



AGROECOLOGICAL CROP PROTECTION TO MEET FUTURE CONSUMER DEMANDS

Agroecological Pest Management in Citrus

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1. Summary

- The 'A Lighter Touch' (ALT) programme aims to transition the crop production approach of the New Zealand horticultural industries from agrichemical based pest management to agroecological crop protection.
- Citrus has been selected as a model system for all perennial tree and vine crops in New Zealand.
- The concept of integrated pest management (IPM), biological control and agroecological enhancements are briefly explained.
- Eight key arthropod pests of citrus in New Zealand were identified: Australian citrus whitefly (Orchamoplatus citri), Black citrus aphid (Toxoptera aurantii), Citrus bud mite (Aceria sheldoni), Citrus flower moth (Prays nephelomima), Citrus rust mite (Phyllocoptruta oleivora), Greenhouse thrips (Heliothrips haemorrhoidalis), Kelly's citrus thrips (Pezothrips kellyanus) and Lemon tree borer (Oemona hirta).
- A literature search was undertaken to identify the natural enemies / biological control agents (BCAs) of these pests that are already present in New Zealand orchards and/or are available from commercial suppliers. These included parasitoids, predators (ladybirds, lacewings, hoverflies, mites, pirate bugs carabid & staphylinid beetles), nematodes, and biopesticides (entomopathogenic fungi and microbes).
- A number of the predators are generalists and attack several of the pests of interest. At a genus and family level the same groups of BCAs occur very regularly in the literature for control of a diverse range of arthropod pests in many different crops.
- For each BCA, a literature search was undertaken to determine the agroecological enhancements required to boost their populations and fecundity. The list of plants included: phacelia, buckwheat, alyssum, lucerne and ryecorn along with groups including the Apiaceae, grasses and clovers.
- The range of plants in the literature that were beneficial for the BCAs also frequently reported the same species or groups of plants.
- It therefore appears that the suite of recommendations in this report for controlling citrus arthropod pests may be equally effective in other perennial tree and vine crops, and could be trialled in these with a few adjustments specific to the crop.
- The optimal list of agroecological amendments for conservation biocontrol has been 'filtered' such that the recommendations can be practically and economically implemented in an orchard, e.g., tall species cannot be planted where machinery access is required, and species, principally grasses, cannot be planted under the trees or they will compete too strongly with the trees and reduce vigour and yield.
- The recommendations are:
 - That the agroecological enhancements focus on perennial plants to reduce the cost and provide year round benefits.
 - That a range of perennial clovers plus alyssum are planted under the trees as these should not compete with trees, rather they could benefit the trees through supply of nitrogen. Alyssum is included here as it is a tough hardy perennial that can compete with the clovers and provides year round, high quality nectar and pollen available to all BCAs.
 - In the inter-row, a highly diverse pasture mix be planted including seven species of grasses, the legumes lucerne, white clover and Persian clover and the forbs dandelion, plantain, yarrow, and marigolds (Tagetes erecta).

- Ideally a sequential sowing of annuals in strips between the understory and inter-row will be planted using a strip / zone tillage approach, including buckwheat, coriander, crimson clover, dill, phacelia and ryecorn.
- Some additional interventions may be required including the use of pheromone mating disruption and use of Bacillus thuringiensis (BT) for citrus flower moth, the control of ants which are mutualists of sap sucking pests and which will defend the pests from the BCAs, and the release of BCAs from commercial suppliers to give an initial boost to orchard BCA populations.

2. Introduction

The *A Lighter Touch* (ALT) programme aims to transition the crop production approach of the New Zealand horticultural industries from agrichemical based pest management to agroecological crop protection. As part of milestone MS6.2 "Enhancing agroecosystems for classical biological control" citrus has been chosen as a model crop for all the perennial crops in a project to demonstrate agroecological enhancements to control key arthropod pests. These are:

- Australian citrus whitefly (Orchamoplatus citri)
- Black citrus aphid (Toxoptera aurantii)
- Citrus bud mite (Aceria sheldoni)
- Citrus flower moth (Prays nephelomima)
- Citrus rust mite (Phyllocoptruta oleivora)
- Greenhouse thrips (Heliothrips haemorrhoidalis)
- Kelly's citrus thrips (*Pezothrips kellyanus*)
- Lemon tree borer (*Oemona hirta*)

2.1 Defining three main biocontrol approaches and agroecology

There are three main categories within biocontrol:

- Importation / 'Classical' biocontrol (IBC)
- Augmentation biocontrol (ABC)
- Conservation biocontrol (CBC)

Importation or 'classical' biocontrol is where an exotic pest (typically an insect or weed species) is controlled by finding a natural control species from the host's country of origin and importing the species to manage the pest. The success rate of full control of the pest is about 10%, some level of control about 50% and about 40% of the time there is no benefit. IBC is typically very expensive due to the substantial amount of host specificity testing required to ensure the imported biological control agent (BCA) does not become a pest itself, i.e., target non-pest species.

Augmentation biocontrol is where the BCA already naturally exists in the environment, but their population needs to be boosted by supplemental release for better management of the pest. Augmentation depends on the supplemental BCAs being reared in large numbers at commercial providers. There are two types of augmentative biocontrol:

- Inoculative release
- Inundative release

An inoculative release is where a small population of BCA's are released with the aim that they will multiply and increase their populations. This is the typical approach used in glasshouses. An inundative release is where large, to very large populations of the BCA are released to 'swamp' the pest. The use of microbial pesticides (biopesticides) such as *Bacillus thuringiensis* (BT) is considered to be inundative ABC.

Conservation biocontrol is where the BCA already exists in the environment, but at population levels that are too low to achieve proper management of the pest. Their populations and control effectiveness are boosted by habitat manipulation, for example planting flowering plants that provide food and shelter. This habitat manipulation can be described as an agroecological enhancement.

"Agroecology is based on applying ecological concepts and principles to optimize interactions between plants, animals, humans and the environment while taking into consideration the social

aspects that need to be addressed for a sustainable and fair food system. By building synergies, agroecology can support food production and food security and nutrition while restoring the ecosystem services and biodiversity that are essential for sustainable agriculture. Agroecology can play an important role in building resilience and adapting to climate change." FAO¹.

2.2 Agroecological enhancements

Agroecological enhancement will mostly involve conservation biocontrol (CBC) techniques, e.g., the introduction of plants that provide resources such as shelter, nectar, alternative prey / food sources and pollen (SNAP) (Gurr *et al.*, 2017; González-Chang *et al.*, 2019) although other approaches, e.g., ant control, may be considered or be required for the other modifications to work effectively. Augmentation biocontrol (both inoculative and inundative) may also be used if required if there are insufficient beneficial insects present in the orchards. These introductions may be a result of the monitoring component of integrated pest management (IPM) showing that pest populations are increasing while BCAs populations are not. Importation biocontrol is outside the scope of this project, but where there is potential for IBC this is mentioned.

To identify what the best agroecological enhancements are to support biological control of insect pests found in citrus, this report undertakes a literature review to first provide a brief overview of each pest's lifecycle and ecology pertinent to agroecological enhancements, including any plants that are alternative hosts to pests. Second it identifies which beneficial insects that attack each pest are already present in New Zealand, both those already present in orchards or the wider environment, and that are available from BCA suppliers. Third it will identify what agroecological enhancements in the orchard are likely to enhance the fitness and longevity of those BCAs and therefore result in improved pest management, while also considering changes to the system that may enhance the fitness and longevity of the pest. The agroecological enhancements for the BCAs of each pest will then be combined into a single set of recommended enhancements for all the pests / BCAs in the citrus orchard system and any additional measures required.

It should be noted that this is a very broad brush approach to agroecological management of pests. Typically a biological control program (IBC, ABC and/or CBC) for a single pest - crop combination would require many years, even decades of research, to achieve a result. This however, results in a full and detailed understanding of the pest-crop ecosystem and ensures that the pest management techniques are fully reliable. This project is taking the opposite approach of looking for a set of agroecological enhancements, based on current knowledge, that offer the best chance of enhancing and promoting biological control of insect pests in the orchards and achieving a reduction in the pests' pressure. The outcomes are therefore much less predictable than a targeted approach, but potentially offer the biggest gains for the least cost, but at higher risk of failure and unexpected outcomes, e.g., enhancement of pests.

¹ <u>http://www.fao.org/agroecology/home/en/</u>

3. Agroecological Enhancements

The potential agroecological enhancements for pest management are exceptionally broad. This section will give a brief overview of the main approaches. The methods work either via the 'enemies' hypothesis' / 'top-down' or the 'resource concentration hypothesis' / 'bottom-up'. In top down regulation of the pests enemies are enhanced so they suppress the pest more, however with bottom-up, the pests ability to find and feed on its host is inhibited (González-Chang *et al.*, 2019).

Intercropping is where one or more crop and/or non-crop species are grown together to achieve reduced pest, disease and weed occurrence. The level of mixing ranges from highly intimate where the species are similar, e.g., annual seed crops, with the seeds mixed and sown together, through to agroforestry systems where the widely spaced tree rows are highly separated from the pasture or crops between them.

Trap crops are non-crop species that attract the pest away from the main crop. They can be planted as intercrops, typically as strips, or around the perimeter of the field. The trap crop may be sufficiently attractive that its presence keeps the pest permanently off the crop, or, the pests may be controlled while in the trap crop, e.g. by agrichemicals (including those not permitted in the cash crop), physical destruction, e.g. mowing and ploughing under, or vacuuming them up. Alternatively, with 'dead-end' crops, the trap plants are attractive to the insect pest but their offspring cannot survive on them and thus die.

Repellent plants are non-crop plants that repel a pest, typically by emitting volatile chemicals, and are typically planted as an intimate intercrop.

Push-pull is where trap crops and repellent plants are combined as intercrops to both push the pest out of the crop and attract it to the trap crop (Hassanali *et al.*, 2008).

Camouflage crops are typically non-crop plants planted among the crop plants to camouflage the crop from its pests, both visually and olfactory, e.g., white clover growing underneath cabbages reduces the contrast of the crop against bare soil, i.e., a brown - green contrast, thereby reducing aphid infestations (Finch & Collier, 2000, 2003).

Refuges / shelter are mostly non-crop plants, predominantly perennial plantings, that provide refuge i.e., protection for BCAs, for example from their predators, or shelter, both year round, and overwinter. Beetle banks (Collins *et al.*, 1997) are a well-known example, consisting of a slightly raised strip in a field which is sown in tussocky grasses provide overwintering shelter for predatory beetles. Refuges can also be created from dead plant material, e.g., straw, in the form of soil covering mulches or in containers to protect both the plant material and BCAs from the weather.

Floral resources provide food in the form of pollen and nectar to BCAs. e.g., The floral resources may provide energy (nectar) and nutrients protein, minerals and vitamins (pollen)which can dramatically increase the lifespan and fecundity of the BCAs.

Alternative food / hosts is where food or hosts is provided, e.g., pollen for predatory mites and noncrop aphid species for parasitoids, to boost BCAs populations, longevity and/or fecundity.

Banker plants are non-crop plants that host alternative food / prey hosts, e.g., lucerne hosts non-cereal infesting aphids which provide food and hosts for a range of aphid BCAs which can then move into the cereal crop next to the lucerne and control cereal aphids.

Mutualist management mutualism is one of the six forms of symbiosis in ecology (mutualism, commensalism, parasitism, neutralism, amensalism, and competition) and is where two or more species interact and all gain a net benefit. A key example in perennial crops is the mutualism between ants and sap sucking pests where the ants' benefit from the honeydew produced by the sap sucking insects (which is a waste product) and the insects benefit from the ants protecting them

from their natural enemies, such that the ants can be described as 'farming' the sap sucking insects. The pest's mutualist(s) may prevent effective biocontrol of the pest, even though appropriate BCAs are present, such that by eliminating the mutualist, biocontrol of the pest increases sufficiently to achieve the required level of control.

Mulching:

Fertilisation/Green manures:

Irrigation:

4. Pests and their biocontrol agents

This section provides information on each pest's lifecycle and ecology relevant to their agroecological management and then lists the known BCAs of each pest and a short recommendation as to the best options for management. As a number of BCAs will manage multiple pests (i.e., they are generalists), the final section lists all the BCAs for all pests with their known ecological enhancements. Then an overall plan for the ecological enhancements and other techniques e.g., use of commercial BCAs (ABC) is proposed.

