Effectiveness of a mass release of the mealybug predator *Cryptolaemus montrousieri* in a Queensland apple orchard.

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This publication has been compiled by Peter Nimmo of Horticulture and Forestry Science, Department of Agriculture and Fisheries.

Summary

The tuber mealybug, *Pseudococcus viburni*, has a worldwide distribution, being found in South Africa, United States of America, South America, New Zealand, Europe, Bangladesh, China and Australia. Tuber mealybug is found on all parts of the plant and is considered to be the most important of the underground mealybug pests in Australia. Tuber mealybug became an important pest of apples and pears at Stanthorpe, Queensland in 1993. The longtail mealybug *Pseudococcus longispinus*, previously a pest only in southern states, is now present in many Queensland apple orchards. Mealybug infestations on pome fruit result in the development of black sooty mould which grows on the sticky honey dew at the stem and calyx ends of the fruit. Such fruit is unmarketable as fresh fruit. Infested fruit is rejected for export to overseas countries as export standards require nil live insects. Control of tuber mealybug and longtail mealybug in pome fruit relies mainly on insecticides with generally unsatisfactory results. The wasp parasitoid, *Pseudaphycus maculipennis* was introduced into Queensland orchards and follow up work needs to be done to confirm the status of this biological control species. *Cryptolaemus montrouzieri* (*Cryptolaemus*) also can be effective in biological control of mealybugs in apple orchards and is commercially available. The differences between the two pest species of mealybug are difficult to establish morphologically and it would highly advantageous to develop a PCR assay to aid in accurate identification. A PCR assay was developed and show great promise in accurately identifying the two mealybug pest species. Correct identification is important in selecting effective control measures for each species.

Mass releases of *Cryptolaemus* reduced mealybug populations but not sufficiently to satisfy the expectations of the fresh produce market. Further field research investigating the use of adult beetles instead of larvae, different timings of releases and more use of control blocks is likely to demonstrate better mealybug control using *Cryptolaemus.* The PCR analysis was highly promising and further work is required to prove the rigour of an accurate assay.

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Background

A different approach to the escalating mealybug problem is required, as the more insecticides are used in an attempt to reduce mealybug damage, the worse the situation becomes. A successful implementation of biological control using *Cryptolaemus* has the potential to lessen the impact of mealybugs on orchard profitability and provide an environmentally friendly, cost effective means of control. The mealybug predator *Cryptolaemus* will be released into Queensland apple orchards and the impact on pest mealybugs evaluated. The major benefit to the apple and pear industry of a successful implementation of *Cryptolaemus* will be the effective control of mealybugs.

Two species of mealybugs, tuber mealybug (*Pseudococcus vibirni*) and longtail mealybug (*Pseudococcus longispinus),* infest Australian apple orchards and cause damage to the fruit. Control of mealybugs is notoriously difficult because their waxy secretions reduce penetration by insecticides. In apples and pears, mealybugs also hide in the calyx end of the fruit, meaning infestations often go unnoticed until the population is above action thresholds. A mealybug's ability to secrete itself in the calyx greatly decreases exposure of the insect to insecticides.

The two mealybug species have a very wide range of host plants, including apples, pears, grapes, citrus, stone fruit, gerbera, gladioli, potato, passionfruit, dahlia, ginger, Jerusalem artichoke, alfalfa and cacti. Mealybug infestations on apples and pears results in the development of black sooty mould that grows on the sticky honey dew secreted by the mealybug at the stem and calyx ends of the fruit. Such fruit is unmarketable, and are rejected for export to overseas countries. Current control of mealybugs relies mainly on insecticidal chemical control. This is not always effective as the insects can become resistant to the insecticides. The use of chemical controls can result in outbreaks of secondary pests (e.g. mites and scale insects).

