Natural enemies of almond pests in Australia, and the potential for a biocontrol program

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1.0 Summary

This report evaluates the potential for developing a biocontrol strategy for controlling insect pests in almonds. The report reviews current literature pertaining to carob moth and carpophilus beetle biocontrol (Part one) and presents the findings from a field survey on natural enemies of almond pests conducted in May and June 2018 in the Sunraysia region of Victoria (Part two). Information from the review and the field survey is then synthesised and discussed, and recommendations are made with a view to addressing knowledge gaps, additional survey work, research focus, and prioritising next steps.

The review of the published and grey literature identified 59 species of natural enemies worldwide for carob moth. Of these, ten species are present in Australia including four parasitic wasps (*Antrocephalus mitys, Venturia canescens Trichogramma carverae* and *Goniozus jacintae*), two predacious mites (*Pyemotes ventricosus* and *Blattisocius tarsalis*), a predatory bug in the genus *Orius,* three generalist predators (*Forficula auricularia*, *Mallada signata* and *Dicranolaius bellulus*) and one entomopathogenic nematode (*Heterorhabditis bacteriophora)*. The literature reports far fewer natural enemies of *Carpophilus* species in Australia, identifying one wasp, the larval parasitoid *Anisopteromalus calandrae,* and a few generalist predators. A parasitoid wasp, *Brachyserphus abruptus*, has shown some promise as a biocontrol agent to control *Carpophilus* elsewhere in the world, but is not present in Australia. As this insect is a generalist (attacks a wide range of host insects), it is unlikely to be considered for introduction into Australia (i.e. classical biological control). At the genus level, there were 22 genera identified for carob moth and three genera for *Carpophilus* known in Australia. The review also identified the potential for using entomopathogenic fungi (insect fungal diseases) and nematodes as promising biological control candidates for use in particular against *Carpophilus* beetles.

Field survey work identified insect species present in almond orchards that would be candidates for conservation biocontrol of carob moth and carpophilus beetle. The surveys took the form of (i) field trapping of insects (using passive airborne insect traps and sticky traps) and (ii) collection of infested mummy nuts and conducting nut examination and insect emergence tests in the laboratory. Whilst Macquarie Island traps did not catch a significant number of insects over the sampling period, sticky traps caught a diversity of insect species. Field trapping for airborne insects (sticky straps and Macquarie Island traps) collected a greater diversity of insect species compared to mummy nut sampling, but many of these insects were not predatory. Due to the considerable diversity of insects trapped and time and cost constraints of the project, morphological and molecular identification was carried out to genus level (but not species) for most specimens. Results of the emergence trials revealed two tentative natural enemies attacking either carob moth or carpophilus beetle.

The report discusses the findings of the literature review and field study with a view to developing a biocontrol strategy through conservation or augmentative biocontrol (classical biocontrol is also discussed, though not deemed feasible with current knowledge of potential agents). Recommendations are made with a view to addressing knowledge gaps, additional survey work, research focus, and prioritising next steps. We recommend additional natural-enemy (emergence test) sampling of mummy nuts and developing almond fruits (particularly at hull split) as key predatory and parasitoid

species may have been absent or in low abundance at the time of sampling. Survival of natural enemies in the field is strongly influenced by a suitable habitat, and while it is appreciated that the economies of scale in almond production may limit the provision of these sites, the possibility of providing an improved habitat for natural enemies should at least be explored. At the very least, growers and industry must be aware that these insect communities are undoubtedly contributing to the control of pest populations, and the use of broad-spectrum insecticides will reduce or remove these beneficial insects, and as a consequence significantly exacerbate the pest problem.

There is a considerable opportunity to develop augmentative biocontrol (mass release of biocontrol agents) for almond pests. *Trichogramma* parasitoid releases for biocontrol of carob moth are currently being trialled by almond growers, and we report here on additional research that may assist in the effectiveness of a *Trichogramma* biocontrol program. This includes evaluating *T. pretiosum* and *T. carverae* augmentative releases, and field surveys for other egg parasitoid species. The literature and survey identified one wasp species (*Antropochephalus myti*) thought to be present in Australia (no recent records) that is being used elsewhere in augmentation biological control programs for lepidopteran pests and could be studied as a potential for inoculative release.

Entomopathogenic fungi (EPF) have considerable potential as agents for almond pest biocontrol, and we highly recommend this as an area of research that warrants further exploration. EPF research could begin with bioassays screening the effectiveness of different strains of the fungus *Beauveria bassiana* on *C. nr dimidiatus* mortality, including field efficacy trials. Autodissemination (auto-inoculation) is a method by which diseases are spread through a pest population by a vector, often the pest species itself. This method may prove an effective way of using EPFs to control both *C.* nr *dimidiatus* and carob moth, where insects remain hidden within the developing almond fruit or mummy nuts. Project AL16009 is currently developing a new attractant for *C. nr dimidiatus* as part of an "attract and kill" mass trapping strategy, which could be used for disseminating EPFs at key times in the pest lifecycle. The use of entomopathogenic nematodes should also be explored, and could begin with developing methods to collect, culture, and screen two species identified in this review as being present in Australia; and a field survey to identify other *Carpophilus* attacking nematode species in Australia.

2.0 Aims of this report

This report details work conducted by Agriculture Victoria to explore the feasibility of developing and implementing a biological control program as a component of an almond IPM strategy. The work was carried out as a component of Project AL16009 "An Integrated Pest Management Strategy for the Almond Industry" and fulfills reporting milestone 103. The feasibility study drew on current knowledge of the biological control of the two major almond pests, carob moth and carpophilus beetle, through a literature search (Part one) and conducted an insect trapping and nut sampling survey in the Sunraysia region of Victoria and Riverland of South Australia (Part two), with the aim of identifying potential biocontrol agents inhabiting the Australian almond agro-ecosystem. The report discusses the overall potential for different biocontrol strategies (conservation, augmentative, classical) in almonds and includes recommendations for next steps and future work.

3.0 Background

Australia's one billion dollar almond industry is currently suffering from high levels of damage from insect pests. Losses in 2017 as a result of the two key pests, carpophilus beetle and carob moth, were estimated at $12.1 million (Madge, unpublished). Both of these pests attack developing nuts at the "hull split" stage, when they can easily enter into the fruit and feed on the kernel (Hossain 2018; Madge *et al.* 2015). Current management strategies such as improved crop hygiene, pesticide applications, and mass trapping strategies have so far proven to be ineffective at controlling these insects, with carpophilus beetle in particular escalating to unprecedented levels of kernel damage in recent years (Hossain 2018). Losses due to these insects are widely seen by the almond industry as unsustainable, and the industry has recognized the urgent need for the development of an effective Integrated Pest Management (IPM) program for insect pests of almonds through this current research program. Biocontrol is a fundamental component of any IPM strategy and has been identified as a specific goal in the program (Goal 5: A better understanding of pest species and their natural enemies). The goal aims to address knowledge gaps in almond pest biocontrol through (i) a review of the published and grey literature to identify current knowledge on biocontrol of carob moth and carpophilus beetle, and (ii) conducting a winter field survey to identify natural enemies in Australian almond orchards. These two aims are addressed in Parts one and two of this document respectively.

4.0 Part One. Literature review exploring potential for biocontrol of almond pests

4.1 Biocontrol strategies used to control insect pests.

The use of natural enemies to reduce impacts of invertebrate pests (biological control) has long been recognized as a viable alternative to insecticides, whose overuse is now associated with negative environmental, ecological and financial consequences. In undisturbed ecosystems, a suite of natural enemies (e.g., parasitoids, predators and pathogens) limit the impact of herbivorous insect pests by maintaining their populations below outbreak levels, in what is termed natural biological control. Natural biological control of insect pests occurs via complex ecological interactions involving a diverse range of species existing in equilibrium (Tscharntke *et al.* 2008). In modern agriculture, this equilibrium is disrupted through extensive monoculture, which decreases biodiversity in the agro-ecosystem. Moreover, as herbivorous insects are more likely to find and remain on host plants that are growing in dense or pure stands (such as monocultures) this further increase the likelihood of pest outbreaks (Root 1973).

Biological control is an environmentally sound, effective and sustainable insect management option that is considered central to integrated pest management (Orr 2009). Natural enemies as biological control agents include predators, parasitoids, and pathogens. Predators consume many prey during their lifetime and include insects such as lady beetles and lacewings. Parasitoids largely comprise wasp species and some flies, where the immature stages develop on or within the pest insect host (usually the egg or larval stage), ultimately killing it. Pathogens are disease-causing organisms including bacteria, fungi, and viruses, which kill or debilitate their host and are relatively specific to certain insect groups.

Biological control is generally divided into three slightly overlapping techniques or categories: conservation biocontrol, augmentative biocontrol and classical biocontrol (Eilenberg *et al.* 2001). Each of these techniques can be used either alone or in combination. **Conservation biological control** involves human actions aimed at protecting and stimulating the performance of naturally occurring natural enemies in the local environment (Seastedt 2014). Unlike other biological control strategies, conservation biological control does not require the introduction or augmentation of natural enemies. Conservation biocontrol aims to reverse negative effects associated with agricultural intensification on beneficial insects that naturally exist in agricultural fields. Such strategies may include reduction in the extensive use of pesticides, and establishment of beneficial habitats that provide resources such as shelter (refugia), food sources (e.g. nectar, alternative prey/hosts, and pollen) that are critical to the survival of locally available natural enemies (Gurr *et al.* 2017). The "Greening Waipara" program in New Zealand potentially provides the most successful application of conservation biological control in an intensive agricultural production system. In this program, a multifunctioning habitat management strategy uses native ground-cover plants, to enhance biodiversity and consequently suppression of lepidopteran pests complemented with erosion management, filtration of winery effluent (Landis *et al.* 2012). Owing to the success of this program, vineyards are actively marketing the aesthetic appeal for ecotourism (Gillespie and Wratten 2012).

In **augmentative biological control** (ABC), natural enemies (parasitoids, predators or micro-organisms) are mass-reared for release in large numbers either to obtain immediate control of pests in crops with a short production cycle (inundative biological control) or for control of pests which have several generations within crops with a long production cycle (inoculative biological control) (Parnell *et al.* 2016; van Lenteren 2012). It is important to distinguish between inoculation and inundation biological control since the practical approaches and ecological implications of these two strategies differ (Eilenberg *et al.* 2001). In inundative release, biological control agents need to contact and kill a sufficiently high proportion of the pest population to give economic control before dispersing or becoming inactive (Eilenberg *et al.* 2001). The focus here is therefore on the released population and not their progeny. By contrast, inoculative biological control aims to release self-sustaining natural enemy populations to support pest suppression over several growing seasons (Eilenberg *et al.* 2001). In this case efforts must be made to provide the biological control agents with necessary resources to facilitate their multiplication and establishment.

Augmentative biocontrol is applied in many agricultural systems worldwide as part of IPM programs to manage pests in fruit and vegetable crops, cereals, maize, cotton, sugarcane, soybean, grapes and many greenhouse crops (van Lenteren 2003; Cock *et al.* 2010). As a recent example, implementation of an ABC program has led to complete replacement of chemical pesticides by opportunistic native predatory bugs (Hemiptera: Miridae), to control an invasive pest (the South American pinworm *Tuta absoluta*) in the Mediterranean basin (Urbaneja *et al.* 2012). Another successful ABC program, this time using microbial agents (entomopathogens) controlled the cotton bollworm, *Helicoverpa armigera* in Brazil. In 2012 this pest caused extensive damage to corn, cotton and soy, as pesticides were not effective due to resistance or were simply not available. Emergency approvals of the entomopathogenic bacterium *Bacillus thuringiensis* and baculovirus products provided farmers with the only effective control method at the time (Pratissoli *et al.* 2015).

