seeded with carob moth eggs, 2014.

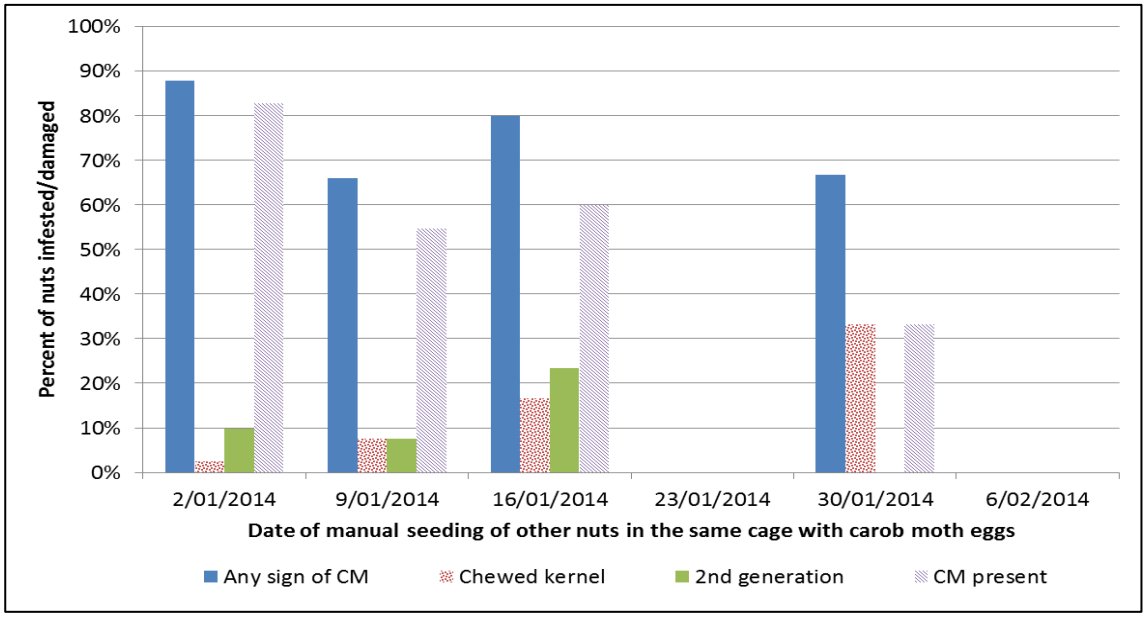

Figure 50. Infestation and kernel damage levels in almonds not directly manually seeded with carob moth eggs, 2014. Note: in the cages seeded on 23 Jan and 6 Feb, all nuts received eggs.

In 2015, 212 nuts were seeded with carob moth eggs over seven weeks as listed in Table 4.

Table 4. Date and number of nuts seeded with carob moth eggs, 2015.

Date of seeding	12/01/2015	19/01/2015	27/01/2015	2/02/2015	9/02/2015	17/02/2015	23/02/2015	Total
Nuts seeded	21	28	31	29	31	37	35	212

The absence of any signs of infestation in nuts from cages that were not seeded at all with carob moth eggs indicates that natural oviposition was not a factor in this trial (Figure 51).

An unexpected aspect of the 2015 result is the drop observed in the percent of seeded nuts showing signs of carob moth infestation with later seeding dates. We would have expected a high percentage of seeded nuts to show signs of infestation for most of the seeding dates, apart maybe from the latest date which allowed the

least time for eggs to mature and hatch and larvae to feed and develop before the nuts were put into cool storage.

The drop seen in levels of infestation may indicate unsuccessful hatching and establishment of larvae, possibly due to environmental conditions. For example, low levels of egg survival have been reported under constant conditions of low relative humidity (10%RH), especially at elevated temperatures (34°C) (Gothilf 1969). Under ambient summer conditions of 28°C-33°C, the expected incubation period for carob moth eggs is approximately 5-8 days (Al-Izzi, Al-Maliky et al. 1985). During the seven days after the seeding of nuts with eggs on 2 Feb 2015, the maximum daily temperature exceeded 38°C on three days while relative humidity dropped to 15% (Figure 52). All three subsequent seeding dates were also followed by periods of hot dry weather, each period including at least two days exceeding 34°C and another of 40°C or above combined with a relative humidity of 12-14%. Under such conditions, some mortality of eggs and young larvae could be expected and may help to explain the apparent drop in infestation success noted above.

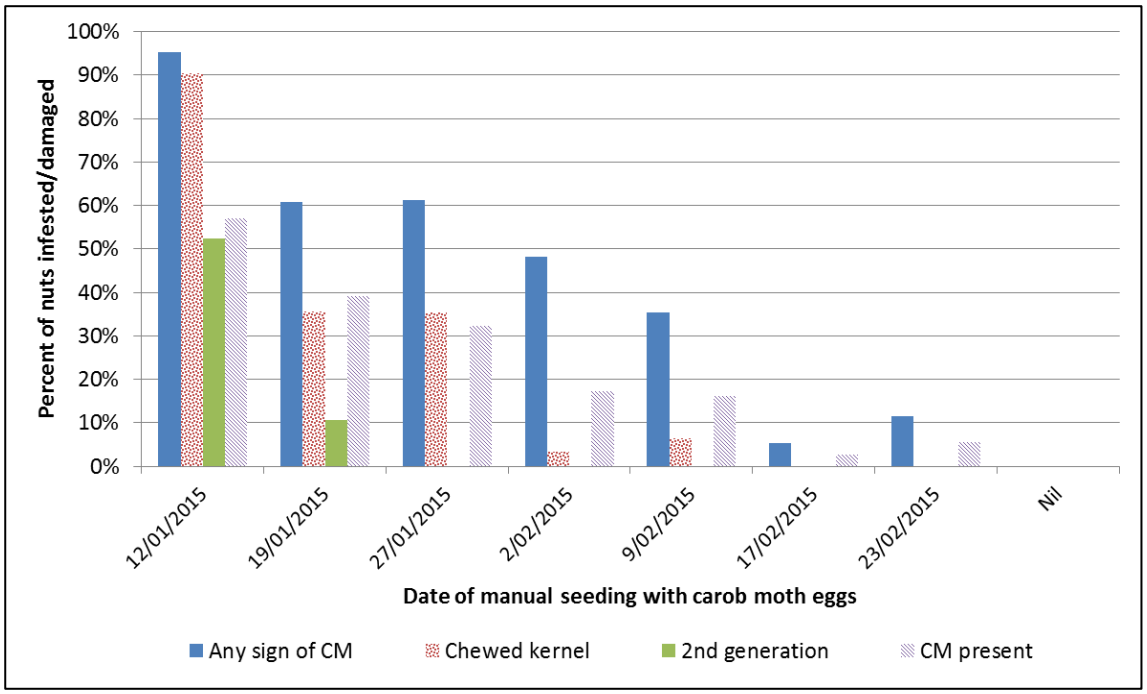

Figure 51. Infestation and kernel damage levels of almonds manually seeded with carob moth eggs, 2015.

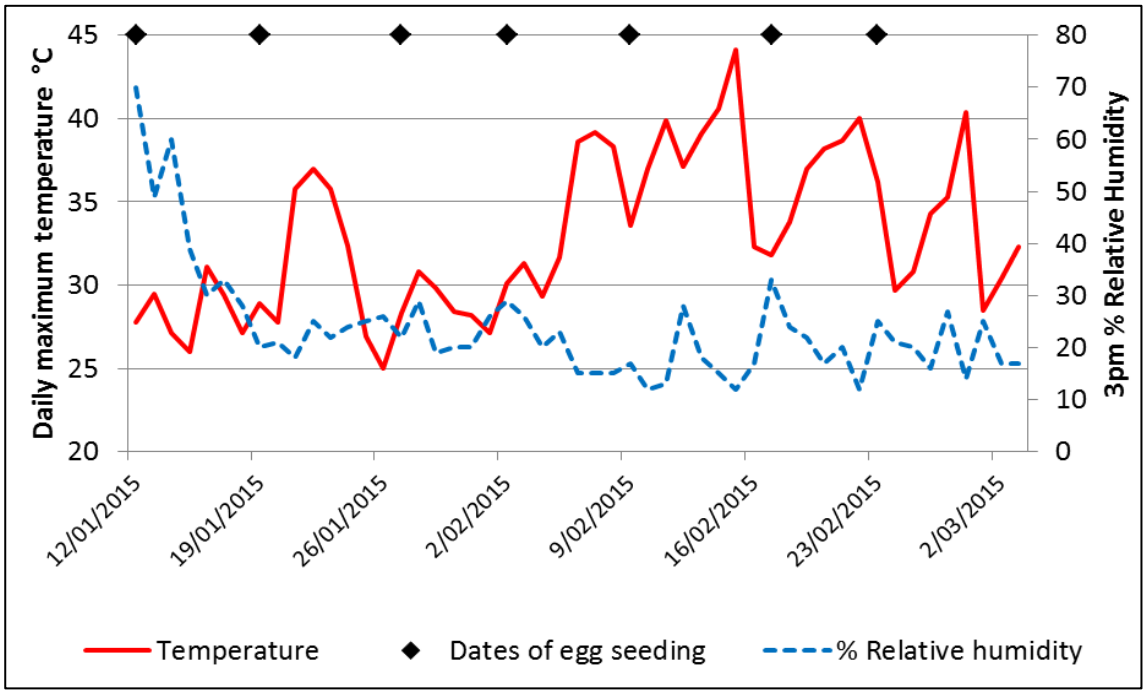

Figure 52. Daily maximum temperature and 3pm relative humidity during the 2015 oviposition trial.

The results in terms of kernel damage appeared as could be expected, in that damage levels were lower the later the nuts were seeded with eggs. These results do however need to be viewed with caution, given the unexpected drop in overall signs of infestation from earlier to later seeding dates, as discussed above. The observed decline in kernel damage with later seeding dates may simply be due to the apparent decline in infestation success rather than to the seeding dates themselves. What these data do show is that a significant level of kernel damage (6%) was recorded from nuts that were seeded with eggs as late as three weeks before harvest. This indicates that protection of the crop from oviposition will be required at least up to that point, if rapid harvest and processing or post-harvest disinfestation is guaranteed. If delays are likely, as is usually the case, protection against oviposition would be desirable, if not necessary, up to the point of harvest itself.

For a significant proportion of the Australian almond industry, current practice for managing carob moth involves a pesticide application at hull split. Two insecticides are currently available for this use under permits from the Australian Pesticides and Veterinary Medicines Authority. Both products, Altacor® (chlorantraniliprole) and Prodigy™ (methoxyfenozide) are ovi/larvicides and are stated to have an effective residual life of 14-21 days after application (Anon 2012; Anon 2015). In the Sunraysia region, it is not uncommon for 40 days to elapse between the start of hull split and the start of harvest, and for a single variety, harvest may continue for several weeks. A single hull split application of either insecticide therefore leaves a gap of at least three weeks prior to harvest, during which the crop is susceptible to infestation and damage by carob moth, as shown above. Two obvious solutions, a repeat spray or earlier harvest are limited in feasibility, the former by cost and the latter by the maturity of the crop. Combinations of alternative approaches to managing carob moth, such as mating disruption, mummy destruction, biological control or possibly spring applications of insecticide are likely be required.

The appearance of a 2nd generation of carob moth in the cages was also as would be expected (i.e. only in the early-seeded cages), as the predicted generation time based on degree-day requirements was approximately eight weeks during January and February. The later seeding dates would not have allowed enough time for development of the seeded eggs to the point of pupation and moth emergence.

On the experimental technique, seeding almond nuts with carob moth eggs on ‘egg cards’ at hull split appears to be a satisfactory technique to induce infestation of nuts, but it seems that field trials using this technique should be located in orchards of low natural populations to minimise the chance of natural infestation of the same nuts by the wild population. It should also be remembered that high temperatures and low humidity may reduce the level of infestation success given their expected impact on egg and larval mortality.

Given the expected generation time (8 weeks) for carob moth around the time of hull split, if trials such as those reported above are operated for a longer period, a second generation of eggs can be expected to be produced naturally within the cages, resulting in infestation of additional nuts or secondary infestation of already seeded nuts. This can be undesirable from an experimental point of view.

Conclusions

Single applications of insecticide at hull split to protect almonds against infestation by carob moth will typically leave new crop nuts exposed to infestation for a considerable period prior to harvest. Additional approaches to managing carob moth, such as mating disruption, mummy destruction, biological control or possibly spring applications or repeat post-hull split applications of insecticide are likely to be required to achieve optimum protection of the crop.

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A review of the impact of Altacor® (Rynaxypyr®; chlorantraniliprole) on beneficial invertebrates.

Aim

Review the scientific and technical literature documenting the effects of the insecticide chlorantraniliprole on beneficial species of invertebrates.

Introduction

Carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae* is an economically significant pest of a wide range of tree crops globally. It has been a minor or sporadic pest of almonds in Australia for many years, but became a significant kernel quality issue for the industry after the unusually wet summers of 2009/10 and 2010/11. It is possible that the growth in populations of the pest during those wet seasons was associated with an increase in numbers of 'mummy' nuts (nuts remaining on trees after harvest). Mummies are an important food resource for carob moth, and often arise from nuts that are affected by hull rot, a fungal disease that develops during wet summer conditions.

In response to concerns regarding increased risk of crop damage from carob moth and the lack of available control options, the Almond Board of Australia (ABA) obtained emergency use permits for Australian producers to apply an insecticide that is used against navel orangeworm in almonds in USA. Subsequently, this project (AL12004) undertook to assess the potential impacts of that insecticide on beneficial invertebrates such as predators and parasites in almond orchards. The aim of this aspect of the project was to inform producers of any potential risks that use of the insecticide may pose to biological control systems operating in almond orchards.