4.1 Australian citrus whitefly (Orchamoplatus citri)

4.1.1 Lifecycle and ecology

The Australian citrus whitefly (ACW) has five distinct life stages: egg, crawler, nymph (four instars), pupa and adult (Figure 1).

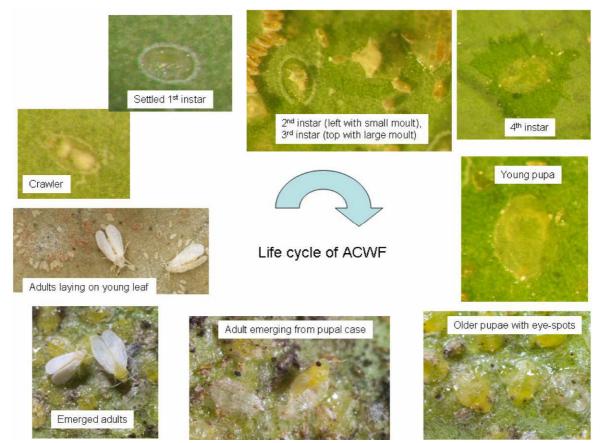


Figure 1. The different life stages of Australian citrus whitefly. From (Jamieson & Chhagan, 2008).

In New Zealand, there is one generation a year. It overwinters as the 4th instar / pupa (the pupa sits within the shell of the 4th instar), on the underside of citrus leaves. Adults hatch in October, egg laying starts in November with the periods for the rest of the lifecycle varying between the Auckland and Gisborne regions (Table 1 and Table 2). However, the overall lifecycle is fundamentally the same in both regions.

Table 1. The main period when each life stage of Australian citrus whitefly was present on leaves in Kerikeri mandarin orchards. From (Jamieson & Chhagan, 2008).

	Oct.	Nov.	Dec.	Jan.	Fe	b.	Mar.	Apr.	
Pupa									
Adults									
Eggs						·			
Crawlers									
Nymphs									

Orange shading indicates adults present in yellow sticky traps but not on monitored leaves.

Table 2. The main period when each life stage of Australian citrus whitefly was present in Gisborne mandarin orchards. From (Jamieson & Chhagan, 2008).

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	
Pupa								
Adults	?							
Eggs Crawlers								
Crawlers								
Nymphs								

? = monitoring did not begin until late October so the beginning of adult emergence in Gisborne is unknown.

The adults are the main mobile life stage, the crawlers can only move a short distance from where their eggs were laid and the nymphs are non-mobile and scale like (Figure 1). The ACW has been recorded feeding on other host plants including natives, sometimes in considerable numbers². It is unclear how far the adults can disperse, but, considering the reasonably rapid spread in New Zealand since its discovery in 2000, and that it can feed on a range of non-citrus plants it can be assumed that it is widespread and adults can move hundreds of meters if not kilometres, especially if blown on prevailing winds.

The main problem caused by ACW is the production of copious honeydew which is a food source for black moulds which inhibit photosynthesis and reduce crop quality. The crop plants will also suffer a direct vigour loss due to ACW feeding on the sap. Overseas the ACW is a key vector of viruses. The biocontrol program therefore needs to minimise the populations of the juvenile stages on the crop plants to achieve success. As the ACW is likely to be ubiquitous in the environment due to its large host range managing the population as a whole is unlikely to be successful. Therefore the management programme needs to focus on reducing the populations in the crop.

4.1.1.1 Further information sources

https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Australian-citruswhitefly---Orchamoplatus-citri.html

4.1.2 Biocontrol agents

There are no known parasitoids of ACW in New Zealand². Work was undertaken to identify ACW parasitoids in the whiteflies native Australia (Jamieson *et al.*, 2010) and then import the best candidates. The parasitoids were successfully imported into New Zealand but attempts to rear the parasitoids in containment were unsuccessful (Chhagan *et al.*, 2013). As noted in the reports, the importation and release of one or more ACW parasitoids is the approach most likely to achieve the most effective long term control of ACW.

There are currently no microbial bio controls (biopesticides) using entomopathogenic fungi or bacteria used against ACW in New Zealand. Anon, (2016) listed *Beauveria bassiana* as a product not currently registered in New Zealand that may control ACW. Internationally more than 20 species of

² <u>https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Australian-citrus-whitefly---</u> <u>Orchamoplatus-citri.html</u>

entomopathogenic fungi are known to infect whiteflies (Gao *et al.*, 2017), with some of these, such as *Beauveria bassiana* having 'broad spectrum' insecticidal properties, i.e., able to infect and kill a diverse range of insect species, and therefore are likely to be effective against ACW but which may also kill beneficial insects as well. It would be a significant advantage to be able to use an entomopathic fungi based biopesticide, especially if they are specific to whitefly and/or are benign to BCAs as a supplementary measure to the agroecological enhancements of ACW predators. From a marketing and export perspective an effective biopesticide would be a preferable alternative to synthetic insecticides for ACW control. Identifying possible biopesticides against ACW is beyond the scope of this report, but it is recommended that this option is pursued further.

There are four generalist and one specialist predators of ACW present in New Zealand: two ladybirds, two lacewings and a *Cybocephalus* species². All originate from Australia. The ladybirds are the Steelblue ladybird (*Halmus chalybeus*) and Citrus whitefly ladybird (*Serangium maculigerum*). The lacewings are the Australian variable lacewing (*Drepanacra binocula*) and the Tasmanian lacewing (*Micromus tasmaniae*). The *Cybocephalus* species is called 'citrus whitefly predator' and is believed to be *Cybocephalus aleyrodiphagus*².

The two ladybirds lay eggs next to ACW colonies in spring and summer. Both the larvae and adults fed on whitefly eggs and instars, but not adult whitefly. They also feed on a wide range of other prey species. The adult ladybirds can fly, and can disperse considerable distances, while the larvae can move quite rapidly on the plant, but, as they can only walk, they can only move across the plant they are on and touching plants. The larvae pupate on the plant in a sheltered location. The ladybirds overwinter as adults, singly or in small clusters, in leaf folds and other nooks and crannies above ground in trees and shrubs. There are several generations a year, especially in warmer climates².

More is known in general about the Tasmanian than the variable lacewing but both are considered to breed all year due to having a low, lower development temperature of 5-6°C so they have multiple generations a year, potentially more than seven generations in citrus growing areas due to warmer climate. Adults can live for 50 to 140 days. Both the adult and larval lacewings feed on ACW and a wide range of other insects, and the adults also need to feed on nectar and pollen. Adult lacewings can fly and move some distance, which the larvae can only move around the plant they are on. There are a number of predators and parasitoids of the lacewings recorded but it is not clear how much of an impact these have on lacewing populations, especially compared with intraguild competition for prey².

The citrus whitefly predator overwinter as adults, lays its eggs next to ACW colonies and has two generations in a year. It preferentially eats ACW and other whiteflies but not other pest species. Both adults and larvae feed on ACW eggs and instars but not adult whitefly. It spins a cocoon on the leaves that may be covered with whitefly eggs and larval skins and pupates inside the cocoon. Adult citrus whitefly predators are good fliers but the larvae can only walk so stay on the same plant².

All five species are considered good candidates to benefit from CBC / agroecological enhancements, particularly the provision of alternative prey, especially for lacewings, when few ACW are present. This could be via banker plants, e.g., lucerne, which hosts the blue-green aphid (*Acyrthosiphon kondoi*) and pea aphid (*Acyrthosiphon pisum*) which cannot host on citrus. Lacewings will also benefit from the provision of floral resources.

It is unknown if New Zealand ants farm and/or protect ACW, but, as ACW produces honeydew and are protected from citrus whitefly predator by ants in Australia (Kirejtshuk *et al.*, 1997) and other whitefly species are attended by ants³ (Queiroz & Oliveira, 2001), it would be valuable to determine if ACW is protected by ants in New Zealand, as this will determine if ant control is likely to be valuable for ACW control.

³ <u>https://www.pestnet.org/fact_sheets/sugarcane_whitefly_245.htm</u>

4.1.3 Further information sources

<u>https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Citrus-whitefly-ladybird-</u> --Serangium-maculigerum.html

https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Steelblue-ladybird---Halmus-chalybeus.html

https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Australian-variablelacewing---Drepanacra-binocula.html

https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Tasmanian-lacewing----Micromus-tasmaniae.html

https://nzacfactsheets.landcareresearch.co.nz/factsheet/InterestingInsects/Citrus-whiteflypredator---Cybocephalus-species-1.html

4.1.4 Recommendations

With the clear annual lifecycle of the ACW and that it is the immature stages that cause crop harm, biocontrol needs to be focused on the egg to 4th instar stages. Adults appear not to be targeted by most of the BCAs and are ubiquitous in the wider environment. Due to the considerable reproductive capability of ACW, it will be critical to have BCA's already present in the orchard to target ACW as soon as the adults start laying in the spring, to prevent populations from increasing. As generalist predators the ladybirds and lacewings that can be most easily maintained on alternative prey, e.g., on banker plants, in the absence of ACW are therefore likely to be key. Although if the whitefly specialist *Cybocephalus* populations can be built up and maintained it may also play an important role as it will then only target whitefly and not other non-pest insects. This could be achieved by providing overwinter shelter, e.g., straw filled weather proof boxes, and banker plants for non-citrus whitefly. Overwinter shelter for the ladybird species could also be valuable in retaining them in the orchard. Banker plants that would host non-citrus whitefly over the winter period could be more challenging to achieve.

As part of the wider ALT program it may be valuable to look at means of directly reducing the growth of black sooty mould on the plants using biological approaches. One option could be milk and milk derivatives that have successfully controlled mildews on grapes and cucurbitacea e.g., (Bettiol, 1999; Crisp *et al.*, 2006; Bettiol *et al.*, 2008), and also both bacterial and fungal based biopesticides as some of these produce strong antibiotics that would inhibit the black mould and some fungi are mycoparasites i.e., fungi that parasitise other fungi, and may therefore control the mould. Identifying these is beyond the scope of this report, but it is considered worth pursuing and could be studied using some basic orchard based small scale trials of possible products.

4.2 Black citrus aphid (Toxoptera aurantii)

There appears to be some confusion over the taxonomic name of the black citrus aphid with both *T. citricida* and *T. aurantii* being used, with common names including brown citrus aphid, black citrus aphid and oriental citrus aphid. However, both *T. citricida* and *T. aurantii* have similar biology, and are very difficult to distinguish, so the confusion has no material difference for this report.

4.2.1 Lifecycle and ecology

Like most aphids, the black citrus aphid reproduces asexually (parthenogenesis) i.e., no mating / no males are required, and they also produce live young (viviparity). Colonies are therefore exclusively female.

The nymphs pass through four stages before they mature in 6-8 days at 20-25°C, and start giving birth soon after they are mature. They can produce 5-7 nymphs a day, and 50 in a life time. The

newly born nymph already contains the embryos of the next generation within her, allowing for an exceptionally fast rate of reproduction, e.g. several thousand aphids could be produced within three weeks from one female aphid, if none of them were destroyed by natural enemies.

Two adult forms exist: winged and wingless, which is again very common in aphids. The winged forms are produced when a colony becomes crowded or the food quality declines, so the winged forms can leave the colony to start new ones, flying up to 30 km. The winged forms are smaller and produce a smaller number of nymphs than the wingless forms.

The black citrus aphid mostly attacks young foliage as older foliage is too tough. *T. aurantii* has a wide host range while *T. citricida* has a narrow host range (Carver, 1978).

Ants are known to farm black citrus aphid⁴ so ant control is likely to be an important component of a CBC approach.

4.2.1.1 Further information sources

https://idtools.org/id/citrus/pests/factsheet.php?name=Black%20citrus%20aphid

https://www.pestnet.org/fact_sheets/citrus_aphids_249.htm

Carver, M. (1978). The black citrus aphids, *Toxoptera citricidus* (Kirkaldy) and *T. aurantii* (Boyer de Fonscolombe) (Homoptera: Aphididae). Australian Journal of Entomology, 17(3), 263-270. <u>https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1440-6055.1978.tb00156.x</u> Available for free.

4.2.2 Biocontrol agents

Bellamy *et al.*, (2020) did not identify any BCAs for black citrus aphid in the literature, however, they did identify a number of generalist predators of other aphid species which are present in New Zealand, all of which may predate black citrus aphid. These include:

- Whirligig mite (Anystis baccarum)
- Two-spotted ladybird (Adalia bipunctata)
- Large spotted ladybird (Harmonia conformis)
- Harlequin ladybird (Harmonia axyridis)
- Transverse ladybird (*Coccinella transversalis*)

In addition there are further generalist BCAs that attack aphids and are present in New Zealand including:

- Carabid beetles (Carabidae)
- Chamaemyiidae ⁵
- Pirate bugs (Anthocoridae)
- Hoverflies (Syrphidae)
- Lacewings (Neuroptera) (Singh & Singh, 2016).