Classical biological control involves the introduction of a biological control agent of exotic origin to permanently control a pest, which is usually also exotic (invasive) (Hajek 2004; Van Driesche *et al.* 2008). Almost all of today's agriculture is

based on introduced crops, especially in Australia and New Zealand. Consequently, a high proportion of important arthropod pests in the agricultural sector tend to be introduced (exotic) (Waterhouse and Sands 2001). While native insects can evolve to become natural enemies of exotic pests, in most cases this process takes several years, and the insects do not result in effective pest control. It is therefore not surprising that in Australia, classical biological control is the most well documented of all three approaches discussed above. An example of this is provided by the parasitoid wasp *Trissolcus basalis*, the biological control agent for the once very widespread green vegetable bug, *Nezara viridula*. The bug is now suppressed by the wasp in much of south-eastern New South Wales and Victoria to the extent that it has become an uncommon pest (Waterhouse and Sands 2001). The BIOCAT database records all deliberate introductions of insects for biological control of insect pests since the 1890s. Cock *et al.* (2016) recently updated BIOCAT to indicate that classical biological control has led to the partial or complete control of at least 226 invasive insects and 57 invasive weed species worldwide since 1888.

4.2 Implementation of biocontrol into IPM.

The different biological control approaches described above can successfully contribute to suppression of invertebrate pests in modern agricultural systems through an effective IPM strategy. However, all three approaches draw on a number of complex ecological and behavioral processes which, if not well studied prior to program implementation, may present unsuccessful results (Tscharntke *et al.* 2016; Chaplin-Kramer *et al.* 2011; Veres *et al.* 2013). If biocontrol options are explored without sufficient scientific depth or rigor they can fail unnecessarily, representing a considerable waste of time and resources. Biocontrol research needs to encompass the identification of effective natural enemies and the resources they require to be successful (Gurr *et al.* 2017). Protocols specific to each approach, such as augmentative biological control (Cock *et al.* 2010) and classical biological control (Kenis *et al.* 2017) have been developed, but the general stepwise process is shown in Figure 1.

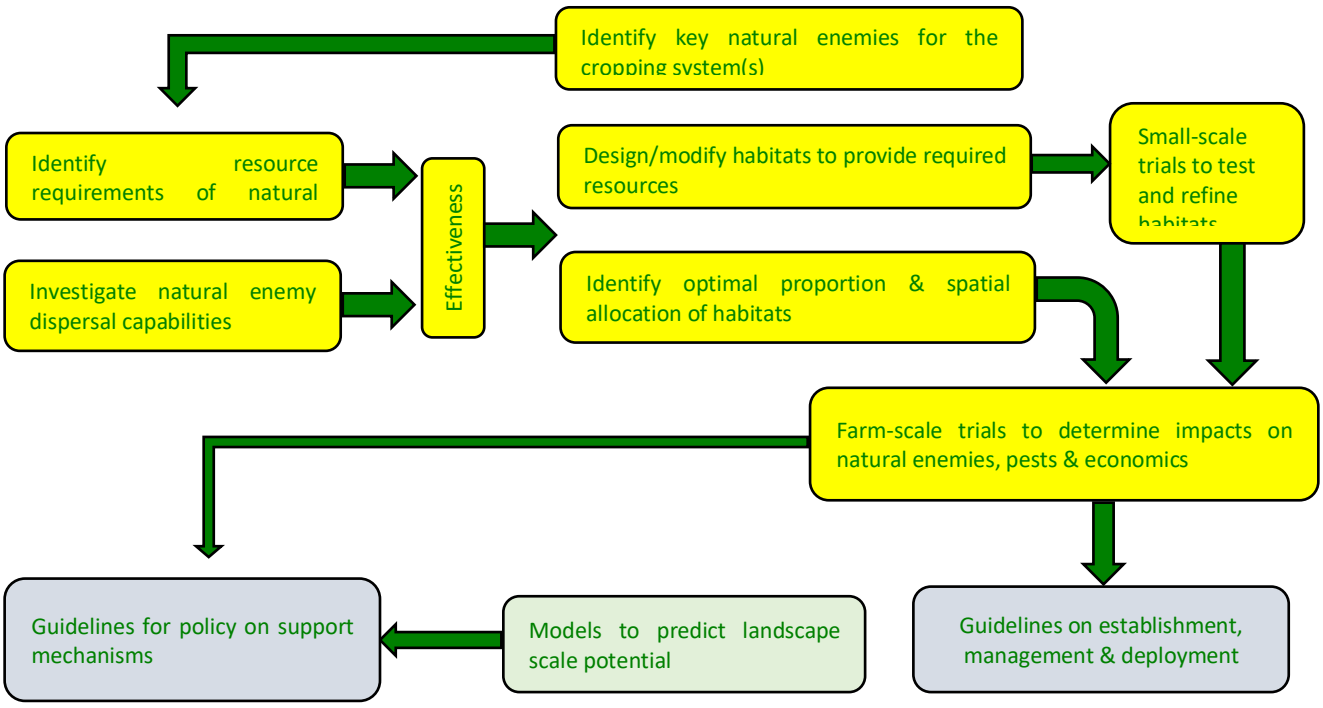

Figure 1. Generalised schematic summary of the stepwise process followed while developing and implementing a biological control program. Figure provided by John Holland (Farmland ecology, Game & wildlife Conservation Trust, UK).

4.3. Biocontrol of carob moth in almonds

The carob moth, Ectomyelois (Apomyelois) ceratoniae Zeller (Lepidoptera: Pyralidae) is a highly polyphagous pest of fruit and nut crops, reported to attack up to 43 plant species across 18 plant families. The worldwide list of susceptible hosts includes twenty-one species of economic importance, the susceptible hosts being orchard species, with families such as Fabaceae and Rosaceae (to which almonds belong) being the most susceptible (Perring *et al.* 2015). In Australia the pest is known to attack carob, almonds, oranges, apples, figs and pomegranates although carob beans and almonds are considered the major host fruits (Madge *et al.* 2015). Native to the Mediterranean, carob moth is currently distributed widely throughout 58 counties of the world (Perring *et al.* 2015). It is unclear when this insect was introduced in Australia, where it is now widespread, however a report by Michael (1968) describes the carob moth as a pest of economic importance limited to almond crops and only occasionally attacked other fruits in Western Australia.

In almonds, economic damage is caused by carob moth larvae feeding on almond kernels, which renders nuts unsuitable for sale through direct damage and the indirect spread of fungal infections (Madge *et al.* 2015). Damage caused by carob moth larvae also negatively impacts export of susceptible hosts due to phytosanitary restrictions (Van Achterberg *et al.* 2017). In almonds, the species is multivoltine, completing at least three generations each year in the Australian almonds growing regions (Madge *et al.* 2015). Adult moths emerge from overwintering pupae in spring, in a generation that extends from early/mid-September until early December. Female moths in this generation lay their eggs (oviposit) on

inner hull, shell or exposed kernel of mummy nuts (Gothilf 1984, Madge *et al.* 2015). The second generation begins to emerge in mid-December and lasts until early February, with oviposition occurring on mummy nuts and new hull-split fruits. On new fruits, eggs are inserted into the splitting portion of the almond hull, and hatching larvae, concealed in the fruit, feed on the inner hull, outer shell and kernel, passing through five larval stages before pupating usually within the fruit (although some pupate in tree crevices or on the ground). The third generation emerges from mid-February to late April and oviposits on mummy nuts and new crop nuts that have not yet been harvested or that remain on trees after harvest. Evidence of a fourth generation of moths emerging from late April to Late May is sometimes seen. Eggs laid on mummy nuts or residual new crop nuts in mid-late Autumn, develop into larvae that will overwinter in the nuts and emerge as the next spring generation.

- *Natural enemies as biocontrol agents*

As this cosmopolitan species is a pest of many crops in many countries, a wide range of natural enemies, including parasitoids, predators and entomopathogenic nematodes have been reported in published reviews (e.g. Nay 2006, Madge 2013, Perring *et al.* 2015, Madge *et al.* 2015, Memari *et al.* 2016). Here, we have summarized this information in easy to read tables (Section 4.5, Tables 1 and 2), which cover 59 species of natural enemies: 48 hymenopterans (parasitic wasps), two dipterans (parasitic flies), three acari (predacious and parasitic mites), three hemipterans (predatory bugs), and three nematodes (entomopathogenic nematodes). Among the 48 species of parasitic wasps identified, only two (*Antrocephalus mitys* and *Venturia canescens*) are known to occur in Australia, although the occurrence of *A. mitys* cannot be confirmed due to lack of records of this species in the Australian Plant Pest Database (APPD). Ten of the parasitic wasp species belong to genera that currently do not occur in Australia while 36 belong to genera known to be present in Australia (Table 2). None of the parasitic fly taxa (both genus and species) occur in Australia, two of the predacious mites are present and none of the entomopathogenic nematode species are present. Thus, many biological control agents for carob moth in other countries do not occur in Australia. In addition, Madge *et al.* (2015) reported the collection of the parasitic wasps *Trichogramma carverae*[1] from carob moth eggs and *Goniozus jacintae* from lepidopteran larvae (most likely carob moth), both in almonds from Australian orchards. A predatory, bug in the genus *Orius* and several other predators such as European earwigs (*Forficula auricularia*), green lacewing (*Mallada signata*) and the red and blue beetle (*Dicranolaius bellulus*) were also observed in Australian almond orchards.

Despite the high diversity of arthropod natural enemies associated with the carob moth, adoption of biocontrol control programs in almond growing regions is limited to a few examples (Gothilf 1969, Gothilf 1978, Gothilf 1984). The use of the egg parasitoid, *Pentalitomastix plethoricus,* resulted in 12-15% parasitism of carob moth eggs in Israel (Gothilf 1978). Mass releasing of parasitoid species *Goniozus legneri* (up to 32,000 adults)*, Copidosomopsis plethorica* (up to 3.5 million adults), and *Diadegma* species (about 1500 adults) into almond plantations in Israel have also been used to control carob moth, with parasitism rate of about 30.8% and 9% recorded for *G. legneri* and *C. plethorica* respectively (*Diadegma* was not recovered one year after the release, presumably due to the relatively small number of adults released). *Diadegma*

[1] Recent molecular work in Project AL16009 has drawn into question whether this species is *T. carverae* or *T. pretiosum*

is also reported to attack carob moth in almonds in Australia, albeit at low densities (Dr. E.F. Legner-University of California, personal communication in Gothilf and Mazor 1987).

Where the success of carob moth biological control is considered across all host crops, the highest rate of parasitism of carob moth eggs was reported using *Trichogramma cordubensis* (64%), followed by *Phanerotoma flavitestacea* (50%) and *Trichogramma embryophagum* (19%) (Perring *et al.* 2015). Although these particular species do not occur in Australia, closely related species belonging to the *Trichogramma* and *Phanerotoma* genera are present. *T. carverae* a species already associated with almonds in Australia is reared for mass releases against a range of lepidopteran pests and is commercially available in Australia[1]. Field releases of *T. carverae* in Australian almond orchards to control the carob moth are being performed in conjunction with project AL16009, through collaboration with mass release trials initiated by growers. Further fieldwork surveying specifically for egg parasitoids of carob moth (esp. *Trichogramma*) is highly recommended, both within almond orchards and other carob moth crop and non-crop hosts to determine if a more suitable species might be locally present. A species of *Diadegma* (*Diadegma semiclausum*) used to a control cruciferous (cabbage family) pest, the diamondback moth (*Plutella xylostella*), is also commercially available in Australia. Laboratory and (if promising) field screening trials to evaluate the potential for inundative release of this agent are recommended.

