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Final Report

Ecology and management of *Sigastus* weevil in macadamias

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Contents

List of abbreviations

Abbreviation	Meaning
µL	microlitre
AMS	Australian Macadamia Society
Anon.	anonymous
APVMA	Australian Pesticides and Veterinary Medicines Authority
BB	*Beauveria bassiana*
BB Sig	*Beauveria bassiana* from *Sigastus* weevil
BLK	block (in orchard)
BOLD	Barcode of Life Data Systems
BWB	banana weevil borer
Cont.	continued
cm	centimetre
cm^2	centimetre square
CTH	Centre for Tropical Horticulture Alstonville
cv.	cultivar
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAFQ	Department of Agriculture and Fisheries Queensland
DNA	Deoxyribonucleic acid
EC	Emulsifiable concentrate
e.g.	Example given
ENP	Entomopathonogenic nematodes
et al.	et alii = and others
EW	elephant weevil
F1	first filial generation
FNQ	Far North Queensland
FRW	Fuller's rose weevil
FSB	fruitspotting bug
g	gram
GIS	Geographic Information Systems
ha	hectare
Hort Innovation	Horticulture Innovation Australia Limited
IP	Intellectual property
IPM	Integrated Pest Management
IRG	Industry reference group
Kg(s)	Kilogram(s)
L	litre
lb	pounds (US)
m	metre
mg	milligram

List of abbreviations (cont.)

ml	millilitre
MNB	macadamia nutborer
n	sample size
NIH	Nut in husk
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
oz	ounce (US)
Pers. comm.	Personal communication
qts	quarts (US)
QLD	Queensland
RNA	Ribonucleic acid
RNAi	Gene silencing or RNA interference
SC	suspension concentrate
SCU	Southern Cross University
SL	soluble concentrate
sp.	species
spp.	species (plural)
UAE	United Arab Emirates
US	United States of America
WG	water dispersible granule

Summary

Sigastus weevil has emerged as an important pest for the Australian macadamia industry in recent years. Sigastus weevil was first found infesting macadamia nuts on the Atherton Tableland in Far North Queensland in 1994/1995. Crop loss in unsprayed orchards could be up to 30% (Fay et al., 1998).

Sigastus weevil is now known to be distributed across the NSW Northern Rivers and Atherton Tablelands in Far North Queensland. While there have been isolated incidences of *Sigastus* weevil in Gympie area, Bundaberg, Mid North Coast NSW and Glasshouse Mountains still remain free of Sigastus weevil distribution (Bright, 2015).

As part of this study, background information was collated including a literature review. As very little has been published on *Sigastus* weevil, the literature review was extended to encompass chemical, biological and cultural control of selected important weevil pests, including pecan weevil *Curculio caryae* (Horn), red palm weevil *Rhynochphorus ferrugineus* Oliver, banana weevil borer *Cosmopolites sordidus* (Germar) and elephant weevil *Orthorhinus cylindrirostris* (Fabricius) (Coleoptera: Curculionidae).

The literature review completed as part of this project revealed commonalities in management approaches in the major weevil pests.

The biology and ecology of *Sigastus* weevil were investigated including its life-cycle and distribution. Genetic diagnostics established that specimens of the NSW and North Queensland belong to the same species.

A series of insecticides were screened in the laboratory for their effectiveness and the IPM compatibility of effective chemicals was assessed. Of the new chemistries, sulfoxaflor and acetamaprid are promising. Sulfoxaflor is highly toxic to bees any use will need to be restricted to a time when no bees are active in the orchard. There are more insecticides that need to be screened.

During this study the efficacy of a number of biopesticides was also investigated. This included a locally found strain of *Beauveria* sp., as well as commercial products. The *Beauvaria bassiana* strain collected from macadamia orchards is the most promising fungal option tried to date and it appears to be able to penetrate into the dropped infested nutlets. Again, there are more commercial products that need to be investigated. Chemicals and entomophagus pathogens also need to be further evaluated in the field.

With regards to cultural control we found that the removal or destruction of infected nuts on the ground was crucial for sustainable management.

Management of out of season nuts to break the life-cycle and stop the population and infection was also considered an important management tool.

Keywords

Macadamia, *Sigastus* weevil, life-cycle, *Bauveria* sp., entomopathogens, biological control, chemical control, cultural control, orchard hygiene.

Introduction

Sigastus weevil (*Sigastus* sp.) belongs to the order of Coleoptera and family of Curculionidae. The species name of the Sigastus weevil infesting macadamia in NSW and Queensland still remains to be identified.

This pest has been known since the mid 1990's, and it was being managed with routine sprays and removal, mulching or solarising of the infested nut (Fay, 1998). The original distribution was confined to the Atherton Tablelands in Queensland. It has since become an important pest for the Australian macadamia industry in other areas, particularly in northern NSW. The increased incidence is thought to be related to the push to go "spray free" in the industry, which is contributing to the expansion of this pest. In areas with good hygiene and good spray coverage, the impact is minimal. The problem areas appear to be where there is a lot of out-of-season cropping, where the weevil larva developing inside nutlets are likely to emerge (high shade, no collection or removal of nuts), where growers are neighbouring abandoned orchards which harbour high levels of the pest, and where poorer spray coverage is likely (e.g. older mature orchards with 9-15m tall trees). Sigastus weevil is long lived and spring emerging weevils can easily live until the following winter. The breeding potential is only limited by the available young nut that develops in the vicinity.

Weevils cause significant early nut drop and the larvae feed and develop on kernels on the ground, rendering the nuts unmarketable. The adult weevils are able to feed on bark, leaf and husk. They are capable flyers that spend periods "dormant" outside the orchard in neighbouring vegetation between the cropping cycles. It was recently estimated that Sigastus weevil was responsible for 15 % loss of crop (Industry Reference Group, October 2014) which equates to around $15m per annum in a season when damage is serious. Original Queensland figures suggest 30% on farm losses were not uncommon when untreated (Fay et al., 1998; Gallagher et al., 2003). Weevils are difficult to control by foliar insecticides as they spend most of their life-cycle hidden inside the developing nut. Insecticide applications are required to manage the adult populations, but good coverage is essential and many farms are unable to manage them in this way with existing equipment. To minimise the losses, good crop hygiene is the only alternative at present for those growers. Systemic pesticides may give longer residual control in the short term but they can also cause residue problems and potential food safety issues.

The aim of this research project was to explore the potential of entomopathogens in the management of *Sigastus* weevil, in comparison to the conventional insecticides and the hygiene practices in macadamia orchards currently adopted. The project addresses three priority areas of IPM in horticulture: sustainable pest management, market access and food safety.

One promising option is the use of entomopathogens such *as Metarhizium* spp. and *Beauveria* spp. and pathogenic nematodes. M. anisopliae isolates have been found that demonstrate excellent efficacy against *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) banana weevil borer (BWB) and some have been commercialised. *Beauvaria bassiana* is considered as the most effective biopesticide to use against and *Orthorhinus cylindrirostris* (Fabricius) (Coleoptera: Curculionidae) elephant weevil (EW). Several entomopathogenic fungal species and nematodes have shown potential for controlling *Pantomorus crevinus* (Bohemian) (Coleoptera: Curculionidae) Fuller's rose weevil (FRW) larvae in the laboratory. An advantage of entomopathogens is that they are often not species specific and products developed for one weevil species may also be used to control other weevil species.

There is already a successful formulation of *Beauveria* sp. for control of coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae), in commercial use in South America. The tropical nutborer *H. obscurus* (Fabricius) is also an issue in macadamia in Brazil and Hawaii and

other *Hypothenemus* species in Australia that attack field nuts. These could also be susceptible to a new *Beauveria* product. NSW DPI investigations have shown early results of a strain of *Beauveria* sp. collected from field collected beetles, being effective on Sigastus weevil. Preliminary screening showed high mortality of *Sigastus* weevils under laboratory conditions when applied to a feeding surface or as a topical droplet.

This study needs further investigation through more extensive laboratory screening of *Beauveria* sp. and initiating field testing.

A critical part of Sigastus weevil management also has to be orchard hygiene. Larvae develop in nuts that have dropped on the ground which need to be managed and/or removed before adults emerge and start a new cycle.

This study also undertook life-cycle studies including the nut size limits for oviposition and longevity of the weevil.

For the purpose of this report *Sigastus* sp. weevil (also known as macadamia seed weevil) will be referred to by its common name *Sigastus* weevil.

Methodology

1. Literature review (Appendix 1, section 1.1.)

To obtain more detailed knowledge on *Sigastus* weevil, a comprehensive literature review was undertaken. This included the following components:

1.1. Biology of *Sigastus* weevil (Appendix 1, section 1.1.1.)

1.2. Ecology of *Sigastus* weevil (Appendix 1, section 1.1.2.)

- Hosts
- Life-cycle
- Damage
- Natural enemies

1.3. Pest Management (Appendix 1, section 1.1.3.)

There has been very little published on *Sigastus* weevil. In order to get a better understanding about management options a review was undertaken on key weevil pests in other horticultural crops that have been more extensively studied in the past. The selected weevil pests included pecan weevil, red palm weevil, banana weevil borer and elephant weevil. The pest management section included the following:

- Chemical control
- Biopesticide and nematodes
- Pheromone trap
- Cultural control
- IPM

2. Species identification, biology and life-cycle (Appendix 1, section 1.2.)

2.1. Species identification (Appendix 1, section 1.2.1.)

Specimens from different areas were collected to establish if there is more than one species. The regions included Atherton Tablelands, Bundaberg and different regions from NSW. Taxonomists from Museum of Victoria and Australian National Insect Collection (ANIC) were consulted and samples of Sigastus specimens were sent the NSW DPI Molecular Systematics Unit at Wagga Wagga for comparison with each other and the reference material on the BOLD and GenBank databases.

2.2. Biology and life-cycle (Appendix 1, section 1.2.2.)

By dissecting out freshly laid eggs from nuts and placing them in cell trays, the hatching time could be determined. Using this information, when we understood which stage the larva was in, the expected emergence time from ground samples could also be estimated. Infested nuts were collected from different orchards and brought back to the insectaries at Wollongbar Primary Industries Institute (WPII). To determine minimum and maximum lifespan in the immature stage, emerged weevils were collected daily and kept at ambient temperatures in a shaded brick room (20>Tmax >35) during the period.

Freshly emerged adults were separated and placed in 750ml Vacola jars with ventilated lids and fed freshly picked and unsprayed macadamia racemes (similar to Appendix 1, Figure 1.2.2-1). Each female was given access to a male for at least 2 months but after the male died the female remained alone. The adult survivorship and oviposition in the nut was recorded each week.

3. Current distribution (Appendix 1, section 1.3.)

The distribution of Sigastus weevil was established by using records from consultant samples, grower feedback from their farms and through direct surveys from the Macadamia Benchmarking project.

Mapping was developed from additional properties including extensive surveys by the NSW DPI entomology team and also reports from crop consultants, using the classic damage marks on green nuts as an indicator of weevil occurrence. Further outbreaks in subsequent years were also identified using this method, as well as the inclusion of an extra question on the Macadamia Benchmarking Project survey. Each year 150 Northern Rivers growers are contacted to be involved in the Benchmarking project which compares productivity and quality of nuts produced. We utilised this survey to ask growers whether they or their consultant had encountered Sigastus weevil on their property in the past year. This was recorded, and results were overlayed on a Google map image (see Figure 3.1).

4. Chemical control (Appendix 1, section 1.4.)

4.1. Screening of chemicals (Appendix 1, section 1.4.1.)

It was necessary to develop an assay technique that could show the pest mortality rate in each life cycle stage. To measure ingested mortality, methods used included topical application to measure knockdown (1µL dorsally) and ingestion of treated nut tissue (dipped nutlets) for adults Field collected adult weevils were used for the screening.

We also investigated the survival of freshly laid eggs from nutlets that had been dipped in various insecticides.

4.2. Evaluation of entomopathogens (Appendix 1, section 1.4.2.)

4.2.1. Laboratory culturing of *Beauveria* and *Metarhizium* (Appendix 1, section 1.4.2.1.)

Fungal isolates

The isolates were obtained from either soil samples or dead insects, including isolations from dead Sigastus weevils collected in New South Wales. Cultures were stored at 4°C and -22°C on agar slants of malt extract agar (*Beauveria* isolates) and Sabouraud's Dextrose Agar (SDA) (*Metarhizium* isolates).

Temperature characterisation

Thermal growth characteristics of isolates were determined by measuring radial growth on SDA plates over 14 days at a range of temperatures (15°C; 20°C; 25°C; 30°C and 35°C). Surface radial growth was recorded using two cardinal diameters, through the X and Y axes on days 7 and 14.

Growth media comparison

The preference for the isolate's different agar for growth and sporulation on different media was investigated similarly to the thermal growth characteristics above. The isolates were grown on.