Background

After observing high levels of infestation and kernel damage by carob moth during the 2011 harvest, the industry decided to take management action to protect future crops. The only response available for the 2012 harvest was a pesticide application at hull split. This option was made possible by the emergency use permit (PER13233 superseded by PER14415) from the Australian Pesticides and Veterinary Medicines Authority (APVMA) arranged by the ABA. This permit allowed for a maximum of two applications of Altacor® (350 g/kg chlorantraniliprole; Du Pont™) with the first application being at 1-5% hull split.

Prior to this, there had been little need for lepidopteran pesticides on almonds and none of the pesticides already approved for use on almonds were suitable for carob moth management. The only registered products at that time were a mating disruption pheromone for codling moth and oriental fruit moth and spray oil for mites. Minor use or emergency use permits were also in force for pesticides against mites, aphids and plague locusts.

Whenever a pesticide is used, especially when a 'new' product is introduced into a production system, there are always concerns regarding potential off-target impacts such as suppression of beneficial invertebrates, that may lead to pest resurgence or development of secondary pests when the natural enemies of those pest species are affected. As part of its research on carob moth, Project AL12004 initially proposed to commission bioassay studies to investigate the impacts of chlorantraniliprole on beneficial invertebrate species found in almond orchards. However, before those commissions were initiated, a review of literature found that a significant amount of new research on the impact of chlorantraniliprole on beneficial invertebrates had been recently published. This new information, which is the subject of this report, was considered relevant enough to almonds to satisfy the project objectives without proceeding with the bioassay component.

Chemistry

Chlorantraniliprole has the chemical name '3-Bromo-N-[4-chloro-2-methyl-6- (methylcarbamoyl)phenyl]-1-(3-chloro-2- pyridine-2-yl)-1H-pyrazole-5-carboxamide' and formula $C_{18}H_{14}N_5O_2BrCl_2$ (Anon 2008).

It is an anthranilic diamide insecticide which acts by interrupting normal muscle contraction, leading to paralysis and death, and is classed as a Group 28 (Ryanodine receptor modulator) insecticide (Insecticide Resistance Action Committee 2015).

Relevance of beneficial invertebrate species

The species represented in the studies summarised below include parasitic wasps and predatory mites, bugs, ladybirds, lacewings and earwigs present in Australia and known to play a role in biological control of a range of pests including or similar to those found on almonds (largely mites, moths and aphids). Some, including European earwig (*Forficula auricularia*), transverse ladybird (*Coccinella tansversalis*), green lacewing (*Mallada signata*), trichogramma wasps (*Trichogramma spp*) and minute pirate bugs (*Orius* spp) have been observed repeatedly on almonds during this project. The range of invertebrate families and species covered by these studies provides considerable confidence that the findings in relation to the impact of chlorantraniliprole will be broadly applicable to Australia's almond production systems.

Effects of chlorantraniliprole on beneficial species: Published studies

Table 18 below summarises the key findings from a number of published Australian and international studies on the impact of chlorantraniliprole (active ingredient of Altacor®) on a range of beneficial invertebrates.

The following points are relevant to the interpretation of Table 18:

1. The APVMA permit for use of Altacor® on almonds limits the use to two applications per season and 280g Altacor® per application. As Altacor® contains 350g/Kg chlorantraniliprole, a single application will equate to 98g a.i./ha or 0.0653g a.i./L (assuming a spray rate of 1500L water/ha). These doses can be compared with values in the 'Dose rate' column of the table.
2. The International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC) rates pesticides according to their impact on beneficial species such as biological control agents. These ratings (Table 17) are determined by field or laboratory bioassays and refer to the mortality or reduction in capacity of the beneficial species, that is caused by the pesticide (Boller, Vogt et al. 2005).

Table 17. IOBC Rating of pesticide impacts on beneficial species.

	Rating	% mortality/reduction
Field bioassays	harmless or slightly harmful	0-50%
	moderately harmful	51-75%
	harmful	>75%
Laboratory bioassays	harmless or slightly harmful	<30%
	moderately harmful	30-79%
	harmful	80-99%
	harmful	>99%

These ratings can be compared to the values in the 'Effect of chlorantraniliprole' columns of 'Mortality', 'Reduction in reproduction', 'Development and behaviour' and 'Longevity reduction'.

Table 18. Effect of chlorantraniliprole on beneficial invertebrates: Published studies.

						Effect of chlorantraniliprole					
Beneficial species		Host/prey	Life stage tested	Dose rate (a.i.)	Formulation	Mortality	Reduction in reproduction	Development & behaviour	Longevity reduction	In Australia	Reference
Acari (mites)											
Phytoseiidae	*Amblyseius herbicolus*	Mites	adults	0.5g/L	200SC	ns				✓	(Reis, Franco et al. 2011)
	Amblyseius swirskii	"	adults	0.018g/L (25g/ha)	35WG	ns					(Gradish, Scott-Dupree et al. 2011)
	Euseius citrifolius	"	adults	0.5g/L	200SC	ns					(Reis, Franco et al. 2011)
	Galendromus occidentalis	"	eggs, adults nymphs	1.7g/L 1.7g/L	35WG	ns 33.9%	ns			✓	(Lefebvre, Bostanian et al. 2011)
	Iphiseiodes zuluagai	"	adults	0.5g/L	200SC	ns					(Reis, Franco et al. 2011)
	Neoseiulus californicus	"	all	0.035g/L	200SC	ns	ns			✓	(Kaplan, Yorulmaz et al. 2012)
	Neoseiulus fallacis	"	all	1.7g/L	35WG	ns	ns			✓	(Lefebvre, Bostanian et al. 2012)
Hymenoptera (wasps, bees, ants)											
Aphelinidae	*Eretmocerus eremicus*	Whitefly	adults	.018g/L	35WG	ns					(Gradish, Scott-Dupree et al. 2011)
Aphidiinae	*Aphidius rhopalosiphi*	Aphids	adults	3.75g/L	35WG, 20SC	ns	ns			✓	(Brugger, Cole et al. 2010)
Apidae	*Bombus impatiens*	Pollinator	adults	1.0g/L 8.75mg/L	35WG 35WG	ns –spray ns - ingestion	ns –spray ns - ingestion				(Gradish, Scott-Dupree et al. 2010)
	Bombus impatiens	Pollinator	adults	230g/ha		ns					(Larson, Redmond et al. 2014)
	Bombus terrestris	Pollinator	adults	0.4-40mg/L 20-40mg/L	200SC	*		**		✓	(Smagghe, Deknopper et al. 2013)
Braconidae	*Dolichogenidea tasmanica*	Leafroller moths	adults	0.18g/L	35WG	ns				✓	(Brugger, Cole et al. 2010)
Encyrtidae	*Copidosoma bakeri*	Cutworm eggs	adults	230g/ha		ns					(Larson, Redmond et al. 2014)
Eulophidae	*Aphelinus mali*	Aphids	adults	0.12g/L	20SC	ns	ns			✓	(Brugger, Cole et al. 2010)
Ichneumonidae	*Diadegma semiclausum*	Lepidopteran eggs	adults	0.08g/L	20SC	ns				✓	(Brugger, Cole et al. 2010)
Mymaridae	*Angrus nilaparvatae*	Rice planthopper	adults	0.067g/L	Technical	ns	ns				(Liu, Zhang et al. 2012)
Tiphiidae	*Tiphia vernalis*	Scarab larvae	adults	230g/ha			ns				(Larson, Redmond et al. 2014)

Trichogrammatidae	*Trichogramma pretiosum*	Lepidopteran eggs	adults, larvae	0.01g/L	35WG	ns	ns			✓	(Brugger, Cole et al. 2010)
	Trichogramma chilonis	Lepidopteran eggs	larvae	0.1g/L	20SC	ns					(Brugger, Cole et al. 2010)
	Trichogramma chilonis	Lepidopteran eggs	adults	25g/ha	SC	ns					(Preetha, Stanley et al. 2009)
	Trichogramma dendrolimi	Lepidopteran eggs	adults	0.063g/L 0.05g/L 0.061g/L	Tech 20SC 35WG	ns ns ns					(Brugger, Cole et al. 2010)
	Trichogramma galloi		adults			ns (27.5%)	35.5%				(de Oliveira, Antigo et al. 2013)
Hemiptera (bugs)											
Miridae	*Cyrtorhinus lividipennis*	General predator	adults, nymphs	0.04g/L		ns	67%		27%		(Yang, Wang et al. 2012)
	Deraeocoris brevis	Soft-bodied pests (aphids, psyllids)	nymphs, adults	110.4 g/ha[#]	35WG	ns	ns		35-78%		(Amarasekare and Shearer 2013b)
Miridae cont.	*Macrolophus pygmaeus*	General predator	nymphs	0.04g/L		ns		ns			(Martinou, Seraphides et al. 2014)
Anthocoridae	*Orius armatus*	Thrips, mites	adults, nymphs	0.03g/L		ns	ns			✓	(Broughton, Harrison et al. 2014)
	Orius insidiosus	Thrips, mites, aphids, caterpillars	adults	0.018g/L	35WG	ns					(Gradish, Scott-Dupree et al. 2011)
			adults	75g/ha	35WG	ns					(Roubos, Rodriguez-Saona et al. 2014)
Pentatomidae	*Podisus nigrispinus*	Lepidoptera	3rd instar nymphs	0.13g/L	200SC	ns (25% after 20 days)					(De Castro, Corrêa et al. 2013)
	Supputius cincticeps	Lepidoptera	3rd instar nymphs	0.13g/L		ns (30% after 20 days)					(De Castro, Corrêa et al. 2013)
Coleoptera (beetles)											
Carabidae	*Harpalus pennsylvanicus*	General predator		230g/ha		ns					(Larson, Redmond et al. 2014)
Chrysomelidae	*Chrysochus auratus*	Weed (biocontrol)	adults	100g/ha	35WG	ns					(Crozier and Cutler 2014)
Coccinellidae	*Coccinella transversalis*	General predator	larvae	0.02g/L?	?	ns				✓	(Broughton, Learmonth et al. 2011)
	Cryptolaemus montrouzieri	General predator	adults	0.0315g/L	35WG	12%				✓	(Thomson and Hoffman 2009)
	Hippodamia convergens	Aphids	adults	75g/ha	35WG	ns					(Roubos, Rodriguez-Saona et al. 2014)

	Hippodamia variegata	General predator	larvae	0.02g/L?	?	50%		Adults 60% lighter		✓	(Broughton, Learmonth et al. 2011)
	Dalotia coriaria	General predator	adults	0.0315g/L	35WG	ns				✓	(Thomson and Hoffman 2009)
Neuroptera (lacewings)											
Chrysopidae	*Chrysoperla carnea* *Chrysoperla johnsoni*	General predator	adults & 2nd instar larvae	110.4 g/ha#		Adults 100% Larvae ns		Larval-adults survival reduced 66-77%	89-94%		(Amarasekare and Shearer 2013a)
	Chrysoperla externa	General predator	3rd instar larvae	0.6g/L	SC	ns	ns	ns			(Joao Zotti, Dionel Grutzmacher et al. 2013)
	Chrysoperla rufilabris	Mites, thrips, aphids, mealybugs	adults	75g/ha	35WG	ns					(Roubos, Rodriguez-Saona et al. 2014)
	Mallada signata	General predator	larvae	0.02g/L?	?	15-20%		Reduced pupation time		✓	(Broughton, Learmonth et al. 2011)
Dermaptera (earwigs)											
Forficulidae	*Doru luteipes*	Lepidoptera	adults	0.2g/L	200SC	ns					(Campos, Picanço et al. 2011)
	Forficula auricularia	General predator	adults	0.0315g/L	35WG	ns				✓	(Shaw and Wallis 2010)

ns no significant effect observed

* 79% mortality when pesticide fed via sugar water

** reduced defence behaviour when pesticide applied directly or via pollen

Worst-case scenario where the insects, cages and food were all treated with insecticide

As can be seen from Table 18, relatively few instances of negative impacts of chlorantraniliprole on beneficial species were found during the studies. Of those, several are likely to be of minor if any concern in almonds (Table 19), given the relatively low level of impact observed and the very high dose rates used in the impact assessments compared to the rate applicable to almonds.

Table 19. Negative impacts of chlorantraniliprole, likely to be of very minor concern.

Beneficial species	Life stage tested	Dose rate (a.i.)	Mortality	Reduction in reproduction	Development & behaviour	Longevity reduction	Dose rate compared to Altacor® label rate
Galendromus occidentalis	eggs, adults nymphs	1.7g/L 1.7g/L	ns 33.9%	ns			2600%
Trichogramma galloi	adults	157.5g/ha	ns (27.5%)	35.5%			160%
Podisus nigrispinus	3rd instar nymphs	0.13g/L	ns (25% after 20 days)				199%
Supputius cincticeps	3rd instar nymphs	0.13g/L	ns (30% after 20 days)				199%

Of more concern are the impacts summarised in Table 20, where the observed levels of impact are high or the dose rate used was relatively low compared to that applicable to almonds. These findings indicate that where chlorantraniliprole is used in almonds routinely, even if only once a year but for several years in succession, it would be prudent to monitor for negative impacts on beneficial species to allow for early detection of disturbances to the pest/beneficial balance of those orchards.

Table 20. Negative impacts of chlorantraniliprole, of some concern.