The efficacy of carob moth biological control agents may depend on the host plant (crop) that is being targeted. For example, *Pentalitomastix plethoricus* was released into almonds and carob plantations, and after several years, the parasitoids were recovered in almonds but not in carobs (Perring *et al.* 2015). Multiple factors can influence the establishment of the biocontrol agents, including nutrition, presence of predators, microclimate, and cultural practices. The host plant itself can have a direct influence on parasitoid survival: *Trichogramma embryophagum* reared on carob moth eggs collected from apples (*Malus pumila*) were more than twice as effective (45% compared to 19%) as parasitoids reared on moth eggs collected from other plants. Parasitoid species may also differ in their effectiveness in different areas of an orchard: in dates, *Habrobracon hebetor* was more effective in dates on the ground, whilst *Phanerotoma ocularis* had higher rates of parasitism in date bunches (Perring *et al.* 2015). This highlights the importance of a robust research program associated with biocontrol agent selection, release and evaluation, which is tailored to both the pest (host) being controlled, the host plant (crop) being attacked.

- *Bacillus thuringiensis and other pathogens for biocontrol*

The bacterium *Bacillus thuringiensis* (Bt) has been evaluated for carob moth control (see Perring *et al.* 2015). Whilst high mortality was observed in laboratory conditions, efficacy often failed in the field. High rates of mortality (95%) of fourth instar larvae were recorded in laboratory conditions using a high concentration of bacterial cells, but unfortunately mortality was much lower at rates that would be practical for field use. At best, field efficacy was at 82% control in a trial on pomegranates, achieved after four applications. As with natural enemies, crop specific effects may influence efficacy of Bt. In navel oranges, 53% control with *B. thuringiensis* subspecies *kurstaki* was achieved; however in dates, *B. thuringiensis* subsp. *kurstaki* had little impact on the densities of carob moth larvae. Insecticidal activity of the biosurfactant of *Bacillus subtilis* was found to be effective for carob moth control. A report of microsporidian

(microsporidia) found in the Malpighian tubules of carob moth collected from walnuts, had a 48% infection rate in larvae, pupae, and adult moths. However, there was no further work to develop this pathogen as a biological control agent (Perring *et al.* 2015).

4.4. Biocontrol of carpophilus beetle in almonds

- *The importance of identifying Carpophilus* near *dimidiatus.*

The taxonomy of the *Carpophilus* species that is causing damage to almonds in Australia is currently underway. This difficult process began with morphological and molecular work in a previous project (Hort Innovation AL 15004; Hossain 2018), together with expert international opinion. Agriculture Victoria have temporarily named this insect *Carpophilus* near *dimidiatus*, because of its morphological similarity and close genetic identity to *Carpophilus dimidiatus* (Hossain 2018). However, Agriculture Victoria taxonomists are confident that this almond pest species, while similar, is not *Carpophilus dimidiatus*. Further taxonomic resolution is currently underway, which requires obtaining reference specimens of nut-attacking *Carpophilus* beetles from other countries (such as *Carpophilus truncatus*) that may be the same species, and then conducting molecular identification on these insects. Consequently, little is known about the origin, ecology, and behavior of *C.* near *dimidiatus,* including natural enemies and diseases that affect this insect within Australia and elsewhere in the world. Knowing the worldwide distribution, and in particular native origins, of this insect would be a crucial step in developing a biocontrol program, as the biocontrol agents specific to this insect are most likely to occur in the pests native home range. Consequently, this literature search focused on published information on biocontrol of other *Carpophilus* species, with the aim of assimilating knowledge that may assist in developing a biocontrol program for *C.* nr *dimidiatus*.

- *Natural enemies of Carpophilus species*

Carpophilus beetles belong to the Nitidulidae family, commonly known as sap beetles, souring beetles, or simply "carpophilus beetle". Thirty species of *Carpophilus* are considered serious pests worldwide (Bai *et al.* 2017). Chemical control of *Carpophilus* species is particularly difficult due to the cryptic behavior of both adults and larvae (Williams *et al.* 1992), which are often concealed within their host fruit (including nut crops). In southern Australia, carpophilus beetles such as *C. davidsoni*, *C. hemipterus* and *C. mutilates* are pests of stone fruit. These species attack and feed on ripening fruit, which can result in substantial fruit losses (James and Vogele 2000).

There is surprisingly little published information on biocontrol of carpophilus beetle, or on natural enemies of these insects. Emekei and Moore (2015) provide a recent review of parasitoids and predators of *Carpophilus* species. Further than that, a review by Williams *et al.* (1984) describes hymenopterous parasitoids that attack beetles of the Nitidulidae family (to which *Carpophilus* belongs). One Nitidulidae parasitoid, *Brachyserphus abruptus* is being used to manage Nitidulidae pests worldwide, but not in Australia (Williams *et al.* 1992). In the USA, *B. abruptus* reduced the number of Nitidulidae larvae by 18% in strawberry farms (Williams *et al.* 1995). This species also parasitised sap beetle larvae in

sweet corn (Emekei and Moore 2015). In laboratory studies in the USA, *B. abruptus*, were successfully reared on three *Carpophilus* species (*C. hemipterus*, *C. freemani*, *C. lugubris*) and six other insect species. Field collections of *B. abruptus* were also made from *C. hemipterus*, *C. lugubris*, and four other species. This parasitoid does not exist in Australia, however. Given its lack of host specificity, it would be highly unlikely to be considered (and approved) for classical biological control of *Carpophilus* species in Australian almonds.

In Table 1, we present information on parasitoid wasp species (Hymenoptera) that have been shown to attack *Carpophilus* species around the world (collated from Williams *et al.* 1984). Of these, only the pteromalid wasp, *Anisopteromalus calandrae*, exists in Australia. *Anisopteromalus calandrae* attacks late larval stages and early pupae of beetles residing inside seeds and cocoons (Schöller *et al.* 2006).

Vertebrate frugivores may also contribute to the control of *Carpophilus* in orchards, particularly if they are attracted to (and prefer) fermenting fruits, which include those infested with larvae. In almonds, fallen mummy nuts that remain on the ground after harvest are a rich source of food and shelter for *C. nr dimidiatus* and carob moth, enabling pest populations to survive (and escalate) between growing seasons. Feeding by frugivorous vertebrates, particularly flocks of birds such as crows, cockatoos, galahs and regent parrots (frequently reported by growers), and even emus (as observed by the authors of this review), can lead to substantial reduction of mummies, and thus contribute significantly to improved orchard hygiene and pest control (Emekei and Moore 2015).

- *Entomopathogenic Fungi (EPF)*

Entomopathogenic fungi (EPF) have considerable potential for use as biocontrol control agents. These microbes naturally occur in soils throughout the world, acting as parasites and diseases of arthropod species. Indeed, EPF are the most abundant type of microorganisms that infect insects, with approximately 60% of insect diseases being caused by these fungal pathogens (Faria and Wraight, 2007). EPF can directly infect insects by penetration of their mycelium into the insect cuticle. The fungi then grow in the haemocoel of the attacked insect, eventuating in death (Pedrini *et al.* 2007; Gabarty *et al.* 2014). Dead insects act as sources of further fungal infestations, with fungal mycelium growing outward and then sporulating on the outer cuticle (Wakefield *et al.* 2013).

EPF in the genera *Beauveria, Metarhizium* and *Lecanicillium* have been commercially used in agriculture as mycopesticides: products based on living fungal propagules intended to control pests through inundative or inoculative applications (Faria and Wraight, 2007). A strain of *Beauveria bassiana* has recently been registered in Australia for use against thrips, whiteflys, aphids, and mites (APVMA, 2018). This particular strain (*Beauveria bassiana* strain PPRI 5339) was isolated from larva of the tortoise beetle, *Conchyloctenia punctate* (Coleoptera: Cassidinae) and can attack other beetles such the *Eucalyptus* snout beetle *Gonipterus scutellatus* (APVMA 2018). Another strain of *Beauveria bassiana* (strain AF-4), from the USA, was reported to cause 90% mortality of adult *Carpophilus lugubris* beetles within three days of exposure in field studies (Dowd and Vega 2003). This review found no record of the use of *B. bassiana* (or any other mycopesticide) for control of other *Carpophilus* pests, nor field studies on any *Carpophilus* species in Australia, which represents an important knowledge gap in a potential new control technology for this pest.

Adoption of EPF for insect pest management remains relatively low (Skinner *et al.* 2014). A number of constraints may be responsible for the low adoption rates (see Sinha *et al.* 2016), including i) the short shelf-life of viable inoculum, ii) the lag period (2–3 weeks) that is generally required to kill the target insects, iii) Incompatibility with other IPM tactics, such as use of fungicides and arthropod biological control agents, (iv) EPF sensitivity to environmental (especially climatic) conditions, such as relative humidity and UV light, and (v) suitability for mass culture and availability of commercial formulations.

Entomopathogenic fungi have recently been developed as plant endophytes. This has the advantage of overcoming climatic obstacles such as susceptibility to ultraviolet (UV) light and low moisture (Jaber and Ownley 2018; Vega 2018). EPF can not only survive in host plants but play critical roles in endophytism, plant disease antagonism, plant growth promotion, and rhizosphere colonization (Vega 2008). EPF could be inoculated directly into host plants to protect them against pests. Various inoculation techniques (e.g., foliar sprays, soil drenching, seed soaking, injections) have been developed to introduce EPF into plants. Inoculation of beneficial EPF has been so far attempted in thirty-eight plant species in 19 families (Vega, 2018), and could be explored further as a possible pest management tool for *Carpophilus* beetles in almonds.

- *Entomopathogenic Nematodes (EPNs)*

Nematode species in more than 30 families are associated with insects and other invertebrates (Kaya and Stock 1997). The major focus of research and development has been on nematode species in seven families, Mermithidae, Tetradonematidae, Allantonematidae, Phaenopsitylenchidae, Sphaerulariidae, Steinernematidae, and Heterorhabditidae, through their potential application as biological control agents of insects (Kaya and Stock 1997). Indeed, several (EPNs) have been used to control several species of agricultural pests (Georgis 2006). Commercial successes are documented in crops such as citrus (*Diaprepes* root weevil) (McCoy *et al.* 2002), greenhouses and glasshouses (black vine weevil) (van Tol *et al.* 2006). The juvenile nematodes infect insect hosts by penetrating the hemocoel through mechanical and enzymatic action. Shortly after entry into their host, the nematodes release symbiotic bacteria and together overcome the host immune system, which rapidly leads to death. The nematodes feed upon the rapidly multiplying bacteria, reach sexual maturity, mate, and produce two or more generations within the insect cadaver before emerging as infective juveniles in search of fresh hosts (Glazer *et al.* 1999).

EPNs have potential for biocontrol of *Carpophilus* in almonds. Steinernematid and heterorhabditid nematodes have been found to effectively reduce sap beetle larval populations in laboratory trials and the field (Vega *et al.* 1994; Glazer *et al.* 1999). *Steinernema riobravis,* which is distributed throughout North, Central and South America*,* caused high mortality suggesting that commercial products of this nematode could potentially be used against *Carpophilus* species (Vega *et al.* 1994). Table 5 summarises information on EPNs that attack nitidulid beetles. Importantly, two species of steinernematid (*S. feltiae* and *S. glaser*) are present in Australia (Table 5). Field tests with four strains of *Heterorhabditis* applied at the rate of 100 infective juveniles (IJs)/cm^2 in a date palm orchard in Israel failed to suppress adult emergence of *U. humeralis*, while a similar test applied at 75 and 150 Ijs/cm^2 in a fig orchard yielded a reduction in adult emergence at both concentrations by 50–70%. A mermithidnematode parasite, *Hexamermis* sp., which can induce infection rates of up to 89%, was found promising in controlling adult dusky sap beetles, *C. lugubris*, in strawberry fields. However, as these

nematodes are difficult to mass produce, conservation methods may be more appropriate for mermithids than augmentation (Emekei and Moore 2015).