Orchardists in the Stanthorpe region of Queensland reported mealybugs as their most serious orchard management problem in 2014. Considerable time and money is currently spent by growers dealing with the aftermath of mealybug damage to fruit, Backpackers are even being employed to clean the sooty mould out of the calyx and stem end of pome fruit using toothbrushes. In addition, a wide range of insecticides are being applied in a manner that is not only ineffectual in preventing mealybug infestation, but disruptive to integrated pest management (IPM).

Current practice in Australian apple and pear orchards is to apply insecticides early (prior to flowering) to kill mealybug crawlers moving on the bark. In most situations, follow up are required at regular intervals throughout the growing season. A scientific assessment of the effectiveness of a mass release of commercially available *Cryptolaemus* predators will provide "cleaner and greener" alternatives to the largely unsuccessful chemical assault that is the basis of current control methods.

The apple and pear industry in Australia prides itself on the early and widespread adoption of IPM. The use of mating disruption to control codling moth, *Cydia pomonella,* was developed in Queensland and is now common practice across the apple growing world. Predatory mites, insect and disease prediction models, pest monitoring services, and disease resistant varieties are some of the advances that have significantly reduced dependence on chemical control of pest and diseases.

A successful implementation of biological control using *Cryptolaemus* has the potential to lessen the impact of mealybugs in orchards and on orchard profitability and provide an environmentally friendly, cost effective means of control.

Project Objectives

- Evaluate the effectiveness of *C. montrouzieri* as biological control agent of mealybug pests in apples. A different approach to the escalating mealybug problem is required, as the more insecticides are used in an attempt to reduce mealybug damage, the worse the situation becomes. The use of a bio-control agent such as *Cryptolaemus* will require careful consideration of all current pesticide usage, facilitating a back to basics approach to IPM.
- Determine the status of the wasp parasite (*Pseudaphycus maculipennis*). *P. maculipennis* (released 1995) are specific to the tuber mealybug and their current status needs to be established to take advantage of their considerable potential for biological control.
- Investigate the potential of cheap, fast PCR assay as a non-morphological means of identifying the two very similar *Pseudococcus* species.
- Maintain the high level of IPM that exists in the Queensland pome fruit industry. The emergence of mealybugs as a serious pest of apples and pears threatens to undo much of the work that has been done in implementing effective IPM. The major benefit to the apple and pear industry of a successful implementation of *Cryptolaemus* will be the effective control of mealybugs, thereby improving the productivity and profitability of apple and pear production.

Methodology

- Field trial site
  - A 25 hectare apple orchard at Pozieres in Queensland's Granite Belt District.
  - Block 1 consisted of 10 rows of the sundowner variety an area of approximately 0.5 ha. Block 1 was part of a larger contiguous block of Sundowner apples.
  - Block 2 consisted of 4 rows of gala apples covering approximately 0.5 ha. Block 2 was surrounded by granny smith and pink lady apples.
  - All trees in both blocks would be considered to be planted at high density (2,500 trees/ha). Both blocks were under protective netting.
- Predator release
  - Three releases of *Cryptolaemus* were applied to Block 1 and Block 2. The first release took place on November 9, 2016 with the second release occurring 14 days later on November 23. A third release was made on the 29th December.
  - All releases consisted primarily of the larval stage of the beetle predators with some additional adult beetles.
  - Releases targeted a rate of 1600 beetle larvae per hectare (8 tubes containing approximately 200 larvae per tube).
  - Releases were made by manually placing the cardboard containing *Cryptolaemus* larvae, from the tubes, on to the apple trees.
  - While predators were released over an area of approximately 25 hectares, sampling for mealybugs and predators was confined to the more manageable discrete areas of Block 1 and Block 2.
- Leaf assessment
  - Leaf assessments of mealybugs, pest mites, mite predators and *Cryptolaemus* infestations were made at seven day intervals commencing 4 December 2015 and continuing until 20 April 2016.
  - Sampling consisted of removing 20 leaves from each of 10 trees per block.
  - Block 2 consisted of 4 rows of Gala apple trees that were bounded by Granny Smith and Pink Lady varieties. Leaves were placed in paper bags returned to the lab and refrigerated until examined under the microscope within 24 hours. Numbers of mealybug (crawlers and adults), two-spotted mite, European red mite, *Typhlodromus pyri, Phytoseiulus persimilis and Cryptolaemus* were recorded per leaf.
- Fruit assessment
  - The presence of mealybug on fruit was assessed in Block 1 on 20 February 2016 by removing 50 fruit per tree from the leaf assessment trees. The fruit was examined under the microscope and the number of mealybugs present both on the outside of the apple and inside the calyx was recorded.
  - Ten fruit were non-destructively inspected on 100 trees in block 1 at harvest (17 May 2016) and the presence or absence of mealybug was recorded.
  - On the 11th February one tree from each of the ten rows in Block 1 was examined for two minutes and the number of *Cryptolaemus* larvae or adults observed, recorded.
- *P. maculapennis* survey
  - Apples were sampled from an area orchard on the Applethorpe Research Facility known to be infested with Tuber mealybug. The Tuber mealybug recovered from the apples were examined at a comfortable height for evidence of parasitism by *P.maculapennis.*