Spore Production

A liquid culture was first grown to inoculate solid media. Solid media of oats, rice or millet were initially investigated for spore production and inoculated with 15ml of the liquid culture for each fungus. Rice was used for *Metarhizium* production and oats were used for *Beauveria* production. The inoculated media were kept in mushroom spawn culture bags and were incubated for seven days at 28°C. The cultures were broken up and left for further 10 days of growth. Bags were opened and left to air dry for 3-4 days. Spores were harvested from the dried grain and stored at 4°C.

4.2.2. Screening of entomopathogens (Appendix 1, section 1.4.2.2.)

Once 100 *Sigastus* weevil adults were available, a series of 10 replicates of 10 individuals was used to compare survivorship of those exposed to the test chemistries with untreated control of demineralised water. Insects were initially kept in 750ml disposable, rectangular, plastic food containers with breathing holes (Appendix 1, Figure 1.4.1.-2), These caused a problem with *Beauveria* sp. infection. We therefore changed to 750ml glass Vacola jars with gauze lids (Appendix 1, Figure 1.4.1.-2).

Macadamia nuts with *Sigastus* weevil eggs were dipped into the insecticide mixtures and stored in labelled plastic cell trays (12 cells per tray), at ambient temperature (25^{o} C). The number of individuals that were alive or dead, their developmental stage and the presence of any fungal growth on the bodies (Appendix 1, Figure 1.4.1.-3 C) was recorded.

Field applications have been made at the CTH "Sink block", in April 2016 where the population has been monitored closely. A few other select sites have been inoculated with fungal spores, to see if any evidence of field infection will present.

Spores have been successfully isolated and cultured on oat meal agar (Appendix 1, Figure 1.4.2.-1). at DPI Wollongbar and at DAFQ Ecosciences Precinct Brisbane (Diana Leemon). We are attempting to source other material that maybe active in the field, for evaluation when the insects are available to test.

4.3. Field assessment (Appendix 1, section 1.4.3.)

The field trial involved weighing and collecting all infested nuts to find a minimum figure for dropped nut under each tree in the unsprayed CTH "Sink block" fortnightly from July 2015 to January 2016, and allocating the cause of nutfall (Appendix 1, Figure 1.4.3.-1).

No pesticides have ever been applied in this area and parasitoids for FSB and *Cryptophlebia ombrodelta* (MNB) were used at the first sign of activity in October 2015

Development of the spray timing to combat the pest emergence period

By collecting the nut drop and determining the impact the various pests are having at a particular time in the season, it was possible to build a temporal treatment picture for each pest (Appendix 1, Figure 1.4.3.-1).

Outputs

- A shortlist of best chemical options, including label extensions for existing registrations in macadamias for *Sigastus* weevil control, based on laboratory screening.
- Characterisation of the local *Beauveria bassiana* strain for testing against *Sigastus* sp.
- Establishment of a comparative demonstration plot with the Beauveria treatments and standard chemistries at NSW DPI CTH Alstonville site
- An initial monitoring strategy for *Sigastus* weevil
- A map detailing the occurrence of *Sigastus* weevil across the major macadamia production regions (see Figure 3.1)
- Determination that the North Queensland and NSW populations are the same species
- A final report detailing:
    - The species occurring in NSW and Queensland
    - Initial efficacy data on the currently registered and future FSB chemistry impact on *Sigastus* weevil evaluated
    - Preliminary findings on the efficacy of indigenous and commercially available *Beauveria* species
    - Recommendations for incorporating *Sigastus* into an IPM strategy for Macadamia

Publications

Australian Macadamia Society (AMS) (2014) Fact Sheet 12 Sigastus weevil pest Information and management options.

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Maddox, C. (2014) Can we make *Sigastus* pest management easier? *AMS News Bulletin* **42**(2), 42-43.

Maddox, C., Pretorius J., Bright J, Huwer R, Robertson D, Purdue I, Janetzki A and Dawes M (2015) Spray patterns and the changing pest pressure for Australian macadamia production.7th International Macadamia Symposium August 2015 Skukuza South Africa- SAMAC www.samac.org.za/index.php/info/international

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Outcomes

1. Literature review (Appendix 2, section 2.1.)

The literature published on *Sigastus* weevil was very limited and therefore needed to be extended to other major weevil pests, which has produced a comprehensive overview on weevil pests and potential management options.

1.1. Biology of *Sigastus* weevil (Appendix 2, section 2.1.1.)

The *Sigastus* weevil, causing damage in macadamias, belongs to the genus *Sigastus* Pascoe (Curculionidae: Molytinae: Hplonychini) (Fay *et al.*, 1998), but is so far undescribed and not identified.

Fay *et al.* (1998) established that female weevils start oviposition into nuts about 4-6 weeks after nut-set. The female scarifies the husk and lays a single egg into it (Fay *et al.*, 1998). After oviposition the nut stalk is chewed to induce drop. After shell hardening, the nuts are no longer suitable for oviposition, but adults continue to feed on the husk. Adults also feed on young leaves (Fay *et al.*, 1998).

Larvae consume entire kernels and pupate within the nuts. The development time from egg to adult is approximately 6 weeks. The larger the nut, the bigger the adult will be. Larger nut size also decreases the development time and increases the survival rate (Fay *et al.*, 1998).

1.2. Ecology of *Sigastus* weevil (Appendix 2, section 2.1.2.)

Hosts

Juniper and Britton (2010) reported that *Sigastus. casaurinae*, *S. facicularis* and *S. fuscodorsalis* have been reared on galls of *Eucalyptus* and *Casaurina* and the flowers/fruit of *Syzygium armstrongii* and *S. suborbiculare* respectively (Juniper and Britton, 2010). One undescribed *Sigastus* sp. was reared on *Syzygium hemilamprum* and the second undescribed *Sigastus* sp. is the pest in macadamias (Juniper and Britton, 2010).

Life-cycle

Little is known about how long each stage (egg, larva, pupa to adult) takes to develop. Lack of sufficient information about the life-cycle makes the management of *Sigastus* weevil difficult.

Damage

Fay *et al.*, (1998) reported that the crop loss in an unsprayed orchard may be up to 30%.

Favorite conditions for *Sigastus* weevil are the following (AMS, 2012):

- Abandoned and untreated orchards are the major source.
- Long flowering season, or out of season flowering with a range of crop stages.
- Dark orchards.
- Warm winter months.
- Native vegetation.

Natural enemies

As part of a study by the Cooperate Research Centre for Tropical Rainforest Ecology and Management (Anon., 2002), 5 species of wasps have been reared from *Sigastus* weevil infected nuts.

In summary:

The literature review gave us some basic information on *Sigastus* weevil, their biology, different species found in Australia, their host (*Syzygium* spp., *Eucalyptus* spp., *Casuarina* spp. and *Macadamia* spp. Further, preferred conditions for the weevil and records of natural enemies were mentioned.

1.3. Pest Management (Appendix 2, section 2.1.3.)

1.3.1. Chemical control (Appendix 2, section 2.1.3.1)

Sigastus weevil

Fay *et al.* (1998) tested the efficacy of carbaryl (125 g/100L), methidathion (125ml/100L) and beta-cyfluthrin (50ml/L). Three days after application mortality was 100% in the carbaryl and methidathion treatments and 86.7% in the beta-cyfluthrin treatment (Fay *et al.,* 1998). Fay *et al.* (1998) recommend methidathion as initial spray, coinciding with the first nut drop, followed by spray applications for macadamia nutborer.

Pecan weevil

In the US, the recommended chemicals for the control of pecan weevil are different synthetic pyrethrins, carbaryl (carbamate) and phosmet (organophosphate) (Appendix 2, Table 2.1.3.1.1) (Ree *et al.*, 2011; Lee *et al.*, 2013; Wells *et al.*, 2016).

Red palm weevil

A list of recommended chemical recommendations for red palm weevil control in coconut and date palms in different countries, is shown in Appendix 2, Table 2.1.3.1.2. (Faleiro, 2006). Imidachloprid and fipronil are also used in prophylactic and curative applications to control the red palm weevil (Kaakeh, 2006; Al-Shawaf *et al.*, 2010; Al-Dosary, 2016).

Banana weevil borer

Chemicals that are currently registered in Australia to control banana weevil borer by the *Australian Pesticides and Veterinary Medicines Authority* (*APVMA*) are listed in Appendix 2, Table 2.1.3.1.3 (APVMA, 2016).

Collins *et al.* (1991) reported on resistance to 4 organophosporous insecticides (pirimiphos, prothiophos, chlorpyrifos and ethoprophos) and evidence of cross-resistance to oxamyl.

Different botanical extracts were also tested and established that they possessed limited insecticidal properties, but the impact on oviposition needs further investigation (Tinzaara *et al.*, 2006).

A study in South Africa tested 5 different insecticides (Appendix 2, Table 2.1.3.1.4) against the banana weevil borer, with fipronil and imidacloprid showing the best control (De Graaf, 2006). A study in Cameroon found that terbuphos was also highly effective (Mongyeh, *et al.*, 2015).

Elephant weevil

In the laboratory trials, foliar applications of indoxacarb, imidacloprid and clothianidin resulted in the greatest mortality of the weevil. In field trials, indoxacarb and imidacloprid gave the highest control Murdoch (2010).

1.3.2. Biopesticide and nematodes (Appendix 2, section 2.1.3.2.)

Pecan weevil

Past studies showed that low concentration of *Beauveria basiana* could kill larvae and adults of the weevil in the field (>75% mortality) (Neel and Sikorowski, 1972; Shapiro-Ilan *et al.*, 2008; Mulder *et al.*, 2012).

A survey on entomopathogenic nematodes (Shapiro-Ilan *et al.*, 2003) showed that entomopathogenic fungi were more common (76%) than nematodes (28%).

Red palm weevil

As part of a larger review on palm weevil control, Faleiro (2006) collated work on biological control that was done over time, which are listed in Appendix 2, Table 2.1.3.2.1. The different biological control agents included bacteria, viruses, enotmopathogenic nematodes (ENP), fungi, flies and wasps (Faleiro, 2006). Neither of the different biological control options gave outstanding control by itself (Faleiro, 2006).

Banana weevil borer

In a study by Fancelli *et al.* (2013) a number of *B. bassiana* isolates, were screened in the laboratory and field conditions in Brazil, resulting in a highly effective strain being identified.

Elephant weevil

Murdoch (2010) tested entomopathogens for the control of the elephant weevil in blueberries. The commercial product Mycoforce and *Beauveria bassiana* var. EWB gave the best results, comparable with synthetic insecticides (Murdoch, 2010).

In summary:

The literature showed commonalities in chemical control of major weevil pest. Generally carbaryl, methidathion, fipronil and imidacloprid gave good results. A resistance to organophosphates has been shown in banana weevil.

The entomopathogenic fungi *Beauveria bassiana,* has been used in the major weevil species selected with varying success.

1.3.3. Pheromones and pheromone traps (Appendix 2, section 2.1.3.3.)

Pecan weevil

The study by Mulder *et al.* (2003) did not show any advantage of any pheromone tested.

Red Palm weevil

In a comprehensive review, Faleiro (2006) reports of the development of pheromone traps for the red palm weevil, baited with a pheromone and ethyl acetate lure. Later, it included food bait (e.g. dates, coconut petioles and sugar cane) mixed in 1L of insecticide (0.05% Carbofuran) (Anon., 1998, Oehlschlager, 1998, Faleiro, 2006). A number of pheromones have been tested over time. These are listed in Appendix 2, Table 2.1.3.3.1. and a suitable trap density of 1 trap per ha has been established (Faleiro and Satarkar, 2003b; Faleiro, 2006). Pheromone traps for the red palm beetle have been successfully integrated into IPM systems in a number of countries (Faleiro, 2006).

Al-Dosary *et al.* (2016) suggests that the addition of insect repellents to pheromones could be useful for sustainable management of the red palm weevil.

Banana weevil borer

De Graaf (2006) investigated options for trapping weevils in South Africa. Cosmolure proved to be the most effective of the different traps (DeGraaf, 2006).

A study by Tinzaara *et al.* (2007a) showed that fermented banana pseudostem tissue was as attractive as the pheromone but more attractive than fresh pseudostem tissue (Tinzaara *et al.*, 2007a). Volatiles from pseudostem tissue and pheromone lure showed a synergistic effect in attracting weevil in the laboratory, but not in the field trials (Tinzaara *et al.*, 2007a).

In summary:

The literature showed that pheromone traps have been successfully developed for banana weevil borer and red palm weevil.

The addition of plant tissue has shown good results in the control of banana weevil borer.

Pheromone traps have been successfully integrated into IPM for the red palm weevil

1.3.4. Cultural control (Appendix 2, section 2.1.3.4.)

Sigastus weevil

Fay *et al.* (1998) considered that the sweeping of fallen nut into the interrows and solarisation to kill larvae (between mid-September and mid-December), is an important part of the management of the weevil.

Banana weevil borer

DeGraaf (2006) investigated cultural control options for the banana weevil borer in South Africa. Covering of the mat with soil and moving debris to the inter-row; was the only effective treatment which reduced the damage parameter most closely related to yield, by 14.18%.