4.5 Tabulated information on biocontrol agents for carob moth and carpophilus beetles.

Table 1. Parasites and predators that have been reported to attack carob moth in the published literature, including species kown to be present in Australia. Green text (+) indicates species occurring in Australia; blue text (*) = genus present in Australia.

Order	Family	Species	Distribution and description	Additional notes	Literature source
HYMENOPTERA	BETHYLIDAE	*Goniozus** *gallicola* Fouts	Israel: egg parasitoid	Parasitisation of carob moth on acacia pods was extremely low	Neunzig 1979 cited in Perring *et al.* 2015; Gothlif 1969
		*Goniozus** *legneri* Gordh	Israel: egg parasitoid	Up to 30.8% parasitisation	Aleosfoor *et al.* 2014 Gothilf and Mazor 1987 Zaviezo *et al.* 2007; Ehteshami *et al.* 2013 cited in Perring *et al.* 2015
		*Goniozius** *emigrata (syn. Perisierola emigrate)* Rohwer	Hawaii: egg parasitoid		Bridwell 1919a cited in Gothlif 1969; Thompson 1946; Zimmerman 1958 cited in Perring *et al.*2015
	BRACONIDAE	*Apanteles** *angaleti* Muesebeck	Iraq: larval parasitoid		Al Maliky and Al Izzi 1990 cited in Delvare *et al.* 2011
		*Apanteles** *lacteus* Nees	Israel: larval parasitoid		Gothlif 1969
		*Apanteles** *laspeyresiella* Papp	Larval parasitoid		Norouzi *et al.* 2009 cited in Perring *et al.* 2015
		*Apanteles** *myeloenta Wilkinson*	Turkey, Cyprus, Iran: larval parasitoid	41 - 77% relative frequency of parasitoid species. *A. myeloenta* was the most prevalent species among the collected parasitoids in this 2-year study. Highest rate of parasitism (6.8%).	Kishani-Farahani *et al.* 2012; Farahani *et al.* 2012; Farahani and Goldansaz 2013 Anon 1953 cited in Gothlif 1969 Haeselbarth 1983 cited in Delvare *et al.* 2011 Sobhani *et al.* 2015
		*Apanteles** *sp.*	Israel, Cyprus	Found in small numbers on carobs	Gothlif 1969 Anon 1953 cited in Gothlif 1969
		*Apanteles** *ultor*	Iraq: larval and pupal parasitoid	10 – 50% of host larvae parasitised. Most dominant and widely distributed parasite of *E. ceratoniae* in Iraq.	Al-Maliky and Al-Izzi 1986 Al-Maliky *et al.* 1988

		*Ascogaster** *sp.*	Iraq: pupal parasitoid	Small numbers on carob moth, collected from pomegranate.	Al-Maliky and Al-Izzi 1986
		Habrobracon hebetor (Say)(synonyms: *Bracon hebetor*+ Say, *Habrobracon** *brevicornis* Wesmael) Note: This species is found on the AFD species list (ABRS 2009) as *Bracon hebetor* Say. There are some records of *Habrobracon hebetor* in Australia according to the APPD but it is not clear where those specimens were collected in Australia and could not find literature to support its presence in Australia.	Larval parasitoid Morocco, Cyprus, Iran: larval parasitoid	Found in small numbers on carobs 14 - 43% relative frequency of parasitoid species, over 2 years. Small numbers on carob moth, collected from pomegranate. *B. hebetor* preferred *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) over *Apomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae).	Gothlif 1969 Thompson 1946 cited in Gothlif 1969 Kishani-Farahani *et al.* 2012 Anon 1953 cited in Gothlif 1969 Al-Maliky and Al-Izzi 1986 Bouka *et al.* 2001 cited in Delvare *et al.* 2011 Saadat *et al.* 2014, 2016.
		*Bracon** *lactus* Wesmael (Mediouni-BenJemaa 2005			Cited in Madge 2013
		*Bracon** *mellitor* Say	Larval parasitoid		Nay 2006 Bridwell 1919b; Thompson 1946 cited in Perring *et al.* 2015
		*Chelonus** sp.	Larval parasitoid		Farahani *et al.* 2010c cited in Perring *et al.* 2015
		Hypomicrogaster suffolciensis Morly	Larval parasitoid		Cited in Madge 2013
		Bracon! *melitor (syn. Microbracon pembertoni)* Bridwell	Hawaii: larval parasitoid		Bridwell 1919b cited in Gothlif 1969 Zimmerman 1958 cited in Perring *et al.* 2015
		*Phanerotoma** *carobivora* van Achterberg and Thackeray	South Africa: larval parasitoid	Mean percentage of parasitism varied 2-30% between host plants and sampled localities. Study on Citrus and Pecans.	Van Achterberg *et al.* 2017
		*Phanerotoma** *dentata* Panzer	Israel,Cyprus: larvae larval parasitoid		Thompson 1946; Anon 1953 cited in Gothlif 1969 Aubert 1966 cited in Delvare *et al.* 2011

		Phanerotoma leucobasis* Kriechbaumer *(Syn. Phanerotoma flavitestacea* Fischer; *Phanerotoma ocularis* Kohl)	Egypt, Morocco, Israel: egg parasitoid	0 to 37.7% parasitisation in carobs.	Gothlif 1969 Gothlif 1978 Madkouri 1978; Biliotti and Daumal 1970; Daumal *et al.* 1973 cited in Perring *et al.* 2015 Doumandji-Mitiche and Doumandji 1982 cited in Perring *et al.* 2015 Bouka *et al.* 2001; Mesbah *et al.* 1998; Gothilf 1969b, respectively, cited in Delvare *et al.* 2011 Khoualdia *et al.* 1996; Bouka *et al.* 2001 cited in Perring *et al.* 2015
		Phanerotoma sp.*	Iraq: larval parasitoid	Small numbers on carob moth, collected from pomegranate.	Al-Maliky and Al-Izzi 1986 Thompson 1946 cited in Gothlif 1969
		Rhogas testaceus Reinch (Spinola)	Cyprus: larval parasitoid		Anon 1953 cited in Gothlif 1969
	CHALCIDIDAE	*Antrocephalus mitys*+ Walker (synonyms: *Halticella mitys* Walker, *Stomatoceras bergeraci* Girault) Note: *Stomatocerus bergeraci* was recorded in Australia in Girault 1921 and is recorded in the AFD. But there are no records of *Antrocephalus mitys* from the APPD.	Israel: larval and pupal parasitoid	Found in small numbers on carobs	Gothlif 1969 Girault 1921
		Brachymeris sp.	Iraq: pupal parasitoid	Small numbers on carob moth, collected from pomegranate.	Al-Maliky and Al-Izzi 1986
		Brachymeria aegyptiaca* Masi	Israel Iraq: pupal parastiod	Found in small numbers on carobs, collected from pomegranate.	Gothlif 1969; Al-Maliky and Al-Izzi 1986; Masi 1931 cited in Delvare *et al.* 2011
		Brachymeria ceratoniae* Delvare	Pupal parastiod		Delvare *et al.* 2011 cited in Perring *et al.* 2015

		Proconura persica Delvare	Pupal parastiod		Delvare *et al.* 2011 cited in Perring *et al.* 2015
		Psilochalcis ceratoniae Delvare	Pupal parastiod		Delvare *et al.* 2011 cited in Perring *et al.* 2015
	ENCYRTIDAE	*Copidosomopsis** *plethorica* Caltagirone	Israel: egg parastiod	2 to 3 years following release in almonds parasite found established with 12-15% total emergence. - In carob plantations, 3 to 5 years after release parasitoid not recovered.	Gothilf 1978; Gothilf and Mazor 1987
	EULOPHIDAE	*Pedobius* sp.	Larval parasitoid		Cited in Madge 2013
	FORMICIDAE	*Pogonomyrmex californicus* (Buckley)	USA, California: larval predator	The predation of carob moth by the native ant species in the USA has been recorded, the California harvester ant, *P. californicus.*	Nay and Perring 2005 cited in Perring *et al.* 2015
		*Solenopsis** *aurea* Wheeler	USA, California: larval predator	The predation of carob moth by the native ant species in the USA has been recorded, the fire ant *S. aurea.*	Nay and Perring 2005 cited in Perring *et al.* 2015
	ICHNEUMONIDAE	*Campoplex** *tumidulus* Grav.	Iran: larval parasitoid	6 - 12% relative frequency of parasitoid species, identified from one site (out of 3) over 2-year study.	Kishani-Farahani *et al.* 2012
		*Diadegma** sp.	Larval parasitoid	Known natural enemy of carob moth at low densities in Australia.	Gothilf and Mazor 1987. Gothilf 1969a; cited in Perring *et al.* 2015
		*Diadegma** *oranginator* Aubert	Egypt, Israel		Aubert 1964 cited in Delvare *et al.* 2011 Aubert *et al.* 1984 cited in Delvare *et al.* 2011
		Herpestomus arridens Gravenhorst	Israel: larval parasitoid	Parasitisation on acacia pods was extremely low	Gothlif 1969
		*Pristomerus** *vulnerator* Panzer	Israel: larval parasitoid	Found in small numbers on carobs	Gothlif 1969
		*Temelucha** *decorate* (Grav.) Cremastinae	Iran: larval parasitoid	3 - 22% relative frequency of parasitoid species, over 2-year study.	Kishani-Farahani *et al.* 2012
		Venturia canescens+ (Gravenhorst) (synonym *Nemeritis canescens*)	Iraq, Israel, Iran: larval parasitoid	3 - 29% relative frequency of parasitoid species, over 2-year study.	Kishani-Farahani *et al.* 2012

		Note: Records of this species in the APPD and ALA. Also listed in the AFD.			Aubert *et al.* 1984 cited in Delvare *et al.* 2011 Al-Maliky and Al-Izzi 1986 cited in Delvare *et al.* 2011
	PTEROMALIDAE	*Anisopteromalus calandrae** *(Syn. Anisopteromalus mollis* Ruschka; *Pteromalus calandrae* Howard; *Neocatolaccus australiensis* Girault; *Pteromalus oryzae* Cameron) Note: Records of this species are found in the APPD (from NT and Tas). Also listed in the AFD.	Israel: larval parasitoid	Found in small numbers on carobs	Gothlif 1969
	TRICHOGRAMMATIDAE	*Trichogramma** sp.	Israel: egg parasitoid	Hundreds of carob moth eggs were collected in carob plantations, only three of these eggs were parasitised by *Trichogramma* sp.	Gothlif 1969
		*Trichogramma** *bourarachae* Pintureau and Babault	Tunisia: egg parasitoid	Pomegranate Collected on *E. ceratoniae* eggs in the field. No parasitism under laboratory conditions.	Herz *et al.* 2007 Ksentini *et al.* 2013
		*Trichogramma** *brassicae* (Bezdenko)	Iran: egg parasitoid	Pomegranate Mass-produced *Trichogramma brassicae* (Bezdenko) began in Iranian pomegranate orchards in the 1990s but have not yet had satisfactory effects.	Moezipour and Shojaei 2008 cited in Mirjalili and Poorazizi 2015 Poorjavad *et al.* 2011
		*Trichogramma** *cacaeciae* Marchal	Tunisia: egg parasitoid	Pomegranate Date palms Collected on *E. ceratoniae* eggs in the field. *T. cacoeciae* accepted eggs of *E. ceratoniae* under laboratory conditions.	Herz and Hassan 2006 Pizzol *et al.* 2005 Ksentini *et al.* 2013
		*Trichogramma** *cordubensis* Vargas and Cabello	Algeria, Morocco: egg parasitoid	64% parasitism rate	Herz *et al.* 2007 Idder *et al.* 2013 cited in Perring *et al.* 2015.