- DNA extraction
  - DNA was extracted from mealybug specimens from Block 1 and specimens collected from the Applethorpe Research Station and multi-locus sequencing analysis carried out. The DNA was also sent to MacroGen (South Korea) for sequencing.

Results

Mealybug and *C. montrouzieri* populations present on apple leaves in Block 1 (sundowner) and Block 2 (gala).

Crawler, late instar and adults of mealybugs were present on apple leaves throughout the sampling period. No tuber mealybug egg masses were observed in any samples from Block 1 or Block 2, suggesting that the majority of mealybugs were longtail mealybug. Block 1 had higher numbers of mealybugs throughout the sampling period (Figure1). Very low numbers of *C.montrouzieri* were observed in leaf samples from Block 1 and Block 2 (Table 1.) No *C.montrouzieri* were recorded in three counts prior to 23 December and none in eight samples after the 19 February. The only insecticide applied to either block post flowering was a single application of spirotetramat to Block 1 on February 8. High numbers of mealybugs were present in the initial leaf counts (4 December) suggesting the very hot spring weather facilitated early crawler movement following a large overwintering population.

Figure 1 – Mealybug leaf infestation Block 1 & 2

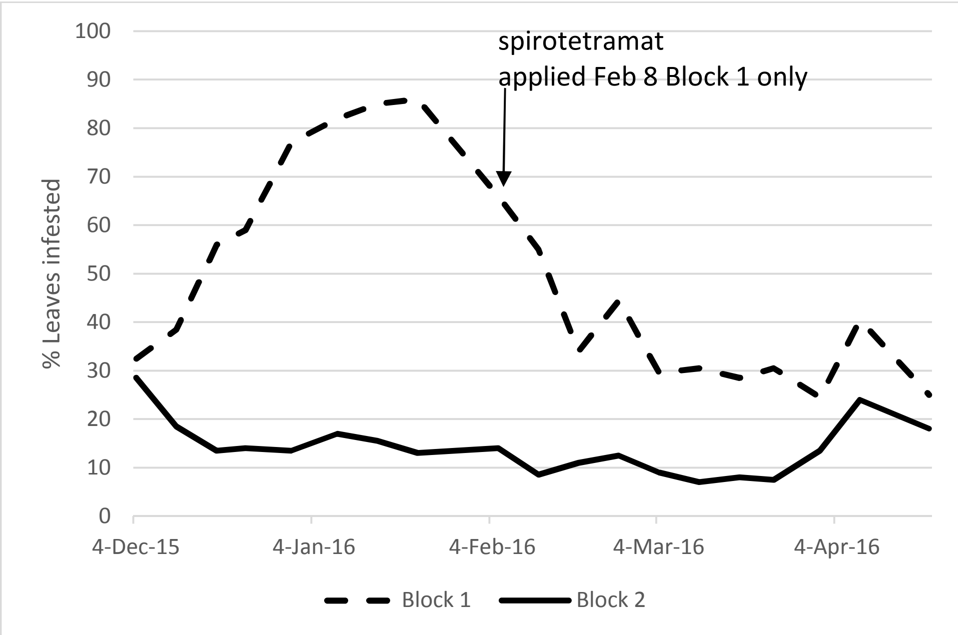


Table 1 – Low numbers of mealybug predators recorded on leaf samples

400 leaves	Date							
	23 Dec	31 Dec	8 Jan	15 Jan	22 Jan	5 Feb	12 Feb	19 Feb
Number of *Cryptolaemus*	1	0	0	1	2	0	1	2
Average *C. montrouzieri* per tree = 0.04								

Visual assessment of the *Cryptolaemus* predator population.

The incidence of *Cryptolaemus* was assessed on 11 February 2016 by counting the number of beetle larvae and beetles per tree in a two minute period could be identified on the 10 sample trees of Block 1. Mean counts from 10 trees from block 1 are presented in Table 2.

Table 2 – Number of Cryptolaemus per tree

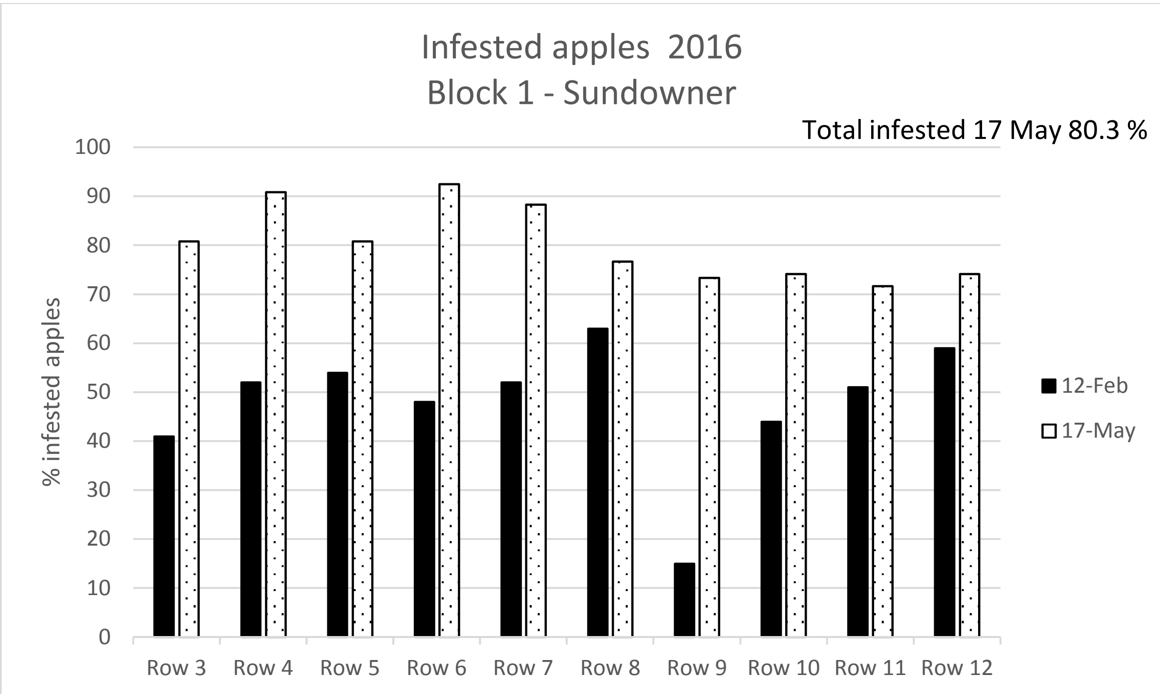
	Block 1									
	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9	Row 10	Row 11	Row 12
Beetle or larvae	2	4	5	1	3	2	0	1	3	1
Average C. montrouzieri per tree = 2.2										

Mealybug fruit incidence

One hundred fruit was sampled from each of 10 trees in Block 1 on 12 February 2016. The percentage infested fruit is presented in Figure 2.