Hoverflies, lacewings and carabids are well known to be highly effective predators of aphids and have been part of a range of CBC of aphid programs.

Bellamy *et al.*, (2020) also identified a number of parasitoids of other aphids which are present in New Zealand. The following also parasitise black citrus aphid:

Diaeretiella rapae (Singh & Singh, 2015).

Aphelinus abdominalis⁶

⁴ <u>https://idtools.org/id/citrus/pests/factsheet.php?name=Black%20citrus%20aphid</u>

⁵ https://b3.net.nz/bcanz/browse.php?by=agent&index=L

⁶ http://www.plantprotection.altervista.org/listinsect/aphelinusabdominalis.html

Aphidius matricariae⁷

Lysiphlebus testaceipes (Starý et al., 1988; Singh & Singh, 2016)

While it is noted that some of the references are not particularly strong, they can be taken to indicate that a number of parasitoids present in New Zealand will attack black citrus aphid at some level. In addition the CABI invasive species Compendium⁸ lists the following aphid parasitoids as parasitising *T. citricida*, so it is considered likely they will also parasitise *T. aurantii*:

- Aphelinus asychis
- Aphelinus gossypii
- Aphidius colemani

All three are present in New Zealand⁹ and *A. colemani* is commercially available.

In summary, there are a diverse range of aphid BCAs present in New Zealand that are good targets for CBC, the key ones being:

- Ladybirds (Coccinellidae)
- Carabids
- Hoverflies
- Lacewings
- Parasitoids

4.2.3 Recommendations

As black citrus aphid has no resting stage, has a wide host range, feeds only on new citrus growth, and as citrus are grown in the warmest areas of New Zealand, it is likely to be ubiquitous in orchards and the wider environment meaning that infestation can occur anytime. CBC / agroecological enhancements therefore need to ensure BCAs are also present year round. Generalist predators are likely to be important as they can maintain their populations on alternative prey when aphid populations are low. Aphid specialists would therefore likely need alternative prey to maintain their populations in the absence of citrus aphids, but, as specialists they often have better prey finding abilities, especially at low prey populations, while generalists, especially if there is plenty of non-aphid prey may not target aphids.

4.3 Citrus bud mite (Aceria sheldoni)

4.3.1 Lifecycle and ecology

The citrus bud mite is usually found on the flowers and buds of lemons and navel oranges. They are a pale cream colour, have an elongated cigar-like shape and are extremely small with adults being less than 0.15 mm in length (Figure 2).

⁷ <u>https://www.cabi.org/isc/datasheet/6283</u>

⁸ https://www.cabi.org/isc/datasheet/54271#tonaturalEnemies

⁹ https://www.landcareresearch.co.nz/tools-and-resources/collections/new-zealand-arthropod-collectionnzac/databases-and-holdings/hymenoptera/checklist-of-new-zealand-hymenoptera/version-6/



Figure 2. Citrus bud mite adults, nymphs and eggs (Department of Primary Industries and Regional Development).

All life stages of the citrus bud mite occur within the citrus buds. Eggs are laid within buds and flowers and then hatch into nymphs, which have two stages before becoming adults. The citrus bud mite has multiple generations per year with each generation taking 10-30 days and is temperature dependant. Adult abundance peaks from January-March and then again in from May-June while egg abundance peaks a month earlier than adults (New Zealand Citrus Growers Inc., 2021).

4.3.2 Biocontrol agents

Predatory mites from the Phytoseiidae and Stigmaeidae family are known to feed on the citrus bud mite (Collyer, 1964; McCoy *et al.*, 1996; Van Leeuwen *et al.*, 2010; Vacante & Bonsignore, 2016). Potential biocontrol agents include the mites *Amblydromalus limonicus*, *Amblyseius largoensis* and maybe *Agistemus novazelandicus* and *Phytoseiulus persimilus*. The latter is commercially available but its ability to attack this pest is unknown. The small ladybird, *Stethorus bifidus* is also known to consume mites on citrus (Collyer, 1964) but has not been confirmed to prey on citrus bud mite.

4.3.3 Recommendations

The lack of research on the ecology of the citrus bud mite means agroecology management is limited to improve the availability of wind dispersed pollen e.g. from grasses and increasing the abundance of alternative prey of the generalist predatory mites. Both of these aspects can be targeted using the habitat management approach described above for Kelly's citrus thrips.

4.4 Citrus flower moth (Prays nephelomima)

There is exceptionally little published research worldwide on citrus flower moth (CFM), with most of it having been conducted in New Zealand for Citrus NZ around the identification of the CFM sex pheromone (Z-7-tetradecenal) and its potential as part of a management system, either by trapping males at high levels and/or pheromone disruption (Dale *et al.*, 1976; Somerfield, 1977; Jamieson *et al.*, 2003; Gibb *et al.*, 2005; Jamieson *et al.*, 2006; Jamieson *et al.*, 2008; Pyle & Jamieson, 2015) plus the Citrus NZ internal research reports on which the journal publications are based (Jamieson & Gibb, 2005; Chhagan *et al.*, 2008; Chhagan *et al.*, 2009; Chhagan *et al.*, 2010; Chhagan & Page-Weir, 2017). Very little published information was found on the basic biology and ecology of CFM.

However, CFM is very closely related to the citrus blossom moth (*Prays citri*), with only small differences in genitalia between the species (Gibb *et al.*, 2005). *P. citri* is found in Europe and the

Middle East while CFM is endemic to Brunei, Australia and the Western Pacific. It is unclear if they become separate species due to geographical isolation (allopatric speciation) or if this is an example of sympatric speciation (Gibb et al., 2005). Due to their very close genetic and phenotypic similarities information on P. citri has been used to supplement the information on CFM. However, there is still only a small amount of published information on P. citri biology and ecology. There is no published information on overwintering, i.e., does the moth overwinter as diapausing late-instar larvae like codling moth (Cydia pomonella), or does it continue its lifecycle year round. There is also a lack of information regarding female moth dispersal (i.e. does she stay on or close to the tree she fed on as a larva (as does codling moth), or does she disperse some distance from her host tree). A significant amount of the research on P. citri is in non-English language publications, some of which are in non-Latin based languages which cannot be machine translated. Much of it is also in obscure and dated journals with no online access. Having a much fuller understanding of the CFM biology and ecology would be of considerable help in designing more effective biocontrol systems. Codling moth is considered to be a useful comparison species for CFM as it is well studied, is a moth with a caterpillar that tunnels inside the crop plant (fruit vs. flower) and is managed with pheromone based IPM strategies.

4.4.1 Lifecycle and ecology

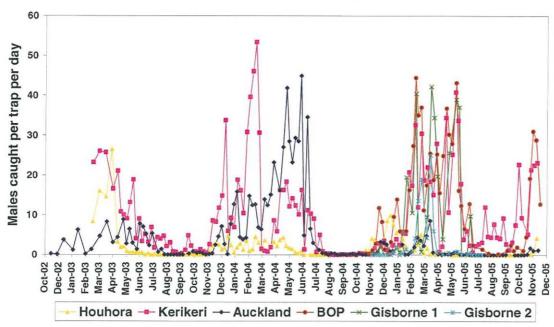
CFM is a pest because the larvae feed on and damage the buds and young flowers, which in high enough populations causes yield losses. Research has also shown that in the absence of flowers, CFM larvae hatching from eggs laid on fruit can cause 'rind spots' which results in fruit being downgraded or becoming unmarketable. Prior to about 2000, CFM was not considered a pest in New Zealand as it only attacked flowers, and, the numbers were sufficiently low, that the amount fruit loss was considered to be a useful alternative to manual thinning (Jamieson & Gibb, 2005). Interestingly this change from a useful thinning agent to a pest has also occurred in South Africa for P. citri around the same time (Moore & Kirkman, 2014). Finding out whether this is purely a coincidence, or if there is a common cause could be informative, though it is difficult to see how this could be determined after some twenty years have passed. CFM is difficult to control by any means, i.e., chemical and biological, as the larvae bore into the flowers and fruit so are protected from insecticide sprays and also generalist predators. It is likely to be one of the more challenging pests to control in an IPM system because of this. A comparison can be made with organic production systems, where codling moth in apples is not controlled by the increased biodiversity and BCAs and therefore needs interventional controls such as pheromone disruption and/or trapping, in addition to the use of biopesticides such as *Bacillus thuringiensis* (BT) and *Cydia pomonella* granulovirus.

CFM almost exclusively attacks citrus, and while the CABI Crop Protection Compendium¹⁰ lists white sapote (*Casimiroa edulis*), broad-leaf privet (*Ligustrum lucidum*) and sapodilla (*Manilkara zapota*) as alternative hosts, it does not cite any references to the primary literature. Within citrus there is a clear preference for lemons and limes (Gazia, 2018). It is therefore expected that citrus are its near exclusive host in New Zealand and therefore as there are no native citrus in New Zealand, it should exist entirely on cultivated plants, both in commercial orchards and in home gardens. This potentially means that a concerted effort at control in the short term (i.e., several years) could bring long-term population declines in the longer term. But this partly depends on how far CFM disperses between generations on the previous seasons' population if dispersal is minimal, while regular and long distance dispersal would mean that season to season populations would be weakly linked. With a strong linkage, it would be expected that good control in one season would lead to smaller populations the following season, meaning that ongoing good control would lead to highly suppressed populations and therefore minimal damage, i.e., creating a virtuous cycle. Weak inter-

¹⁰ <u>https://www.cabi.org/cpc/datasheet/43910</u>

season population linkages would mean that good control one season would have little benefit the next season. This would require all orchards that are close enough for CFM to travel between them would have to be part of the management programme, as any orchard that is not part of the program and which has high CFM populations would be a source of re-inoculation for the managed orchards.

New Zealand research into flight behaviour and generations using pheromone based trapping of males (Jamieson & Gibb, 2005; Chhagan *et al.*, 2008) found that depending on location and crop species, e.g., lemons vs. mandarins, that moths were trapped all year round, peaking between November to June. Figure 1 shows the number of males trapped across a number of sites in New Zealand over two years with indications of multiple overlapping generations. Overseas reports indicate that there are also multiple generations, i.e., there are peaks in male numbers caught in traps over a seasons, with the number of generations varying considerably e.g., as few as three and as high as sixteen, again depending on crop species and climate¹⁰. This contrasts with codling moth which overwinters as diapausing late-instar larvae in the ground under its host tree, and which has clear generations where there are large peaks of moths followed by periods of no adult moths. That there are multiple but overlapping generations indicates that CFM does not have a resting stage, like codling moth, rather the speed of growth and reproduction slows down in colder weather. Generational timing could also be driven by the periodic flowering of many citrus species, which would create a pulse of egg laying and then a pulse of emerging moths.



Citrus Flower Moth Phenology 2002-2005

Figure 3. Mean number of male CFM adults caught in pheromone traps at all monitored orchards. From (Jamieson & Gibb, 2005).

In New Zealand, ICFM trapping has been based on catching male moths attracted to traps with female pheromone lures. There is no published information on the behaviour of the female moths. Jamieson & Gibb (2005) also noted there is often a lack of correlation between insecticide sprays and number of males caught in traps. However, for there to be a clear causal link and therefore a correlation between spraying and male catch rates, the males would have to remain in the orchard in locations on the citrus trees that exposed them to the insecticidal sprays. However, we have no information on where either female or male moths' roost. It is quite possible that the male moths are not roosting in the orchards, e.g., the open nature of the orchards may make the moths feel too exposed during daylight, so they may move to much denser, darker, vegetation outside the orchard.