		*Trichogramma** *embryophagum* Hartig	Iran: egg parasitoid	Pomegranate The Qum strain of *T. embryophagum* was the most promising candidate to be considered as a biocontrol agent against *E. ceratoniae*.	Poorjavad *et al.* 2014 Mirkarimi 2000; Doumandji-Mitiche and Doumandji 1982; Doumandji- Mitiche and Idder 1986; Karami *et al.* 2011 cited in Perring *et al.* 2015
		*Trichogramma** *evanescens* Westwood	Tunisia: egg parasitoid Iran: egg parasitoid	Collected on *E. ceratoniae* eggs in the field. *T. evanescens* accepted eggs of *E. ceratoniae* under laboratory conditions (but to a lesser extent than T. cacoeciae). Pomegranate	Ksentini *et al.* 2013 Poorjavad *et al.* 2014
		*Trichogramma** *oleae* (Voegele & Pointel)	Tunisia: egg parasitoid	Collected on *E. ceratoniae* eggs in the field. No parasitism under laboratory conditions.	Ksentini *et al.* 2013
		*Trichogramma** *principium* Sugonyaev & Sorokina	Iran	Pomegranate	Poorjavad *et al.* 2011
DIPTERA	TACHINIDAE	*Clausicella suturata* Rondani	Southern Europe, Israel: larval parastiod	Internal solitary parasite 0 to 29.2% parasitisation in carobs. Parasitisation on acacia pods was extremely low.	Gothlif 1969; Gothlif 1978 Kugler and Nitzan 1977 Farahani *et al.* 2009 cited in Perring *et al.* 2015
		Fischeria bicolor Robineau-Desvoidy	Larval parastiod		Farahani *et al.* 2009; 2010c cited in Perring *et al.* 2015
ACARI	ACEOSEJIDAE	*Melichares tarsalis* Berlese (oophage*)	Egg predator		Cited in Madge 2013
	BLATTISOCIIDAE	*Blattisocius tarsalis*+ (Berlese)	Libya: egg predator		Bitaw *et al.* 1988 cited in Perring *et al.* 2015
	PYEMOTIDAE	*Pyemotes* (*Pediculoides*) *ventricosus*+ Newport	Israel: larval parasites	Parasitisation on acacia pods was extremely low (two instances feeding on carob moth larvae). Only one larva parasitised by the mite in citrus.	Gothlif 1969
HEMIPTERA	ANTHOCORIDAE	*Buchananiella** *continua* B. (oophage)	Predators		Cited in Madge 2013
		Cardiasthetus fasciiventris Garb. (oophage)	Predators		Cited in Madge 2013

		Cardiasthetus nazarenus Reuter (oophage)	Predators		Cited in Madge 2013

Table 2. **Entomopathogenic nematodes (EPNS) used as biological control agents against the carob moth.** Green text (+) indicates species occurring in Australia

Nematode species	Mortality Rates	Additional notes	Literature source
Heterorhabditis bacteriophora+	LC50 value of 426.92 IJ/larva (low virulence)	Laboratory trial-2cm diameter plates.	Memari *et al.* 2016
Steinernema carpocapsae	76.5%; LC50 value of 2.05 IJ/larva 26.65% mean mortality rate	Laboratory trial-2cm diameter plates. Field test, inhibitory/repellence effects in the field of saprophytic fungi within the infested pomegranates.	Memari *et al.* 2016
Steinernema feltiae	79.75% Mortality Rates; LC50 value of 2.02 IJ/larva 10.89% mean mortality rate	Laboratory trial-2cm diameter plates. Field test, inhibitory/repellence effects in the field of saprophytic fungi within the infested pomegranates.	Memari *et al.* 2016

Table 3. Parasitoid wasps (Hymenoptera) of *Carpophilus species.* Extracted from (Williams *et al.* 1984). Green text (+) indicates species occurring in Australia; blue text (*) = genus present in Australia.

FAMILY	*Genus species*	Plant and host insect species	Distribution and additional notes	Literature source
BETHYLIDAE	*Pseudisobrachium foutsi (Syn. Pseudisobrachium flavinervis* Fouts)	Dried fruit beetle: *Carpophilus hemipterus*	USA	Cotton and Good 1937; Fouts 1928; Simmons *et al.* 1931 (cited in Williams *et al.* 1984); Azevedo 2008.
BRACONIDAE	*Peristenus nitidus (Syn. Microctonus nitidulidis* Loan)	Strawberries and other fruits: Sap beetles, *Stelidota geminata* (Say)	USA: Adults Parasitoids were introduced to Israel from the USA. No evidence of their establishment was ever recorded.	Blumberg 2008 Connell 1980; Weiss 1979; Weiss and Williams 1979a; Weiss and Williams 1979b; Weiss and Williams 1980; Weiss *et al.* 1978 (cited in Williams *et al.* 1984)
ENCYRTIDAE	*Cerchysiella** *abilis* (Syn. *Zeteticontus abilis* Silvestri)	*Carpophilus* sp., *C hemipterus* (L.)	Australia, Central America, Eritrea, Europe, Ghana, Hawaii Kenya, Pakistan, South America Seycheles, West Indies	De Santis 1964, Ghesquiere 1951; Silvestri 1915; Thompson 1943 (cited in Williams *et al.* 1984)
	*Cerchysiella** *insularis* (Syn. *Zeteticontus insularis* Howard)	*Lobiopa insularis* (Castelnau) *Carpophilus* sp. and several Nitidulid larvae	Argentina, Dominica Grenada USA Trinidad	Bennett and Baranowski 1981; De Santis 1964; De Santis 1967; Kozlov 1971; Noyes 1979; Parker *et al.* 1953; Subba Rao 1972; Taniguchi 1977; Yaseen 1976 (cited in Williams *et al.* 1984)
	*Cerchysiella** *utilis (Syn. Zeteticontus utilis* Noyes)	*Carpophilus hemipterus* and *Carpophilus mutilatus* *Carpophilus hemipterus*	Israel: larval parasitoids. *Z. utilis* was always quite rare in the field, its contribution to the reduction of sap beetles is probably not significant. Israel, Kenya.	Blumberg *et al.* 1984. Blumberg 2008. Gerling and Ben-Mordechai 1981; Noyes 1982; Taniguchi 1977 (cited in Williams *et al.* 1984)
ICHNEUMONIDAE	*Allophrys* sp.	Several Nitidulid larvae	Trinidad	Horstmann 1970a; Horstmann 1970b; Horstmann 1971; Horstmann 1981 (cited in Williams *et al.* 1984)
PROCTOTRUPIDAE	*Brachyserphus abruptus* (Say)	*Stelidota strigosa* (Gyllenhall)	Brazil, Canada, Costa Rica, Mexico, USA:	Ashmead 1893; Brues 1916; Eastham 1929; Krombein *et al.* 1979; Muresebeck *et al.* 1951; Riley and Howard 1892; Ruhl 1921; Townes and Townes 1981;

				Washburn 1918; Williams 1932 (cited in Williams *et al.* 1984).
		Date palm, Strawberries Sweet corn: *Carpophilus hemipterus* (L.), *C. freemani* Dobson, *C. lugubris* Murray, *Stelidota geminata* (Say), *S. octomaculata* (Say), *S. ferruginea* Reitter, *Glischrochilus quadrisignatus* (Say*), Lobiopa insularis* (Casteinau), and *Haptoncus luteolus* (Erichson).	Percent parasitism in 1st instar *C. hemipterus* averaged 65% and for 2nd instar 45%. After parasitism, larvae of *C. hemipterus* surviving to become adults averaged 0.6% for 1st instar, 9.3% for 2nd instar, and 90% for 3rd instar. Has potential as a biological control agent, since it can be cultured and produced in large numbers on nitidulids reared on artificial diet. It appears in the field too late to be of value in some cases. Only nitidulid parasitoid, *B. abruptus*, being used in pest management programs (R. N. Williams, pers. comm.) (Vega *et al.* 1994). Parasitoid of larvae were introduced from the USA to Israel. No evidence of their establishment was ever recorded. 18% parasitism	Williams *et al.* 1992. Vega *et al.* 1994 Blumberg 2008 Williams *et al.* 1995 Alston *et al.* 2014 (cited in Emekei and Moore 2015)
PTEROMALIDAE	*Anisopteromalus calandrae*[+] Howard	*Carpophilus obsoletus* Er. - Stored grain	Formosa	Cotton and Good 1937; Okuni 1928 (cited in Williams *et al.* 1984).

Table 4. Entomopathogenic nematodes (EPNS) which attack members of Nitidulidae Green text (+) indicates species occurring in Australia; blue text (*) = genus present in Australia.

Nematode species	Insect host	Distribution and additional notes	Literature source
Heterorhabditidis sp.	*Urophorus humeralis*	Nematodes applied at 100 IJs/cm^2 in the laboratory resulted in a drastic reduction in emergence of the beetles (70 - 90%). Applications in date palm orchards resulted did not impact insect emergence. Application in fig orchards resulted in 50 - 70% reduction in insect emergence.	Glazer *et al.* 2007.
Heterorhabditis sp. with the	*Urophorus humeralis* *Carpophilus hemipterus*	Laboratory applications of different strains of *heterorhabditid* to beetles resulted into moderate levels of mortality (35 - 65%). The IS -12 strain showed poor virulence (< 35% mortality) against larvae of *U. humeralis* as well as larvae and pupae of *C. humipterus*.	Glazer *et al.* 1999.
Steinernematid sp. 1. *S. carpocapsae* Weiser (All strain) 2. *S. feltiae*+ (= bibionis) (Filipjev) (SN strain) 3. *S. glaser*+ Steiner (biosys 326) 4. *S. riobravis* (biosys 355)	*Carpophilus hemipterus*	For *S. carpocapsae*, no differences in larval mortality, only IJ concentrations of 400 per larva caused a significantly higher larval mortality of approximately 13%. For *S. feltiae*, larval mortality ranged from 6 to 17%. *S. glaseri*, there were no significant differences and the mortality levels were below 6% for all IJ concentrations tested. *S. riobravis* caused 80 to 88% larval mortality at 200 and 400 IJs per larva.	Vega *et al.* 1994.
Howardula truncata	*Carpophilus* sp.	Susceptible host stage: Grubs	Rukminidevi and Rao. 1982 (cited in Rahaman *et al.* 2000.)
Howardula multilatus	*Carpophilus* sp.	Susceptible host stage: Grubs	Devi *et al.* 1991. (cited in Rahaman *et al.* 2000.)

5.0 Part Two: Field survey of natural enemies of almond pests

5.1 Methods

Natural enemy surveys were conducted in May 2018 to identify insect species that would be candidates for conservation biocontrol of carob moth and carpophilus beetles. The surveys took the form of (i) field trapping of insects and (ii) collection of infested mummy nuts for nut examination and insect emergence tests. Two trap designs, passive traps for airborne insects and sticky traps, were used for field trapping. The passive traps were a modification of Macquarie Island traps, which have been shown to collect comparable numbers of insects to water and sticky traps (Farrow and Greenslade 2013). Both traps are suitable for diverse insect groups and have been recommend specifically for wind-dispersed insects where they are currently being used in surveillance operations in Australia (Finlay *et al.* 2018). Mummy nuts were harvested to sample for juvenile/immobile parasitoids that might not be caught in traps designed for air-borne insects.