Beneficial species	Life stage tested	Dose rate (a.i.)	Mortality	Reduction in reproduction	Development & behaviour	Longevity reduction	Dose rate compared to Altacor® label rate
Cyrtorhinus lividipennis	adults, nymphs	0.04g/L	ns	**67%**		27%	61%
Deraeocoris brevis	nymphs, adults	110.4 g/ha#	ns	ns		**35-78%**	113%
Cryptolaemus montrouzieri	adults	0.0315g/L	12%				48%
Hippodamia variegata	larvae	0.02g/L	**50%**		Adults **60%** lighter		31%
Chrysoperla carnea *Chrysoperla johnsoni*	adults & 2nd instar larvae	110.4 g/ha#	Adults **100%** Larvae ns		Larval-adults survival reduced **66-77%**	**89-94%**	113%
Mallada signata	larvae	0.02g/L	15-20%		Reduced pupation time 22%		31%

ns no significant effect

Worst-case scenario where the insects, cages and food were all treated with insecticide

Effects of chlorantraniliprole on beneficial species: Industry internal studies

Table 21 below lists the outcomes of a series of unpublished internal DuPont studies on the toxicology of chlorantraniliprole. These studies comprised part of the information that was reviewed by the United States Environmental Protection Agency during the registration process for the insecticide.

In Table 21, the 'Endpoint Type' refers to the type of impact that the treatment was observed to have on the test species of invertebrate. The 'Effects Value' is the dose or rate of chlorantraniliprole relating to the observed impact.

Codes for the 'Endpoint Type' are:

- EC_{50}: Median effect concentration. Concentration (e.g. g/L) that adversely affects half of the tested animals
- ER_{50}: Median effect rate. Rate (e.g. g/ha) that adversely affects half of the tested animals
- LC_{50}: Median lethal concentration. Concentration (e.g. g/L) that kills half of the tested animals
- LD_{50}: Median lethal dose. Dose (e.g. g) that kills half of the tested animals
- LR_{50}: Median lethal rate. Rate (e.g. g/ha) that kills half of the tested animals
- LR_{100}: Lethal rate. Rate (e.g. g/ha) that kills all of the tested animals
- LOAEC: Lowest observed adverse effect concentration. Lowest tested concentration that has an observable adverse effect on the organism
- LOEC: Lowest observed effect concentration. Lowest tested concentration that has an observable effect on the organism
- NOAEC: No observed adverse effect concentration. Highest tested concentration that does not show any adverse effect on the organism
- NOAEL: No observed adverse effect level. Highest tested dose level that does not show any harmful effect on the organism

Several of the 'Effects Values' relate to very high rates of the insecticide compared to the rate applicable to almonds (e.g. 750 g/ha = 7.6 x almond rate), so the impacts observed in those trials are unlikely to be seen in almonds.

Negative impacts were observed on honeybees, but the recommended application time in almonds (1-5% hull split) would obviously avoid the period of intense bee activity around bloom. An application targeting the spring emergence of carob moth (which typically starts in week 1-2 of September) should also pose little threat to bees because the application itself should occur during the peak of moth activity in October.

Impacts that could be of concern because of their effects at rates less than or similar to those relevant to almonds, include the *Chrysoperla* (green lacewings), *Coccinella* (lady birds), *Episyrphus* (=*Euplotes*) *balteatus* (hoverfly), *Orius* spp (minute pirate bugs) and *Typhlodromus* pyri (predatory mite). As mentioned above, the potential for negative impacts of chlorantraniliprole on these beneficial groups indicates the desirability of monitoring for such impacts in almond orchards where the insecticide is used routinely.

Table 21. Effect of chlorantraniliprole on beneficial invertebrates : Internal DuPont studies (Anon 2008)

Test Material Identification	Nature of Tested Material	Registrant Study ID	Test Species	Test Type	Endpoint Type	Effects Value Based on A.S.	Units of Active Substance
Chlorantraniliprole 20SC	Formulated Product	DuPont-18423	*Aphidius rhopalosiphi*	Mortality and reproduction	LR 50 and ER 50	>750	g. chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-12405	*Aphidius rhopalosiphi*	Mortality and reproduction	LR 50 and ER 50	>750	g. chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-12753	*Apis mellifera (Honeybee)*	Semi-field	NOEC	156.16	g. chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-14387	*Apis mellifera (Honeybee)*	Acute oral	LD50	>0.119	mg. chlorantraniliprole/bee
Chlorantraniliprole 35WG	Formulated Product	DuPont*-14387	*Apis mellifera (Honeybee)*	Acute contact	LD50	>0.100	mg. chlorantraniliprole/bee
Chlorantraniliprole 20SC	Formulated Product	DuPont-14388	*Apis mellifera (Honeybee)*	semi-field	NOAEC	52.5	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-14706	*Apis mellifera (Honeybee)*	semi-field	NOAEC	52.5	g. chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-16269	*Apis mellifera (Honeybee)*	Acute	Mortality <4%	112.5	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-16271	*Apis mellifera (Honeybee)*	semi-field	NOAEC	>60	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-16272	*Apis mellifera (Honeybee)*	semi-field	NOAEC	>60	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-17208	*Apis mellifera (Honeybee)*	semi-field	NOAEC	60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-17247	*Apis mellifera (Honeybee)*	semi-field	LOAEC (mortality and decreased flight intensity)	>60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-17248	*Apis mellifera (Honeybee)*	semi-field	LOAEC (mortality and decreased flight intensity)	60	g. chlorantraniliprole/ha
Chlorantraniliprole Technical	Technical	DuPont-17582	*Apis mellifera (Honeybee)*	Acute oral	LD 50	>0.0274 >104.1	µg/bee in water µg/bee in acetone chlorantraniliprole/bee
Chlorantraniliprole 20SC	Formulated Product	DuPont-18085	*Apis mellifera (Honeybee)*	semi-field	NOAEC	60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-18086	*Apis mellifera (Honeybee)*	semi-field	LOAEC (mortality and decreased flight intensity)	60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-18087	*Apis mellifera (Honeybee)*	semi-field	NOAEC	60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-18426	*Apis mellifera (Honeybee)*	Acute oral	LD 50	>114.1	µg. chlorantraniliprole/bee
Chlorantraniliprole 20SC	Formulated Product	DuPont-17301	*Chrysoperla carnea (Green lacewing) larvae*	Mortality Reproduction	EC50 LOEC	120 120	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-19746	*Coccinella septempunctata (Lady bird beetle)*	Mortality Reproduction	LOAEC LOAEC	60 60	g. chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-17300	*Coccinella septempunctata (Lady bird beetle) larvae*	Mortality Reproduction	EC50 LOEC	<120 120	g. chlorantraniliprole/ha
Chlorantraniliprole	Technical	DuPont-14398	*Eisenia fetida (Earthworm)*	Acute	LC 50	>1000	mg chlorantraniliprole /kg soil dry weight.
IN-EQW78	Technical metabolite	DuPont-15389	*Eisenia fetida (Earthworm)*	Acute	LC 50	>1000	mg IN-EQW78/kg soil dry weight.
Chlorantraniliprole 35WG	Formulated Product	DuPont-16694	*Eisenia fetida (Earthworm*	Reproduction Growth	NOAEC	350	mg chlorantraniliprole /kg soil dry weight.

Table 21 Cont.

Test Material Identification	Nature of Tested Material	Registrant Study ID	Test Species	Test Type	Endpoint Type	Effects Value Based on A.S.	Units of Active Substance
IN-EQW78	Technical metabolite	DuPont-17093	*Eisenia fetida (Earthworm*	Reproduction Growth	NOAEC	1000	mg IN-EQW78/kg soil dry weight.
IN-F6L99	Technical metabolite	DuPont-17631	*Eisenia fetida (Earthworm)*	Acute	LC 50	632.5	mg IN-F6L99/kg soil dry weight.
IN-ECD73	Technical metabolite	DuPont-17632	*Eisenia fetida (Earthworm)*	Reproduction Growth	NOAEC	1000	mg IN-ECD73/kg artificial soil dry weight
IN-GAZ70	Technical metabolite	DuPont-17633	*Eisenia fetida (Earthworm)*	Reproduction Growth	NOAEC	1000	mg IN-GAZ70/kg soil dry weight
Chlorantraniliprole 35WG	Formulated Product	DuPont-18817	*Eisenia fetida (Earthworm)*	Acute	LC 50	>350	mg chlorantraniliprole/kg drysoil
Chlorantraniliprole 20SC	Formulated Product	DuPont-18818	*Eisenia fetida (Earthworm)*	Acute	LC 50	>200	mg chlorantraniliprole/kg drysoil
Chlorantraniliprole 20SC	Formulated Product	DuPont-16532	*Episyrphus balteatus (Hoverfly)*	Mortality	LR100	120	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-18082	*Episyrphus balteatus (Hoverfly)*	Mortality Reproduction	LR 50 ER 50	12.6 13.3	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-19747	*Episyrphus balteatus (Hoverfly)*	Mortality 1st treatment Mortality 2nd treatment Reproduction	<control >control NOAEL	60 60 60	G chlorantraniliprole/ha twice with 7-day interval
Chlorantraniliprole 35WG	Formulated Product	DuPont-18084	*Episyrphus balteatus (Hoverfly)*	Mortality Reproduction	LR50 ER 50	4.64 >4.4	g chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-20303	*Episyrphus balteatus (Hoverfly)*	Mortality 1st treatment Mortality 2nd treatment Reproduction	<control <control NOAEL	60 60 60	g chlorantraniliprole/ha twice with 7-day interval
IN-EQW78	Technical metabolite	DuPont-16531	*Folsomia candida (Springtail)*	Reproduction	EC 50 NOEC	>100 100	mg IN-EQW78/kg dry soil
IN-ECD73	Technical metabolite	DuPont-17083	*Folsomia candida (Springtail)*	Reproduction	EC 50 NOEC	>100 100	mg IN-ECD73/kg dry soil
Chlorantraniliprole	Technical	DuPont-18730	*Folsomia candida (Springtail)*	Reproduction	EC 50 NOEC	0.48 0.39	mg chlorantraniliprole /kg dry soil
Chlorantraniliprole	Technical	DuPont-19748	*Hypoaspis aculeifer (mite)*	Reproduction	NOAEC	100	mg chlorantraniliprole /kg dry soil
Chlorantraniliprole 20SC	Formulated Product	DuPont-18081 RV1	*Orius laevigatus*	Mortality and reproduction	LR 50 & ER 50	>120	g chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-12406	*Typhlodromus pyri*	Mortality and reproduction	LR 50 and ER 50	>750	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-14704	*Typhlodromus pyri*	Mortality and reproduction	LR 50 ER 50	>750	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-17312	*Typhlodromus pyri*	Population study	NOAEC	750	g chlorantraniliprole/ha
Chlorantraniliprole 35WG	Formulated Product	DuPont-14705	*Typhlodromus pyri*	Population reduction (transient)	LOAEC	52.5	g chlorantraniliprole/ha
Chlorantraniliprole 20SC	Formulated Product	DuPont-18424	*Typhlodromus pyri*	Mortality and reproduction	LR 50 and ER 50	>750	g chlorantraniliprole/ha

Note: The author apologises for the quality of this table. It is reproduced here as originally published to avoid any chance of transcription errors.

Conclusions

Overall, chlorantraniliprole had relatively low impact on beneficial invertebrates, as observed in the published and unpublished studies summarised above. There are however some impacts that may have potential to disturb the balance between pest and beneficial species in almond orchards and compromise naturally occurring biological control systems. It would be prudent therefore to monitor for such impacts.

Regardless of the apparent safety of insecticides, it is almost always preferable to minimise the input of biocide chemistry into production systems because of the risks, including long-term, of known as well as unforseen negative impacts. For this reason, while keeping chlorantraniliprole as a useful tool, alternative options for carob moth management need to be pursued, such as mummy reduction which provides multiple benefits for pest and disease management in almonds.

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DRAFT PAPER FOR PUBLICATION

Active radius and optimum number of pheromone traps for reliable monitoring of carob moth populations

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Introduction

Volatile attractants create an active space around a source point. The active space is the area in which the semiochemical concentration is above a behavioural threshold, thereby eliciting orientation by a target insect towards the source point (Baker and Roelofs 1981, Baker et al. 1985, Byers 2009). The active space of a trap that relies on a plume of volatiles for attraction is represented in two dimensions as the area of a circle centred on the trap. Any overlap of the active spaces indicates competition between the traps. The point at which two traps cease interfering with each other is the point at which the circumferences of the two circles meet but do not overlap. The number of traps/ha required to reliably monitor a moth population in an orchard depends on the active space of the trap, the behaviour of the target moth species and spatial distribution of the moth population in the orchard.
The aims of the work reported here are to determine the optimal spatial density of traps required to reliably monitor a carob moth population, and to estimate the active space of traps in orchards.