In summary:

Solarisation of infected nuts for *Sigastus* weevil and hygiene for banana weevil borer have been noted as important cultural control methods.

1.3.5. IPM (Appendix 2, section 2.1.3.5.)

Pecan weevil

An IPM strategy investigated for pecan weevil (Reid, 2002; Reid and Mulder, 2003; Ree *et al.,* 2011). Ree *et al.* (2011) are taking a trap and spray approach, using monitoring as triggers for insecticide treatments (Ree *et al.* (2011).

A study by Shapiro-Ilan *et al.* (2011) showed that a synergistic effect of carbryl and an antagonistic of cypermethrin on *B. bassiana*. Both chemicals had a synergistic effect on the nematode *Steinernema carpocapsae* (Weiser) in controlling weevil larvae and an additive effect in control of adults (Shapiro-Ilan *et al*., 2011).

Red palm weevil

In a comprehensive review, Faleiro (2006) looked at different options for control of the red palm weevil, including chemical control, biological control, monitoring and lure and kill, host plant resistance in different palm species and varieties, and male sterile technique. The pheromone based strategy offers a sustainable approach (Failero, 2006). Effective biological control combined with host plant resistance was also suggested as an important part of an IPM approach for this weevil (Failero, 2006).

As part of cultural management, Al-Dosary *et al.* (2016) considered sanitation (Abraham *et al*., 1998; Al-Ajlan, 2008), varietal preference or host plant resistance (varieties sugar content are more susceptible and varieties with higher calcium content inhibit growth and development of the weevil) (Farazmand, 2002; Faleiro, 2006; Al-Ayedh, 2008; Al-Dosary *et al.*, 2016), plant density, pruning of fronds and removal of off-shoots as an important part an IPM strategy. Gene silencing or RNA interference (RNAi) are considered a potential future path to develop resistant plants (Niblett and Bailey, 2012; Al-Dosary *et al.*, 2016). Furthermore, a strict quarantine regime is very important (Al-Dosary *et al.*, 2016). Al-Dosary *et al.* (2016) give a good summary of case studies of area wide management, which are listed in Appendix 2 Table 2.1.3.5.1.

Banana weevil borer

The study by Tinzaara *et al.*, (2007b). showed that infected weevils successfully transmit the fungal pathogen to healthy individuals. Mortality due to *B. bassiana* is significantly higher where the pathogen was applied in combination with the pheromone (Tinzaara *et al.*, 2007b).

In summary:

The literature review noted that IPM approaches for weevil pests included IPM compatible insecticides, biological control, orchard hygiene, host plant resistance and the use of pheromone traps in combination with entomopathogenic fungi.

2. Species identification, biology and life-cycle (Appendix 2, section 2.2.)

2.1. Species identification (Appendix 2, section 2.2.1.)

The DNA barcodes show that there is no species difference between the samples from the sites and some haplotypes present are found in both Alstonville and Tolga (QLD). Unfortunately there was no previous record of the *Sigastus* genera on the GenBank or BOLD databases (Appendix 1, Figure 1.2.1.-3). This has been updated now, and the Juniper and Britton (2010) samples would be a worthwhile comparison.

2.2. Biology and life-cycle (Appendix 2, section 2.2.2.)

Egg hatching time is 6 (+/- 1) day at 25^{o} C. Emergence rate of the adult weevils from infested nut >10mm diameter ranges between 30-70% (Appendix 2, Figure 2.2.2.-1). The time from egg to adult takes 40 (+/- 8) days depending on temperature (Appendix 2, Table 2.2.2.-1). The longevity of the weevil is 100-150 days conservatively in the field and over one year in the laboratory (Appendix 2, Table 2.2.2.-2). A female lays over 300 eggs during their lifespan and average between 10-20 eggs per week (Appendix 2, Table 2.2.2.-2).

Adults' emergence rises during November, drops back through December, then rises again in January as the second generation begins to emerge (Appendix 2, Figure 2.2.2-2). The emergence rate from the field nut collected averages 50% when the size is >10mm (Appendix 2, Figure 2.2.2-2).

Trees with out-of-season nut can enhance the *Sigastus* weevil egg production within a plot by a factor of 4 (68/tree vs 270+/tree between July and January) and give a continuing supply of new weevils (Appendix 2, Tables 2.2.2.-3 and 2.2.2.-4). The weekly collection of fallen nuts from under the trees, prevented most of the second generation from establishing.

The take home message is that the infected nut on the ground in late winter/spring is crucial in the build up and damage caused by the Sigastus weevil population. Where possible we should be removing that infected nut in spring and trying to limit how much of that crop is available to Sigastus weevil by maintaining a good nut set in spring.

In summary:

Sigastus weevils from the different areas are the same species. Life-cycle and biology of the weevil are better understood and described. The offspring of the first generation is important and needs to be controlled.

3. Current distribution (Appendix 2, section 2.3.)

Mapping was developed from grower and consultant information, grower survey and the AMS Benchmarking project. Records were overlayed on a Google map image, Figure 3.1 showing the expanding distribution of the pest over time.

The red area represents the initial outbreak. The orange represents the distribution 1 year later and green area represents the currently known distribution area.

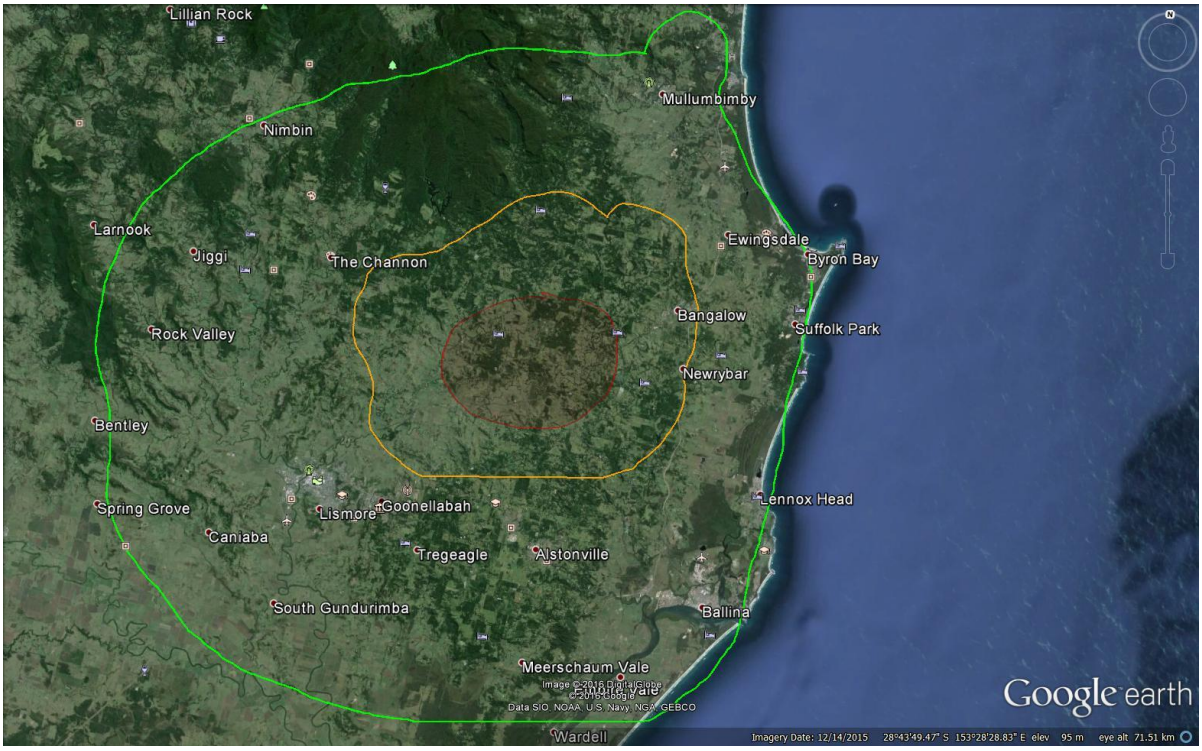

Figure 3.1: Expanding distribution of *Sigastus* weevil; red = initially infected area, orange = distribution after 1 year and green = current distribution.

In summary:

Sigastus weevil is widely distributed in the NSW Northern Rivers macadamia growing area, but is yet to establish in Central Queensland.

4. Chemical control (Appendix 2, section 2.4.)

4.1. Screening of chemicals (Appendix 2, section 2.4.1.)

Pesticide assays

Topical doses of 1µl confirmed the grower perception of a poor knockdown effect, where nothing applied gave a complete kill at the registered rates. The order of efficacy (90% mortality maximum) of the currently registered products is beta-cyfluthrin 0.1ml/L (2x rate), carbaryl 1.3ml/L, acephate 0.8gm/L, methidathion 1.25ml/L, then diazinon 1.3ml/L and trichlorfon 2ml/L. Of the soon to be registered chemicals for fruitspotting bug, sulfoxaflor 0.4ml/L was more toxic to *Sigastus* weevil than the acetamaprid mixture 0.8ml/L. The unregistered foliar compounds tested so far show methomyl 2ml/L and Bifenthrin 0.5ml/L were the most notable.

Better mortality results were achieved with dipped food assays, showing how important coverage is for *Sigastus* weevil. All registered options gave mortality figures between 85-100%. Sulfoxaflor and the acetamaprid mix also gave 100% mortality. Of the new and unregistered options tested, methomyl 2ml/L, bifenthrin 0.5ml/L, were 100% effective, and the carboxamide, chlorantranilliprole and cyantranilliprole, and DC143 could all work if coverage is optimal and allowable rates adjusted.

In summary:

Good coverage is the key to control here. Trichlorfon is the least effective of the available chemicals. Of the new chemistries, sulfoxaflor and acetamaprid are promising as a rotational chemical for beta-cyfluthrin, methidathion and acephate. If stronger foliar controls are needed, methomyl 2ml/L and bifenthrin 0.5ml/L are the likely candidates of the older chemistries.

4.2. Evaluation of entomopathogens (Appendix 2, section 2.4.2.)

4.2.1. Laboratory culturing of *Beauveria* and *Metarhizium* (Appendix 2, section 2.4.2.1.)

Four different isolates of entomopathogenic fungi were investigated. These included one strain of *Metarhizium anisopliae* (M16) known to have a wide host range and very good spore production characteristics, and three other isolates all identified as different strains of *Beauveria bassiana*, (B24, B27 and Bbsig) including the fungus infecting the *Sigastus* weevil.

All isolates had the highest growth rate on Sabouraud's Dextrose agar but sporulated best on oatmeal agar.

4.2.2. Screening of entomopathogens (Appendix 2, section 2.4.2.2.)

Once the organism was collected from the field a series of limited field laboratory and field experiments showed it was active under high humidity conditions. Within the laboratory weevil colonies it would be lethal within 10 days, in the field it was very difficult to see any evidence of reduced activity.

Culturing the organism to increase the active spore load and stabilize it, was attempted successfully at NSW DPI, using oat meal agar. Diana Leemon at DAFQ also found a successful culturing medium.

Despite the limited replication at this stage, we have a very promising result. The background mortality rate is normally 50% for field nutlets with *Sigastus* weevil eggs. *Isaria* sp., *Metarhizium* sp. and *Beauvaria bassiana* were tested. *Beauvaria bassiana* from *Sigastus* weevil gave the most effective control (about 100% mortality). Pulse® and Synertrol® additions to the entomopathogen had a synergistic effect and resulted in the highest mortality of the developing weevil larva in nut.

In summary:

The *Beauvaria bassiana* strain collected from infected nuts in macadamia orchards is the most promising fungal option tried to date and it appears to be able to get into the dropped nutlets. More commercial and experimental strains have been accessed and trials are ongoing.

Development of the spray timing to combat the pest emergence period

The correct time to reduce the impact of the adults on the developing nuts is variety dependent. Orchards with good control are targeting the period when the adults are beginning to drop nutlets and when the emergence of adults is at reaching a maximum. The of out-of-season nutset increases *Sigastus* weevil breeding potential by a factor of 4. Management of the weevil population causing the spring nutdrop is very important orchard hygiene is crucial.

In summary:

Targeting the adult initial nut drop within the season, then the period of maximum weevil emergence is being successfully practiced in NSW. The removal of the spring infested nut is also key in limiting the impact on the main crop up until late December.

First field trials have been set up and chemicals applied, but data collection could not be completed within the timeframe of the project.

Evaluation and Discussion

Literature review

The literature review gave a good overview of what is known about *Sigastus* species and management of other selected weevil pests. It showed that current chemical control of *Sigastus* weevil management is in accordance with the chemical control of other weevil pests. However, it is important, that new and softer chemicals will be tested for efficacy in the future.

The review shows that entomopathogens have been tested for other species with varying results. This will have to be confirmed for *Sigastus* weevil.