- *Field trapping*

Insect traps were set up at four sites/orchards, two in South Australia (SA) (Renmark and Loxton) and two in Victoria (VIC) (Robinvale and Nangiloc). The orchards in Renmark and Nangiloc are relatively small with cover crops (ryegrass and clover) as part of the cropping system, while the orchards in Loxton and Robinvale are much larger and predominantly monocultures (Table 5). Pairs of traps (Macquarie Island trap and sticky trap) were set up at each location. Macquarie Island traps ware deployed 2.2 to 2.4 m above the ground on a star picket (Fig. 1a). Trapped insects were collected into an insect chamber filled will a killing solution (40-50% propylene glycol and one tablespoon of borax). Sticky traps comprising three transparent A4 acetate sheets covered with an insect adhesive (*Tanglefoot*) were positioned at regular intervals on a 4 m high post placed in close proximity to mummy nuts on the Nonpareil variety of almond trees (Fig. 1b). Nonpareil is the dominant commercial variety of almonds. This variety has a softer shell compared to other varieties and consequently higher levels of pest infestation compared to hard-shelled varieties such as Carmel. Traps were placed in close proximity to Nonpareil trees to maximize chances of trapping pests and natural enemies. Insect sampling was conducted over a one-month period and traps were serviced weekly: samples were collected from insect chambers of Macquarie Island traps and acetate sheets containing trapped individuals collected from sticky traps and replaced with fresh sheets. Insects were removed from sticky traps by soaking field collected acetate sheets in kerosene to dissolve the tanglefoot, although this was later found not to be the best method due to degradation of DNA. Sheets were soaked for a maximum of two minutes after which invertebrates were then rinsed in ethanol and placed in 1.5 ml Eppendorf tubes filled with 100% ethanol. Insect identification was carried out by insect diagnosticians at the AgriBio Centre, Bundoora, Victoria using molecular and morphological techniques.

Table 5. Locations and descriptions of orchards surveyed for natural enemies and pests of carob moths and carpophilus beetles.

Location	GPS data	Orchard characteristics	Cover Crop: Notes
Renmark, SA	34°13'9.38"S; 140°42'27.28"E	• < 50 ha • Sprinkler irrigation	Ryegrass / clover, recently seeded 2-3 weeks prior to trap deployment-c.a. 25% ground cover.
Loxton, SA	34°28'7.35"S; 140°38'21.57"E	• 640 ha • Sprinkler irrigation • Almond Varieties: Nonpareil, Carmel, Price, Peerless and Monterey	None
Robinvale, VIC	34°45'34.85"S; 142°56'36.99"E	• 800 ha • Drip irrigation • Nonpareil, Carmel	None
Nangiloc, VIC	34°30'20.73"S; 142°20'27.46"E	• 180 ha • Sprinkler irrigation	Ryegrass / clover

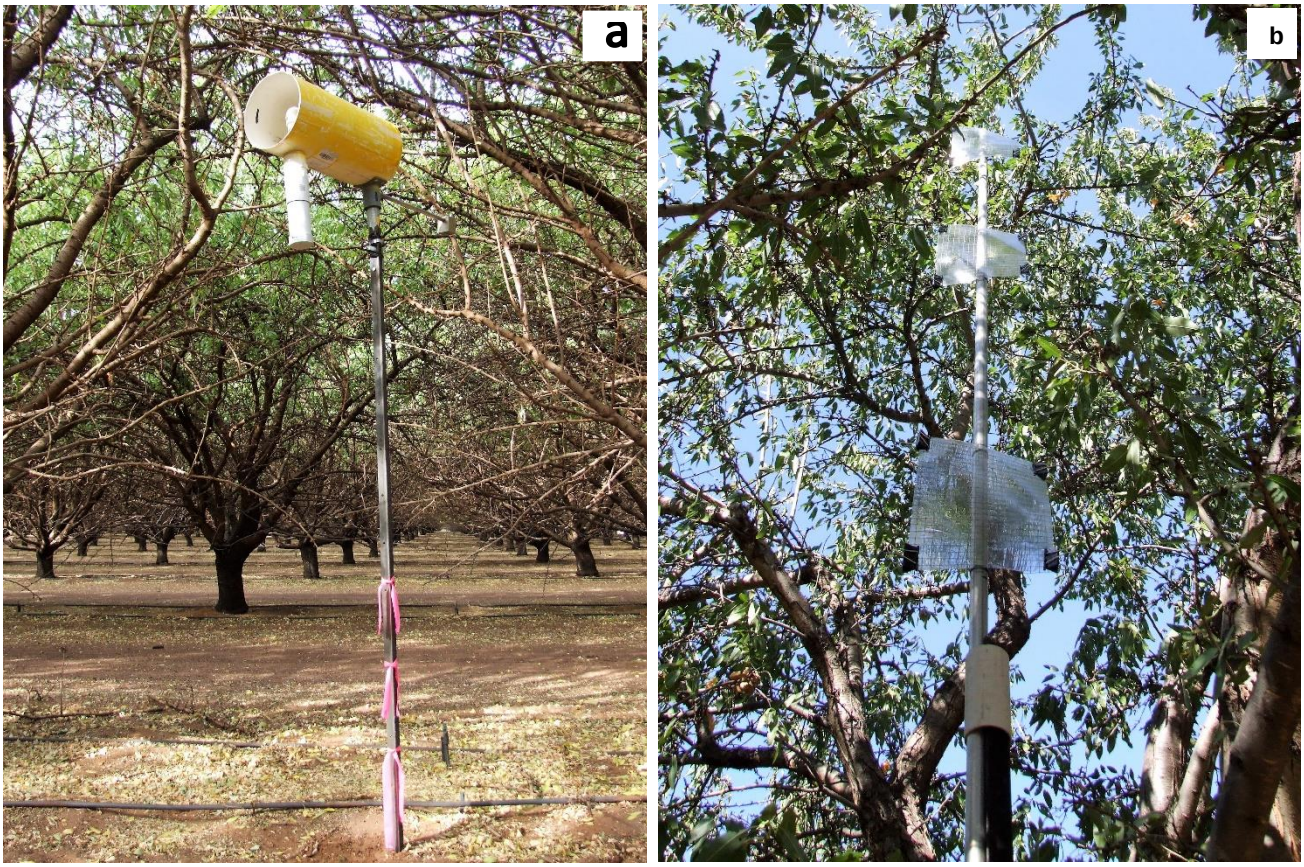

Figure 1. Trapping methods used to sample natural enemies and pests of carob moth and carpophilus beetles in surveyed orchards. (a) A modified Macquarie Island trap was used to trap air-borne insects that passively flew into the yellow cylinder at the top and collected in the attached white insect chamber filled with an insect killing solution. (b) Sticky traps trapped and retained air-borne insects by means of an adhesive applied on A4 acetate sheets. (Photos: D. Clements, AVR).

- *Harvesting mummy nuts*

Mummy nuts were collected from trapping sites on two sampling occasions (i) upon trap deployment date and (ii) two weeks after trap deployment. One hundred and fifty mummy nuts were collected per collection date and location, sampling 50 nuts at two canopy heights: high (≈ 75% up in the canopy), low (≈ 25% up into canopy), and 50 nuts on the ground. An extendible pole was used to harvest mummy nuts from high elevations (by gently knocking nuts from trees). Ground collections were made directly beneath the trees being sampled. All collections were obtained from the same row of trees in which traps were deployed. Prior to the second collection, a subsample (n = 10) of mummy nuts from each site and elevation were destructively sampled to confirm infestation by the two pests. Trees near traps were not sampled to minimize interference with traps. Collected mummy nuts where kept separate by canopy height, date and site of collection in transparent disposable plastic containers (22 x 11 x 16 cm), thus six plastic containers per site. Mummy nuts collected from each height were divided into two and kept in separate paired containers (25 nuts per container) before being transferred into cages in controlled environment rooms (25°C, RH = 61%, 16:8 L:D) at the AgriBio Center, Bundoora, Victoria.

- *Rearing and emergence trials*

Containers of mummy nuts were covered with a fine cloth mesh (0.3 mm) and placed into four *Bugdorm* rearing cages (76 x 29 x 28 cm), one rearing cage per site. All cages were fitted with a sticky yellow trap to collect emerging insects (Fig. 2). Cages were checked every two to three days for emergence of larvae and adults. Insects that emerged were collected and preserved in ethanol (70-100%) for identification. Emergence trials were terminated after one month (when all carob moth and *Carpophilus* beetle adults were expected to have emerged). At the end of each trial, mummy nuts were either destructively sampled or gently tapped on a bench top to dislodge any insect stages that had not emerged into the cage.

In addition to these mummy nut surveys, potential biocontrol agents were also collected during other research activities. Eight wasps emerging from mummies which were collected during establishment of a carob moth culture, three wasps were collected from mummies that were placed in tullgren funnels as part of the previous Agriculture Victoria *Carpophilus* project (AL15004; Hossain 2018), and one wasp was collected during destructive sampling of mummy nuts.

Figure 2. Rearing and emergence cages used in mummy nut surveys. (A) Rearing containers from each site were placed inside cages (six containers per cage) and insect emergence was monitored. (B) Rearing containers were covered in 0.3 mm cloth mesh to prevent escape of small parasitoids (especially egg parasitoids). Photos: D. Clements, AVR.

- *Morphological and molecular Identification*

Wasp specimens collected on sticky traps and reared from mummies in cages were first sorted from all other Orders using a stereo microscope. The aim was to acquire DNA barcoding sequence data for each individual wasp and to identify these to family level (at least) using molecular methods. Molecular methods were employed primarily due to the specimens being in relatively poor condition (often as a result of removal from sticky traps) and difficult to identify based on morphological traits. Also, due to the numbers of insects collected and time constraints, a molecular approach was considered the most time effective method to estimate the range of families collected: many of the wasp families require specialist consultation for morphological identification to genus and species, as the wasp families can be very diverse and speciose, often with very similar looking species in some groups or very tiny specimens that require slide mounting and careful examination under a compound microscope. As this takes considerable time for each insect, it was not always possible in the timeframe of this study.

Wasp specimens examined morphologically were sorted to morphotaxa. These insects were grouped depending on the external features: mainly body shape, size and colour, antennal segments, wing shape and venation, following characteristics in Naumann (1991), Goulet & Huber (1993). Once families and tentative genera could be matched to specimens through DNA sequence data, those specimens were re-examined morphologically to verify they concurred with the purported families.

- *Barcoding Methods*

Three wasp specimens were obtained from cage emergence experiments (mummy nuts) and around 50 were removed from the sticky traps. We used standard DNA barcoding methods (Ratnasingham & Hebert 2007). DNA extraction, PCR amplification, & DNA sequencing DNA was extracted from ethanol preserved wasps. Genomic DNA was extracted from ethanol-preserved specimens, usually from a leg (destructively) or from the whole body (non-destructively) when insects were smaller then 1 mm or in particularly poor condition. Samples were then returned to 100% ethanol and kept for morphological identification studies. DNA was extracted using DNeasy kit (Quiagen) as per the manufacturer's protocol. PCR was conducted in 25 µL volume containing 2 µL of DNA, 0.2 µL Taq Polymerase, 1.25 µL 10 µM primers, 2.5 µL 2.0 mM dNTPs and 2.5 µL 10X buffer. The primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') was used (Folmer *et al.* 1994) and amplification conditions were 1 cycle, 95 °C (5 min), 40 cycles, 94 °C (45 s), 49 °C (45 s), 72 °C (45 s), and 1 cycle 72 °C (1 min). Specimens that did not initially amplify were re-tested using LCO1490/HCO2198 primers in combination with internal wasp specific primers (Meyer & Hoy 2007). PCR products were identified on 2% agarose gel electrophoresis with SYBR Safe staining under UV light. Product was sent

to Macrogen (Korea) for purification and sequencing. Universal DNA primers LCO 1490 and HCO 2198 were originally designed from three coding and six anticoding strands by comparing highly conserved regions of mitochondrial cytochrome c oxidase subunit I (COI) genes across taxa. These primers have been successful in amplifying a 710-bp fragment of highly conserved regions of the COI gene for more than 80 invertebrate species from 11 phyla. Resulting sequence reads were blasted against BOLD database (http://www.boldsystems.org/libhtml/docs/bold.pdf) for matching sequences. Individual insects were barcoded to family level.