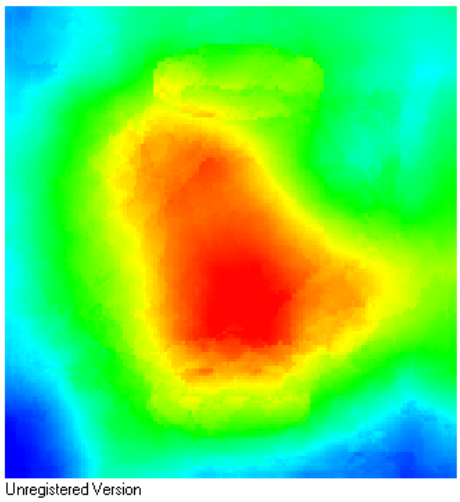
Materials and methods

A block of almonds in an orchard at Lake Cullulleraine in North Western Victoria, Australia with a history of infestation by carob moth was selected as the study site. Tree rows were 7.2 m apart and trees within the rows were spaced approximately 5.5 m apart. The experimental plot was approximately 2.33 ha in size. One delta trap baited with a one–week old carob moth lure (ISCAlure-Ceratoniae™ , ISCA Technologies Inc., Riverside, California USA) was placed 1.5-2.0 m above ground on a branch in the outer edge of the tree canopy every 14.4 m in every 2nd row until 10 traps had been placed in each of 10 trapped rows. This resulted in a grid of 100 traps in which each trap was approximately 14.4 m away from its nearest neighbour either across or within rows. The location of all traps was recorded as GPS coordinates in UTM format. The trapping period commenced 20/03/2015 and finished 24/04/2015. Lures in the traps were not refreshed during this time. Traps were inspected each week for presence of moths.
The number of moths captured in each trap and the GPS referenced location of each trap, was used in the geostatistical software Vesper 1.6 (Australian Centre for Precision Agriculture, The University of Sydney, NSW, Australia) to produce a spatial prediction of the carob moth population density in the orchard at each sampling date. There were high numbers of zero catches due to spatial variation in population density, so the data were log(x+1) transformed before fitting a variogram, using a linear-with-sill model, to the spatial data for moth catches using 50 lags, 50% lag tolerance, 100m maximum distance , with number of pairs at each lag used as weights . The point of inflexion, or the start of a sill, in the variogram indicates the distance at which traps cease to influence each other. If traps are identical then the distance indicated by the point of inflexion is twice the radius of the active space.
To determine an optimal sample size (number of traps) that may reasonably adequately represent the average catch in the population of 100 traps, we generated 200 random samples each of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 traps using simple random sampling without replacement using GenStat statistical software. The arithmetic mean of each of these 200 random samples was then computed. The distribution of these 200 mean values for different sample sizes was summarized using box plots for total catch.

Results and Discussion

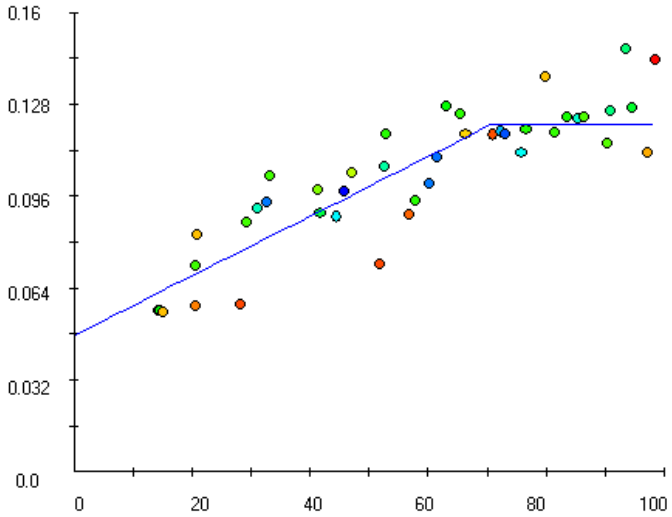
The predicted spatial distribution for log10(x+1) transformed total catch (Figure 1) indicates considerable variation in the moth population distribution in the orchard block. Moth numbers were highest around the edges (deep blue) and lowest in the middle (red).

Figure 1: Predicted log (x+1) moth numbers determined by kriging based on the total catch in each trap. Numbers are highest in dark blue areas and gradation from high to low follows the sequence dark blue-blue-green-yellow-red.



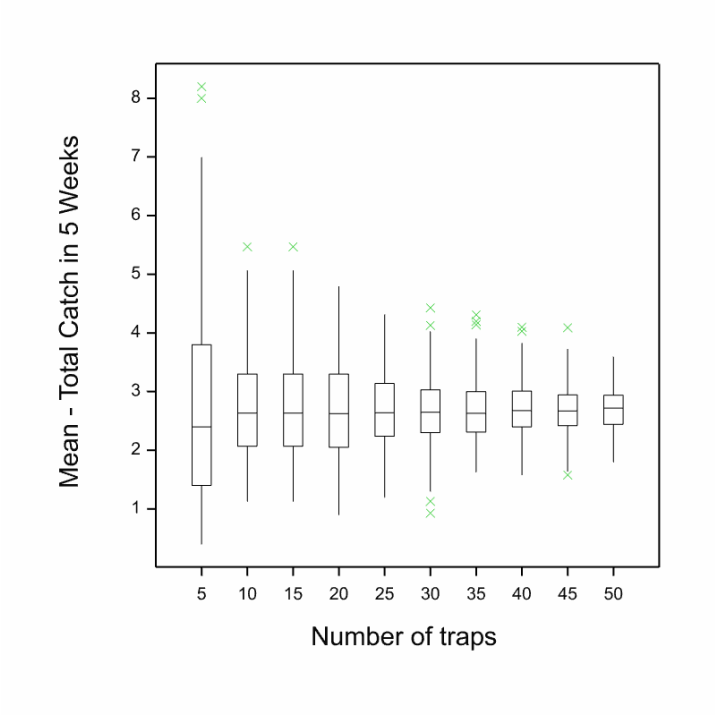
The inflexion point in the linear-with-sill variogram is the start of the sill on the fitted variogram model (Figure 2) and indicates a separation distance of approximately 70 m and therefore an active radius of about 35 m. This suggests that a single trap may be adequate to monitor an area of 3848 m2 and therefore 2.6 traps/ha could be adequate to monitor the moth population.

Figure 2: Variogram generated from log10(x+1) transformation of total catch in each trap. Horizontal axis is distance in metres. Colours assigned to dots indicates number of data points, with dark blue being the highest number and red being the lowest number of points. The fitted model was linear with sill.



Examination of the box plots of means resulting from the random sampling based simulation (Figure 3) suggests that 12.9 traps/ha (30 traps/2.33 ha = 12.9 traps/ha) may be optimal for estimating population means. This could be interpreted as meaning that the active space is 775 m2 (10000 m2/12.9 traps) and the active radius is therefore 15.7 m (√(775/pi) which is just under half that calculated from the variogram. The discrepancy between the results of the two methods may highlight the danger of relying on spatial predictions based on low densities of highly aggregated, as opposed to randomly distributed, populations to determine active space. On the other hand, use of random sampling of traps without providing some form of limitation regarding nearest neighbours may also bias results towards higher numbers of closer (i.e. auto-correlated or spatially dependent) traps. This would have the effect of ignoring overlap of active spaces and therefore over-estimating the number of traps required per unit area. Further inspection of Figure 3 reveals that the median value is similar for all sample sizes of 10 or more, and that the estimates of the means generally are within ± 1 moth/trap of each other. For all practical pest management purposes this would be acceptable accuracy.

Figure 3: Box plots of mean total catch derived from random sampling without replacement, of 5-50 traps. Each box plot depicts the sampling distribution of 200 means for each sample size.



Conclusions

A density of 12.9 traps/ha is likely to be too expensive for regular monitoring programs but may be necessary to map the initial infestation hotspots within an orchard. Once hotspots have been identified it may be feasible and cost-effective to monitor those hotspots with a lower trapping density but care would be required to ensure that processes are in place to enable detection of new hotspots developing in areas previously considered to have low pest prevalence. In the real world four evenly distributed traps/ha (based on 10 traps/2.33 ha= 4.35 traps/ha rounded to 4 traps/ha) would probably be sufficient to both detect presence of, but not delimit, hotpots and to estimate the mean population represented by numbers of moths caught in traps, in order to develop action thresholds.

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Investigating potential female attractants.

Aim

To screen potential attractants for female carob moth and identify candidates for further investigation.

Introduction

The carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae* is a widespread pest of numerous fruit and nut crops globally and has caused significant levels of kernel damage in some major Australian almond producing districts since the 2011 harvest. In almond orchards, carob moths survive over winter mostly as slowly developing larvae in mummy nuts (nuts remaining on trees after harvest). In early spring they complete their development, pupate and start to emerge as adult moths. From that time until hull split in the current season crop, oviposition, larval and pupal development in carob moth is restricted to mummy nuts. From the start of hull split onwards (typically early January), oviposition and subsequent development of carob moth occurs in both mummies and current season nuts. Kernel damage in the new crop occurs when carob moth larvae burrow into and feed on the kernel.

Effective management of carob moth requires reliable monitoring systems to allow producers to determine the need for, optimum timing of, and impact of applied control measures. Currently a sex pheromone mimic is available for use in traps to monitor the activity of male carob moth (ISCA Technologies 2014), but no effective female lure appears to have been developed or made available for commercial use. A lure for females would be a valuable tool for researchers and producers alike, as it would assist the development of economic thresholds for carob moth and would allow the monitoring of the real risk to current season almond crops – female moths and their oviposition activity.

Females and males of many insect species, especially beetles, can be attracted by aggregation pheromones, and the aggregation of numerous individuals can serve various purposes such as to enhance the chance of mating or provide protection from predators. In contrast, most pheromone communication in lepidopteran species, including moths, involves only the females producing sex pheromones to attract males for mating. Attractants for female carob moths will therefore be most likely associated with the females' search for suitable egg-laying sites, which in turn relates to suitable food resources for the larvae once they hatch.

This aspect of project AL12004 sought to carry out preliminary screening of a range of potential attractants (baits) for female carob moths to identify candidates for more detailed future investigation with a longer-term view to developing a female lure.

Materials and methods

The trials reported here were conducted in the laboratory using culture-reared moths and in an almond orchard located in the Sunraysia region of Victoria, Australia.

2012/13 Glasshouse experiment

Trial design

Candidate bait materials were tested individually for their attractiveness for oviposition by female carob moth in a three way choice situation. In each trial, the test bait was compared with a blank 'control' and a 'standard' bait of kernels from almond mummies that had been infested with carob moth. Mummy kernels were chosen as the standard as they are known to attract oviposition by carob moth, and to be useful in an orchard situation any new bait would need to outcompete mummies in attractiveness.

The trials were located in a glasshouse maintained at 25 °C and under natural light conditions and were performed in a 0.6 x 0.6 x 1.6 m high insect cage. The cage was regularly stocked with newly emerged carob moths of both sexes, sourced from a culture maintained by this project. Because the moth population in the cage varied over time, comparisons of relative attractiveness of baits can only be made between the blank, almond standard and test bait within each trial, not between test baits from different trials.

In these trials, navel orangeworm (NOW) traps were used to hold the bait materials and provide a suitable substrate for oviposition. These traps were developed specifically for that purpose, for use in monitoring oviposition by NOW (*Amyelois transitella*), a close relative of carob moth, in Californian almond orchards (Kuenen et al. 2008). The NOW trap consists of an 8 x 4 cm diameter black plastic tube with three 2.5 cm

diameter holes covered with fine plastic mesh. The outer surface of the tube is finely ridged and provides a surface that the moths find attractive for laying eggs. One end of the trap is sealed and the other has a cap which can be removed to access the bait.

For each trial, three traps were hung at the same height in the cage. One was left empty as a blank control, one was filled with mummy kernels and one was filled with the test bait. Every 2-3 days the traps were inspected using a dissecting microscope and the number of eggs on each trap was recorded. The eggs were then removed and the traps returned to the cage. Newly laid, white eggs and maturing pink eggs were recorded separately. Each bait was tested for at least ten days.

Baits

The bait materials tested were old pistachio kernel, pomegranate fruit, fresh dates and anthracnose-infected avocado fruit. The rationales for testing these materials were:

- pistachio, pomegranate & dates: During the review of literature for the project, numerous references were found to these nuts and fruits being attractive to female carob moths (e.g. Mehrnejad 1993, Mirkarimi 2002, Nay 2006).
- avocado with anthracnose: Carob moth has been reported to be attracted to fruit and other food substrates infected with fungus (Cosse et al. 1994, Levinson & Gothilf 1965) and to oviposit on unsplit almonds if the nuts are infected with anthracnose (Gothilf 1984). It is possible that the female moth is attracted by volatile compounds released as a result of fungal infections. Anthracnose on both avocados and almonds is caused by two species of the fungus *Colletotrichum* (*C. gloeosporioides* and *C. acutatum*) (Freeman et al. 1998). Although different strains of these species may infect avocado and almond differentially, it was considered worth testing anthracnose-infected avocado because it was readily available and could be easily cultured if necessary.

2014 Glasshouse experiment

Trial design

The 2014 trials used the same equipment and procedure as in 2013 apart from the bait receptacles and the duration of each trial.

Due to the inconvenience of cleaning carob moth eggs from the NOW traps during the 2012/13 trials, different bait receptacles were used in 2014. These were white confectioners paper bags, measuring 19 cm x 13.5 cm. To aid the counting of eggs laid on the bags, the bags were printed with a 2 cm square grid. Before adding bait material, the bags were folded tightly then unfolded. The resulting creases provided desirable areas for oviposition.

As in 2013, for each trial three bags were hung at the same height in the moth cage. One was left empty as a blank control, one contained mummy kernels that had been infested with carob moth and one contained the test bait. The bags were removed after 3-4 days, inspected for eggs and the number of eggs recorded. During two of the trials, the bags were duplicated. i.e. two bags of each treatment (blank, mummy kernel, test bait) were hung in the cage.