At harvest, 10 fruit were examined from 120 trees in Block 1 for the presence or absence of *Cryptolaemus* (Figure 2). Mealybug number on fruit increased over the three months from late summer to harvest. Sundowner is a very late harvested variety and like other late varieties (e.g. pink lady) is very susceptible to mealybug damage.

Figure 2 – Mealybug infested fruit

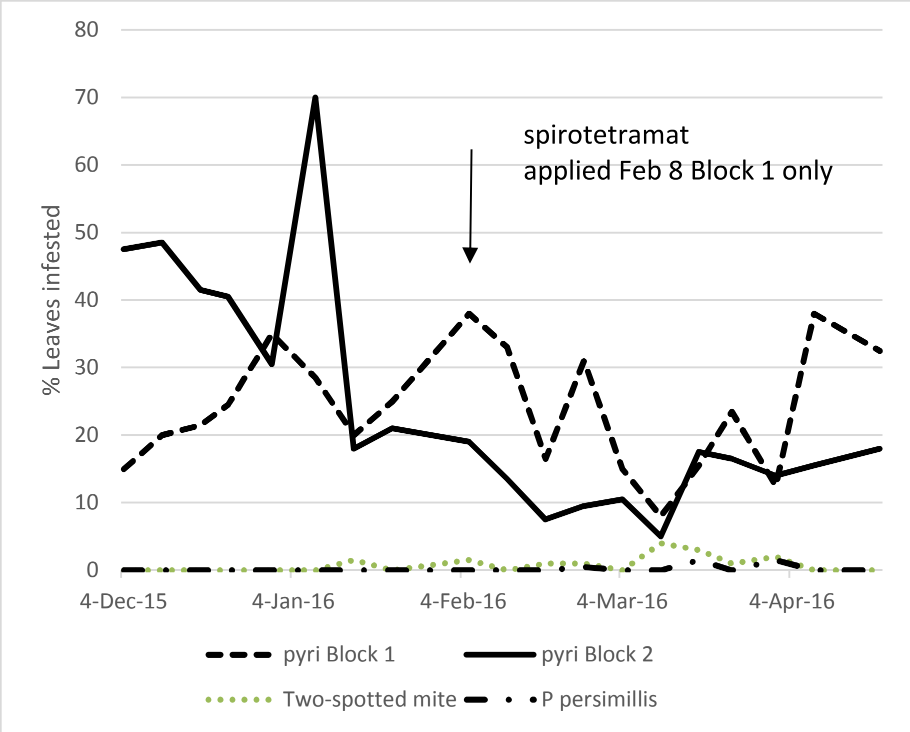


Assessment of pest and predator mite populations

The percentage of leaves with any stages of the pest *Tetranychus urticae* (TSM) and the mite predator *P. persimilis* in Block 2 and any stage of the mite predator *Typhlodromus pyri* in Block 1 and

Block 2 are presented in Figure 3. Despite a lack of pest mites to feed upon *T. pyri* numbers remained high for the sampling period providing good control of pest mites. However a TSM population was increasing towards the end of the season before the timely arrival and predation of *P. persimilis*.