5.2 Results

Macquarie Island traps did not catch any natural enemies over the sampling period, and thus results from these traps are not included in this report. Sticky traps caught an abundance and diversity of invertebrates (total catch = 446 insects, summarised in Fig. 3), with many fly species (Diptera) and a number of potentially beneficial wasps (Hymenoptera). Carpophilus beetle and carob moth were not caught by these traps. Significantly fewer insects were caught at the Loxton site compared to Robinvale, Nangiloc and Renmark ($P < 0.005$, χ^2 test, Fig. 3). Flies (Diptera) comprised over 50% of the total catch in all orchards.

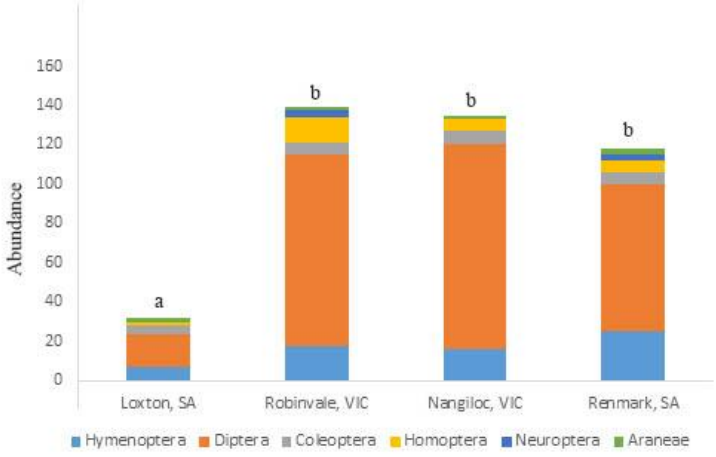

Figure 3. Insect abundance and diversity across orchards. Total number of insects caught on the yellow sticky traps over the duration of the trial. Identified to order level. Significantly fewer insects were caught at Loxton, compared to the other three sites ($P < 0.005$, χ^2 test), bars with common letters are not significantly different.

Results of molecular and morphological diagnostics are presented in Table 6, together with molecular phylogenies (Fig. 7). Identification of trapped insects focused mainly on Hymenoptera, as this family is known to include many species that are natural enemies (parasitoids and predators) of insect pests. A small number of generalist predators including spiders (Aranae) and lacewings (Neuroptera) were identified and included in the analysis. Traps in Renmark and Robinvale captured Neuroptera (lacewings) (Fig 3. Table 6) in addition to the Hymenoptera (wasps) and Homoptera (that were found in the other orchards. The high proportion of beneficial Hymenoptera found on the Renmark property may be due to orchard management practices, and perhaps the availability of understory nectar provided by local flowering plants.

- *Results from emergence trials*

Cage rearing experiments yielded a total of 158 separate samples. A higher proportion nuts infested by carpophilus beetle vs carob moth was found at Nangiloc ($P < 0.001$, χ2 test), and this proportion was significantly different from Loxton, Renmark, and Robinvale (G-test, $P < 0.001$), which all had higher proportions of carob moth ($P < 0.005$, χ^2 test): these latter three sites were not significantly different in their proportions of nuts infested by each pest (G-test, $P > 0.05$)(Fig. 4).

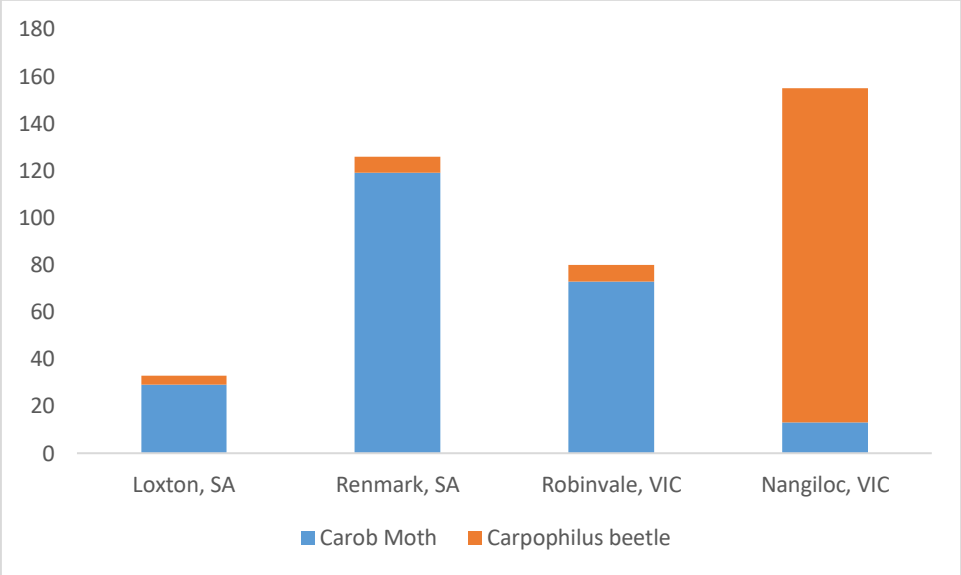

Figure 4. Total number of carob moth (*Ectomyelois ceratoniae*) and carpophilus beetle (*C.* nr *dimidiatus*), collected from mummy nuts in cage emergence experiments for each property. Proportions of carob moth vs carpophilus at Nangiloc were significantly different from Loxton, Renmark, and Robinvale (G-test, $P < 0.001$).

Natural enemies. Insects identified tentatively as natural enemies are displayed in Figure 7 and listed in Table 6. These include earwigs, ants, wasps and spiders. Wasps emerging from these field collected mummy nuts may be natural enemies of either carob moth (most likely) or *Carpophilus* beetle, though further research would be needed to identify which (or both) pest insect is the host.

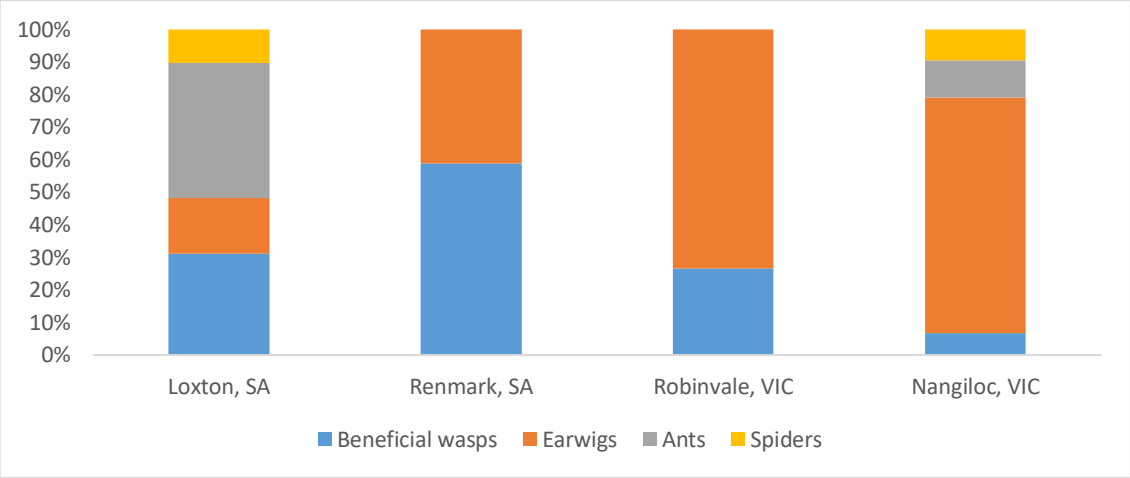

Figure 5. Percentage of beneficial and predatory invertebrates (wasps, earwigs, ants and spiders) identified in the emergence trials.

- *Additional natural enemy collection (Mildura)*

Specimens of potentially beneficial wasps were also reared from mummy nuts collected by field staff at Agriculture Victoria Mildura additional to the trial (Figure 9)

Figure 6. Examples of potentially beneficial parasitic wasp morphotypes collected from almond orchards in Mildura.

- *Identification of tentative beneficial wasps*

At least 34 wasp morphotaxa were collected from sticky traps, mummy samples (emergence trial) and additional collections based on both morphological and molecular data. Around 21 (62%) of morphotaxa could be confidently identified to superfamily or family based on the DNA (barcode) sequences. However, of these morphotaxa, eleven (32%) could not be placed to a family and remain unidentified: there was difficulty amplifying the DNA for these specimens, possibly due to degradation of specimens when collected on sticky traps. As specimens were removed using kerosene, the quality of DNA could have been affected; and in some cases identification of a species might have required DNA primers that were not available.

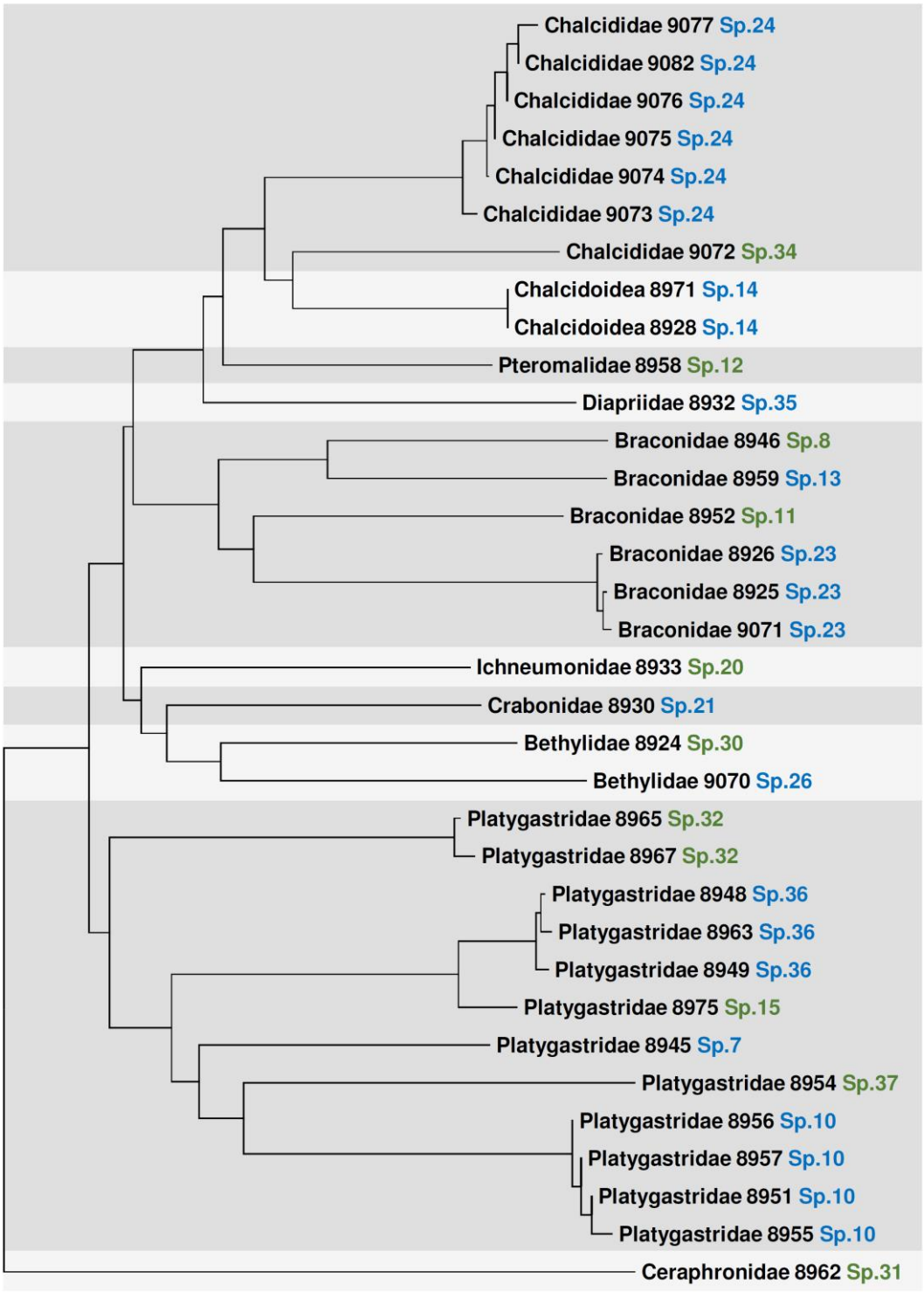

Figure 7. Results from DNA barcoding for potentially beneficial wasps (neighbour-joining tree). Samples analyzed include collection from all three methodologies (sticky traps, emergence trails and opportunistic collections).