Biological control of the pecan weevil *Curculio caryae* has been successful, but this weevil has a different life-cycle to *Sigastus* weevil. *C. caryae* pupates and diapauses in the soil (Mulder *et al.*, 2012) before emerging as adults, which would probably give pathogens and nematodes a better chance to infect the insect. Rather than targeting adult weevils in trees, potentially treatment of the developing larvae in nuts on ground would be a better option.

The literature review did point out the importance of orchard hygiene and this will need to be included in a management strategy for *Sigastus* weevil. Practices such as removal or destruction of infested nut on the orchard floor to break the life cycle can be implemented immediately by the industry, and should be included as part of any future extension efforts in IPM.

There is currently no monitoring tool for *Sigastus* weevil. The literature review shows that pheromone traps are being successfully used for pecan weevil, red palm weevil and banana weevil borer. This is certainly one area that needs to be included in future *Sigastus* weevil research. A monitoring trap would further allow the development of an IPM program. It would be important to establish if pheromones would improve the impact of entompathogens and have a synergistic effect.

Species identification

DNA comparison of *Sigastus* weevil specimen collected in different areas showed that we are only dealing with one species. In the future it might be worthwhile looking at the DNA of the other *Sigastus* species and review the genus *Sigastus*.

Biology and life-cycle

Through this study we gained a much better understanding of the biology of *Sigastus* weevil. We are now able to identify different generations through the season which allows us to determine best treatment times.

Some larval parasitoids had been observed by Ross Blanche CSIRO (Anon., 2002), and Jarrah Coates (2016) found a Doryctine Braconid wasp emerging from weevil larvae (Appendix 1, Figure 1.4.1.-1) but as yet no significant biological control agent has been found. Again, these have only been preliminary investigations and in the future a more targeted survey for parasitoids and parasites is warranted.

Current distribution

We identified the area where the dispersal of *Sigastus* weevil initially started from and were able to follow the expansion of the distribution over time. This gives us a good starting point for calculation of its dispersal capacity. In the late 90's the weevil was identified as an emerging pest in macadamias on the Atherton Tablelands in Far North Queensland (Fay *et al.*, 1998), but the reason why and how this pest has become an issue in the macadamia growing areas of NSW is yet unknown.

Chemical control

- *Screening of chemicals*

The screening trials showed that of the new chemistries, sulfoxaflor and acetamaprid showed promising results. Sulfoxaflor is highly toxic to bees any use will need to be restricted to a time when no bees are active in the orchard. It will be important to build them into a rotation with beta-cyfluthrin, methidathion and acephate that will also cover other pests such as fruitspotting bugs. If stronger foliar controls are needed, methomyl 2ml/L and bifenthrin 0.5ml/L are useful chemicals that could be used as last option.

As this has been a pilot study, it is important to continue screening of new chemicals to have alternative chemical options when older chemicals are getting de-registered.

- *Laboratory culturing of Beauveria and Metarihizium*

Once the best isolates for *Sigastus* weevil control are selected further investigations should be carried out into formulation agents for the entomopathogenic fungi. Microbial agents can be formulated in different ways to improve their delivery and efficacy. A good formulation has the potential to provide numerous benefits for myco-insecticides such as longer storage, easier handling and greater field efficacy, while at the same time maintaining the positive ecological attributes inherent in biological control.

Studies into the best formulation and application strategies for the Macadamia industry will also need to be researched. Usually the most common application system(s) already in use for controlling pests and diseases in an agricultural industry will be the most practical system to use. However the formulation will be critical for transitioning an application method used for chemical insecticides across to the successful use of myco-insecticides.

– *Screening of entomopathogens*

The preliminary tests showed that the *Beauvaria bassiana* strain collected from infected nuts in macadamia orchards is the most promising pathogen option tested so far. An optimum formulation for this pathogen strain still needs to be identified and developed.

We have now developed a suitable screening technique and there are more commercial products that should be tested as part of future research.

Any of the major fungal organisms thrive in confined high humidity, but that is not the situation in the field where the adult *Sigastus* weevil is high in a tree top and environmental conditions do not favour the fungus. The challenge here is to develop a method of delivering the fungus to the weevil that is lethal, with the fungus, collected from infected macadamia nuts, remaining stable for long periods. The most promising direction would be an association with a food lure or pheromone which has been done already with other weevils.

Field trials

Field trials were set up as part of the pilot study, but within the timeframe of this project we were not able to complete data collection and analysis.

What we were able to do as part of the field trial within the timeframe of the project was the development of the spray timing strategy. Targeting the adult initial nut drop within the season, then the period of maximum weevil emergence is being successfully practiced in NSW. The removal of the spring infested nut is also key in limiting the impact on the main crop up until late December. This also demonstrated the importance of orchard hygiene.

In summary, this has only been a pilot study and there are we are still a lot we need to investigate in order to be able to develop a management strategy for *Sigastus* weevil. However, this study showed that there is scope for the development of a successful IPM program using IPM compatible insecticides, entomopathogens, pheromone traps and orchard management, including orchard hygiene (i.e. removal, mulching or solarisation of infected nuts) and management of out of season flowering and nut set. Any management strategy for *Sigastus* weevil will need to fit into a greater pest management strategy for macadamia pests.

From the past we have learned from changes in pest management can create niches for previously minor pests and we need to be careful to consider the whole system.

Recommendations

– It has to be kept in mind that this has only been a pilot study and results from chemical screening and the *Beauveria* sp. investigations are very early indications at most.

  We therefore recommend that screening of chemicals and *Beauveria* sp. isolates and commercial entomopathogens need to continue and be validated in field trials.

- An outcome from the literature review is that monitoring with pheromone traps have been successfully adopted as monitoring tool for other important weevil pests and should be investigated in future *Sigastus* weevil research.

- Options for area wide management, including a combination of pheromone traps and entomopathogens for control appears to be a feasible approach and should be part of future research.

- A clear recommendation from this project that comes from this pilot study is the impact of out of season flowering and nutset on weevil populations. There are several options for non-chemical management of populations by breaking the life cycle of the pest, by management of out of season flowering which restricts feeding and breeding sites in the orchard.

- Out of season flowering needs to be prevented to ensure a break in the *Sigastus* life cycle.

- A small trial at CTH Alstonville also emphasised the importance orchard hygiene and removal of infested nuts, which is paramount for *Sigastus* weevil management.

- A better understanding of the pest life cycle and cultural management options should be part of a wider extension effort across the entire macadamia industry, including those areas that have not been impacted by the pest to date.

Scientific Refereed Publications

N/A

Intellectual Property/Commercialisation

No commercial IP generated

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Appendices

Appendix 1: Detailed Methodology

Appendix 2: Detailed Results

1. Appendix 1: Detailed Methodology

1.1. Literature review

To obtain more detailed knowledge on *Sigastus* weevil, a comprehensive literature review was undertaken. This included the following components:

1. Biology of *Sigastus* weevil

2. Ecology of *Sigastus* weevil

- Hosts
- Life-cycle
- Damage
- Natural enemies

3. Pest Management

There has only been very little published on *Sigastus* sp. In order to get a better understanding about management options a review was undertaken on key weevil pests in other horticultural crops that have been more extensively studied in the past. The selected weevil pests included the pecan weevil, the red palm weevil, banana weevil borer and the elephant weevil. The pest management section included the following:

- Chemical control
- Biopesticide and nematodes
- Pheromone trap
- Cultural control
- IPM

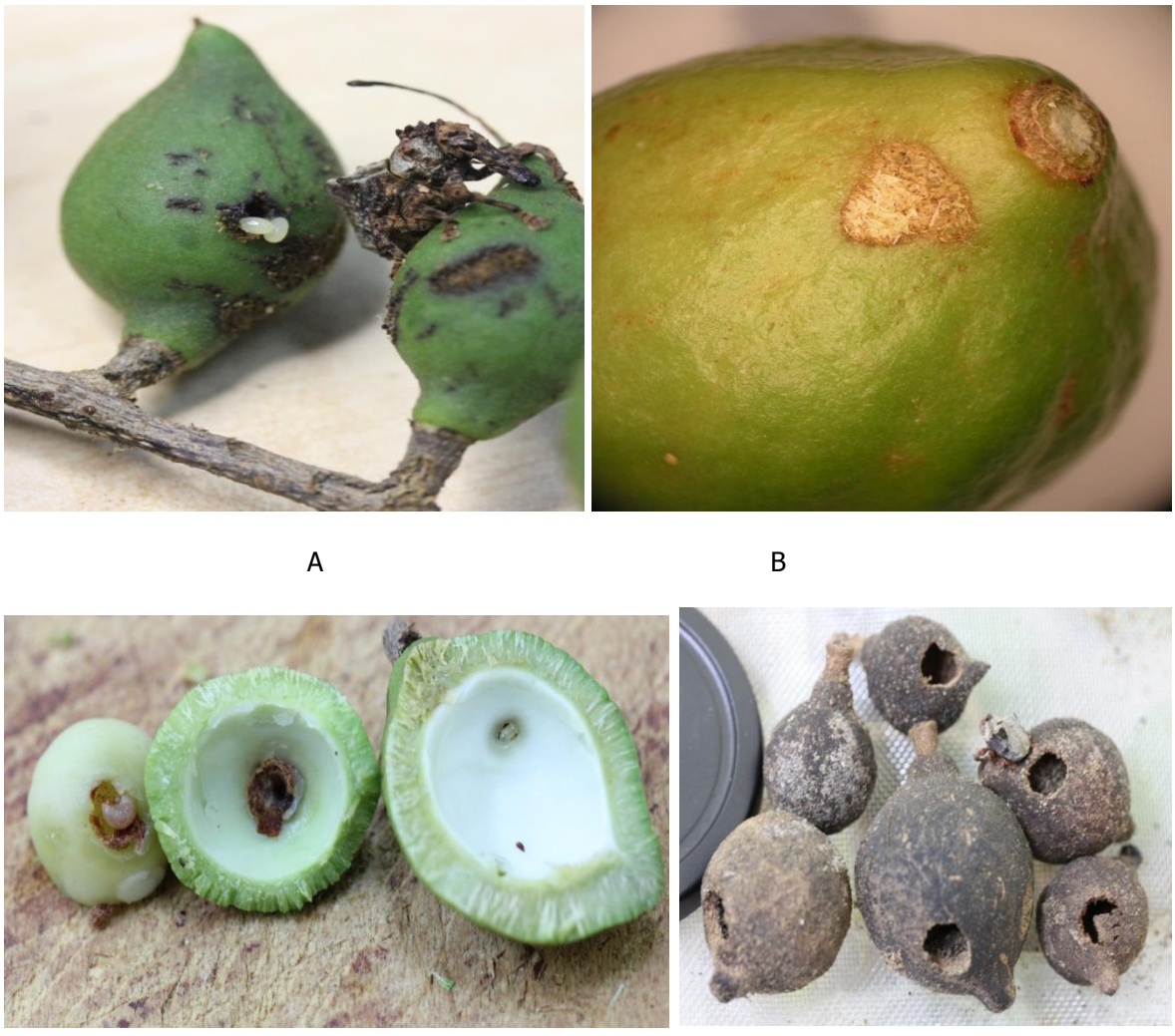
1.2. Species identification, biology and life-cycle

1.2.1. Species identification

Finding a name and DNA barcoding for Sigastus weevil populations

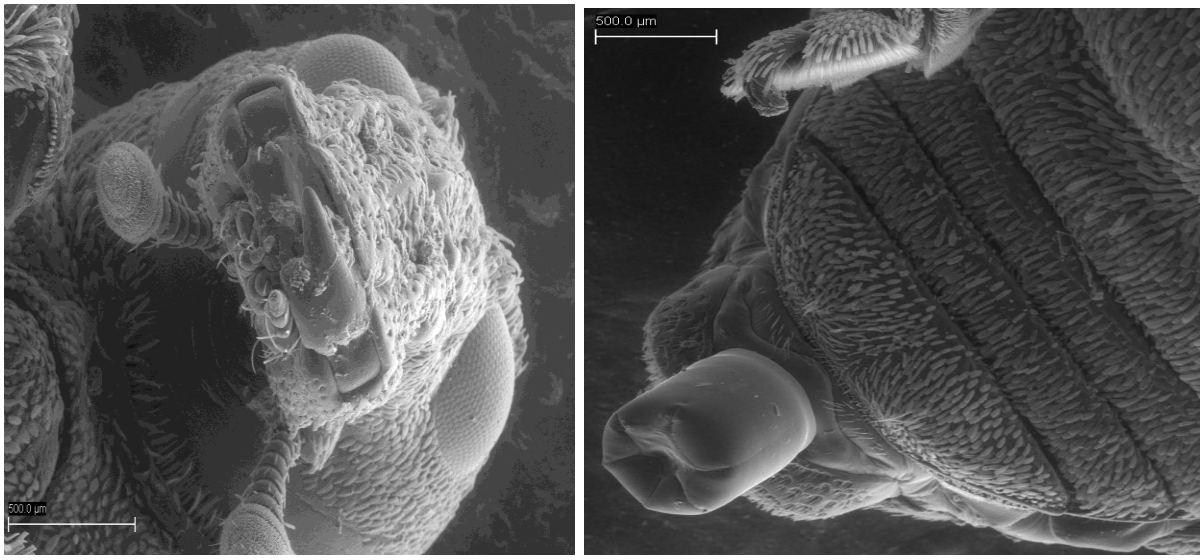
The original identifications gathered by Harry Fay (1995-98) QDPI suggested this was a new species, perhaps even a new genus of weevil for the Australian taxonomists and with that comes significant labelling issues. It is different to the new *Sigastus* species found in *Syzygium* sp. fruit (Juniper and Britton, 2010) and we now know the undescribed *Sigastus* species 2 will feed on *macadamia tetraphylla*, *M. ternifolia* as well as the *M. integrifolia* listed (Fay *et al.*, 1998). The issue will be resolved as the molecular taxonomists improve the areas within the weevil fauna that have been mapped. The genus name used at present is *Sigastus* and we will continue to use it until the taxonomists provide a final reason to change it. The name refers to the weevil that lays its egg within the developing macadamia kernel shell tissue and emerges through the shell margins via a chewed 5-10mm hole (Figure 1.2.1.-1). The most appropriate common name for the pest is probably macadamia seed weevil, as there are many other weevils living on macadamia but none breed within the seed like this one (Figure 1.2.1.-1, Figure 1.2.1.-2, Figure 1.2.1.-3). Specimens from different areas were collected to establish if there is more than one species. Department of Agriculture and Fisheries Queensland (DAFQ) have material collected from Bundaberg-Baffle creek in early 1990