Baits

The bait materials tested included clean almond kernel, reject almond kernel, fresh soy meal diet (Gothilf 1968), used soy meal diet from the carob moth culture, fresh pomegranate skin and old carob pods. ‘Reject almonds’ were obtained from an almond processing plant and were stained and mouldy but not necessarily damaged by carob moth. ‘Used soy meal diet’ was diet that had been used to culture a generation of carob moth larvae, so it was contaminated with frass, pupal cases, webbing and other traces of carob moth. All these bait materials were tested on their own and combined with albumen (egg white). In tests using pomegranate mixed with a range of substances, a pomegranate+albumen mix was reported as being the most attractive to female carob moth (Mansour 1984). Mummy kernels combined with albumen were also tested against the ‘standard’ mummy kernel.

Other bait materials tested were mouldy avocado and banana skin. Ripe banana skin was included as it is a rich source of ethylene and it was considered possible that the attractiveness of splitting almonds to carob moth females could be related to ethylene emissions during the ripening/splitting process.

When it appeared that used soy meal diet was attractive as a bait, a sample was sorted as much as possible into three components – carob moth webbing, pupal cases and diet media without obvious traces of webbing,

moths or pupal cases. These components were then compared in a five-way choice test against each other, homogenised unsorted used diet and a blank control.

Fresh and used soy meal diet were also compared against each other in a three way choice test with a blank control, and a four way choice test with a blank control and the mummy standard.

2014/15 Field tests

Two small field tests were conducted to assess the attractiveness of used soy meal diet to female carob moth in an almond orchard.

The first test was conducted in an area of the orchard that had moderate numbers of mummy nuts and a previous history of carob moth. In this test, four different trap types were used:

- Egg traps - Small paper bags containing used soy meal diet as described for the glasshouse trials.
- Male trap – White plastic delta trap containing a sticky base and carob moth pheromone lure.
- Female trap - White plastic delta trap containing a sticky base and a vented 75 ml plastic specimen vial of used soy meal diet. The vial was attached to the top inside of the trap.
- Blank trap - White plastic delta trap containing a sticky base with no attractant.

Two traps each of the male, female and blank traps, and four egg traps were placed 1.5-2 m high in trees in random order along a single tree row, so that there was one trap in every second tree. The egg and female traps were installed on 30 Oct 2014 and the male and blank traps on 6 Nov 2014.

The delta traps were inspected weekly until 24 Apr 2015. Moths caught in the male and female traps were examined to determine that only the targeted sex was being attracted to each trap type. The egg traps were replaced each week, and the used traps were examined under a dissector microscope to determine the number of eggs present. Egg traps were only included in the test for four weeks.

The second test was located in an area of the orchard that had a larger mummy population. For this test, two male traps and two female traps as described above were installed in separate trees in the same row and monitored from 17 Dec 2014 to 10 Mar 2015.

2015 Plant volatiles sampling

Because carob moths start oviposition into healthy current season almonds only at hull split, it is possible if not likely that they are attracted by volatile compounds emitted by the maturing and splitting nuts. If such compounds could be identified, they may be useful in the development of a female lure. A similar approach was in fact used in the development of the co-attractant currently used in monitoring and 'attract and kill' traps for the carpophilus beetle (Bartelt & Hossain 2006).

To begin investigating this possibility, air samples were collected from immature green almonds on trees, prior to hull split when the nuts have no attraction for carob moth oviposition, and during hull split when the nuts become attractive for oviposition. If chemical analyses detect marked differences between the two sets of samples, those differences may indicate the key compounds involved in attracting oviposition.

Air samples were collected using a positive pressure system in which purified air was pumped into a polyester oven bag that was sealed around an almond nut still attached to the tree (Figure 53). An outlet in the bag contained a glass tube filled with a porous polymer adsorbent matrix (Tenax®) which is specially formulated to adsorb volatile compounds present in the air.

Prior to hull split, air samples were collected from the same five nuts on three consecutive nights, from 16-18 Dec 2014. The pump system operated for five hours each night, starting at dusk. This time was chosen for the collection of samples as it is the main period for oviposition by carob moth (Vetter et al. 1997; Hung et al. 2003). For each air sample from a nut, a blank air sample was also collected, using the same setup but with no nut enclosed in the bag. On the morning following each sampling, the equipment was returned to the laboratory and the sample tubes were labelled, wrapped in aluminium foil, sealed in plastic bags and stored at -20°C. A total of 15 pre hull split samples from nuts and 15 blank samples were collected.

Post hull split air samples were collected from five split nuts and five blank bags over 6-8 Jan 2015 using the same procedure as above.

The same technique was used in the laboratory to collect air samples from fresh and used soy meal diet, in an attempt to identify the compounds responsible for the difference between those two materials in their attraction for oviposition by carob moth.

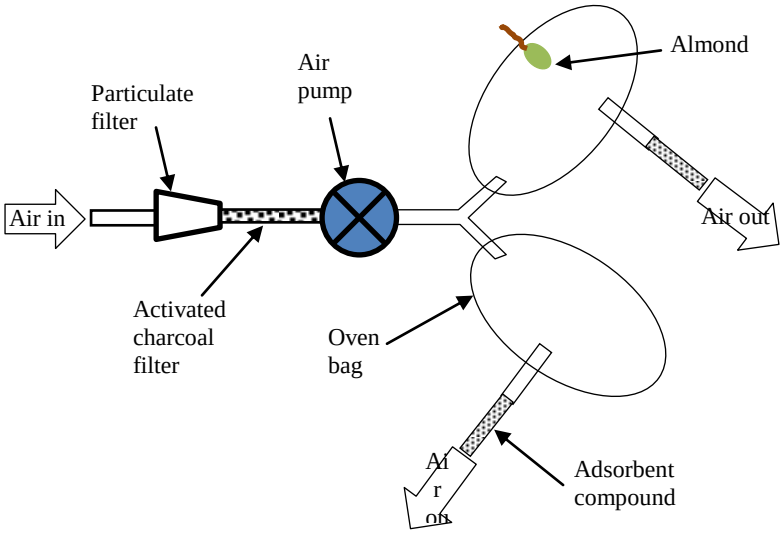

Figure 53. Positive pressure system for collection of air samples.

Data management & presentation

For the testing of bait materials, counts of eggs on traps or bags containing test bait or mummy kernel were adjusted to take account of the level of 'background' or 'chance' oviposition as measured by the number of eggs on blank traps. This was done within each trial, by simply subtracting the number of eggs on the blank trap from the egg count of each of the other traps in that trial.

The percent relative attractiveness of each test bait compared to the mummy standard was then calculated as 100 x (adjusted number of eggs on test bait trap)/(adjusted number of eggs on mummy trap). A relative attractiveness greater than 100% indicates that the test bait was more attractive than mummy kernel.

Results & discussion

2012/13 Glasshouse experiment

As can be seen in Table 22, none of the four test baits performed as well as mummy kernels in terms of attracting oviposition by carob moth.

The NOW traps functioned well in terms of holding the baits and providing suitable oviposition sites, but they were inconvenient to clean as eggs were laid in and through the plastic mesh.

Table 22. Relative attractiveness of test baits compared to mummy kernel, 2012/13.

Test bait	Relative attractiveness
Old pistachio kernel	61%
Mouldy avocado	76%
Pomegranate	4%
Fresh date	17%

2014/15 Glasshouse experiment

Of the 15 bait materials tested, only used soy meal diet outperformed the mummy kernel 'standard' in attracting oviposition by carob moth (Figure 54). The used diet attracted more oviposition in five of seven trials and had an overall attractiveness of 134% of that of mummy kernel. Fresh diet had a relative attractiveness of only 74%, suggesting that the past activity of carob moth in the diet increases its attractiveness as an oviposition site.

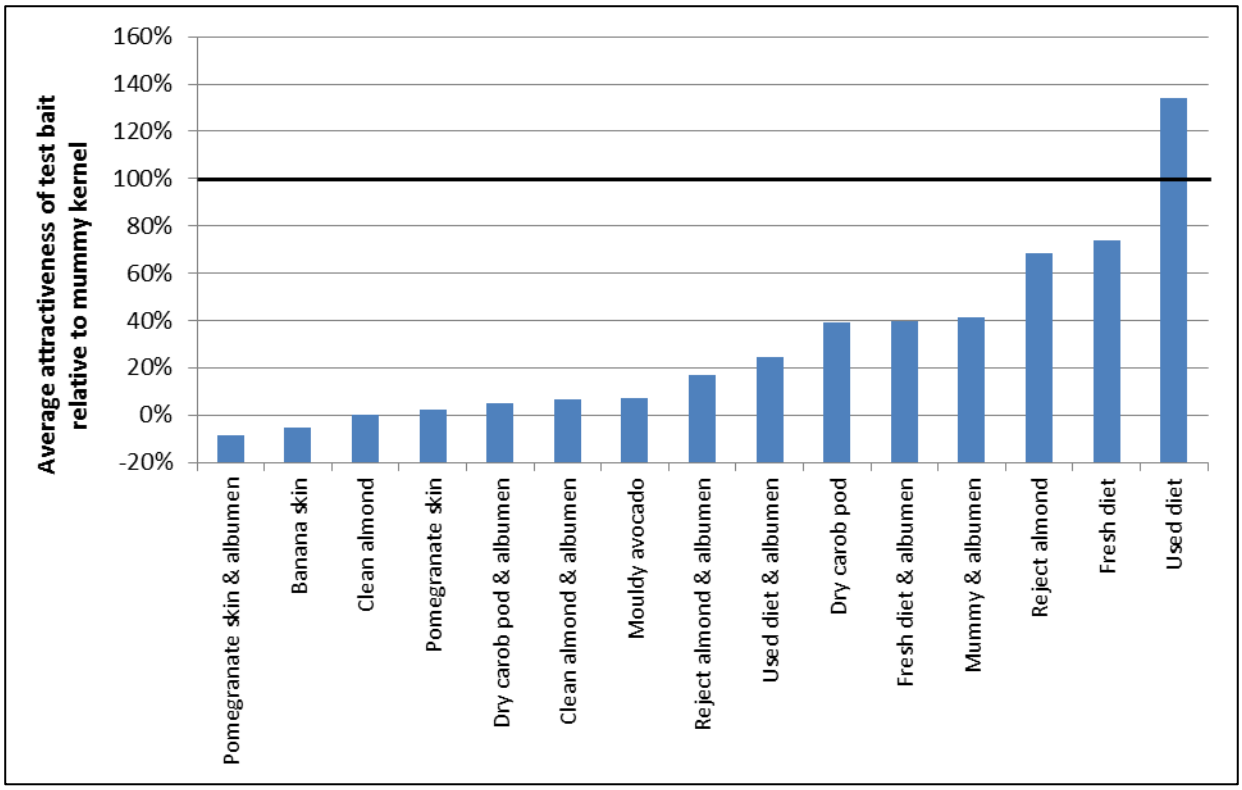

Figure 54. Relative attractiveness of test baits compared to mummy kernel, 2014/15.

The fact that the relative attractiveness of used diet compared to mummy kernel varied widely between trials (54%-1100%) suggests that variability in the 'quality' of that material, such as moisture content or degree of contamination with carob moth frass, webbing, mould and so on is likely to be important in relation to attracting oviposition by carob moth.

This most likely applies also to mummy and reject almond kernels. In a trial involving two bags each of reject kernels, mummy kernels and blanks, the number of eggs laid on the two mummy bags varied more than three-fold. These results indicate that there is scope for identifying key differences between more and less attractive bait materials, which may lead to the identification of specific chemical factors to be assessed as female attractants.

When a sample of used soy diet was separated into its main components, the diet medium itself and carob moth webbing were both of similar attractiveness as homogenised whole used diet, while pupal cases alone appeared less attractive (Table 23).

Table 23. Relative attractiveness of diet components compared to homogenised whole used diet, 2015.

Used diet medium	108%
Pupal cases	78%
Webbing	97%

In the single trial comparing used diet and fresh diet, used diet was four times more attractive than fresh. Another single trial comparing used diet, fresh diet and mummy kernel found used diet to be just over five times more attractive than fresh, but only 27% as attractive as mummy kernel. This highlights again the effect of variability in some key quality of the used diet.

Throughout the experiment, the bags containing test baits attracted more oviposition than the empty 'blank' bags, with two exceptions. These were the bags containing pomegranate skin with albumen, and banana skin, both of which appeared to have a slight repellent effect in relation to oviposition by carob moth.

The average percentage of eggs laid on empty 'blank' bags over the 27 individual tests was 8.5% with a range of 0-18%. The higher proportions of oviposition on blanks tended to occur where the test baits were least attractive.

2014/15 Field tests

Because the inspection interval (weekly) allowed most eggs to hatch, and the clear empty egg cases were difficult to observe on the white bags, the paper bag egg traps were removed from the trial after one month. During that period, a maximum of 19 and average of 3.3 eggs were laid on each trap each week.

Inspections of moths caught in the delta traps found no females in pheromone lure traps and no males in used diet traps. The trapped females were often easily sexed as they tended to release large numbers of eggs onto the sticky base of the traps. Over a 24 week trapping period, only two moths in total were found in blank traps.

In the first 23 weeks of trapping in the area of orchard with a moderate mummy population density, both pheromone and diet traps had weekly catches in the range of 0-8 moths. The overall rate of accumulation of moth catches in diet traps was however only 60% of that in pheromone traps (Figure 55), but given the crude nature of the diet traps, this result is quite promising.