As the moths fly at dusk and at night, and spraying is mostly a day-time activity then it is quite possible males are not being sprayed. This also indicates that where adult moths are present, spraying during dusk / night when they are on the wing with a contact insecticide could dramatically reduce adult moth numbers and therefore also reduce eggs laid. An indication the moths stay in the orchard is the work of Chhagan et al. (2008) which in a mark-recapture study found that male moths did not move very far from their release sites with 83% captured within 100m of the release sites with a maximum of 400m of travel. However recapture rates were very low with only 86 moths recaptured out of 2450 moths released (3.5%). Statistically there is the risk of the uncertainty of small samples (Bishop, 2021) and it also begs the question what happened to the moths that were not trapped. Chhagan et al. noted that there was quite high mortality in the rearing and release process, but even then, the low recapture rates suggests that many moths left the orchard, which contradicts the trapping results that indicate that the moths do not fly very far. One source, an extension article¹¹ states "The moths, which are poor fliers, tend to remain in the same area." but no original research demonstrating this has been found, so its reliability cannot be ascertained. Therefore, a much better understanding of both the male and female moth movements, both short term, i.e., diurnally, and over weeks' even months is required to design the best IPM and agroecological solutions.

The use of pheromones for control of CFM, either by large-scale trapping of males or mating disruption by saturating the orchard with pheromones so the males cannot find females have been studied in New Zealand (Jamieson *et al.*, 2003; Gibb *et al.*, 2005; Jamieson *et al.*, 2008; Chhagan *et al.*, 2009; Chhagan *et al.*, 2010). The research indicated that the methods had potential but that a reduction in damage was not consistently achieved. As each female can lay from 60-156 eggs (at 26°C the average is 110.7 eggs)¹⁰ it would only take a small proportion of the females to still mate under mating disruption or immigrate into the orchard for significant numbers of eggs to be laid and therefore significant resulting damage.

Knowledge regarding CFM flight capacity and movement are critical for the effectiveness of mating disruption. Mating disruption is highly effective in controlling codling moth because the female's mate and lay eggs on the trees close to where they emerged, so, while the males can fly several kilometres¹², if the population of females in an orchard are depleted, then, future populations will continue to be suppressed. However for CFM, if both males and females move considerable distances, i.e., between orchards, then, they will be able to mate in locations outside the mating disruption area, and then the mated females can fly into the disrupted orchard and still lay eggs. This again highlights the need for a better understanding of the male and female moths' behaviour to inform IPM and agroecological control approaches.

Overseas research on *P. citri* has however shown that mating disruption is effective (Sternlicht *et al.*, 1981; Sternlicht, 1982). As noted in the CFM reports for Citrus NZ, mating disruption is most effective at lower populations, over larger areas, and there is a cumulative effect, i.e., a virtuous cycle of continual downward pressure on the populations over several years. It is therefore considered likely that were mating disruption, or mass trapping be used at scale, i.e., across contiguous orchard areas, or whole orchards where they are distant from other orchards, for several years, and in conjunction with other control measures, i.e., *Bacillus thuringiensis* sprays, then CFM should be manageable in the medium term, e.g., three years. There would be value in seeing if mating disruption for *P. citri* is still used commercially in overseas citrus production regions, and if it is, as the pheromone for *P. citri* and *P. nephelomima* are the same, then, *P. citri* twist ties and other pheromone products should be able to be purchased from countries using this technique.

¹¹ <u>http://www.agri.huji.ac.il/mepests/pest/Prays_citri/</u>

¹² https://en.wikipedia.org/wiki/Codling_moth

No information has been found on the food sources of adult CFM. As a moth it is expected to be a nectar feeder, and as the females are listed as being able to live for between 5 to 37 days depending on temperature¹⁰ they would likely need to feed over such a duration to stay alive. As CFM larvae feed on citrus there is a logic that CFM adults feed on citrus flowers but it is also equally possible that citrus flowers are unsuitable for it and that it uses other flowers. With the introduction of floral resources into the orchard for CBC it is possible that CFM adults could benefit from the additional flowers. It will be important to monitor floral resources during times that the moths are active, which is during twilight and night time¹⁰, to determine if CFM adults are frequenting them.

Water stress appears to be a factor in management of *P. citri*. In Sicily, water stressed orchards had total infestation in buds, flowers and set fruits below the economic threshold, while in well-watered orchards the total registered infestation surpassed the economic threshold in the same period¹⁰. While an interesting observation there are practical difficulties in using water stress as a control mechanism including unpredictable precipitation in New Zealand i.e., even if irrigation is withheld then rain at flowering time would reduce or eliminate water stress, and, water stress at flowering could have direct negative impacts on the trees and fruit set and quality. The causal mechanism is unknown, it may be the female moth can detect a lack of turgor in the flowers which inhibits her laying eggs, or, trees could be giving off volatile chemicals that put the females off from laying, to give just two contrasting hypotheses. The technique is probably a long-shot for a viable management technique, but there may be value in re-testing the technique on a handful of trees in dryer climates, e.g., Gisborne to see if the effect is real, and what impact it has on the trees and yield. If those results are positive then further more detailed and expensive research could be considered.

4.4.1.1 Further resources

https://www.cabi.org/isc/datasheet/43910

http://www.sun.ac.za/english/faculty/agri/conservation-ecology/ipm/Documents/Prays citri fact sheet Addison MFA.pdf

4.4.2 Biocontrol agents

There are a number of natural enemies of CFM including the parasitoids *Ageniaspis fuscicollis, Bracon laetus* and *Trichogramma evanescens*¹⁰ (Abo-Sheaesha & Agamy, 2004) which if CFM continues to be difficult to control by other methods could be considered for a classical biocontrol program. However, we have found no published evidence for any parasitoids already present in New Zealand attacking CFM.

Generalist predators such as mites, including *Metaseiulus occidentalis* (Bellamy *et al.*, 2020) and beetles may eat the eggs and young larvae before they bore into the flowers or fruit and become inaccessible. As this project is aiming to enhance a number of these to control other pests, some control of CFM may be achieved as well. However as CFM larvae quickly bore into the flowers and fruit, they are then inaccessible to generalist predators.

Bacillus thuringiensis (BT) has been reported as having a good level of efficacy (Shetata & Nasr, 1998; Moore & Kirkman, 2014) as has *Beauveria bassiana* (BB) (Shetata & Nasr, 1998). BT has been widely used as a biopesticide of lepidopteran larvae for many decades, and has the advantage of being highly specific to butterfly and moth larvae, so it will not harm other BCAs. BB in comparison will attack a wide range of insects including BCAs so it may negatively affect BCAs that are being enhanced as part of this project. It is therefore best reserved as a supplementary control option should an insect pest become problematic. BT has to be eaten by larvae to work, so, as the CFM larvae rapidly bore into the flowers and fruits BT will have to be used preventatively by ensuring there is good coverage of the flowers and fruit so that the newly hatched larvae consume sufficient BT spores, during the short period they are consuming the surface plant layers, to kill them before they cause too much damage.

4.4.3 Recommendations

Of all the citrus pests CFM is one of the more challenging from a conservation biocontrol perspective, due to the limited information on the pest's biology and ecology, the adult moths being mobile and the larvae boring into the plants putting them beyond the reach of most, if not all BCAs. From what is known and taking cues from IPM of codling moth, a longer term focus is required, based on the use of pheromone disruption, coupled with preventative use of BT, with some assistance from generalist predators eating eggs and newly hatched larvae.

Internationally, particularly in southern France and northern Italy 'mesh crop covers' (Merfield, 2017b) are being used against insecticide resistant pests, such as a codling moth, on tree crops (Dib *et al.*, 2010; Chouinard *et al.*, 2016; Chouinard *et al.*, 2017). Beyond being a pest barrier, mesh crop covers also alter the undercover micro-climate and light spectrum, which has had beneficial effects on disease control, crop quality and yield in potatoes (Merfield, 2017a; Merfield *et al.*, 2019). It would be valuable to study the direct effects of mesh crop covers on citrus, and, also test its potential as an insect barrier, especially for pests where CBC and agroecology may be less effective, such as CFM, so the industry develops and understanding of the pros and cons of the approach, which would indicate the profitability or not of mesh under the current pest assemblage, but, also provide options should new and difficult to control pests (such as brown marmorated stink bug (BMSB, *Halyomorpha halys*)) become established in New Zealand. It is understood that New Zealand Apples & Pears are already undertaking such an exercise.

4.5 Citrus rust mite (Phyllocoptruta oleivora)

4.5.1 Lifecycle and ecology

The Citrus rust mite is a moderate pest of lemons, oranges and tangelos in New Zealand and feed on fruits and leaves. They have an elongated cigar-like shape, are yellow coloured and are about 0.15 mm in length (Figure 4). This makes them very difficult to see even with a 10x hand lens. Mites are most common in humid coastal conditions and prefer sheltered sides of fruit, or the underside of leaves (New Zealand Citrus Growers Inc., 2021).

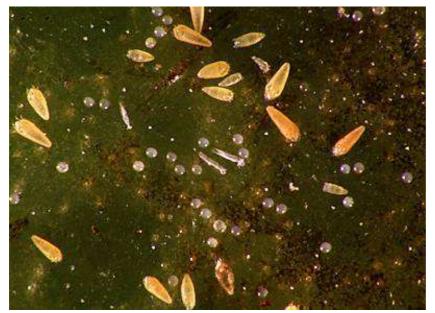
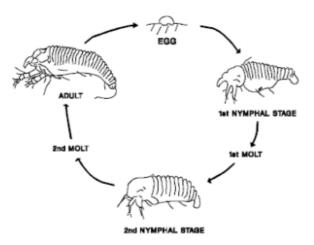
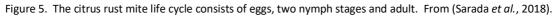


Figure 4. Citrus rust mite adults, nymphs and eggs (New Zealand Citrus Growers Incorporated).

Adults lay spherical eggs in depressions on fruit or leaves which hatch into yellow coloured nymphs that pass through two stages before becoming adults (Figure 5). The adult females live for several weeks laying an average of 30 eggs. Citrus rust mites have multiple generations per year and can

complete a generation in a minimum of 10 days but is dependent on hot humid conditions and so fluctuates from season to season. These mites are generally an issue from January-March on developing fruit (New Zealand Citrus Growers Inc., 2021).





4.5.2 Biocontrol agents

Similar to the citrus bud mite, arboreal predatory mites in six taxonomic families are predators of citrus rust mite but predominantly those from Phytoseiidae and Stigmaeidae (Van Leeuwen *et al.*, 2010; Sarada *et al.*, 2018) which can often successfully manage the pest without miticide or oil applications (New Zealand Citrus Growers Inc., 2021).

4.5.3 Recommendations

Similar to other citrus foliage pests, increasing the ground cover floral resources enhances the abundance of citrus mite biocontrol agents and can result in effective management. For instance, ground cover of flowering Asteraceae plants have been used in orchards to enhance predatory phytoseiidae mites and reduce citrus rust mite abundance (Gravena *et al.*, 1993). Wind dispersed pollen from Rhodes grass has been used to manage citrus rust mite in Australia (Smith & Papacek, 1991) and Israel (Maoz *et al.*, 2014). Furthermore, augmentative release of predatory *Amblyseius* mites have been used in Australia, USA, China and Israel to manage citrus rust mite (Smith & Papacek, 1991; Maoz *et al.*, 2014; Niu *et al.*, 2014). Although similar mites are not commercially available in New Zealand, related predatory mites can be found in citrus orchards around the country.

4.6 Greenhouse thrips (Heliothrips haemorrhoidalis)

4.6.1 Lifecycle and ecology

Greenhouse thrips is a major pest of oranges, mandarins and tangelos but attacks other citrus and has a large host range of at least 30 species in New Zealand (Froud & Stevens, 2004). It is known to feed on both leaves and fruit (Blank & Gill, 1997). Greenhouse thrips is most abundant on citrus fruits late summer and autumn e.g. April-July in Gisborne, but can be found throughout the year. Adult greenhouse thrips are found on fruit and leaves, are 2-3 mm long and have a black body, pale legs and two pairs of pale coloured wings (Figure 6). The wingless larvae have a similar shape to adults, but are smaller and are a pale cream colour. Larval greenhouse thrips often carry around a faecal droplet on the end of their abdomen (Figure 7) and allows them to be distinguished from other thrips found on citrus. Unlike many other species of thrips, pupation occurs in the canopy rather than the soil and the pupae can be seen sheltering in protected areas such as between

touching fruit or leaves (Froud & Stevens, 2004; Martin, 2018; New Zealand Citrus Growers Inc., 2021).



Figure 6. An adult greenhouse thrip. Photo: Phillipa Stevens, HortResearch.



Figure 7. Larvae of greenhouse thrips. Photo: Phillipa Stevens, HortResearch.