Table 6. List of wasp species identified from natural enemy surveys. Method of collection; C = Cage, emergence trials; O = Opportunistic collections; * = specimens identified further to genus based on morphological features. Families highlighted bold contain parasitoids/predators identified from literature to attack either carob moth or *Carpophilus* beetles (see Tables 2 & 4).

Collection Method	Family	Genus/ species/ morphotaxa	VAITC	DNA sequence	Known parasitoid?
C	**Bethylidae**	*Eupsenella*? sp. = sp. 30*	8924	YES	Species in the Bethylidae family attack both carob moth and carpophilus beetles (Tables 2 & 4)
O	Bethylidae	sp. 26	9070	YES	
C, O	**Braconidae**	*Phanerotoma* sp. = sp. 23*	8925, 8926, 9071	YES	At least three species of *Phanerotoma* are known to parasitise carob moth (according to Tables 2)
S	**Braconidae**	sp. 08	8946	YES	Species in Braconidae attack carob moth and carpophilus beetles (Tables 2 & 4)
S	Braconidae	sp. 11	8952	YES	
S	Braconidae	sp. 13	8959	YES	
S	Braconidae?	sp. 09*	8947	NO	
S	Ceraphronidae	sp. 31	8962	YES	
O	**Chalcididae**	*Antrocephalus*? = sp. 24* = sp. 27 & 28	9076, 9077, 9073, 9075, 9074	YES (to family only)	Species in Chalcidae are known to attack carob moth (Table 2)
O	Chalcididae	sp. 34	9072	YES	
S	Chalcidoidea	sp. 14 = sp. 22	8970, 8971, 8928	YES	
S	Crabonidae	*Nitela*? sp. = sp. 21	8930	YES	
S	Cynipoidea?	sp. 04*	8939, 8953	NO	
S	Diapriidae	sp. 35	8932	YES	
S	**Ichneumonidae**	sp. 20	8933	YES	Different species in this family attack carob moth and carpophilus beetles (Tables 2 & 4)
S	Platygastridae	*Gyron*? sp. = sp. 10	8951, 8955, 8956, 8957	YES	
S	Platygastridae	*Idris*? sp. = sp. 15	8974, 8975, 8976	YES (1 specimen)	
S	Platygastridae	sp. 07	8945	YES	
S	Platygastridae	sp. 32	8965, 8967	YES	
S	Platygastridae	sp. 36	8948, 8963, 8949	YES	
S	Platygastridae	sp. 37	8954	YES	

S	**Pteromalidae**	sp. 12	8958	YES	At least two species in the Pteromalidae attack carob moth (Table 2).
S	Scatopsidae	sp. 33	8966	YES	
S	Undetermined	sp. 01	8940	NO	
S	Undetermined	sp. 02	8941	NO	
S	Undetermined	sp. 03	8935	NO	
S	Undetermined	sp. 05	8942	NO	
S	Undetermined	sp. 06	8929	NO	
S	Undetermined	sp. 16	8927	NO	
S	Undetermined	sp. 17	8931	NO	
S	Undetermined	sp. 18	8938	NO	
S	Undetermined	sp. 19	8934, 8954	NO	
O	Undetermined	sp. 25	9080	NO	
O	Undetermined	sp. 29	9079	NO	

6.0 Discussion and recommendations for next steps

Through a combination of literature review and experimental (survey) work in almonds, we have shown that there is clear potential for biocontrol of insect pests of almonds, which should be further explored, developed and implemented as an important component of the almond IPM program.

- *Conservation biocontrol*

Conservation biocontrol focuses on improving the local environment of the agro-ecosystem to support populations of natural enemies. Our field survey identified several families of insects that are present within Australian almond orchards, including predatory insects that may be natural enemies of carpophilus and carob moth. Field trapping for airborne insects (sticky traps) collected a greater diversity of insect species compared to mummy nut sampling, but many of these insect species were not predatory. Morphological and molecular identification of insects is a time-consuming process, and was not possible for all captured insects in the present study. We therefore focused our analysis on beneficial wasps. We found mummy nut sampling a better method for identifying natural enemies, as this method provides more evidence for an association between the (tentatively) beneficial insect and almond pests compared to sticky traps. Two beneficial wasp species (*Antrocephalus mitys* and *Venturia canescens*,) documented in the literature as attacking carob moth are known to be present in Australia. Two other wasp species (*Trichogramma carverae* and *Goniozus jacintae*) also known to attack carob moth were collected from Australian almond orchards in previous studies (Madge *et al.* 2015): these authors also identified the predatory bug *Orius sp.*, European earwigs, *Forficula auricularia,* the green lacewing, *Mallada signata,* and the red and blue beetle, *Dicranolaius bellulus* as natural enemies of carob moth (Madge *et al.* 2015). Two carob moth predacious mites (*Blattisocius tarsalis* and *Pyemotes ventricosus*) identified from the literature are also present in Australia. Only one wasp (*Anisopteromalus calandrae*) known to attack *Carpophilus* beetles was identified in the survey. However, it is likely that several generalist predators of *Carpophilus* beetles are present in almond orchards but were not detected in the current work.

As sampling was conducted only in the winter season (May – June), we recommend additional survey work during the growing season (particularly at hull split, when carob moth and carpophilus beetle attack the new nuts) as key predatory and parasitoid species may have been absent or in low abundance at the time of sampling. The study provided some evidence for variation in insect abundance and diversity between almond orchards. Further work could focus on environmental and cultural practices that might be responsible for these differences in diversity, with a focus on predatory species. The survival of natural enemies in the field is strongly influenced by a suitable habitat, including the presence of refugia, and feeding sites such as floral nectar and pollen (Gurr *et al.* 2017). Whilst it is appreciated that the economies of scale that underpin almond production, including extensive monoculture, may limit the provision of these sites, the possibility of providing an improved habitat for natural enemies should at least be explored. This could include improving the knowledge of the behavioural ecology of natural enemy species identified in almond orchards, and for example providing suitable flowering plant species (especially native plants) or other nectar / feeding sources that are hardy and can be sustained in the almond orchards (with the caveat that it is also import that such plants are selected

carefully to avoid exacerbating the pest problem). **At the very least, growers and industry must be aware that these beneficial insect communities are undoubtedly contributing to the control of pest populations, and the use of broad-spectrum insecticides will reduce or remove these beneficial insects, and as a consequence significantly exacerbate the pest problem.**

- *Augmentative biocontrol and inoculative releases (mass rearing)*

Parasitoid wasps

The literature review details the use of egg parasitoids in the genus *Trichogramma* for controlling carob moth. Whilst the *Trichogramma* species used to control this pest elsewhere in the world are not present in Australia, *T. carvarae* has been reared from field collected carob moth eggs in almonds (Madge *et al.* 2015, and could be the most suitable candidate for biocontrol. The commercial production of *T. carvarae* has been undertaken in Australia, although at the time of this report, the (apparent) *T. carvarae* was identified through molecular methods as being *T. pretiosum*. Initial studies (Agriculture Victoria, in progress) have shown that *T. pretiosum* will successfully attack carob moth eggs in the laboratory but its efficacy, survival and establishment in almond orchards still needs to be evaluated. Further work should be carried out focusing on (i) evaluating *T. pretiosum* augmentative release, (ii) distributing carob moth "egg traps" in orchards (particularly during the growing season) to collect *T. carvarae* for mass culture, and also survey for other egg parasitoid species, and (iii) screening trials comparing lab and field efficacy and survival of *T. pretiosum* vs *T. carvarae*.

Our survey identified one wasp species (*Antropochephalus myti*) that is being used elsewhere in augmentation biological control programs for lepidopteran pests (Pereira *et al.* 2013). This wasp could therefore be considered as a candidate for inoculative release. A common parasitic wasp (*Anisopteromalus calandrae*) commonly used in biological control programs of stored grain pests in Australia could also be considered. Further knowledge is needed in the ecology and host range of these two insects.

Entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs).

Entomopathogenic fungi have considerable potential as agents for almond pest biocontrol, and we highly recommend this as an area of research that warrants further exploration. EPFs are particularly interesting, as these microorganisms have been identified for beetles, including Carpophilus (Dowd and Vega 2003). *Beauveria bassiana* is an ideal organism to begin studies on the use of EPF in almond IPM, particularly for *C.* nr *dimidiatus* control, as strains of this species have been permitted for use as a biopesticide in Australia. We recommend further work focussing on screening the effectiveness of different strains on *C. nr dimidiatus* mortality, and field efficacy trials. EPFs are sensitive to abiotic factors such as temperature, humidity, and UV radiation (Teja and Rahman 2016), and this will be an important consideration in selecting a suitable EPF species or strain for the control of almond pests. Our field survey did not cover insect diseases, and further work looking for EPF that attack carpophilus beetles (or carob moth) in almond orchards might uncover ideal candidate species for screening.

The use of **entomopathogenic nematodes** should also be explored further. Focus could begin with (i) developing methods to collect, culture, and screen the two species identified in this review that are present in Australia (*S. feltiae* and *S. glaser*), and (ii) conducting a comprehensive field survey to identify other nematode species naturally occurring in *Carpophilus* species in Australia, such as stone fruit attacking species (*C. hemipterus*, *C. davidsoni*) and in particular the almond-attacking *C. nr dimidiatus*.

Autodissemination (auto-inoculation) is a method by which insect diseases are spread through a pest population by the use of a vector, often the pest species itself. This method can be particularly useful where the pest is difficult to reach (e.g. by using pesticides or cultural practices). **Autodissemination may prove to be a novel and effective way of using EPFs to control both *C.* nr *dimidiatus* and carob moth, where insects remain hidden within the developing almond fruit or mummy nut.** Project AL16009 is currently developing a new attractant for *C.* nr *dimidiatus* as part of an "attract and kill" mass trapping strategy. These new traps could equally be used for disseminating EPFs at key times in the pest lifecycle. The effectiveness of this devise was demonstrated by Dowd and Vega (2003), who detected 100% prevalence of an inoculated strain of *B. bassiana* in *B. bassiana* infected beetles collected during their study.

- *Classical Biological Control*

Classical biological control is usually only considered when native natural enemies are insufficient to control an exotic pest. Little is known about the native origins of *C. nr dimidiatus*, but it is possible that this insect has been introduced to Australia, which might at least in part explain its escalating populations, as a result of "enemy free space" (Jeffries and Lawton, 1984). The nut-attacking *Carpophilus truncatus* is exotic to Australia and morphologically similar to *C. nr dimidiatus*, and obtaining reference specimens and conducting molecular identification (DNA barcoding) of *C. truncatus* is highly recommended to help resolve whether they are the same species. Further taxonomic resolution is essential to understand *C. nr dimidiatus* worldwide distribution, and would help identify natural enemies from its home range, which may include candidates for classical biological control. The parasitoid wasp *Brachyserphus abruptus,* used to manage several Nitidulidae pests worldwide, is unsuitable for classical biocontrol of *C. nr dimidiatus,* as it is a generalist predator: before an exotic biological control agent is introduced to Australia, it must be rigorously assessed to ensure that it possess no risk to Australian native insects, and this is rarely the case for generalists.

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