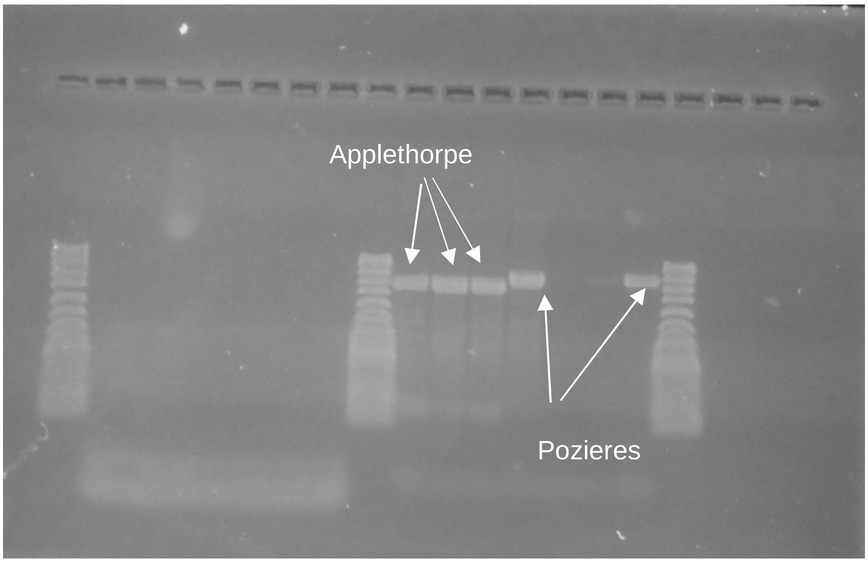
Figure 3 – Seasonal incidence of pest and predator mites Blocks 1 & 2



DNA extraction

The PCR gel plate result from a Total Nucleic Acid (TNA) extraction on 24 February 2016 is shown in Image 1. Three mealybug samples from Applethorpe Research Station and two samples from Block 1 show a difference in this plate of gene expression according to the origin of the sample. The two samples from Pozieres amplify at points consistent with database records for longtail mealybug (95% confidence level) and the thee samples from Pozieres correspond with database entries for tuber mealybug.

Image 1 – PCR gel tuber mealybug and longtail mealybug



Status of the wasp parasite (*Pseudaphycus maculipennis*)

Twenty-six large female mealybugs collected from apple trees at Applethorpe Research Facility. While no wasps were observed characteristic wasp exit holes were observed in three mealybug carcases (mummies) (Image 2).

Image 2 – Mealybug mummy showing exit holes due to parasitism

Conclusions/Significance/Recommendations

Seasonal incidence of mealybugs

Block 1

The incidence of mealybugs increased from 32.5% infested leaves in the first count on December 4, 2015 to a season high of 86% on January 22, 2016 (Figure 1). This is despite two mealybug predator releases in November. These two releases achieved a release rate of approximately 3200 *C. montrouzieri* larvae per hectare. Bugs for Bugs (Mundubbera) is the commercial provider of *Cryptolaemus* and recommend a minimum of 1000 larvae per hectare over two releases. A third release was made on December 29 at the same rate as the first two releases – a total release rate of 4800 larvae per hectare. Recommended release rates and those used in practise are arbitrary and further research is required to find the most effective rate. The percentage of infested leaves finally commenced a steady decline from 22 January, presumably as a result of predation by *C. montrouzieri*. The decline in predator numbers preceded the only insecticide (spirotetramat) applied to the block on 8 February. By 19 February, a combination of predators and the spirotetramat had reduced the population to 34% of leaves infested.

The low incidence of *Cryptolaemus* larvae in Table 1 suggest that leaf sampling is not a good method for assessing the presence of the predator as larvae and beetles were observed regularly on bark and twigs while leaves were being picked. A two-minute inspection of individual trees was trialled (Table 2) and did at least produce numbers that if repeated could produce useful trends in predator incidence. The higher number of *Cryptolaemus* per tree (2.20) in Table 2 (visual assessment) compares favourably with Table 1 (leaf counts) number of *Cryptolaemus* per tree (0.04).

Future research should make greater use of timed inspections as a sampling method and use cardboard bands from the dormant stage of the apple tree through to harvest. Cardboard bands on major limbs and smaller branches with the bands removed and replaced at various stages would have been a useful addition to this project.

The relative abundance of mealybugs on apple leaves was reflected in the percentage infestation of fruit, both in February and at harvest in late May. In Block 1 the mealybug fruit infestation increased from 51% in mid-February to 80.3% with all the infested fruit likely to be unsaleable. This level of damage is unsustainable.