The life cycle of the greenhouse thrips is similar to that of Kelly's citrus thrips (Figure 8) but the former are all females and reproduce by parthenogenesis. Eggs are laid on leaves or fruit beneath the outer layer of plant tissue and therefore cannot be seen and take 15-38 days to hatch at temperatures 16-25°C. The larval period takes 9-23 days at temperatures 16-25°C. Pupation takes around 4-12 days at similar temperatures as above. Greenhouse thrips undergo many generations per year and generally all life stages can be found at any one time. They congregate in sheltered sites such as under the calyx or between touching fruits and leaves or deep inside the canopy unlike Kelly's citrus thrips which do not penetrate the inner canopy (Mound & Walker, 1982; Froud & Stevens, 2004; Martin, 2018; New Zealand Citrus Growers Inc., 2021)

4.6.2 Biocontrol agents

There are several parasitoid wasps that parasitise greenhouse thrips larvae such as *Ceranisus sp.* and *Thripobius javae* (was *semiluteus*) which attacks 1st-2nd instar larval stages (Froud & Stevens, 2004), as well as the egg parasitoid *Megaphragma* sp. Several generalist predators also attack greenhouse thrips, including solitary wasps *Spilomena nozela* and *Spilomena emarginata* which attack larvae and adult stages and the pirate bugs *Cardiastethus consors* and *Cardiastethus poweri*. Additionally, predatory thrips, e.g., *Aeolothrips fasciatus*, arboreal predatory and parasitic mites e.g. *Adactylidium spp*. have also been recorded attacking the greenhouse thrips (Mound & Walker, 1982). However, the net impact of these biocontrol agents on greenhouse thrips population size has so far been minimal (Froud & Stevens, 2004; New Zealand Citrus Growers Inc., 2021).

4.6.3 Recommendations

It is apparent that the greenhouse thrips prefers high humidity and that increasing airflow within the canopy by pruning can reduce thrips abundance (Martin, 2018; New Zealand Citrus Growers Inc., 2021) and allow greater canopy penetration by biocontrol agents. Furthermore, enhancing the efficacy of known biocontrol agents through habitat manipulation may result in a substantial reduction in greenhouse thrips populations. Recommended habitat manipulation of inter-rows is similar to that of Kelly's citrus thrips but with a greater emphasis on nectar for parasitoid wasps. The addition of clovers and other legumes around the edge or in the inter-rows may be beneficial as they will host pirate bugs (*Orius* spp.) and other generalist biocontrol agents while providing ground cover. Augmentative release of biocontrol agents can also be implemented in a similar way as suggested against Kelly's citrus thrips using predatory mites (Navarro-Campos *et al.*, 2020) and pirate bugs (*Orius* spp.) which are known to consume the greenhouse thrips (Dennill, 1992).

4.7 Kelly's citrus thrips (*Pezothrips kellyanus*)

4.7.1 Lifecycle and ecology

Kelly's citrus thrips damage flowers and fruit, particularly around contact points of touching fruit (Pyle & Stevens, 2004). Lemons, limes and navel oranges are particularly susceptible to Kelly's citrus thrips. The thrips is most abundant on fruits from December-January and March-April but occurs throughout the year in New Zealand. Adults are 2-3 mm long with a brown/black body, dark legs and fringed wings (Figure 8). The wingless larvae are a similar shape to adults, but are smaller and are a lemon or apricot colour (Figure 9). Kelly's citrus thrips can be distinguished from adult male New Zealand flower thrips (*Thrips obscuratus*) which are pale (New Zealand Citrus Growers Inc., 2021) but for distinguishing females, microscopic examination of the wings is required (Stevens *et al.*, 1997).



Figure 8. Adult KCT. Photo: Lisa Jamieson, HortResearch



Figure 9. Kelly's thrip larva. Photo: Lisa Jamieson, HortResearch

The Kelly's citrus thrips life cycle consists of eggs, several larval stages, pupa and an adult stage (Figure 10). Eggs are laid beneath the outer layer of plant tissue, mainly flowers but also fruit and leaves and are not visible. Eggs take 7-72 days to develop depending on the time of year (New Zealand Citrus Growers Inc., 2021).

Larvae are usually found on flowers, developing fruitlets and immature fruit. The larval period takes 10-47 days depending on temperature (New Zealand Citrus Growers Inc., 2021). Pupation occurs in the soil (Baker *et al.*, 2000) and is vulnerable to beneficial soil-dwelling predatory mites (Baker *et al.*, 2005). Adults then move back up in to the tree and can be found on flowers, fruits and leaves. Pollen is a requirement for the successful completion of the life cycle (New Zealand Citrus Growers Inc., 2021).

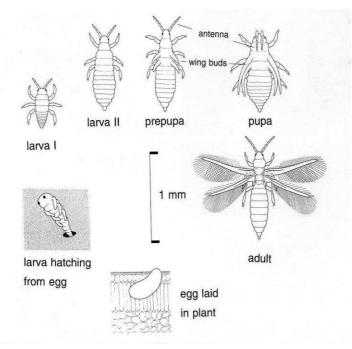


Figure 10. Thrips life cycle consists of eggs, larval stages, pupa and adult stages. From (Plant & Food Research, ©Crop & Food Research Info sheet No3-16).

4.7.2 Biocontrol agents

Predatory mites, particularly those that are soil-dwelling are considered the most effective agents that attack the Kelly's citrus thrips pre-pupal and pupa life stages (Baker *et al.*, 2005; Jamieson & Stevens, 2006; Baker *et al.*, 2011; Colloff *et al.*, 2013). Generalist arboreal predators also include the steel blue ladybird (*Halmus chalybeus*), lacewings (*Drepanacra binocular* and *Micromus tasmaniae*), a predatory thrips (*Aeolothrips fasciatus*) and predatory mites. However, these predators currently do not provide sufficient control of Kelly's citrus thrips on their own. There are no known parasitoids of Kelly's citrus thrips in New Zealand (New Zealand Citrus Growers Inc., 2021).

Although there are limited options for Kelly's citrus thrips management, enhancing the efficacy of predatory mites and other generalist biocontrol agents can be achieved through physical control and habitat manipulation such as mulches, grasses and floral resources. For these management options to be effective, use of pesticides including herbicides and fungicides, must be limited to when they are the only option. This is because many pesticides have been shown to have severe non-target effects on biocontrol agents such as predatory mites, which are generally more sensitive to such products than the target pests (Gunstone *et al.*, 2021).

In a one-year New Zealand mulch trial in a lemon orchard, up to eight predatory mite species were found to be more abundant under a 10 cm layer of organic mulch (25% compost and 75% woody plant material) compared with non-mulched plots. This resulted in a significant increase in Kelly's citrus thrips pupation mortality in mulched understoreys although the Kelly's citrus thrips abundance in the canopy and fruit damage was not affected (Jamieson & Stevens, 2006). Californian research indicates that a reduction in thrips fruit damage may not be observed for at least one year after mulch application (Hoddle *et al.*, 2002). This is congruent with a three year trial in Spain where composting manure was used to increase soil-predatory mite abundance and was associated with reduced Kelly's citrus thrips abundance and fruit damage (Navarro-Campos *et al.*, 2012). Similar results have occurred when using manure in other studies e.g. (Belaam Kort *et al.*, 2020). In addition to providing mulches to increase predatory mite abundance, augmentative release of soil-dwelling predatory mites to reduce Kelly's citrus thrips populations can also be implemented. This has been achieved in Mediterranean citrus orchards with predatory mites and sawdust + bran mulch

(Navarro-Campos *et al.*, 2020). Predatory mites are also commercially available in New Zealand however, their effectiveness has not been tested in augmentative field releases.

Citrus orchards in the Kerikeri and Auckland areas have been found to contain soil-dwelling predatory mites and were shown to have some effect on the abundance of Kelly's citrus thrips. However, mite abundance was dependent on the populations of alternative prey rather than Kelly's citrus thrips (Jamieson et al., 2005). This suggests that providing resources such as plant cover or mulches to improve soil quality could enhance generalist predatory mite abundance and therefore, increase biocontrol of Kelly's citrus thrips. This has been shown in Australian citrus orchards where dense ground cover such as perennial grasses, legumes, and diverse forbs with a deep litter layer have been shown to increase predatory spiders, beetles and most importantly soil-dwelling predatory mites, resulting in significantly reduced Kelly's citrus thrips populations (Baker et al., 2005; Baker et al., 2011; Colloff et al., 2013) to a benefit of up to AU\$ 8,540 per hectare (Colloff et al., 2013). Supporting this, Colloff et al. (2003) found that orchards with low densities of Kelly's citrus thrips had inter-rows of dense and diverse perennial grasses and herbs compared to conventional inter-rows and those with only Lucerne. Conversely, citrus orchard inter-row ground cover in the form of a mixture of Lolium ryegrasses, Lucerne and three clover species significantly increased the abundance of parasitoids, ladybirds and spiders in citrus canopies over two years in Portugal. The ground cover did require cutting twice a year to maintain practical use of the inter rows (Silva et al., 2010).

Pollen is an important resource for most biocontrol agents and has been shown to improve predatory mite reproduction. Predacious mites fed a diet of pollen from bell bean (*Vicia faba*), Pea (*Pisum sativum* cv. Arvense) and white clover (*Trifolium* repens) had increased longevity and fecundity (Grafton-Cardwell *et al.*, 1999). Wind dispersed pollen from maize (González-Fernández *et al.*, 2009) and millet (Maoz *et al.*, 2014) has enhanced Phytoseiid populations in orchards. Increasing the soil organic matter has been shown to increase predatory mite abundance and reduce Kelly's citrus thrips populations. This is thought to occur because of an increase in alternative prey for the generalist mites such as detritivores and fungi (Baker *et al.*, 2005).

4.7.3 Recommendations

The recommendations for KCT are:

- Citrus understories and inter-rows do not need to be the same, multiple options can be adopted in a replicated pattern which may reduce costs and increase biocontrol agent diversity and efficiency.
- Organic mulches can be applied to the citrus understories annually such as a 10 cm layer of 25% compost and 75% woody plant material. This provides shelter and alternative prey for predatory mites.
- A perennial living mulch can also be used in the understories e.g. strawberry clover and red clover, which could be more cost-effective with minimal maintenance compared to mulches. Perennial clovers provide additional benefits to standard organic mulches such as increased activity of multiple biocontrol agents and increased soil nitrogen with minimal competition for nutrients due to deep tap roots (Silva *et al.*, 2010; Kahl *et al.*, 2018). However, cutting may be required if they grow within 20 cm of the lower leaves of citrus trees to maintain air flow and prevent pests from easily climbing between the ground cover and the citrus canopy.
- Inter-rows should have dense ground cover of perennial grasses and/or annual and perennial flowing plants. This will provide resources for multiple biocontrol agents and allow their populations to increase over time. The ground cover should be kept high as possible within practical limitations and have a minimum of 15 cm. Organic matter from these plants should not be removed (unless it is of citrus origin). Floral resources can include buckwheat,

alyssum, coriander, phacelia and marigolds which are the most commonly used plants (González-Chang *et al.*, 2019) due to easily accessible nectar of high quality (Vattala *et al.*, 2006). Legumes such as red and white clover, peas, beans etc. can also enhance mite populations. However, most flowering plants will provide some benefit to biocontrol agents. Annuals e.g. buckwheat, alyssum, phacelia and coriander can be sown on a monthly basis to have continuous flowering from October to May but this varies with species, cultivar and climate. Irrigation may be required to establish the plants.

- In conjunction with citrus understorey and inter-row management, augmentative releases of commercially available biocontrol agents involving the predatory whirligig mite (*Anystis baccarum*), and the pirate bug *Orius vicinus* which also attacks thrips could be released on citrus trees early in the season as a preventative measure or when the Kelly's citrus thrips population is getting too high. The predatory mite *Stratiolaelaps scimitus* (formerly known as *Hypoaspis miles*) is a soil-dwelling mite can attack thrips pupae and is also commercially available. However, *S. scimitus* may attack other mites and it is only active above 12°C, limiting its application to a secondary biocontrol agent during summer in the Northern New Zealand.
- Around the edges of the orchard perennial grasses e.g. cocksfoot and red clover should be grown to promote biocontrol agents and create a physical and visual barrier for low flying pests.

4.8 Lemon tree borer (*Oemona hirta*)

4.8.1 Lifecycle and ecology

Lemon tree borer (LTB) are large 25 mm long brown beetles with an orange spot visible between the base of the wing covers and are endemic to New Zealand (Figure 11). The adult beetles have very long, curved antennae which can be as long as their body. The larvae grow up to 35 mm long and are a pale cream colour with orange gut contents, similarly to a small huhu grub. LTB are generalist feeders that bore tunnels into the branches of trees, attacking a very wide range of species, some 200 across 81 families, (Figure 12) (New Zealand Citrus Growers Inc., 2021).