(Haaksma pers com, Gallagher *et al*. 2003), and anecdotally long-term growers in NSW did believe damage of this type has been present for as long as they can remember, but not on the scale it has been since 2010 (Bright pers com.). Consultation with Ken Walker (Museum Victoria Curator), Rolf Oberprieler (CSIRO weevil expert) suggested that the closest species to the weevils sampled are in the Curculioninae: Haplonychini, Storeini. Photos from Ken and Rolf of *Sigastus fuscodorsalis* (Heller) a northern territory weevil has a colour and size match, but it is smooth, unlike the new weevil (Figure 1.2.1.-1A) and *Haplonyx fasciculatus* (Boheman) another native weevil carries some of the texture but not the markings. A reference sample from the *Haplonyx* genera found in the Global DNA database only shows a 74% genetic proximity to our unknown species, the Scarab cane beetle *Rhopaea magnicornis* was closer than other exotic scolytid weevils (*Xylosandrus* sp.) in the sample tested, so they are not genetically similar to anything presented so far.



A B C D

Figure 1.2.1.-1 A) *Sigastus* weevil on developing macadamia showing adult body sculpture and two eggs. B) The oviposition mark left by the weevil with the plug covering the egg inside the shell. C) The internal view of where the egg is placed and how the larva feed below the plug on the kernel. D) Adult *Sigastus* weevil emergence holes showing how the larvae have removed sufficient softer shell to escape the nut when it hardens.



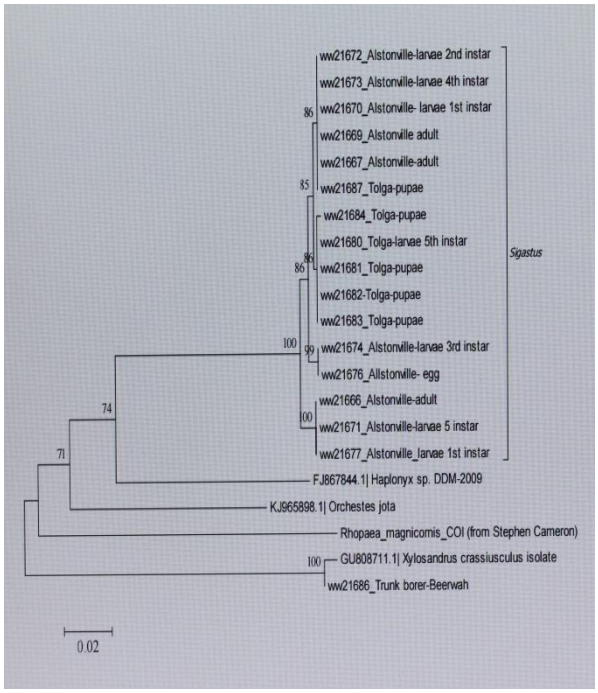
A B

C D

Figure 1.2.1.-2: A) Maxine Dawes SCU Lismore, micrograph of the *Sigastus* weevil mouthparts. B) Maxine Dawes SCU Lismore, micrograph of the *Sigastus* weevil "cookie cutter" ovipositor. C) Multiple oviposition marks made by the macadamia seed weevil are unusual in the field normally only 1 per nut, competition for the kernel in small nut even with nutborer is usually fatal. D) Male and female weevils on macadamia nut, mating is frequent and both sexes have the rough nodules on the elytra and pronotum.

Collections of infested nut (>100 nut with eggs) from the original problem areas in Northern Queensland around Tolga and Atherton were made. These nuts, along with a similar sample taken from CTH Alstonville plots and a field sample brought in from Dunoon were maintained as separate populations at the Wollongbar facility from November 2015 (Figure 1.2.1.-3, Appendix 2, Table 2.2.1.-1). After 1 month, when the first generation had begun to emerge in the cages and new laying was occurring on the host nuts being provided each week, a series of samples were taken to cover egg, larval, pupal and adult stages of the weevil from the Alstonville and Tolga sites. These were sent to David Gopurenko at the Wagga Wagga Molecular Systematics Unit for comparison with each other

and the reference material on the BOLD and GenBank databases.



A B

C D

Figure 1.2.1.-3: A) The DNA sequence comparisons for the *Sigastus* weevils collected from Tolga in Far North Queensland and Alstonville NSW showing very close populations courtesy David Gopurenko NSW DPI molecular taxonomy unit. B) Field cages used to house *Sigastus* weevil colonies from Alstonville (CTH), Dunoon (DUN) and Tolga (FNQ) and a field nut sample to collect parasitoids and measure emergence rates from Tregeagle NSW. C) The weekly change over routine for the cage involving extracting the beetles, collecting the old nut, using ethanol to wipe down the cage and replacing the nutlets. D) The eggs need to be transplanted into clean nut, (because of the damage done by the adults) in order to check on emergence and viability (on going).

1.2.2. Biology and life-cycle

By dissecting out freshly laid eggs from nuts and placing them in cell trays, the hatching time could be determined. When we understood which stage the larva was at, we could use this information to estimate the expected emergence time from ground samples. Infested nuts were collected from many farms during 2013 onwards and growers supplied the site and date of collection. We tracked the emergence period from the time the samples were at the all eggs stage. Ventilated 2 litre containers were used to house the nut samples that ranged from 25-100 nuts. We collected weevils daily to determine minimum and maximum lifespan in the immature stage. During this period they were kept at ambient temperatures in a shaded brick room (20>Tmax >35).

At the same time individual weevils that had freshly emerged were isolated and placed in 750ml Vacola jars with ventilated lids and fed freshly picked and unsprayed macadamia racemes (similar to Figure 1.2.2-1). They were cleaned out with ethanol and given a saturated dental wick of water each week. Each female was given access to a male for at least 2 months but after the male died the female remained alone. The adult survivorship and oviposition in the nut was recorded each week.

1.3. Current distribution

The *Sigastus* weevil distribution map has been developed utilising grower feedback from their farms and through direct surveys from the AMS Benchmarking project.

Mapping was developed from further properties identifying the classic damage marks and extensive surveys by the NSW DPI entomology team and crop consultants. Further outbreaks in later years were also identified using this method, as well as including an extra question on the macadamia Benchmarking survey. Each year 150 Northern Rivers growers are contacted to be involved in the Benchmarking project which compares productivity and quality of nuts produced. We opportunistically used this survey to ask growers whether they or their consultant had witnessed *Sigastus* weevil on their property in the past year. These records were overlayed on a Google map image.

1.4. Chemical control

1.4.1. Screening of chemicals

Background

Since 2012, and probably before then in some farms around Dunoon, a high level of "treatment failure" and re-infestation of crop had been reported from growers. Those areas have larger trees (>10m canopy), steeper slopes, and generally more shaded orchard floor. From Harry Fay's 1995-2001 work, carbaryl, methidathion, and beta-cyfluthrin were all considered to give adequate knockdown of the pest during the nut development phase when nut drop is occurring. The following generation within the nutlets that remain on the tree and on the orchard floor are probably the most difficult to manage.

Removing the infected material is important in reducing the impact the pest has and solarising or mulching were considered the easiest option to improve orchard hygiene (Fay *et al*. 1998). The *Sigastus* weevil problem is worst where the trees are too tall for the sprayer and the likelihood of larvae within infested nut completing development is high. Areas where the property neighbours' orchards are unmanaged are becoming problematic with continual re-infestation. In stark contrast orchards where the coverage and spraying have been well timed to target the emerging *Sigastus* weevil populations best, and orchard hygiene is good, the problem is minor (Pretorius and Mclean pers. comm.).

Methodology

Adult weevils were obtained from the infested field nuts provided by growers and collected on site at CTH Alstonville. It was necessary to develop an assay technique that could show the pest mortality rate in each life cycle stage. Methods used included topical application to measure knockdown (1µL dorsally), ingestion of treated nut tissue (dipped nutlets) for adults, to measure ingested mortality, and the level of emergence from nutlets containing freshly laid *Sigastus* weevil eggs that had been dipped in various mixtures for the immature stages. As with most insects that are not in culture the limiting factor is obtaining the number of individuals necessary to provide enough replicates to make meaningful comparisons. Over the 2 seasons, there was access to enough weevils to work on during the spring nut drop period and again in late summer and autumn if there are significant out-of-season nut set.

The advent of *Beauvaria bassiana* within the *Sigastus* weevil colony made it difficult at times to maintain insect numbers. The colony was split, keeping a clean *Sigastus* weevil population for pesticide assays, while still maintaining the virulent field fungal strain for investigation (Figures 1.4.1.-1 and 1.4.1.-2, 1.4.1.-3).

1.4.2. Evaluation of entomopathogens

1.4.2.1. Laboratory culturing of *Beauveria* and *Metarhizium*

The *Metarhizium* and *Beauveria* isolates used in these studies are stored at the Queensland DAF entomopathogenic fungal culture collection housed at the Ecosciences Precinct (ESP) Dutton Park. The isolates were obtained from either soil samples or dead insects, including a *Sigastus* weevil collected in New South Wales. Cultures are stored at 4°C and -22°C on agar slants of malt extract agar (*Beauveria* isolates) and Sabouraud's Dextrose Agar (SDA) (*Metarhizium* isolates). Isolations form dead insects were carried out by surface sterilising the dead insect with 70% ethanol, washing in sterile deionised water, blotting on sterile filter paper then plating on water agar amended with 0.01 % chloramphenicol.

Temperature characterisation

Thermal growth characteristics of isolates were determined by measuring radial growth on SDA plates over 14 days at a range of temperatures from 25°C to 35°C. Plates of Sabouraud's Dextrose Agar (SDA) in 90 mm petri dishes were prepared by marking X and Y axes on the underside. Spore solutions of 1×10^8 spores/ml in sterile 0.1% Tween 80 were made up for the different isolates of *Metarhizium anisopliae* and *Beauveria bassiana.* Sterile 6 mm disks of filter paper dipped in a spore solution were placed on the agar above the intersection of the axes. Four replicate plates of each isolate were incubated in the dark at different temperatures for 14 days. Surface radial growth was recorded using two cardinal diameters, through the X and Y axes on days 7 and 14. This assay was carried out with the plates incubated at 15°C; 20°C; 25°C; 30°C and 35°C.

Growth media comparison

The preference for the isolates for growth and sporulation on different media was investigated similarly to the thermal growth characteristics above. The isolates were grown on oatmeal agar, Potato Dextrose agar and Sabouraud's Dextrose agar.

Spore Production

Spores were produced via a biphasic process. A liquid culture was first grown to inoculate solid media. The liquid culture consisted of 150ml of sterile yeast peptone broth in 250ml Erlenmeyer flasks inoculated with spores scraped from Oatmeal agar (Difco™) plates. Cultures were grown for 5 days at 28°C on an orbital shaker. Solid media of oats, rice or millet were initially investigated for spore production in 500ml flasks; 100g of each media type was added to a 500 ml flask with 20 ml of water then sterilised. Flasks were then inoculated with 15ml of the liquid culture for each fungus. Further investigations were carried out with larger amounts of solid media. Mushroom spawn culture bags containing 500g steam sterilised rice or 300g steam sterilised oat flakes were chemically sterilised with 60ml 1.5% sodium metabisulphite for 24 hours, then neutralised with 12ml saturated sodium bicarbonate. Each bag was inoculated with 75ml of the liquid culture. Rice was used for *Metarhizium* production and oats were used for *Beauveria* production. Extra sterile water was added to the bags to bring the total moisture to 40%. Inoculated bags were incubated for seven days at 28°C on wire racks; the solid cultures were then broken up and left for further 10 days of growth. Bags were opened and left to air dry for 3-4 days at 19°C in a de-humidified room. Spores were harvested from the dried grain through a series of sieves (1mm, 300µm and 150µm) on an Endicott sieve shaker. Spore powder was stored at 4°C.