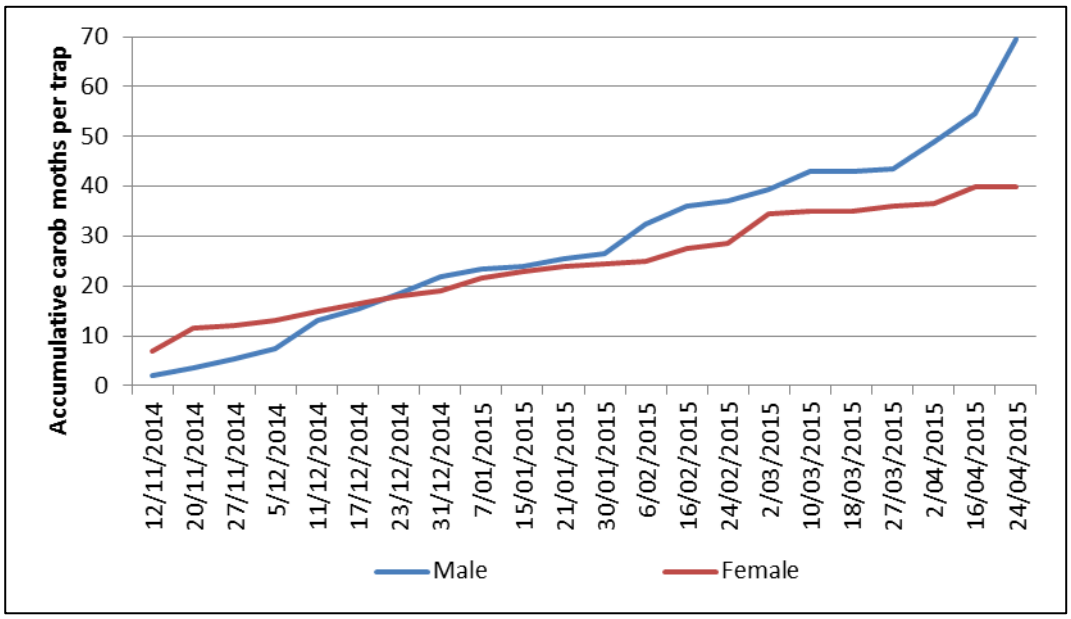

Figure 55. Accumulative catches of carob moth in pheromone (male) and used soy diet (female) traps in an area of moderate mummy population density.

In the last week of trapping, numbers of males increased significantly to an average of 15 per trap (Figure 56). This increase was in line with the expected start of a fourth generation of carob moth. The numbers of females trapped did not increase at the same time, but this is not surprising given the lack of synchrony in peak catches of the twos sexes as seen in Figure 56. Over the 24 week period, traps baited with used soy diet caught an average of 1.7 female moths per week compared to 2.9 males in pheromone lure traps.

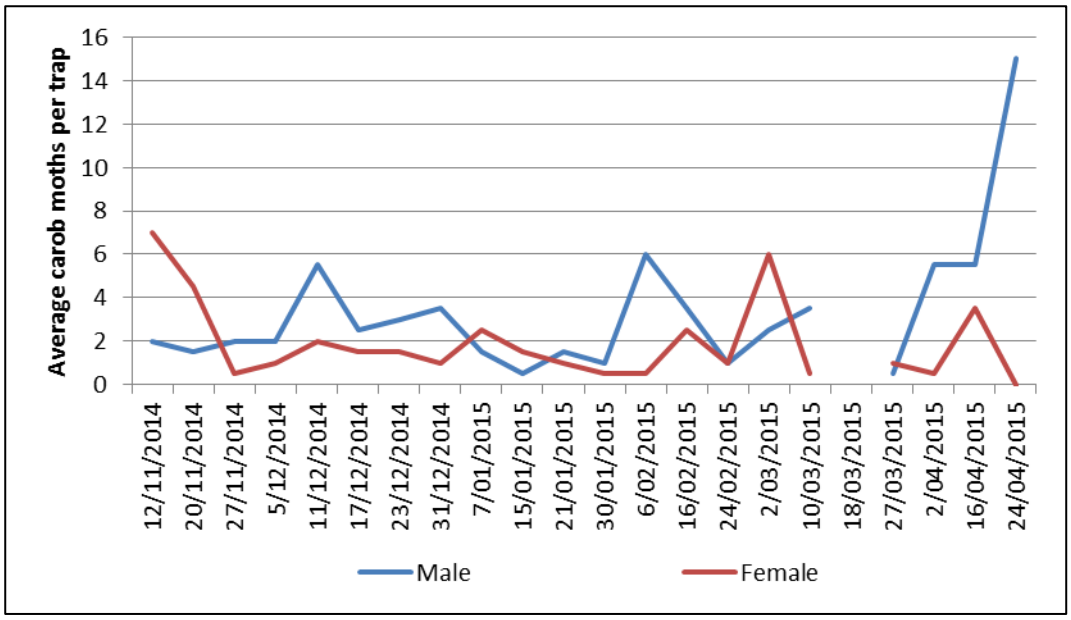

Figure 56. Average catches of carob moth in pheromone (male) and used soy diet (female) traps in an area of moderate mummy population density.

Where mummy populations were greater, the used diet traps caught females at a similar rate as above (average 1.5 per week) while catches of males in pheromone traps were considerably higher at 6.8 per week (Figure 57).

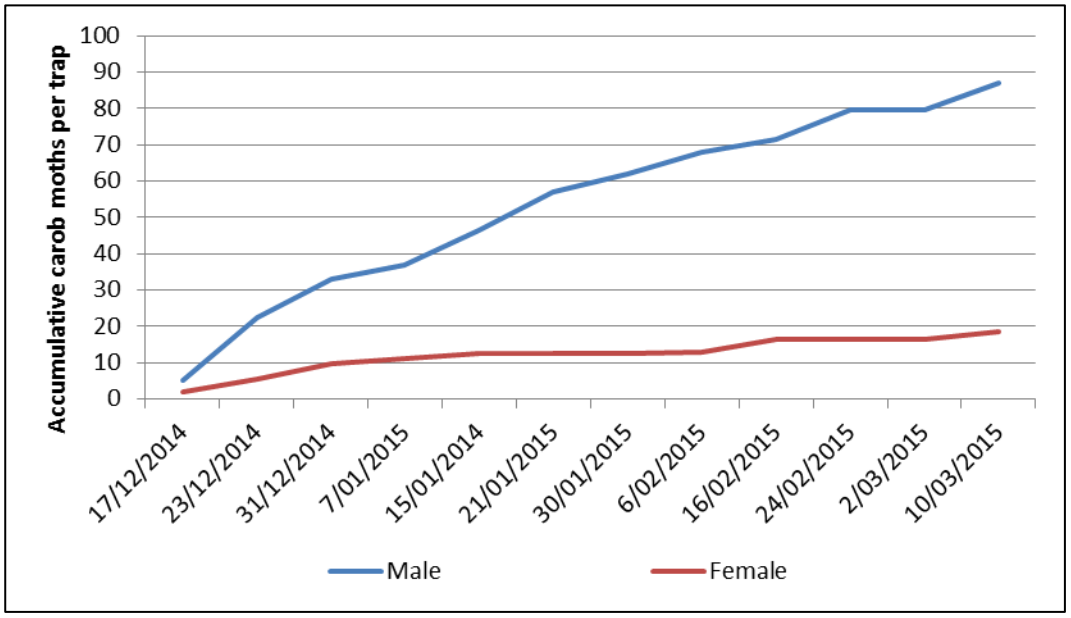

Figure 57. Accumulative catches of carob moth in pheromone (male) and used soy diet (female) traps in an area of high mummy population.

Whether the relatively poorer performance of the diet trap compared to pheromone trap in this location was due to greater competition from mummies or some characteristic of the diet lure itself is a point for further investigation. Also, it is worth noting that the ability of a female lure to compete with high mummy population densities may not be so relevant if the almond industry continues its drive to reduce mummy nuts as an aspect of overall orchard pest and disease management.

2015 Plant volatiles

At the time of writing this report, the samples of volatiles collected from pre and post-hull split almonds and from fresh and used soy diet had not been analysed.

Conclusions

In glasshouse tests of the level of attraction that a range of food materials had for oviposition by carob moth, only one material, used soy meal diet from a carob moth culture, scored more highly than almond mummy kernels.

The variability in relative attractiveness of batches of the diet compared to mummy kernels suggests some scope for identifying the key factors involved in the diet's attractiveness for oviposition.

Preliminary field tests of the diet as a lure in delta traps produced some promising results but this work would need to be followed up by identification and analysis of the attractive factors in the diet to determine the likelihood that they could be developed into an effective lure for female carob moth.

This preliminary work screened a limited range of materials. Because of the potential value of an effective lure for female carob moth, further screening of a wider range of materials is warranted, to pick candidates for more detailed chemical identification and analysis.

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Carob moth laboratory culture

Aim

To provide a source of carob moths for use in laboratory and field trials.

Introduction

HIA Project Al12004 was commissioned to investigate the behaviour and management options for carob moth, *Apomyelois* (=*Ectomyelois*) *ceratoniae*, a widespread pest of numerous fruit and nut crops globally, that has caused significant levels of kernel damage in some major Australian almond producing districts in recent years.

During the project, the need arose for a reliable supply of carob moths for use in laboratory assessments of potential attractants, and in field trials in which virgin female moths were used as 'lures' for male moths. To address this need, a laboratory culture of carob moth was established at the DEDJTR Irymple site, using a simple diet recipe and culture procedure as reported below.

Materials and methods

Source of carob moths

To initiate the culture, we collected almond mummy nuts from orchards in the Sunraysia region of north-west Victoria. In some orchards, up to 50% of mummy nuts are infested with carob moth, so they provided a ready source of the pest. We stored the nuts in 8 x 30 x 40 cm plastic trays on the floor of a 0.6 x 0.6 x 1.6 m 'flight cage' in a glasshouse cell that was maintained at 25°C.

Once the culture was operating, we occasionally introduced additional moths reared from infested mummy nuts from field collections to ensure the maintenance of some genetic diversity within the culture.

Mating, oviposition and egg collection

Moths emerging from the field-collected mummy nuts were free to fly and mate within the flight cage. Fresh eggs were originally collected by caging individual moths from the flight cage under 75 ml plastic specimen vials on a sheet of finely corrugated paper card. Females that had been mated laid their eggs on the card, and at times on the vial itself. Trays of these moths were kept in a controlled temperature (CT) room at 25°C for one to two days, after which the moths were discarded and the cards with eggs attached were used to 'seed' the larval diet. The CT room lighting was programmed to provide a 16 hour day/8 hour night cycle.

Later in the project, we used the flight cage as a test arena for assessing the attractiveness of various baits to female carob moth (as reported in the previous chapter). During these bait tests, egg laying occurred on white confectioners paper bags, measuring 19 cm x 13.5 cm which were used to hold the bait materials. As each bait test was completed, the bags were used as the source of eggs to seed new batches of the larval diet.

Larval diet

For this culture, we selected the simple but effective larval diet reported by Gothilf (1968). This diet is comprised of 43.5% soy bean meal, 43.5% sucrose and 13% water by weight. The diet is prepared by dissolving the sucrose in the water, then mixing in the soy meal. A bulk lot of diet can be prepared and stored refrigerated until needed.

When required for rearing larvae, we placed an approximately 2-3 cm depth of the diet into 1,000 ml rectangular plastic take-away containers.

Larval rearing

To start a new cohort of larvae, we placed fresh eggs from the cards or paper bags described above, onto the surface of the newly prepared diet in the take-away containers. We then covered the diet and eggs in each container with a sheet of paper as per Gothilf (1968) and placed the lids of the containers on firmly. The containers were stored the CT room as described above.

Pupation

When the carob moth larvae were mature and preparing to pupate, they chewed a hole in the paper covering the soy diet and spun a silken tube from the hole down into the diet. At that point, we removed the plastic covers from the containers and placed the containers into 40 cm cubic Perspex insect cages where the carob moth adults subsequently emerged.

Unless the newly emerged moths were required for a specific trial purpose, we collected them and released them into the glasshouse flight cage to continue the culture cycle.

Results & discussion

The soy meal/sucrose diet and rearing technique described here proved to be a simple and effective method for maintaining a productive culture of carob moth for experimental purposes. Up to 350 eggs were laid on each paper bag in the flight cage, with the actual numbers varying according to the moth population in the cage.

We could choose to use just a few of the eggs collected from the flight cage to operate the culture at a low maintenance level, or use all available eggs to increase production relatively easily when required. At 25°C, the time between seeding a batch of diet and moth emergence was approximately one month. This needed to be taken into account when planning future moth and egg requirements.

The use of field-collected mummy nuts to seed and supplement the culture did have some disadvantages. At some point, the egg parasitoid *Trichogramma carverae* was introduced and its population developed to the extent that a significant proportion of eggs collected from the flight cage were parasitised. This necessitated a complete cleaning and disinfestation of the flight cage and glasshouse cell before the culture could be re-established. Separation of the culture and field material, and transfer of moths only, should avoid this issue.

At one point we also experienced some failure in larval development due to the development of mould in the diet. We believe this may have been due to contamination on the paper bags used to collect eggs in the flight cage, as some of the bait materials used in the bags were themselves affected by fungal growth. We could most probably have avoided this issue by paying greater attention to hygiene and avoiding the use of contaminated bags.

Conclusions

The technique for rearing carob moth as described by Gothilf (1968) and approximated in our culture was a simple way to maintain a relatively reliable and easily scalable supply of carob moth for experimental use.

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Other almond pests of concern

During our assessments of numerous almond nut samples for carob moth infestation, we took note of any other invertebrates that appeared to be causing damage of concern, had specimens formally identified and notified the industry accordingly.