Figure 11. Figure 9. Lemon tree borer adult. Photo: Dave Rogers, HortResearch



Figure 12. Lemon tree borer larva and tunnelling damage inside a young branch. Photo: Keith Pyle.

The lemon tree borer has a two-year life cycle with an egg and five larval stages as well as pupa and adult stages. Larval stages are present throughout the year. Adults can be found from October-January, during which they lay eggs. A female can lay over 50 eggs which are laid individually in leaf-stem junctions and in bark cracks. Fresh pruning scars are also used for egg laying (New Zealand Citrus Growers Inc., 2021).

4.8.2 Biocontrol agents

Three parasitoid species are known to attack the larval stages. The most common is the ichneumonid wasp *Xanthocryptus novozealandicus*, known as the lemon tree borer parasitoid which attacks late instar larvae in shallow tunnels and is found throughout New Zealand. The ichneumonid wasp *Campoplex* sp. has only been recorded in Gisborne whereas the Braconid wasp *Apsicolpus hudsoni* has been recorded from Kerikeri, Gisborne and Auckland. Both *Campoplex* sp. and *A. hudsoni* attack early-mid instar larvae (Wang & Shi, 1999) in larger branches unlike *Z. novozealandicus* which has a shorter ovipositor (Wang & Shi, 2001). Higher levels of parasitism (< 54.5 %) have been recorded in unsprayed orchards compared to conventional orchards (< 15 %) (Wang & Shi, 1999), although there is little evidence that parasitism has impacted pest populations (New Zealand Citrus Growers Inc., 2021).

Conventional insecticides are largely ineffective against lemon tree borer because of the larval tunnelling behaviour. Growers are therefore reliant on pruning dying branches and destroying them. Whilst this method is effective, it is time consuming and requires a high level of vigilance (New Zealand Citrus Growers Inc., 2021).

4.8.3 Recommendations

In addition to pruning of dead branches, it is apparent that enhancing the efficacy of the lemon tree borer parasitoid wasps is essential. This may be achieved by simultaneously reducing pesticide use (Wang & Shi, 1999) while providing nectar resources in the citrus inter-rows and surrounding area. Buckwheat, sweet alyssum and coriander are ideal plants for this due to their nectar access and quality (Vattala *et al.*, 2006) as well as their compatibility with habitat manipulation for other biocontrol agents (González-Chang *et al.*, 2019).

5. Biocontrol agents and their agroecological enhancements

This section lists the biocontrol agents (BCAs) that have been identified in the previous section as attacking the key citrus pests, plus some additional generalist predators. It then identifies from the literature what agroecological / conservation biocontrol (CBC) techniques are known to enhance each of the BCAs. The enhancements have been 'filtered' to remove any species that are not commonly available in New Zealand, and those which are likely to be impractical in a citrus orchard due to issues such as height, e.g., sunflowers (*Helianthus* spp.). Then all the enhancements for each BCA are combined into an overall enhancement scheme.

5.1 Ladybirds (Coccinellidae)

Ladybirds are listed in this report as controlling Australian citrus whitefly, black citrus aphid, citrus bud mite, and Kelly's citrus thrips, though as generalist insect predators they may well feed on other pests as well.

Ladybirds are a highly diverse family so generalisations about CBC techniques to boost their biocontrol efficacy will not be applicable to all species. Also in the warmer climates where citrus are grown, it is unlikely that ladybirds will enter full diapause (overwinter dormancy) as occurs in colder climates, so overwintering sites will not be critical, however, all year round refugia and shelter are still likely to be important. For example Ramsden et al. (2015) found that both floral resources and overwintering sites were key drivers of ladybird populations.

Ladybirds require alternative prey, shelter and pollen to maintain high populations and may take advantage of shelterbelts and man-made refugia (Michaud, 2012; González-Chang *et al.*, 2019). Ladybirds are listed as benefiting from the following plants that are commonly available in New Zealand: alyssum (*Lobularia maritima*) (Haseeb *et al.*, 2018), crimson clover, (*Trifolium incarnatum*) (Hooks *et al.*, 2013), Lucerne (*Medicago sativa*) (Harmon *et al.*, 2000; Qureshi *et al.*, 2010), dill (*Anethum graveolens*), coriander (*Coriandrum sativum*) and fennel (*Foeniculum vulgare*) (Lixa *et al.*, 2010; Togni, 2014), dandelion (*Taraxacum officinale*) (Harmon *et al.*, 2000), *Phacelia tanacetifolia* and buckwheat (*Fagopyrum esculentum*) (Irvin *et al.*, 2021).

Beetle banks for ground dwelling beetles (see section 0) have also proved beneficial as ladybird overwintering sites in annual crops (Michaud, 2012). Beetles are also likely to overwinter on trees, both citrus trees and shelterbelts in cracks and other areas protected from the weather. While it is unlikely that the ladybirds in citrus growing regions will go into full diapause, it may be worthwhile putting out some 'ladybird hotels'¹³ to provide artificial overwinter shelter, as this may also be used by other species, e.g., lacewings, and even if they make little practical difference, they may be valuable as a monitoring tool.

5.2 Predatory mites

Predatory mites are listed in this report as controlling thrips and citrus pest mites.

For Kelly's citrus thrips, one of the most serious citrus pests, soil-dwelling mites have been identified as the most effective BCAs that target the vulnerable pre-pupal and pupal stages. Organic mulches can be used to enhance the abundance of these BCAs which provide shelter and alternative prey via increased organic matter as well as a physical barrier to the soil. An example is using a 10 cm layer of 25% compost and 75% woody plant material (Jamieson & Stevens, 2006). However, it may take at least one year before effects are noticeable and requires mulch to be added annually (Hoddle *et al.*, 2002). Adding organic mulches is however, costly and labour intensive, and the large quantities required can result in an oversupply of nutrients, e.g., phosphorus and potassium leading to issues of

¹³ <u>https://www.nhm.ac.uk/discover/how-to-make-insect-hotel-ladybird-lodge.html</u>

nutrient imbalance and losses (Merfield, 2019). Also obtaining sufficient quantities of organic mulch materials can be challenging and costly (Merfield, 2019). For some growers this may be a viable option, but, probably not for most.

A better alternative would be to use a mixture of perennial living plants to form an organic mulch layer in the understories e.g., strawberry clover (*Trifolium fragiferum*) and red clover (*Trifolium pratense*), which would be more cost-effective, require lower maintenance, and avoid all the other issues of organic mulches. Perennial clovers provide dense ground cover and a thick layer of organic matter over time, which provides additional benefits to standard organic mulches. Examples include increased activity of multiple BCAs, weed suppression and increase soil nitrogen and soil structure with minimal competition with the trees for nutrients due to deep tap roots (Silva *et al.*, 2010; Kahl *et al.*, 2018). Cutting may be required 1-2 times a year if they grow within 10-20 cm of the lower leaves of citrus trees to maintain air flow in the canopy, and also to promote ongoing flowering.

Augmentative releases of commercially available BCAs may be valuable, especially when the understory plants are establishing and have therefore only started to provide services to the BCAs, so the BCAs have not had time to build up their populations to levels that will control the pests. The predatory mite *Neoseiulus* (formerly *Amblyseius*) *cucumeris* could be used on citrus trees early in the season. The predatory mite *Stratiolaelaps scimitus* (formerly known as *Hypoaspis miles*) is a soil-dwelling mite can attack thrips pupae. However, it may attack other mites and is only active above 12°C, limiting its application to a secondary biocontrol agent during summer in Northern New Zealand.

The greenhouse thrips prefers high humidity and increasing airflow within the canopy by pruning can reduce thrips abundance (Martin, 2018; New Zealand Citrus Growers Inc., 2021) and allow greater canopy penetration by biocontrol agents. Recommended habitat manipulation of inter-rows is similar to that of Kelly's citrus thrips but with a greater emphasis on nectar for parasitoid wasps. Augmentative release of biocontrol agents can also be implemented in a similar way as suggested against Kelly's citrus thrips using predatory mites (Navarro-Campos *et al.*, 2020).

To enhance the biocontrol of arboreal citrus mite pests, use of ground cover with floral resources, wind dispersed pollen is a key component of this (Smith & Papacek, 1991; Gravena *et al.*, 1993; Maoz *et al.*, 2014). Furthermore, similar to augmentative releases against thrips, *Neoseiulus* spp. have been used to manage citrus rust mite (Smith & Papacek, 1991; Maoz *et al.*, 2014; Niu *et al.*, 2014). Additionally, related predatory mites can also naturally be found in New Zealand citrus orchards.

Around the edges of the orchard tall perennial plants such as cocksfoot (*Dactylis glomerata*) and red clover should be grown to promote biocontrol agents and create a physical and visual barrier for low flying pests.

5.3 Generalist predators

The generalist predators: carabid and staphylinid beetles are listed in this report as controlling black citrus aphid, citrus flower moth and Kelly's citrus thrips.

Carabids and staphylinids as well as harvestmen and spiders are often present in orchards but are overlooked due to nocturnal activity and as their preference for undisturbed non-crop vegetation. The net biocontrol provided by these BCAs can be substantial if sufficient ground cover, plant architecture and alternative prey are provided to increase their abundance (Thomas, 1991; Berry *et al.*, 1996; MacLeod *et al.*, 2004; Bowie *et al.*, 2014; González-Chang *et al.*, 2019). Plants such as perennial grasses e.g. cocksfoot and perennial ryegrass (*Lolium perenne*), lucerne, alyssum, marigold (*Tagetes* spp.), crimson clover and white clover (*Trifolium repens*), buckwheat and coriander are all valuable.

5.4 Hoverflies

Hoverflies are not listed in this report as confirmed predators of any of the pests, but, the larval stages of hoverflies are voracious generalist predators, akin to ladybirds and lacewings, particularly against aphids and other soft bodied pests, so will be an important predator for black citrus aphid, and are also likely to feed on Australian citrus whitefly. They are often a key predator used for biocontrol, and therefore they should be included in this project.

In the absence of pest prey, hoverfly larvae need alternative prey, particularly non-pest aphid species such as those found on legumes and brassicas. Adult hoverflies require easily accessible nectar and pollen to increase their longevity and fecundity. Therefore a range flowering species have been implemented to achieve effective biocontrol (Bowie *et al.*, 1995; Wratten *et al.*, 1995; González-Chang *et al.*, 2019). This includes buckwheat (Laubertie *et al.*, 2012; van Rijn *et al.*, 2013), alyssum and coriander (Pineda & Marcos-García, 2008; Laubertie *et al.*, 2012; Haseeb *et al.*, 2018) and phacelia (Laubertie *et al.*, 2012). Floral resources need to be within 20 m of the crop to ensure the adults feeing on the flowers then lay eggs on the crop. They can be sown as mixtures or strips. Key alternative prey (non-pest aphids) plants include grasses e.g., ryegrass species, cereals e.g. ryecorn (*Secale cereale*), legumes and brassicas.

5.5 Pirate bugs

Pirate bugs are listed in this report as controlling black citrus aphid, greenhouse thrips and Kelly's citrus thrips.

Pirate bugs such as *Orius vicinus* and *Cardiastethus* spp. are voracious predators that consume thrips, aphids, mites, whiteflies and insect eggs (Dennill, 1992). Pirate bugs can be naturally found on orchards such as citrus (Froud & Stevens, 2004). *Orius vicinus* is also commercially available in New Zealand but it should only be released when temperatures are above 16°C as below that it is inactive.

The abundance of pirate bugs are likely to increase when they have access to range of alternative hosts, pollen and shelter such as clovers, other perennial pasture legumes, alyssum, marigolds and ryecorn. (Wearing & Colhoun, 1999; Wearing & Attfield, 2002; González-Chang *et al.*, 2019).

5.6 Lacewings

Lacewings are listed in this report as controlling Australian citrus whitefly, black citrus aphid and Kelly's citrus thrips, but, as the larvae are especially voracious generalist predators (Hopwood *et al.*, 2016) they are likely to attack most of the pests. The adults feed on pollen, nectar and honeydew supplemented with mites, aphids and other small arthropods. Good sources of nectar and pollen are therefore critical to increase lacewing populations. Plants that have been shown to be used by lacewings for floral resources and also as hosts of alternative prey include Lucerne (Robinson, 2009; Depalo *et al.*, 2017) *Poaceae* and Asteraceae (Medeiros *et al.*, 2010), buckwheat (Robinson, 2009; Jacometti *et al.*, 2010), alyssum (Ribeiro & Gontijo, 2017), basil (*Ocimum basilicu*) (Batista *et al.*, 2017) although as Medeiros *et al.* (2010) extracted pollen grains from 21 plant families from *Chrysoperla externa*, it is likely that lacewings feed on a very wide range of plant species.