Block 2

Block 2 (Gala) (Figure 1) had a lower incidence of mealybugs, compared to Block 1 (Sundowner), on leaf samples for the duration of the project. This may have been due to the very heavy Sundowner crop in Block 1 that was not thinned until February, thus providing an ideal protected breeding site for mealybugs. Mealybug numbers in Block 2 declined from a high of 28.5% in the first sample to just over 10% and remained at the level for the rest of the season. No insecticide was applied to this Block 2 after petal fall and although no harvest assessment of mealybug was made, the grower was happy with the level of mealybug control.

Both Blocks 1 & 2 had significant numbers of mealybugs present on leaves when the predators were released. Improved breeding methods by the mealybug predator supplier will allow earlier releases. The weather data in Appendix 1 shows well above average daytime and overnight temperatures during late September and early October enabling accelerated development of mealybug crawlers. This also has implications for insecticidal control as early November has in the past been considered the best window for applying control measures.

Seasonal history of pest mites and predator mites

Both blocks demonstrated the importance of integrated pest management in apple orchards with good control of two-spotted mite and the virtual absence of European red mite achieved by mite predators *Typhlodromus pyri* and *Phytoseiulus persimilis* (Figure 2).

PCR assay for differentiating tuber mealybug and longtail mealybug

Considerable progress has been made towards developing a real time PCR assay that will enable a cost effective method for determining what mealybug is the major pest in an orchard. In Block 1 and Block 2, the mealybugs present on the apples and leaves are assumed to be longtail mealybug. This may not be the case as it is extremely difficult to distinguish between the species particularly in the immature stages that make up the majority of the population. Gene sequences from a range of samples of both species have been expressed and compared to the annotated collection of all publicly available DNA sequences (GenBank). These comparisons gave a set of consistent matches for the two species of *Pseudococcus* under investigation.

P. maculapennis status

The survival of *P. maculapennis* 20 years after its release is confirmed. Further work is required to establish if the apparent absence of tuber mealybug in the Pozieres orchard is due to parasitism by *P. maculapennis* or competition from longtail mealybug. Insecticides are unlikely to be the cause of the scarcity of tuber mealybug.

Key Messages

- *Cryptolaemus montrouzieri* is not a 'silver bullet' (i.e. simple solution providing satisfactory control of mealybugs)
- Insecticides are problematic and have limited efficacy particularly in IPM programmes.
- Effective control of mealybug with a solely chemical control programme is becoming increasingly unreliable.
- Apply insecticides and or *Cryptolaemus* early in the season before mealybug crawlers become established inside the apple calyx and can no longer be controlled (Image 1)

Image 3 – Photo on left shows mealybug inside the calyx on the right

- Accurate identification of apple mealybug species using PCR Assays shows promise
- Identification of mealybug species is important as *Cryptolaemus* appears not to breed as well without the egg masses *of* tuber mealybug present in the orchard.

Where to next

Develop a concept proposal for submission to Horticulture Innovation Australia Limited. The proposal will require clear objectives providing outputs that benefit the apple and pear industry across the growing districts of Australia.

Further research – development work is required to:

- Identify the timing and rate/ha of mealybug predator releases to improve the control of mealybugs
- Identify the optimal timing of insecticides within an IPM program to increase their effectiveness whilst minimizing their impact on biological agents
- Complete development of a real-time PCR assay as a fast, cost effective and accurate alternative to morphological identification of mealybug species
- Provide satisfactory control of the pest mealybugs tuber mealybug and longtail mealybug while maintaining a high level of IPM

Budget Summary

Date	Item	$
27/01/16	*Cryptolaemus*	3672.73
9/03/16	primers	144.80
9/03/16	DNA extraction kit	344
24/03/16	USB card reader	9.09
23/06/16	Bank charges	14.38
23/06/16	DNA sequencing	575.39
30/06/16	bluetooth head set	40.91
Total		4801.30

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