Between October 2011 and February 2015, only one invertebrate species apart from carob moth was found to be causing kernel damage in almonds. Samples of current season nuts collected in the Robinvale district just after harvest 2012 showed a low level of kernel damage that appeared to be caused by a pest other than carob moth, judging by the appearance of the damage. Unfortunately no suitable specimens of the pest causing the damage were available for identification. During assessments of nuts from the same site soon after the 2013 harvest, 7% of current season kernels were damaged by a species of *Carpophilus*, commonly known as the dried fruit beetle. Specimens were collected and provided to the DEDJTR Biosciences taxonomy group who confirmed the pest's identity as *Carpophilus davidsoni*. This 'new' pest of Australian almonds was subsequently flagged as an issue at the ABA Almond R&D Forum, Renmark on 12 Jun 2013.

Once it was apparent that *Carpophilus* was causing kernel damage, some effort was made to gather preliminary information on this pest.

At harvest 2014, while nut samples from a DEDJTR-managed deficit irrigation trial in the Robinvale district were being assessed, records of insect damage to the kernels, including that caused by carpophilus beetle, were kept and provided to the project. Table 24 lists the irrigation treatments of the deficit irrigation trial and Figure 58 shows how the level of carpophilus beetle damage varied between treatments.

As Figure 58 shows, carpophilus damage to kernels was generally much higher in the wetter treatments (W, C, RDI85, SDI85), in contrast with carob moth which tended to cause greater damage in the drier treatments (RDI55, SDI55,SDI70). These results are as could be expected, given:

a) Carpophilus beetles' preference for moist food substrates and breeding conditions.
b) The extended period of exposure of nuts to oviposition by carob moth under the dry treatments, due to earlier hull split in those treatments (refer to the report 'Seasonal phenology and distribution of carob moth in almonds').

Table 24. Irrigation treatments within the deficit irrigation trial.

Irrigation treatment	% of recommended irrigation
C (Control)	100%
W (Wet)	120%
RDI85	85% (50% mid Jan-mid Feb; 100% all other times)
RDI70	70% (50% mid Nov-mid Feb; 100% all other times)
RDI55	55% (50% mid Sep-mid Feb; 100% all other times)
SDI85	85% all season
SDI70	70% all season
SDI55	55% all season

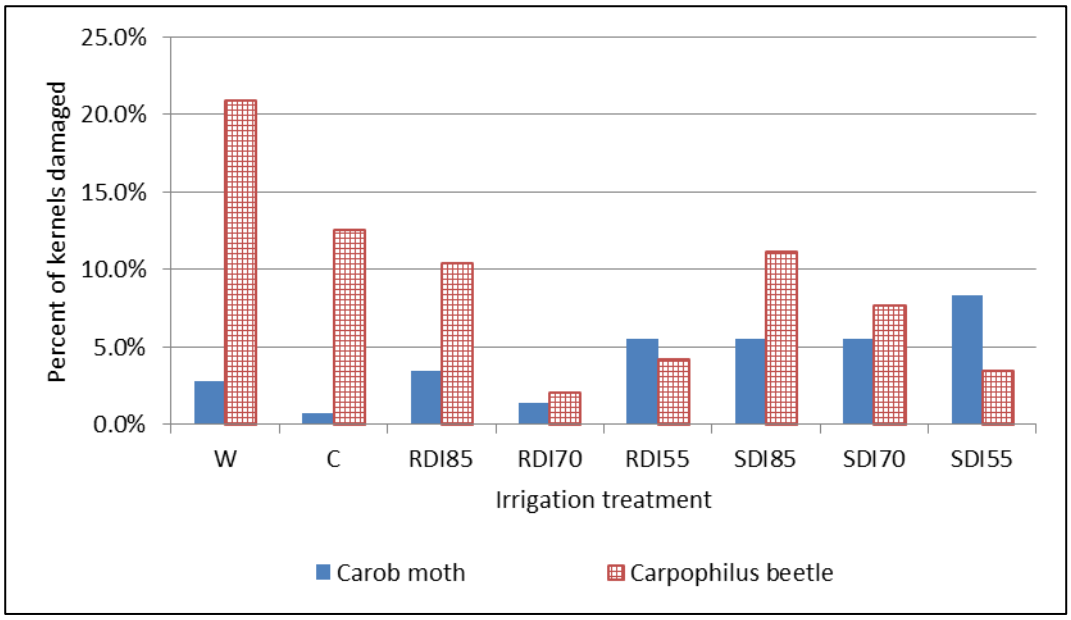

Figure 58. Deficit irrigation treatments and kernel damage by carob moth and carpophilus beetle in new crop nuts at harvest, 2014.

During the 2014 harvest, carpophilus beetle was recognised as causing significant kernel damage across the Robinvale district. This led to the industry establishing a pilot monitoring and 'attract and kill' trapping program for the 2014/15 season.

In 2014/15, we used a series of surveys to follow the development of carob moth and carpophilus beetle infestation of almonds during the critical period from hull split to harvest. The results of these surveys relating to carpophilus beetle are presented below while the carob moth results are included in the chapter 'Seasonal phenology and distribution of carob moth in almonds'. That report should be consulted for details of the structure and conduct of the survey and sample assessments.

At the start of the 2014/15 nut infestation survey (29 Dec 2014), prior to hull split, 73% of the trees surveyed had carpophilus-infested mummy nuts on the ground under them, while only 2% of mummy nuts on the trees were infested (Figure 59). At that time, no current season nuts showed any sign of carpophilus infestation or damage (Figure 60). As can also be seen in these figures, within six weeks the rate of carpophilus infestation of nuts on trees had increased to 25% in mummy nuts and 20% in current season nuts, with 10% of current season kernels damaged by the beetle. This provides some indication of the potential of carpophilus beetle to cause serious damage to almond crops.

It is interesting to note that in contrast with carob moth, carpophilus-infested mummy and current season nuts seemed to be preferentially removed during shaking for harvest, as indicated by the drop in percent of nuts with carpophilus-damaged kernels between 10 Feb (pre-shake) and 26 Feb (soon after shaking).

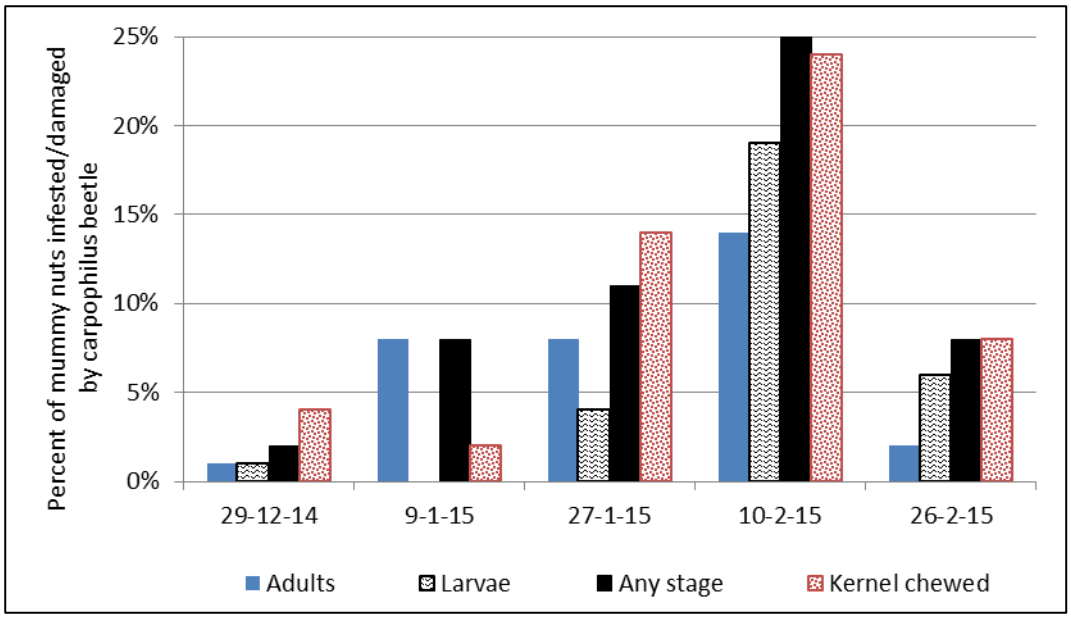

Figure 59. Presence of carpophilus beetle life stages in mummy nuts, summer 2014/15.

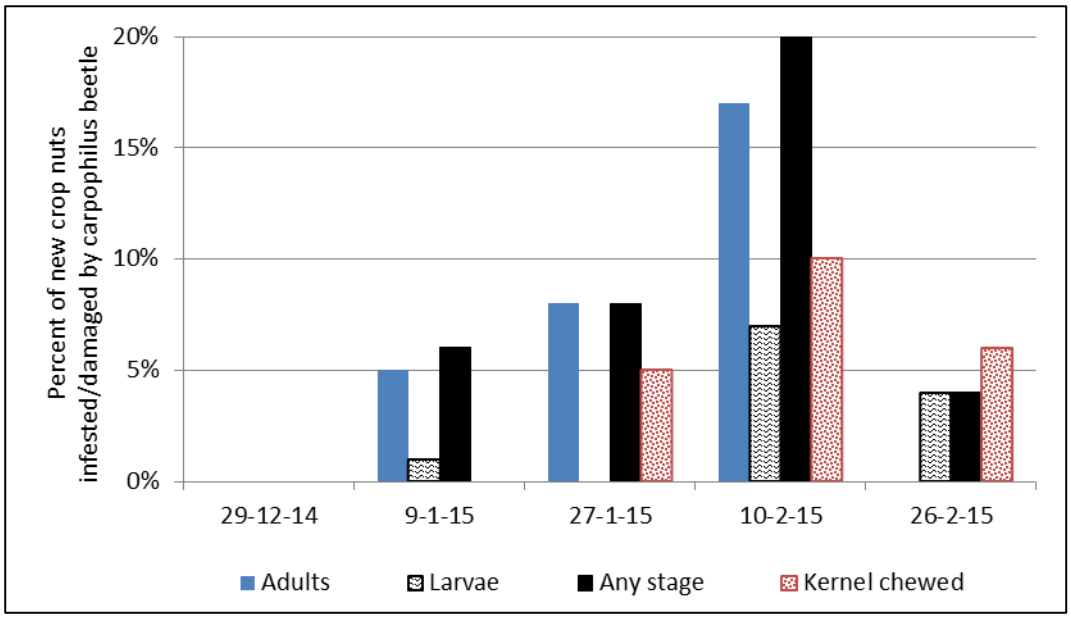

Figure 60. Presence of carpophilus beetle life stages in new season nuts, summer 2014/15.

Visiting scientist

During the review of literature and current research programs on carob moth, consideration was given to potential candidates for a 'visiting scientist' who could make a useful contribution to the project. From several individuals and groups with expertise and experience relevant to this project, two were identified as being of interest for a sponsored visit. One was invited to Australia in 2013. The second is recommended for consideration if an opportunity arises in the future.

2013

Prior to finalising the design for the second year of field trials on applied control of carob moth, the project sponsored a visit to Australia by Dr. Michael Reinke, entomologist and chemical ecologist with ISCA Technologies Inc. ISCA developed and produced SPLAT-EC®, the carob moth mating disruption product being used by the project. This put Dr. Reinke in a unique position to add value to the interpretation of first

year results and design of second year trials, through on-site discussions and inspection of the previous and proposed trial sites.

Dr. Reinke visited the trial site in Sunraysia with the project research team during the week of 26-30 August 2013. The project also arranged for Dr. Reinke to meet representatives of several major almond producers during his visit to the region, for discussions on their approach to pest management and carob moth in particular, their attitude to technology such as mating disruption, and their views on the practicalities, economics and their likelihood of adopting mating disruption as an alternative to insecticide use. While in Mildura, Dr. Reinke also met with representatives of the ABA to discuss the mating disruption research.

Potential future visit

If another opportunity arises for a visiting scientist to contribute to Australian research on carob moth management, the research group led by Prof. Thomas Perring (University of California, Riverside) should be seriously considered. Prof. Perring himself has had research experience with carob moth since 2001, and as at 2015 his group is investigating the biology, ecology and management of carob moth as a pest of dates, with interests and experience in its natural enemies, insecticidal control and mating disruption. The most appropriate invitee from this group would depend on the area of focus of Australian research at the time.

Contact details:
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Discussion

Each of the main chapters presented above contains a detailed discussion relating to the chapter's technical subject. More broadly, in relation to each of the projects objectives:

'Develop a good understanding of carob moth as an almond pest'

By using a range of investigative techniques, we have achieved our objective of developing a good local understanding of carob moth as a pest of almonds in Australia in terms of its geographic and localised distribution, its abundance within and between seasons and properties, to some degree its local suite of natural enemies, the timing and nature of the damage it causes to almond crops and its reliance on mummy nuts as a food resource within the almond orchard system. We have also begun to tease out the relationships between mummy nuts, moth activity and crop damage which will aid any future effort to develop economic thresholds and risk forecasting tools for producers.

'Develop and evaluate strategies to minimise nut infestation by carob moth'

By using local data to highlight the importance of mummy nuts to carob moth, its level of exploitation of that resource, and its potential for population growth and crop damage, we have continually reinforced the message that orchard sanitation is likely to always be the key component of carob moth management.

We demonstrated the potential for mating disruption of carob moth in almonds by adapting the use of a product developed for the USA date industry, but fell short of demonstrating its efficacy and testing its cost-effectiveness in the final year due to very low pest populations and a technical failure. Completing that step is necessary for industry to be able to make a judgement on the technical and economic viability of the mating disruption option.

We have also shown that insecticide application at hull split does provide a degree of crop protection, but that this approach may not be cost-effective in all seasons, highlighting the need for alternative management options as well as seasonal risk assessment tools for producers. We reinforced those points by confirming that protection of new crops is required for almost the entire hull split-harvest period which is beyond the scope of the pesticides currently available for use in almonds.