The Tasman Lacewing (Micromus tasmaniae) is commercially available in New Zealand.

5.7 Parasitoids

Parasitoids are listed in this report as controlling thrips, lemon tree borer, and aphids. However, the parasitoids are even more diverse in their requirements than ladybirds so general statements of their requirements may not apply to specific species. The parasitoids identified in this report include: *Aphelinus abdominalis, Aphidius matricariae, Apsicolpus hudsoni, Campoplex* spp.,

Ceranisus spp., Diaeretiella rapae, Lysiphlebus testaceipes, Megaphragma spp., *Thripobius javae* and *Xanthocryptus novozealandicus*. It is beyond the resources of this report to identify floral resources for each of these parasitoids, and it is considered likely that specific species have not been tested. However, because the parasitoids are small, a few millimeters at maximum and many are sub-millimeter, the key features required of floral resources is that the flowers also have to be small to be accessible to the parasitoids (Bowie *et al.*, 1995; Vattala *et al.*, 2006; Begg *et al.*, 2017). Species such as buckwheat, the Apiaceae (e.g. coriander), alyssum and some clovers are beneficial, while species with deep nectaries e.g., phacelia are not (Bowie *et al.*, 1995; Vattala *et al.*, 2006; Begg *et al.*, 2006; Begg *et al.*, 2017).

5.8 Cybocephalus spp.

There is very little information in the literature on *Cybocephalus* spp. Most simply list that *Cybocephalus* spp. are BCAs in a range of situations and many feed on scale insect pests. The paper first describing *Cybocephalus aleyrodiphagus* (Kirejtshuk *et al.*, 1997), notes that when kept in confinement they "devoured" eggs and juveniles of *O. citri* living for up to four month and produced "numerous eggs", while when fed other whitefly they lived for two months and produced few eggs. Kirejtshuk *et al.* (1997) also noted that the adults fed on bulrush / raupo (*Typha orientali*) pollen, but it was not stated if pollen from other species had be offered and was not eaten. Kirejtshuk *et al.* (1997) also considered that *C. aleyrodiphagus* populations appeared to be synchronised with *O. citri* populations indicating that ACW is the main prey of *Cybocephalus*.

No specific recommendations can therefore be made for agroecological enhancements of *Cybocephalus* spp., but, like most BCAs they are highly likely to benefit from cessation of agrichemical use, will probably benefit from the general increase in plant cover and therefore resting places in the orchard, nectar and pollen from flowers and, may benefit from preventing ants from protecting ACW.

5.9 Ant control

A number of the pests in this report are farmed / protected by ants overseas including for Australian citrus whitefly in Australia (Kirejtshuk *et al.*, 1997) and black citrus aphid¹⁴. Argentine ant (*Linepithema humile*) is present through large areas of the north island and in all the citrus growing areas on New Zealand¹⁵. This species is known to farm and protect sap sucking insects¹⁵. It is therefore considered highly likely that ant control will be essential to ensure BCAs can achieve their maximum control rates. This is likely to require eliminating the ant nests with foraging distance of the CBC plot. It is beyond the resources of this report to provide control strategies, but Manaaki Whenua - Landcare have detailed information at

https://argentineants.landcareresearch.co.nz/control_tools.asp.

5.10 Citrus flower moth mating disruption

Due to the limited BCAs for citrus flower moth, pheromone based mating disruption is likely to be essential (see section 0). Overseas research has shown that mating disruption is effective for *P. citri* (Sternlicht *et al.*, 1981; Sternlicht, 1982). Pheromone twist ties for the citrus blossom moth (*Prays citri*), which has the same pheromone as CFM, are commercially available overseas, e.g., https://www.pestmagazine.co.uk/wp-content/uploads/2020/11/inpest-catalogue.pdf so it should be possible to obtain supplies for the trial. This will however require that the whole orchard and any neighbouring orchards use pheromone disruption as it has to be used over large / contiguous areas of orchard to work, and may require several years to achieve full control of CFM.

¹⁴ <u>https://idtools.org/id/citrus/pests/factsheet.php?name=Black%20citrus%20aphid</u>

¹⁵ https://argentineants.landcareresearch.co.nz/

5.11 Bacillus thuringiensis

In conjunction with pheromone mating disruption for CFM, it is likely, especially in the first year(s) that *Bacillus thuringiensis* (BT) will be required to infect newly hatched larvae before they bore into the flowers and fruit. BT is widely used and available in commercial quantities, e.g., Dipel[®]. BT has a residual period of one to two weeks before it is inactivated by UV light or washed off by rain. Individual product information, and the use of stickers and UV protectants, will determine the reapplication period.

The critical time for protection is likely to be during flowering, so spraying may be able to be limited to flowering time. It is not clear however if CFM is laying on fruit outside of flowering times, so this needs to be investigated.

6. Combined agroecological enhancements

6.1 General overview

Despite the diversity of the pests and their BCAs it is interesting that the same plant species frequently are found to benefit BCAs. This is very helpful as it simplifies the task of creating a combined agroecological enhancement for all pests and BCAs. It is also vital that the enhancements are practical, economical and do not excessively interfere with orchard operations, e.g., harvest, pruning, and spraying (with BCA safe materials), and do not harm tree growth and yield, and ideally, they enhance them.

As noted at the start of this section the recommendations have already been 'filtered' to remove any species that are not commonly available in New Zealand, and those which are likely to be impractical in a citrus orchard due to issues such as height, e.g., sunflowers (*Helianthus* spp.).

6.1.1 Avoiding chemical pesticides

All pesticides, not just insecticides should be considered to be harmful to BCAs. Further work is to be undertaken to identify which pesticides are the most and least harmful. However, as the aim of ALT is to "transition the crop production approach of the New Zealand horticultural industries from agrichemical pest management to agroecological crop protection" then this project should be aiming for nil use of chemical pesticides (including organic certified products, as a number of those are also highly toxic to BCAs e.g., copper, sulphur and pyrethrum) and only use biopesticides where CBC is not achieving sufficient control of pests. In addition, for a number of pests, e.g., lemon tree borer, the spraying of pesticides has clearly been linked to increased pest populations. Stopping spraying of all chemical pesticides is therefore essential in this project. The first year in particular may not achieve a desirable level of control, as agroecological enhancements and BCAs are still establishing and have not reached their full potential, but, as this is an experimental project, and only a small area of orchard, it is vital that agrichemical use is avoided unless long term harm will be done to the trees.

6.1.2 The broad vision

The broad vision is for a mixture of primarily perennial legumes and non-grasses to be grown under the trees where the current herbicide strip is and a diverse grass based pasture with a range of legumes and forbs in the inter-row.

Grasses are considered to have the highest competitive ability, compared with forbs and legumes, against all perennial crops as they have shallow fibrous root systems, which occupy the same soil space as the shallow feeder roots on perennial crops, such as citrus, and the fine fibrous highly effectively penetrate the soil bulk around the tree roots. This is why the grasses are restricted to the inter-rows. Conversely many legumes have tap roots which 'pierce' through the shallow tree feeder roots so competition between legume and tree roots should be minimised. Further because legumes can fix their own nitrogen (N), their root systems are poor competitors as they don't need to compete for N which is the soil nutrient needed is the greatest amounts by plants. Legumes have also been shown to provide N to their intercrops, even to the point of direct transfer via mycorrhizae (Meng *et al.*, 2015) so a legume dominated intrarow under the trees could directly benefit the trees through nitrogen supply, with minimal competition for other nutrients. Water competition may well increase however.

6.1.2.1 A perennial based system

The aim is to also mostly use perennial species, to simplify management, reduce costs, reduce soil disturbance and maintain a year round cover of vegetation. Having most of the vegetation remain all year round is vital for providing refugia for many of the species, i.e., if plants were killed off,

particularly if the soil is then cultivated, and resown, many BCAs would be killed or leave the orchard. Following this logic the perennial plants need to be allowed to grow as much as possible without any management, i.e., mowing. Where mowing is undertaken, it should leave the plants as long as possible, e.g., greater than 20 cm high, as mowing close to the ground, e.g., less than 10 cm is likely to be detrimental to both plants and BCAs. Some strategic mowing may however be beneficial to encourage plants to keep flowering, rather than setting seed. Trial and error and further literature research will be required to best establish how this should be done.

6.1.2.2 Incorporating annuals

A number of beneficial species are annuals and it could be valuable to incorporate them into the system. They could be sown with the perennials at establishment, and then be allowed to seed so they can regenerate, however, it is considered unlikely that the seedbank will even attempt to germinate under a dense layer of perennial vegetation, and if they do, it is highly unlikely that they will be able to establish and thrive.

It is suggested one way to incorporate annuals is to use strip / zone tillage at the junction of intra and inter-rows using a single zone tillage cultivator on a custom toolbar, or having two zone tillers one on either side of the tractor. It is likely that the strips to be zone tilled will need to be herbicided off, principally by glyphosate, about six weeks before zone tillage to allow the sward and its roots to decay. It is quite likely that considering the density of the plantings being considered that the strips my need to be mown, before, and/or after spraying. This will require the use of customised spray and mowing equipment. It is considered that a simple spray rig could be easily built, e.g., a 200 L plastic drum or 20 L plastic container, 12V DC pump and suitable nozzle system, or an ATV all in one sprayer, etc. A lawn mower could also be used rather than build a custom tractor mounted mower.

Another issue with annuals is that they have a defined, and often short, flowering period. It is envisaged that there would need to be a sequential sowing of annual plants, e.g., every month the annual mixture is sown into a zone tilled strip on one side of one inter-row of the trial area, and therefore each month another sowing is made, such that there is always one strip of annuals flowering at any time. This is likely to take some trial and error to work out the best timings, especially considering more rapid summer growth and slower winter growth.

6.1.2.3 The cropping benefits of biodiversity

There is also a substantial evidence base in the ecological literature that increasing plant diversity also increases crop yield (Weisser *et al.*, 2017; Tamburini *et al.*, 2020) as well as benefiting a wide range of other ecosystem system services – of which pest control is the key focus here. Therefore a diversity of plants in the orchard should have an overall benefit for the trees and yield beyond just pest management. This also indicates that where possible and practical more species should be planted rather than less.

6.2 Plant lists

The following plants have been listed as being beneficial to BCAs in this report (Table 3).

Table 3. Plants identified in this report as being beneficial for BCAs, grouped according to type and lifespan.

	Perennials	Annuals	Both
Grasses	Cocksfoot Perennial grasses Perennial ryegrass	Ryecorn	
Legumes	Lucerne Red clover Strawberry clover White clover	Crimson clover	
Forbs	Alyssum Dandelion Fennel	Basil Buckwheat Coriander Dill Phacelia	Asteraceae Marigolds (<i>Tagetes</i>)

Silva *et al.* (2010) selected a mixture of annual ryegrass (*Lolium multiflorum*) perennial ryegrass, strawberry clover, crimson clover, and Persian clover (*Trifolium resupinatum*) to promote nectar and pollen sources in extended flowering periods. This has two additional species than Table 3.

The author C. Merfield has also undertaken research on highly diverse pasture mixtures for dairy pastures and used the following species list (Table 4)

Common name	Scientific name	Lifespan	Туре	Family
Cocksfoot	Dactylis glomerata	Perennial	Grass	Poaceae
Plantain	Plantago lanceolata	Perennial	Forb	Plantaginaceae
Chicory	Cichorium intybus	Perennial	Forb	Asteraceae
Timothy	Phleum pratense	Perennial	Grass	Poaceae
White clover - large	Trifolium repens	Perennial	Legume	Fabaceae
White clover - medium	Trifolium repens	Perennial	Legume	Fabaceae
White clover - small	Trifolium repens	Perennial	Legume	Fabaceae
Alsike clover	Trifolium hybridum	Perennial	Legume	Fabaceae
Red clover	Trifolium pratense	Perennial	Legume	Fabaceae
Brome	Bromus spp	Annuals & perennial	Grass	Poaceae
Prairie Grass	Bromus willdenowii	Perennial	Grass	Poaceae
Parsley	Petroselenium crispum	Biennial	Forb	Apiaceae
Smooth meadow-grass	Poa pratensis	Perennial	Grass	Poaceae
Lucerne	Medicago sativa	Perennial	Legume	Fabaceae
Birdsfoot Trefoil	Lotus corniculatus	Perennial	Legume	Fabaceae
Sheep's Burnett	Sanguisorba minor	Perennial	Forb	Rosaceae
Yarrow	Achillea millefolium	Perennial	Forb	Asteraceae
Tall Fescue	Schedonorus phoenix	Perennial	Grass	Poaceae
Red Fescue	Festuca rubra	Perennial	Grass	Poaceae
Meadow Fescue	Festuca pratensis	Perennial	Grass	Poaceae

Table 4. Highly diverse pasture mixture used by the author C. Merfield.