1.4.2.2. Screening of entomopathogens

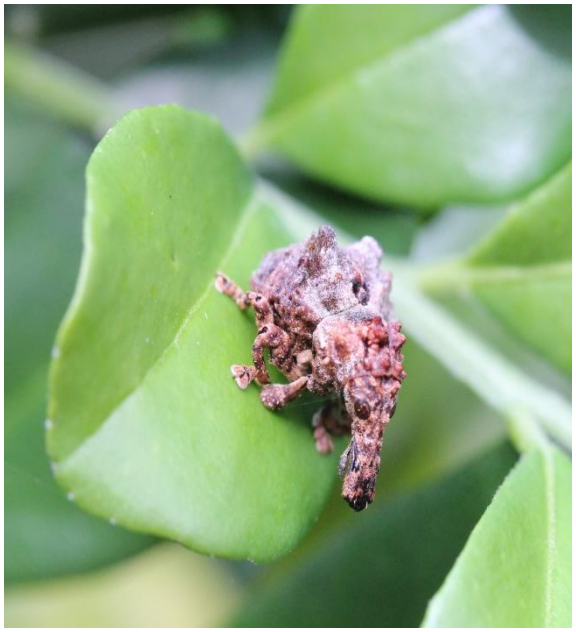
Once 100 *Sigastus* weevil adults were available, a series of 10 replicates of 10 individuals was used to compare survivorship of those exposed to the test chemistries with untreated control of demineralised water. The assay was housed in 750ml disposable, rectangular, plastic food containers with breathing holes (Figure 1.4.1.-2) but this enhanced the *Beauvaria bassiana* activity within most assays so 750ml glass Vacola jars with gauze lids were used instead, which reduced the problem (Figure 1.4.1.-2). Freshly infested nutlets (April 2016) were dipped into the experimental mixtures and stored in labelled plastic cell trays (12 cells per tray), at ambient temperature (25^{o} C) and dissected out after 35 days (when the first adults appeared in the trays), recording the numbers of individual that were alive or dead and what stage they had reached, along with the presence of any fungal growth on the bodies (Figure 1.4.1.-3 C).

Limited field applications have been made at CTH "Sink block", in April 2016 where the population has been monitored closely. A few other select sites have been inoculated with fungal spores to see if any evidence of field infection will present. To date no real activity increase has been detected although we have had much less rainfall than normal in autumn this year and this is not helping. Attempts to improve the spore levels by culturing the *Beauvaria bassiana* are also on going.

Spores have been successfully isolated and cultured on oat meal agar by NSW DPI at the Wollongbar laboratory (Janice Palmer Shane Macintosh Tony Vancov) (Figure 1.4.2.-1) and at DAFQ, Ecosciences Precinct Brisbane (Diana Leemon). We are attempting to source other material that may be active in the field, for evaluation when *Sigastus* weevil is available to test.

A

B

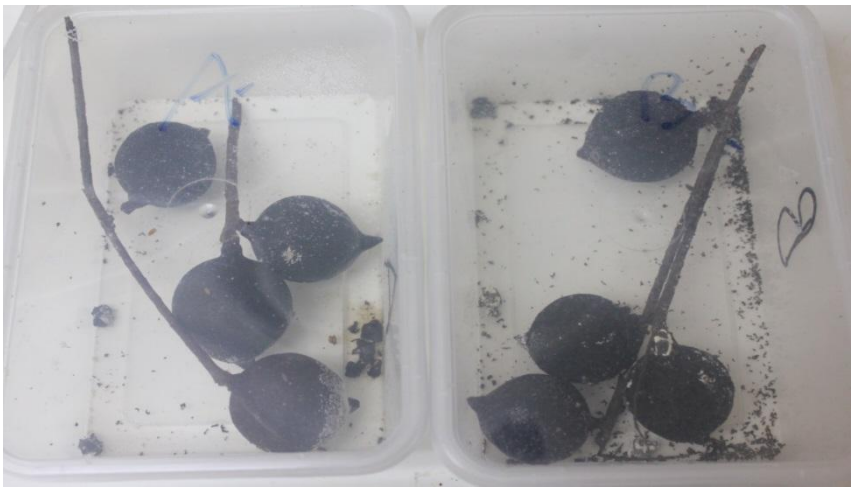


C

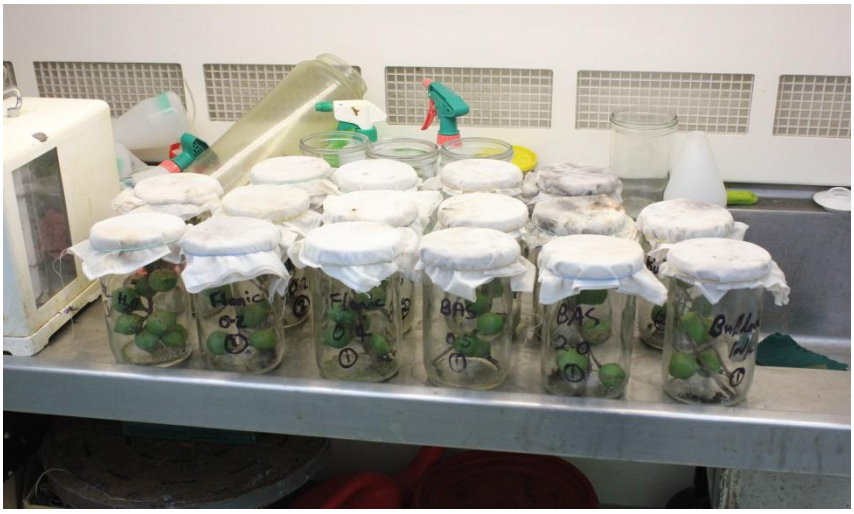
D

Figure 1.4.1.-1: A) *Sigastus* weevil pressure is building in the Tregeagle district closer to Alstonville NSW and under floor grass (left) is being implicated in the level of out of season nut not being collected. B) Does having the thick carpet of grass improve the chances of *Sigastus* weevil larva making it to adult? A question that needs to be answered C) *Sigastus* weevil on *Murraya paniculata* leaf and branches in March 2016 neighbouring the sink block at CTH Alstonville. D) A 10mm long Doryctine Braconid wasp close to *Syngaster* sp. We are still waiting on confirmation of preliminary identification.

A



B



C

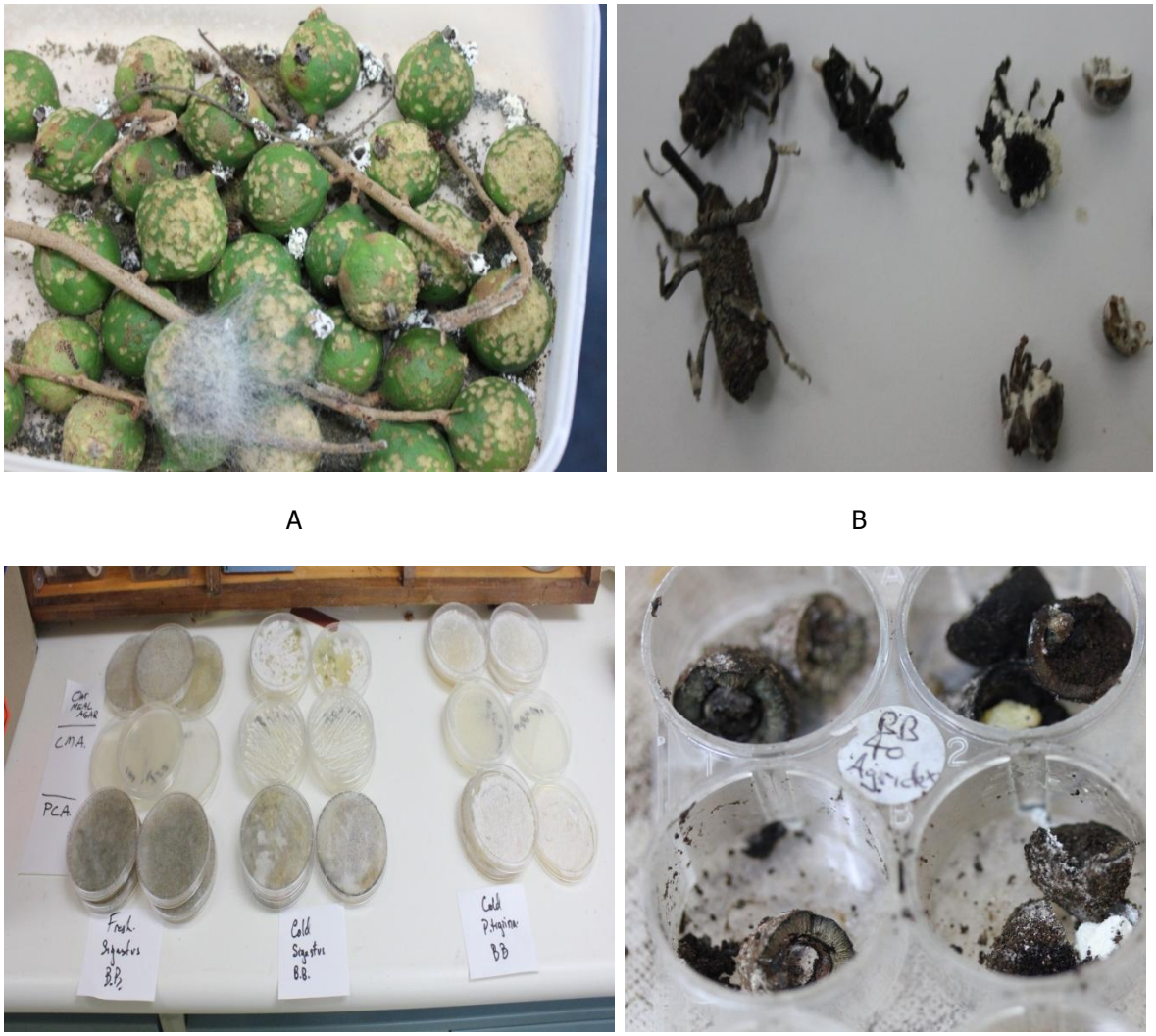
Figure 1.4.1.-2: A) *Sigastus* weevils in replicates of 10 individuals were placed in 750ml plastic food containers with ventilation holes for various pesticide assays with treated macadamia racemes or when doses applied topically to the beetle with clean nuts. The expansion of *Beauvaria bassiana* within the *Sigastus* weevil colony (white weevil dead in right hand container) meant we had to change to a drier system to do the assays. B) Current system is the gauze topped glass Vacola jar. C) *Sigastus* weevil mortality was scored in the assays over the 1-10 day period with much better results using the dipped nut food source than the topical application.

A

B

C

Figure 1.4.1.-3: A) Field trials with *Beauvaria bassiana* spores as a suspension applied in field in Nashua NSW. B) The application of cultured *Beauvaria*, *Isaria* sp., *Metarhizium* has taken place in April 2016 at Alstonville CTH. C) Field *Beauvaria bassiana* infested *Sigastus* weevil from growers Photo courtesy of Shaun James Eureka NSW.



A B

C D

Figure 1.4.2.-1: A) The *Beauvaria bassiana* fungal infection collected from field populations of *Sigastus* sp. B) The spores from the *Sigastus* weevil fungal infection were lethal to a range of other pest species including elephant weevil, banana weevil and the tea tree chrysomelid *Paropsisterna tigrina* C) The culturing done by the NSW DPI team (Janice Palmer) comparing fresh spore with cold stored material and a variety of media D) The *Beauvaria* infested *Sigastus* weevil larvae inside the nuts during the assay using a raw suspension of dead beetles in Agridex® 1ml/L.

1.4.3 Field assessment

The field trial involved weighing and collecting all dropped nut, to find a minimum figure for dropped nut under each tree in the CTH "Sink block" fortnightly from July 2015 to January 2016, and allocating the cause of nutfall (Figure 1.4.3.-1). The impact *Sigastus* weevil had in comparison to other pests was then investigated (only FSB is reported here because of the links with Hort Innovation project MT-10049 – A multi targeted approach to fruitspotting bug management). Only the first harvest has been completed within the timeframe of the project. This shows the likely crop damage inside nut under each tree, the percentage loss to each pest is the number of kernel halves out of the total number of kernel halves examined expressed as a percentage. Each tree had a sample of 30 nuts de husked, dried using the standard AMS drying regimes and cracked out, scoring the kernel defects as per the AMS kernel assessment guide 2011. There is more nut to fall and the numbers will change, as a proportion of the total crop on the tree will be unavailable until

September/October 2016.