'Inform industry of 'best bet' practices for management of the pest'

During the project, we have informed industry of our progress, findings and management recommendations through a range of approaches from national conferences and industry publications to regional forums, farm walks and one-to-one contact with producers. The final suite of publications from this project will continue this effort.

Technology Transfer

The project officers participated in a range of activities during the project, to inform industry of the findings from research trials and subsequent recommendations for carob moth management, and to obtain feedback from industry on their experiences and concerns regarding the pest from a commercial viewpoint. The various activities are described briefly below and listed in Appendix A.

ABA Australian Almond Conferences

Project officers attended the four Australian Almond Conferences held after the start of the project in 2011 and gave presentations on project progress and findings to the later three conferences. As is general practice, useful discussions were held between conference sessions with almond producers, processors, agricultural suppliers and local and international researchers, on the behaviour and management of carob moth as an almond pest.

ABA committee meetings

The project presented its current knowledge of carob moth as an almond pest, findings from the 2011/12 trapping program and the proposed full carob moth research project to the ABA joint meeting of the plant improvement, production, processing and marketing committees in May 2012. Because of the industry membership of the four committees, the information that was presented raised awareness of carob moth and the research program at the manager level with producers who accounted for the majority of Australia's almond crop.

From 2013 onwards, this forum for information exchange became the 'Activated Almond R&D Forum', open to all almond producers (see below).

ABA Activated Almond R&D Forums

Progress in the project's research activities and findings from field and laboratory trials were presented to industry audiences (open to grower, processor and marketer attendance) at 'Activated Almond R&D Forums' in June 2013 and 2014. Each forum provided an opportunity for almond producers to gain updates on, and question, the previous season of research, current recommendations for managing carob moth, and plans for the following season.

Grower/farm manager meetings

Background information on carob moth as a pest, the trapping program that was underway, and potential management options were presented to and discussed with Select Harvests orchard managers at an 'Irrigation & carob moth' workshop organised by Select Harvests in June 2012. This workshop, early in the project, provided upper-level and on-the-ground managers with useful information regarding the use and interpretation of carob moth traps, the seasonal cycle of carob moth in almonds and the impact of their insecticide application during the 2012 hull split.

In October 2014, the project participated in the ABA 'Orchard Walk' program, during which field walks and discussions were held on 13 orchards across the Griffith, Robinvale, Mildura, S.A. Riverland & Adelaide Plains districts. During these sessions, the project leader outlined and discussed carob moth and Carpophilus monitoring and management options with orchard managers and staff. The program provided an excellent opportunity to provide project information to orchard managers/staff who may not ordinarily attend conferences and other forums, and for the project to gain first-hand experience of the pest issues across a broader range of almond producing districts.

In April 2015, the project's knowledge to date of carob moth and carpophilus beetle behaviour, monitoring and management was presented to orchard managers from Olam Orchards Australia at an 'Olam Research Day' workshop. Included in the audience were Olam's almond orchard agronomist, technical services manager and general manager, which helped to ensure that all management levels received the same information and were aware of the issues associated with these pests.

Other industry/technical meetings

In addition to the presentations and discussions held with broad audiences in the activities listed above, smaller but equally important review and planning discussions were held with a range of groups, particularly early in the project. At these meetings, the project officer(s) contributed the project's monitoring experience and current knowledge on carob moth behaviour in almonds, as related to potential management strategies, as well as suggestions on future trials and pest management options. The groups involved in these meetings included:

- Olam Orchards Australia technical services and agronomy officers and DuPont representatives (reviewed outcomes of the 2011/12 DuPont insecticide trials and Olam's commercial insecticide applications) (May 2012).
- Brownport Almonds managers, ABA Executive and IDO, and Dow Chemical Co. representatives (July 2012).
- Olam Orchards Australia's technical services manager, agronomist and private pest management consultant (Dec 2012).
- Select Harvests site manager and agronomist (Dec 2012).
- ABA IDO and technical managers/agronomists from the major almond producers (Aug 2014).

Information sheets/Fact sheets

'Preliminary trapping guidelines for Carob Moth, *Ectomyelois ceratoniae*' (see Attachments). This information sheet was produced in August 2011 and distributed to almond growers who were participating in the carob moth trapping program that season. It provided guidance on the placement and maintenance of traps and identification of carob moth. After the first season of trapping, the guidelines were revised and redistributed to existing and new participants (see Attachments).

ABA fact sheet 'Carob moth: Preliminary monitoring guidelines' (see attachments). Building on the preliminary trapping guidelines, this fact sheet included monitoring of nuts and was published on the ABA internet site in September 2014 for general access by producers.

ABA fact sheet 'Carob moth in almonds' (see Attachments). This fact sheet describes the biology, seasonal behaviour and management options for carob moth in almonds. (Submitted to ABA, May 2015).

'Pocket guide to Carob moth *Apomyelois* (=*Ectomyelois*) *ceratoniae*' (see Attachments). This pocket-sized guide is designed for orchard managers, field staff and others involved in the monitoring and management of carob moth. It carries clear and simple information on the identification of carob moth life stages, its seasonal cycle in almonds and differentiation from two other moth pests found in association with almonds (Submitted to ABA May 2015).

Articles

'Carob moth in Australia' (Australian Nutgrower June 2014). This article described the development of carob moth as a pest of almonds, the role of mummy nuts in the life cycle of the pest, and the monitoring and research program that was underway.

'Does size matter when it comes to identifying carob moth?' (see Attachments). This article was designed to answer industry queries regarding the identity of small moths that were being found at times in carob moth traps, as discussed above in 'Carob moth identification' (submitted to ABA, June 2015 for publication in 'In a nutshell').

General media

In February 2014 the project revised and made technical corrections to a press release "Victorian Government acts to alleviate carob moth", prior to publication on the web site of Peter Crisp MP. Subsequently the project provided information and images to WinTV for a news item on carob moth (21 Feb 2014) and to the Sunraysia Daily newspaper for an article "Moth threat for almonds" (4 Mar 2014).

Other

As a small exercise in increasing general public and grower awareness of the carob moth research program, displays were maintained during the Mildura Field Days over May 23-23 2014 and May 22-23 2015. The displays included photographs of carob moth life stages, infested almonds, pheromone traps and one of the parasitoids reared from carob moth eggs, as well as live specimens for examination by field day attendees. The display for 2015 also included similar information relating to carpophilus beetle.

Recommendations (scientific & industry)

The almond industry needs to identify and prioritise the key drivers of mummy nut development and retention, and investigate options to minimise the development and maximise the removal of those nuts. This is indicated by the importance of mummy nuts to the carob moth life cycle and is likely to be a pre-requisite to minimising the need for applied controls for carob moth in the long term. Addressing the issue of mummy nuts will have broader implications than just carob moth management, as the nuts are also utilised by carpophilus beetle and act as a source of inoculum for important almond diseases.

The key drivers of mummy formation/retention are likely to include such factors as:

- Pathology (e.g. hull rot)
- Physiology (possibly water stress)
- Mechanics (e.g. tree structure preventing adequate transfer of shaking forces throughout the tree)
- Orchard structure & environment (e.g. large orchards not being conducive to bird predation of nuts)

Risk assessment tools including economic thresholds need to be developed to assist commercial decision-making regarding the likely value of any applied controls in any particular season. The apparent variability between seasons in cost/benefit of one-off insecticide applications for example, suggests that reductions in insecticide usage and costs could be achieved if the seasonal risk of carob moth damage could be forecast.

The timeliness of key crop management operations from hull split to processing should be assessed by the relevant sectors of the industry and optimised wherever possible. Kernel damage by carob moth begins within three weeks of hull split and the level of damage can grow rapidly with time. The risk of significant increases in economic loss grows with any delay in harvest or post-harvest disinfestation or processing.

The optimum timing and rate of chlorantraniliprole application against carob moth need to be determined as they are based on USA recommendations for navel orangeworm. This task was beyond the scope of this project. Spring applications are not covered by the current APVMA emergency use permit but should be investigated.

Where chlorantraniliprole-based insecticide is used routinely, some level of monitoring should be undertaken to detect and address any potential longer-term impacts of its use on beneficial invertebrates in the almond agro-ecosystem.

Because of the promising results and potential benefits from mating disruption, the optimum method of use of SPLAT-EC® in almonds needs to be clarified. This will be assisted by determination of the mode of action of the pheromone mimic in disrupting male location of females. Mechanical application of the product also needs to be assessed and demonstrated, as that will determine the economic viability of this approach. The possibility of formulation improvements to extend the field life of SPLAT-EC® should be investigated with the manufacturer.

An effective lure for female carob moth should be pursued, building on the preliminary findings from this project, as it would be of significant value to producers and researchers alike.

In the event that carob moth continues to pose a threat to the almond industry because the issue of mummy nut management cannot be adequately addressed, the sterile insect technique (SIT) could be worthy of consideration, given Australia's experience with that approach for fruit fly suppression.

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- The orchard manager and farm staff who accommodated our mating disruption and insecticide field trials over three seasons and applied the insecticide treatment for the project. This cooperation and contribution to the project by the industry was very much appreciated and without it the trials would of course been impossible.
- The numerous orchard managers who maintained traps on their properties and sent in their moth counts regularly. This was another job for already busy people and their help was also appreciated. Thanks also for allowing us to conduct the nut surveys and trapping trials on particular properties.
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Appendix A Technology transfer

Meetings and presentations

31/1/2012	DPI Irymple. Presented information on carob moth, the trapping program and proposed research project to a visiting group of Spanish growers/researchers/agronomists.
10/5/2012	ABA meeting of plant improvement, production and processing committees, Renmark Golf Club. Presented an outline of carob moth as an almond pest, the trapping program and proposed research project.
16/5/2012	Olam meeting on results of Altacor spray program with DuPont representatives – Olam office, Mildura. Discussed results of DuPont field trials and broader industry spray program and suggested more detailed trials were needed to prove any effect of the sprays.
28/6/2012	Irrigation & carob moth workshop, Select Harvests, Kyndalyn Park. Presented background information on carob moth and the trapping program and discussed management options with Select Harvests orchard managers.
12/7/2012	Brownport Almonds, Carwarp. Discussed carob moth management and monitoring with Brownport Almonds managers, ABA Executive and IDO, and Dow representatives.
8-10/10/2012	14th Australian Almond Industry Conference, Barossa Valley Gave a presentation on carob moth as an almond pest, results from the monitoring program, and the proposed full project.
6/12/2012	Olam, Mildura office. Discussed carob moth and the 2012/13 season with Olam's field manager and agronomist, and Californian consultant.
6/12/2012	Select Harvests, Carina. Discussed carob moth and management options with Select Harvests site manager and agronomist.
12/6/2013	ABA Almond R&D Forum, McCormick Centre for the Environment, Renmark. Presented update on carob moth project to industry audience.
29-31/10/2013	15th Australian Almond Industry Conference, Glenelg. Presented carob moth project findings to producer, supplier and researcher audience.
18/6/2014	ABA Almond R&D Forum, McCormick Centre for the Environment, Renmark. Presented update on carob moth project to industry audience.
5/8/2014	DEPI Mildura. Discussed carob moth project progress and directions for mating disruption and other aspects, with ABA IDO and industry technical managers/agronomists.
14-17/10/2014	ABA Orchard Walk program, Griffith, Robinvale, Mildura, Riverland & Adelaide Plains. Contributed to orchard walk program with ABA IDO. Discussed carob moth and Carpophilus monitoring and management options with orchard managers. Included 13 orchard walks.
28-30/10/2014	16th Australian Almond Industry Conference, Glenelg. Presented carob moth project findings to producer, supplier and research audience.
28/4/2015	Olam research day, Euston Club, Euston. Presented current knowledge of carob moth and carpophilus beetle behaviour, monitoring and management in almonds to Olam orchard managers.

Internal & external publications

*Madge, D. (2011) Preliminary trapping guidelines for carob moth *Ectomyelois ceratoniae*. 3pp. Distributed to producers involved in the carob moth trapping program, from August 2011 onwards.

*Madge, D. (2012) Preliminary trapping guidelines for carob moth *Ectomyelois ceratoniae*. Revised. 4pp. Distributed to producers involved in the carob moth trapping program, from September 2012 onwards.

Madge, D., Taylor, C. and Williams, D. (2014) Carob moth in Australia. Australian Nut Grower, 28(2): 18-19.

*Madge, D., Taylor, C. and Williams, D. (2014) Carob moth in almonds: Preliminary monitoring guidelines. ABA Fact Sheet. 4pp. Published 30/9/2014

*Madge, D., Taylor, C. and Williams, D. (2015) Carob moth in almonds. ABA Fact Sheet. 4pp (submitted to ABA for approval for internal publication)

*Madge, D. (2015) Pocket guide to carob moth. (submitted to ABA for approval for publication)

*Taylor, C. and Madge, D. (2015) Does size matter when it comes to identifying carob moth? (submitted to ABA for approval for publication in 'In a nutshell')

*Madge, D. and Taylor, C. (2015) Seeking a lure with allure for the female carob moth. (submitted to ABA for approval for publication in Australian Nutgrower)

* Published or submitted copy attached.

Media

Publication date	Project contribution
18/2/2014	Revised and corrected a press release "Victorian Government acts to alleviate carob moth", published on the web site of Peter Crisp MP.
21/2/2014	Provided information and images to WinTV for a news item on carob moth.
4/3/2014	Provided DEPI Communications Branch with images for a Sunraysia Daily article "Moth threat for almonds".