Table 4 gives an idea of the level of diversity being used in pastures. Some of these species would be valuable to include e.g., yarrow as it is the Asteraceae which Medeiros *et al.* (2010) found significant

quantities of pollen of in hoverflies, while others are unsuitable e.g., chicory as while it is also in the Asteraceae, its flower stalk grows up to two meters tall so would impede machinery access or grow into the tree canopy. This list also indicates the species that are commercially available in New Zealand, though some could only be supplied by specialist commercial seed retailers.

Based on the above the following recommendations are suggested for this project.

6.2.1 Intra-row - under tree

- Red clover
- Strawberry clover
- White clover large leaved cultivars
- Alsike clover
- Birdsfoot Trefoil
- Alyssum
- Plantain

The mixture is principally clovers, all species of which produce dense tall foliage which will outcompete weeds. Lucerne is not included as it is considered that its open foliage will be swamped and out-competed by the other clovers. Alyssum is included as it is considered an excellent floral resource, it is perennial, very hardy and can also grow quite large, so should be able to compete with the clovers. Plantain is added for diversity and it should be able to hold its own with the clovers. It is suggested that the sowing density should be light, so individual plants can achieve a good size, which is felt to be important to allow the alyssum to be able to grow large enough at establishment to then hold its own.

6.2.2 Inter-row

- Lucerne
- White clover
- Persian clover
- Dandelion
- Plantain
- Yarrow
- Marigolds (Tagetes erecta)
- Cocksfoot
- Perennial ryegrass
- Timothy
- Smooth meadow-grass
- Tall Fescue
- Red Fescue
- Meadow Fescue

The mixture is principally grasses, with a high level of species diversity, to try and get a good and long duration supply of pollen, and for general biodiversity. Many CBC papers find lucerne to be beneficial for a range of BCAs so it should be a strong proportion of the mix. The legumes have been limited due to the clover dominant intrarow area, but some legumes are required for N fixation. Persian clover is an annual, so as per previous comments it is in theory not suited to permanent pasture, but, annual clovers have evolved to be in pasture, and they have large seeds which makes for large strong seedlings so it may be able to persist. Plantain is again added for diversity as it is in a different family to the other species. Dandelion is in the Asteraceae, and, personal observations of the author C. Merfield is that it is a good nectar source, being particularly visited by butterflies, though it has not been determined if seed is available. Yarrow is also in the Asteraceae and it may hold its own better in the mixture than dandelion as it can grow as tall as the grasses. Marigolds (*Tagetes*) are also in the Asteraceae family and Silveira *et al.* (2009) and Souza *et al.* (2019) both found the Mexican marigold (*Tagetes erecta*) enhanced CBC in onion and sweet pepper crops respectively. They will also bring colour to the strip to make a clear visual statement.

6.2.3 Annual flower strips

As discussed above, it is considered unlikely that the annual spp. listed in Table 3 will be able to germinate in established swards. A strip tillage approach was suggested (section 0) or any other technique that can create a 'space' for the annuals and allow for their establishment and flowering. If annuals are planted the recommended species are:

- Buckwheat
- Coriander
- Crimson clover
- Dill
- Phacelia
- Ryecorn

All of these plants can grow to be quite substantial, e.g., 0.5 m high, particularly when they go to flower, so, the sowing rate needs to be reasonably low to allow individual plants to achieve sufficient size. With the cultivation associated with strip tillage there is a likelihood that weeds may establish with the sown plants, however, unless these are known to cause real and actual harm, e.g., hosting citrus pests or diseases, they should be considered 'non-crop plants' adding to overall biodiversity (Gurr *et al.*, 1998). A number of studies have also looked at the CBC value of 'weeds' e.g., (Gurr *et al.*, 1998; Togni, 2014 ; Begg *et al.*, 2017; Gontijo, 2019), and, with the dense plant foliage both under the trees and in the inter-row, it is unlikely that many annual weed plants will be able to become populous.

6.2.4 Establishment

The herbicide strip under the trees is likely to be quite hostile to seed establishment, due to it being compacted and having reduced soil biology due to the absence of a diversity of plants covering the soil over many years. Considerable thought needs to be given to how to maximise seed establishment and subsequent growth, as some papers report significant establishment problems. Testing potential techniques on small areas, rather than just applying seed across the whole CBC area, is likely to ensure a viable technique is determined without establishment failures across the whole area. Possible options include light cultivation, e.g., with a 'spring tine weeder' so to loosen the top centimetre or two of soil without damaging the tree roots. Hydro seeding may be another option, but this would need to be tested to ensure all the plant species germinate successfully using this technique.

Establishing the new inter-row should be more straight forward on the assumption that there is an existing mown grass strip. This can be simply sprayed off, and then ideally the seeds drilled into the dead sward to ensure good germination.

There may be a need for irrigation during the establishment phase, and, if the orchard is using drip or other irrigation that does not wet the whole orchard floor, then an alternative will be required if there is insufficient rain / soil moisture. Sumi rain

<u>https://www.perennial.co.nz/collections/irrigation/products/sumi-rain</u> is one option, that would give very even and gentle watering, i.e., would not dislodge soil and seeds.

6.2.4.1 Legume inoculation

For legumes to be able to fix atmospheric nitrogen, the particular species of bacteria they form a symbiosis with need to be present in the soil. For common legumes, i.e., white clover, the bacteria is present in all soils, however, for other species, e.g., lucerne, if inoculated plants have not been growing in the soil in the last five to ten years it is likely that the seed will need to be inoculated with the correct bacteria for that legume species. Advice needs to be sought from the seed suppliers, or other knowledgeable parties as to which species need inoculation at sowing.

6.2.5 Maintenance

Correct ongoing maintenance of both the inter- and intra-row is essential. It is vital that they are not regularly mown and/or mown short. As flower production is a key objective for most of the species, including the grasses, the vegetation needs to be allowed to become quite long / tall. This is quite at odds with the expected aesthetic of orchards, but, with time, the new aesthetic should come to be appreciated. In the wine industry, the use of buckwheat and phacelia in the inter-row in the "Greening Waipara" program became a significant part of the marketing strategy (S.D. Wratten pers. comm.). Likewise, the agroecological approach here could be a valuable part of a marketing strategy so concerns around expected aesthetics need to be allayed.

However, while regular low mowing will be highly detrimental, there may well be a need for strategic mowing to 'reset' the plants if they move from flower to seed production. Some seed production and shed may be valuable for the plant communities to self-regenerate, but, at the same time it is important to ensure the plants produce flowers through the part of the season when pests are most present. This may require the plants to be 'topped' e.g. the top third mown off. The best approach will need to be established through trial and error, and using small areas which are manually cut, e.g. shears, weed eater, will be the best way to establish the best techniques, rather than just treating the whole area at once.

It is also likely that a sequential approach to topping will be required, e.g. topping every third or fourth row, at one time, so as not to remove all the flowers in the orchard in one go, but to leave the majority of the plants with flowers, and when the topped rows are re-flowering, to then top another third / fourth of the rows.

Ideally all mowing's should be left in situ to contribute to the build-up of a detritus layer and soil organic matter. However, if the mowing's are quite thick and they could suppress the plants they may need to be removed.

Depending on the climate it is possible that the CBC plants will need watering. It is likely the plants will also increase the overall water demand in the orchard. As discussed for establishment, if the tree irrigation does not irrigate the whole orchard floor, an alternative irrigation system may be required, e.g. K-line. However, many of the plants are deep rooted, and with the associated increase in soil organic matter and therefore water holding capacity, it is hoped the plants will be able to cope with a reasonable amount of soil moisture deficit if irrigation is only being focused on the trees.

6.2.6 Augmentative inoculation

Aphidius colemani, Phytoseiulus persimilus, Anystis baccarum, Stratiolaelaps scimitus, Neoseiulus cucumeris, Orius vicinus and Micromus tasmaniae are listed in this report as commercially available BCAs in New Zealand that may have value in being released as inoculative augmentation biocontrol, particularly during the establishment phase of the trial. It is recommended that the value of this is discussed further with Plant & Food scientists on the project and also the commercial suppliers of the BCAs to decide on the value of such actions, also taking into account the economic cost and how quickly the BCAs are likely to self-introduce and build up their populations to sufficient levels.

6.2.7 Augmentative inundation

Bacillus thuringiensis (BT) has been recommended for control of the newly hatched larvae of citrus flower moth (CFM) (see sections 0 and 0). Depending on the crop and therefore when it flowers, and the BT product and adjuvants used it is recommended that a spray program is developed to ensure that trees are protected with BT during the key times that CFM eggs will be hatching.

6.2.8 Pheromone mating disruption

The use of pheromone mating disruption using twist ties has also been recommended for CFM (section 0). This could also be based on the flowering times of the crop and when CFM are expected to be laying eggs. A trapping program could also be used to determine when the moths are flying. If this is to be undertaken, then a commercial supplier of pheromone twist ties for CFM will need to be identified, and, the area for disruption, including potentially neighbouring orchards, will need to be determined.

6.2.9 Ant control

As discussed in section 0 an ant control program is considered highly likely to be required so this should be built into the project. It would still be valuable to undertake a survey to determine if ants are farming / protecting pests and if so which ants. It will be essential to monitor for the effectiveness of the ant control measures, which will likely capture if ants are farming pests or not.

6.2.10 Ladybird hotels

As discussed in section 0 it may be valuable to build some ladybird hotels to see if they are used and if so to give an indication of ladybird and other BCA populations.

7. Conclusions

There is an interesting convergence apparent in this report. Despite starting with a list of highly diverse arthropod pests, including thrips, whitefly, aphid, mite, moth and a beetle, there was a considerable level of communality among the BCAs that could control them, particularly the generalist predators. Then the plants that had been identified as being important for those BCAs showed an even greater level of communality, with the same species regularly occurring, such as phacelia, buckwheat, alyssum, lucerne and ryecorn along with groups such as the Apiaceae family, grasses and clovers. There may be a certain amount of 'follow the leader' in these plants being selected due to the conservative nature of research as scientists are more likely to test plant species that have proved successful in previous research, rather than start with a random selection. However, there are also studies, such as Medeiros et al. (2010), who extracted pollen grains from 21 plant families from a hoverfly species, which found there was a very clear difference in the amount of pollen from different plants, indicating clear preferences by the insect which can then inform which plants to use. Even if there is a level of group think, it is clear from the research that the 'usual suspects' in terms of plant species, clearly provide benefits to the BCAs, and many studies then follow that through to shown reductions in pest levels, including below IPM economic thresholds. This indicates that while this report has focused on a suite of pests many of which are specific to citrus, the BCAs that predate or parasitse them, also manage a wide range of other pests from many cropping systems, especially at when grouping the BCAs at genus or family level, e.g., ladybirds and parasitoids. This strongly indicates that this suite of the best overall performing beneficial plants and habitat modifications should also support the BCAs inhabiting all perennial tree and vine crops.

The ecological literature also increasingly shows that highly simplified crop ecosystems, especially where there is frequent use of agrichemicals, not just insecticides, but also herbicides and fungicides, actually creates an environment where pests, diseases and weeds can flourish, and by reintroducing carefully selected biodiversity, and using targeted biological rather than chemical pesticides, the whole system is more robust and resilient, and able to withstand challenges such as climate change, and help address challenges such as biodiversity loss and nutrient leaching (Weisser *et al.*, 2017; Tamburini *et al.*, 2020).

If the habitat modifications recommended in this report are then combined with similar approaches to all the needs of the orchard, e.g., nutrient management, weed and disease control, crop health, yield and quality, then the perennial crop sector in New Zealand can truly say it has changed to agroecology.

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