Due to close proximity to neighbouring houses, no pesticides have ever been applied to the "Sink block" and parasitoids for FSB and *Cryptophlebia ombrodelta* (MNB) were used at the first sign of activity in October 2015. Every 3 weeks an *Anastatus* sp. card was released from 23/10/2015 (10 total) in this block and *Trichogrammatoidea cryptophlebiae* cards were also released fortnightly to manage MNB (16 cards total Maddox *et al* 2002). This was done to limit the crop loss from these pests and allow *Sigastus* weevil (which has no known biological control agent at present) to cause maximum damage to the most unprotected crop for our measurement. Losses to macadamia nutborer are not reported here but were minimal once the parasitoid established in November. Losses to FSB were significant despite the continual releases during the season.

Development of the spray timing to combat the pest emergence period

Some key information is required in order to manage a pest like *Sigastus* weevil effectively. With a long and protected life-cycle, knowing how and why the population builds within your orchard and not others is the starting point. From there you can find when the population is most susceptible to spraying (minimise the impact on developing nut), when the most adults are out there, and when the developing larval population needs to be collected and destroyed. The exact timing is variety dependent but we can get an idea of the *Sigastus* weevil timing using our unsprayed Macadamia "Sink block" planting at CTH Alstonville for *Amblypelta nitida* (FSB) trap cropping to assess if a *Sigastus* weevil infestation is present. By collecting the nut drop and determining the impact the various pests are having at a particular time in the season, it is possible to build a temporal treatment picture for each pest (Figure 1.4.3.-1).

A B C

Figure 1.4.3.-1: A) Fortnightly comparison between nut drop caused by *Sigastus* weevil oviposition (top left), *Cryptophlebia ombrodelta* (MNB) larvae feeding (top right) and the feeding damage by *Amblypelta nitida* (FSB) (below). Each fortnight all dropped nutlet under each of the 35 trees were collected, weighed and sorted by causal factor (a subsample of 100 nutlets during the heavier period) then cut to determine proportions. B) The trial site from the western boundary of the no spray Centre for Tropical Horticulture (CTH) Alstonville macadamia sink block showing the 13 trees in row1 down to the density plot below and the residential boundary. C) *Sigastus* weevil, *Scirtothrips albourmaculatus, Hypotheneumus sp.*, *Cryphalus subcompactus* and *Ulonemia decoris* have been caught on simple flight traps to get a guide to flight periods for various pest species at this trial site, in Rous NSW and Bundaberg QLD.

2. Appendix 2: Detailed Results

2.1. Literature review

2.1.1. Biology of *Sigastus* weevil

The weevil had been undescribed and was identified belonging to the genus *Sigastus* Pascoe (Curculionidae: Molytinae: Hplonychini) (Fay *et al.*, 1998). Gallagher *et al*. (2003) lists *Sigastus* weevil as a pest only affecting the Atherton Tablelands.

Fay *et al.* (1998) undertook biology studies on *Sigastus* weevil. Female Sigastus weevils begin laying eggs in macadamia nuts about 4-6 weeks after nut-set. The female scarifies an area of 3-4mm in diameter in the husk and lays a single egg into it (Fay *et al*., 1998). The egg is either lodged within the husk or intrudes into the surface of the kernel. After oviposition the nut stalk is chewed about half through to induce drop. Generally, a single egg is laid per nut, although up to three eggs (or larvae) were observed in a nut representing different oviposition dates. Although a large proportion of nuts within a panicle could be onto, it is rare to find that all are affected. Nut fall normally occurs a few days after the oviposition. Mating was observed on nut panicles. After the nuts hardens (around mid-December) they are no longer suitable for oviposition, and adult weevils start feeding on the green surface of the husk, sometimes even completely removing the epidermis. Adults also feed on young leaves (Fay *et al*., 1998).

Larvae consume entire kernels before pupating within the nuts. Adults chew exit holes on the husk and come out. The development time from egg to adult is approximately 6 weeks depending on temperature. Nut size can affect the size of the adult emerged from it. Normally the larger the size of the nut, the bigger the size of the adult. This is evident from the diameter of the emergence hole. Also the development time is shorter and the survival rate is higher in larger nuts (Fay *et al*., 1998).

2.1.2. Ecology of *Sigastus* weevil

Hosts

The usual host of this group of weevils was considered to be *Eugenia* spp. and *Ficus* spp. In a study by Juniper and Britton (2010) they reported on 5 *Sigastus* species including *S. casaurinae* Lea, *S. facicularis* Pascoe and *S. fuscodorsalis* and 2 undescribed species of *Sigastus* species. *S. casaurinae*, *S. facicularis* and *S. fuscodorsalis* have been reared on galls of *Eucalyptus* and *Casaurina* and the flowers/fruit of *Syzygium armstrongii* and *S. suborbiculare* respectively (Juniper and Britton, 2010). One of the undescribed *Sigastus* sp. was reared on *Syzygium hemilamprum* (Juniper and Britton, 2010). The second undescribed *Sigastus* species in their survey was identified as the pest species in macadamias (Juniper and Britton, 2010).

Life-cycle

Little is known about how long each stage (egg, larva, pupa to adult) in the life-cycle takes to develop under different temperatures. Lack of sufficient information about the life cycle makes the management of *Sigastus* weevil difficult.

Life-cycles of other weevils could be good references for studies on *Sigastus* weevil. Pecan weevil *Curculio caryae* has four periods during which they are potentially vulnerable to control: (a) as adults

emerging from the soil, (b) as free-living adults while feeding and ovipositing in the nut, (c) as larvae exiting the nut and burrowing into the soil, and (d) as larvae, pupae or adults, during their 2-3 years subterranean period (Smith *et al.*, 1993).

Damage

Adult weevils chew on the husks and petioles of the nut, causing the nuts to fall from the tree. Eggs in the developing nuts or fallen nuts will hatch and the larvae remain inside the nuts, consuming the whole kernel. Crop loss in an unsprayed orchard may be up to 30% (Fay *et al.*, 1998).

Seasonal change of population

Seasonal change of population is still unknown. However, it is believed that *Sigastus* weevil population in macadamia orchards increases following the flowering and nut growing season (Anon., 2014).

Favorite conditions for *Sigastus* weevil are the following:

(a) Abandoned and untreated orchards are the major source.

Abandoned orchards provide an unsprayed host for *Sigastus* weevil to breed up and then move on to neighbouring orchards.

(b) Long flowering season with range of crop stages.

Long flowering season will result in a wide range of crop development stages (e.g. match head, pea size and full size nuts) occurring within the same tree at the same time. This will advertently provide a continuous food source over a longer period of time for the *Sigastus* weevil to build up the population.

(c) Dark orchards.

Summer sunlight (solarisation) can kill *Sigastus* larvae and pupae inside the fallen nuts on the orchard floor. Older orchards with bigger canopy will block the sunlight onto the floor, hence will create a suitable environment for the weevil population to increase.

(d) Warm winter months.

Mild to warm winter temperature will favour *Sigastus* weevil to develop and shorten their life cycle. This will lead to an increase of *Sigastus* weevil populations early in the season.

(e) Native vegetation.

Orchards close to native vegetation, especially when surrounding with Brush Cherry and other rainforest, have a higher risk of *Sigastus* weevil damage (AMS, 2012).

Natural enemies

As part of a study by the Cooperate Research Centre for Tropical Rainforest Ecology and Management (Anon., 2002), 5 species of wasps have been reared from *Sigastus* weevil infected nuts. There was no clear identified link between tropical rainforest and the weevils and their parasitoids (Anon., 2002).

2.1.3. Pest Management

There has only been very little published on *Sigastus* sp. In order to get a better understanding about management options, a review was undertaken on key weevil pests that have been more extensively studied in the past in other horticultural crops. The selected weevil pests included the pecan weevil, the red palm weevil, banana weevil borer and the elephant weevil.

Pecan weevil

The pecan weevil *Curculio caryae* (Horn) (Coleoptera: Curculionidae), is a major pest in pecans, native to North America. The general distribution of the pecan weevil is "west from New York to Iowa and south to Oklahoma, Texas and Georgia" (Gibson 1968) east of the Rocky Mountians (Mulder *et al.*, 2012). The host range of the weevil includes all North American *Carya* spp. but it also attacks walnut *Juglans regia* L. (Mulder *et al.*, 2012).

Red palm weevil

The red palm weevil *Rhynochphorus ferrugineus* Oliver (Coleoptera: Curculionidae), is a key pest of a number of palms including date palms (*Phoenix dactylifera* L.), Canary Island date palm (*Phoenix canaiensis* hort. Ex Chabaud) and coconut palms (*Cocos nucifera*, L.) (Shulka *et. al.*, 2012). The weevil is native to South and South-East Asia (Nirula, 1956a,b) but spread to the Middle East (Abraham *et al.*, 1998), Europe including Italy (Sacchetti *et al.* 2005; 2006) and Spain) (Barranco *et al.* 1996a,b) and also the Caribbean Islands (Roda *et al.*, 2011) and USA (Anon., 2016a) and Australia (Shulka *et. al.*, 2012; Anon., 2016).

Banana weevil borer

The banana weevil borer *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is a major pest of bananas. It is native to Malaysia and Indonesia and it occurs in major banana growing regions in Central Africa, Central America, Brazil, The west Indies, Eastern Australia and many Indian and Pacific Ocean Islands (Treverrow, 2003). Its hosts are *Musa* spp. including bananas plantain and manilla hemp (Treverrow, 2003).

Elephant weevil

The elephant weevil *Orthorhinus cylindrirostris* (Fabricius) (Coleoptera: Curculionidae) is native to Australia (Murdoch *et al.*, 2014). Its host range includes native hosts (i.e. hickory wattle, *Acacia falcata* and rough-barked apple *Angophora floribunda*) and several horticultural crops like citrus, blueberries and grapevine (Murdoch *et al.*, 2014).

2.1.3.1. Chemical control

Sigastus weevil

Fay *et al.* (1998) reported on *Sigastus* weevil as an emerging pest in macadamias in north Queenland. They tested the efficacy of carbaryl (125 g/100L), methidathion (125ml/100L) and beta-cyfluthrin (50ml/L). Three days after application mortality was 100% in the carbaryl and methidathion

treatments and 86.7% in the beta-cyfluthrin treatment (Fay *et al.,* 1998). Fay *et al.* (1998) recommend methidathion as an initial spray, coinciding with the first nut drop, followed by spray applications for macadamia nutborer.

Pecan weevil

In their 2016 commercial pecan spray guide, the University of Georgia suggests carbaryl (43% by wt.) at a rate of 4-5qt per acre (which is approximately 9.35-11.69L/ha) (Wells *et al.*, 2016). Texas A&M also suggest carbaryl (Sevin 80S) at a rate of 1.25-3.0 pounds per 100 gallons (which is approximately 150-360g/100L)(Ree *et al.*, 2011). The publication by the Oklahoma Cooperative Extension Service provides a list with recommendation for chemical control of pecan weevil (Lee *et al.*, 2013) (Table 2.1.3.1.1). The recommendations cover different synthetic pyrethrins, carbaryl (carbamate) and phosmet (organophosphate) (Table 2.1.3.1.1) (Lee *et al.*, 2013).

Table 2.1.3.1.1: List of insecticides recommended for control of pecan weevil by Oklahoma State University in 2013

Insecticide name	**Active**	**Rate / acre**	**Rate / ha**		**Group**
			Min	**Max**	
Ammo 2.5 EC	Cypermethrin	3-5 oz	210.16g	350.27g	3
Asana XL	Esfenvalerate	4.8-14.5 oz	336.25g	1015.77g	3
BathroxidXL	Beta-cyfluthrin	2.0-2.4 oz	140.11g	168.13g	3
Battalion 0.2EC	Deltamethrin	12.8-21.1oz	896.68g	1478.12g	3
Hero	Zeta-cypermethrin and bifenthrin	10.3oz	721.55g		3
Imidan 70WSB4	Phosmet	2.0-3.125lbs	2.24kg	3.50kg	1B
Mustang-MAX	Zeta-cypermethrin	3.2-4.0 oz	224.17g	280.21g	3
Proaxis	Gamma-cyhalothrin	2.56-5.12 oz	179.34g	358.67g	3
Sevin 80S	Carbaryl (80%)	2.5-6.25 lbs	2.80kg	7.01g	1A
Sevin XLR+	Carbaryl (44.1%)	2-5 qts	5.22L	13.06L	1A
Silencer	Lambda-cyhalothrin	2.56-5.12 oz	179.34g	358.67g	3
Warrior	Lambda-cyhalothrin (11.4%)	2.56-5.12 oz	179.34g	358.67g	3
Warrior II	Lambda-cyhalothrin (22.8%)	1.28-2.56 oz	89.67g	179.34g	3

In Uganda crude extracts of neem (*Melia azedarach* L.), mexican marigold (*Tagates* spp.), water hyacinth (*Eichornia crassipes*, Martius) and castor oil (*Ricinus communis* L.) were investigated as potential control for banana weevil borer (Tinzaara *et al*., 2006). Investigations included mortality, settling responses and oviposition in the laboratory. Not all extracts showed significant effects on weevil mortality compared to controls (Tinzaara *et al*., 2006). There was no significant difference in weevil settling responses on corms treated with extracts after 1 h and 72 h of observation. Oviposition was significantly lower on corms treated with *M. azedarach*, *Tagetes* spp and *R. communis* compared to controls (Tinzaara *et al*., 2006). The data indicates that botanicals possess limited insecticidal properties but the potential of *M. azedarach*, *Tagetes* spp and *R. communis* to prevent oviposition needs further investigation (Tinzaara *et al*., 2006).

Red palm weevil

Stem injections of chemicals are part of the management of the red palm weevil. Faleiro (2006) undertook a review of the red palm weevil management.

A list of chemicals used in different countries for red palm weevil control in coconut and date palms is shown in Table 2.1.3.1.2.

New generation insecticides belonging to the neonicotinoid (imidachloprid) and phenylpyrazole (fipronil) groups are used in prophylactic and curative applications to control the red palm weevil (Kaakeh, 2006; Al-Shawaf *et al.*, 2010; Al-Dosary, 2016).

Banana weevil borer

Collins *et al.* (1991) reported on resistance to 4 organophosporous insecticides (pirimiphos, prothiophos, chlorpyrifos and ethoprophos). There was evidence of cross-resistance to oxamyl but not carbofuran, isazofos or isophenphos.

There are a number of chemicals that are currently registered in Australia to control banana weevil borer by the *Australian Pesticides and Veterinary Medicines Authority* (*APVMA*). Registered chemicals are listed in Table 2.1.3.1.3 (APVMA, 2016).

On Martinique two rates of the nematicide oxamyl were compared with a reference program (rotation of several nematicides: cadusafos, aldicarb, fosthiazate and fenamifos) and untreated control in their control of nematodes and the banana weevil borer (Chabrier *et al.*, 2004). Oxamyl was comparable in controlling nematodes but inadequate in controlling banana weevil borer (Chabrier *et al.*, 2004).

Table 2.1.3.1.2: List of chemicals used for red palm weevil control in coconut and date palms in different countries over time (from Faleiro, 2006)

No.	Chemical tested	Crop	Country	Reference
1	Methyl demeton	Coconut	Sri Lanka	Kirthisinghe (1966)
2	1% Carbaryl isobenzene, Dimethoate	Coconut	India	Mathen and Kurian (1967)
3	1% Carbaryl WP (20–30 g in water)	Coconut	India	Mathen and Kurian (1970)
4	1% Carbaryl or PyroconE after plugging holes	Coconut	India	Kurian and Mathen (1971)
5	0.2% Fenthion, 1% Carbaryl, (0.2% Methyl demeton phytotoxic)	Coconut	India	Lakshmanan *et al.* (1972)
6	0.2% Fenthion, 1% Carbaryl	Coconut	India	Subba Rao *et al.* (1973)
7	1% Trichlorphon	Coconut	India	Abraham *et al.* (1975)
8	1% Gamma BHC (Lindane), Diazinol, Dimethoate, Malathion	Coconut	Philippines	Abad and Callego (1978)
9	10ml Monocrotophos or 5ml Monocrotophos + 5ml Dichlorvos per infested palm	Coconut	India	Muthuraman (1984)
10	Monocrotophose	Coconut	India	Rajmanickam *et al.* (1995)
11	Marshal, Primicid and Rogodial	Date palm	UAE	El-Ezaby (1997)
12	2% Metasystox, Trichlorphon, Supracid and Salut	Date palm	Saudi Arabia	Anon. (1998) and Vidyasagar *et al.* (2000)
13	Diazinon, Dimethoate, Chlorpyriphos, Carbaryl, Oxamyl, Carbosulphan, Imidacloprid, Fipronil and Methidathion	Date palm	Spain	Hernandez-Marante *et al.* (2003)
14	Dichlorvos and Imidacloprid (infusion, 100–150 cc every 3 weeks until infestation disappears)	Date palm	Israel	Anon. (2004)
15	10,000ppm of Chlorpyriphos, Diazinon, Phenthoate and Methomyl	Date palm	Egypt	Anon. (2004)

Table 2.1.3.1.3: Chemicals registered for banana weevil borer in Australia (from APVMA (https://portal.apvma.gov.au/pubcris) on 23 May 2016)

Chemical	**Group**	**Formulation**	**Rate**	**Comments**
Bifenthrin	3A	100g/L	Stool treatment: 250-330ml/100L twice per year or 660ml/L once per year Band treatment: 250ml/100L twice per year **Monitoring Program:** Stool treatment: 330ml/100L Band treatment: 250ml/100L	
Cadusafos		100g/kg	30g/ stool 3 times per year (per hand) or 2.00kg/100m row (single row,3 times/year 4.00kg /100m row double row, 3 times/year	
Chlorpyrifos	1B	500g/L	1 or 1.8L/100L	
Clothianidin	4A 23	200g/L	3ml/ pseudostem or 4.5ml spray per pseudostem in a total water volume of 10ml	
Diazinon	1B	800g/L	125ml/100L	NSW and WA only
Fipronil	2B	200g/L	150ml/ 100L; 0.75ml/ stool	
Imidachloprid	4A	50g/kg	18g/m (single row); 36g/m (dual row bed)	
Spirotetramat (ISO) Imidacloprid (Movento Energy)	4A 23	120g/L Imidacloprid and 120g/L Spirotetramant	7.5ml undiluted or up to 10ml diluted/ per stool	
Terbufos	1B	150g/kg	20g/ tree or 2.00kg/100m row (single row, single sucker plantation 3.00kg/ row single row, double sucker plantation 4.00kg /100m row double row, single sucker plantation	QLD only

A study in Cameroon investigated the efficacy of insecticides with different modes of action on banana weevil borer in a laboratory and field trial (Mongyeh, *et al.*, 2015). In the laboratory and field trial terbuphos, fipronil achieved 100% mortality (Mongyeh, *et al.*, 2015). Imidacloprid showed a moderate effect (<20% mortality) in the control of the weevil while Bromorex® (botanical, with pepper and chilli extracts as activeingredients) had no significant effect on weevil mortality (Mongyeh, *et al.*, 2015). Bromorex® also had no ovicidal effect, while terbufos, fipronil and imidacloprid completely inhibited larvae emergence from eggs.

A study in South Africa tested 5 different insecticides (Table 2.1.3.1.4) against the banana weevil borer (De Graaf, 2006). In this study fipronil and imidacloprid showed the best control against the banana weevil borer, minimising damage to the periphery, cortex and central cylinder of the rhizome and significantly reduced adult density (De Graaf, 2006).

Table 2.1.3.1.4: Chemical groups, trade names, formulations, active ingredients and gram active ingredient of chemicals evaluated against *Cosmopolites sordidus* in South African study from October 2003 to October 2005 (from De Graaf, 2006)

Chemical group	**Trade name (formulation)**	**Active ingredient (a.i.)**	**Gram active ingredient (g.a.i.)/plant**
Pyrethroid	Talstar (EC)	Bifenthrin (100g/L)	0.015
Organophosphate	Dursban (WG)	Chlorpyrifos (750g/kg)	0.125
Phenyl pyrazole	Regent (SC)	Fipronil (200g/L)	0.01
Chloro-nicotinyl	Confidor (SC)	Imidacloprid (350g/L)	0.245
Oxime carbamate	Vydate (SL)	Oxamyl (310g/L)	0.5

Elephant weevil

Murdoch (2010) investigated a number of chemical control options for the elephant weevil, including indoxacarb; imidacloprid methomyl and clothianidin. In the laboratory trials foliar applications of indoxacarb, imidacloprid and clothianidin resulted in the greatest mortality of the weevil. In field trials, indoxacarb and imidacloprid gave the highest control, but also the entomopathogenic fungi Mycoforce and *Beauveria bassiana* var. EWB.

Current practice is the use of bifenthrin twice a year, during flights (Rocchetti (Costa Berry Exchange), pers. comm., 2016).

2.1.3.2. Biopesticide and nematodes

Pecan weevil

Earlier field experiments by Tedders *et al.* (1973) showed that the entomopathogenic nematode *Neoaplectana dutkyi* achieved 67% control, while the entomopathogenic fungi *M. anisopliae* achieved 59.3% control and 61.5% with *B. bassiana*.

Mulder *et al.* 2012 investigated the biological control of the pecan weevil. *Metarhizium anisopliae* (Metschnikoff) and *Beauveria basiana* (Balsamo) Vuillemin had been tested in earlier studies, which showed that low concentration of *Beauveria basiana* could kill larvae and adults of the weevil in the field. A survey on entomopathogenic nematodes (Shapiro-Ilan *et al.*, 2003) showed that entomopathogenic fungi were more common (76%) than nematodes (28%). This study also showed that fungal infections are dependent on soil micronutrient levels.

Trunk applications of *Beauveria bassiana* achieved >75% mortality of pecan weevil, but it takes about 7 days to kill the weevils, during which time they can still cause damage (Mulder *et al.*, 2012).

C. caryae has a different life cycle to *Sigastus* weevil*. C. caryae* pupates and diapauses in the soil (Mulder *et al.*, 2012) before emerging as adults, probably giving pathogens and nematodes a better chance to infect the insect.

Red palm weevil

As part of a larger review on palm weevil control Faleiro (2006) collated work on biological controls that were done over time (Table 2.1.3.2.1). A number of different biological control agents were tested in different countries over time (Faleiro, 2006). These included bacteria, viruses, enotmopathogenic nematodes (ENP), fungi, flies and wasps (Faleiro, 2006). Neither of the different biological control options gave outstanding control by itself (Faleiro, 2006).

Banana weevil borer

The objective of a study by Fancelli *et al.* (2013) was the selection of effective strains of *Beauveria bassiana* for controlling *Cosmopolites sordidus* (Germ.) in plantain farms (cv. Terra) of the "Recôncavo" and southern regions in the state of Bahia, Brazil. Thirty two *B. bassiana* isolates were screened in the laboratory and the 3 isolates (CNPMF 407, CNPMF 218, and CNPMF 416) were selected and evaluated under field conditions in plantations located in the counties of Mutuípe and Wenceslau Guimarães (Fancelli *et al.*, 2013). The population of *C. sordidus* was monitored every 15 days by using pseudostem traps (Fancelli *et al.*, 2013). The efficiency of the 3 *B. bassiana* strains was compared to chemical control (carbofuran, 4g/trap) and untreated control (Fancelli *et al.*, 2013). Carbofuran caused around 90% mortality of adult within 12 months. The *B. bassiana* strain CNPMF 218 was the most efficient in controlling *C. sordidus* adults, leading to 40% reduction of the weevil population within 12 months.

Elephant weevil

Murdoch (2010) tested entomopathogens for the control of the elephant weevil in blueberries, which included the commercial product, *Aspergillus parasiticus* var. EWB (elephant weevil borer). The commercial product Mycoforce and *Beauveria bassiana* var. EWB gave the best results in the laboratory screening and also gave adequate control in the field, comparable with synthetic insecticides (Murdoch, 2010).

Table 2.1.3.2.1: List of biological control studies on red palm weevil (from Faleiro, 2006)

No.	Biological control agent	Live stage attacked	Country	Reference
1	Bacteria—*Pseudomonas aeruginosa*	Larvae	India	Banerjee and Dangar (1995)
2	Bacteria—*Bacillus* sp., *Serratia* sp. and coryneform group	Larvae and adults	India	Dangar and Banerjee (1993)
3	Bacteria—*Bacillus sphaericus*, *B. megaterium* and *B. laterosporus*	Larvae	Egypt	Salama *et al.* (2004)
4	Bacteria—*Bacillus thuringiensis*, *B. sphaericus*	Larvae and adults	Egypt	Alfazariy *et al.* (2003) and Alfazariy (2004)
5	Yeast (isolated from haemolymph)	Larvae and adults	India/Egypt	Dangar (1997) and Salama *et al.* (2004)
6	Virus—cytoplasmic polyhedrosis Virus	All stages	India/Egypt	Gopinadhan (1993), Gopinadhan *et al.* (1990), Alfazariy *et al.* (2003) and Alfazariy (2004)
7	(EPN)—*Heterorhabditis* spp.	Pupae and adults	Egypt	Shamseldean and Abd-Elgawad (1994)
8	(EPN)—*Steinernema abbasi and Heterorhabditis indicus* with antidesiccants	Larvae and adults	UAE, Egypt	Abbas *et al.* (2000, 2001a,b)
9	(EPN)—*Teratorhabditis palmarum, Steinernema* sp., *H. indica*	Larvae, pupae and adults	India	Sosamma and Rasmi (2002)
10	(EPN)—*H. indica, Steinernema* sp. and *S. glaseri*	Larvae and adults	India	Banu *et al.* (2003)
11	(EPN)—*H. indica* (Saudi Arabian strain)	Larvae and adults	Saudi Arabia	Saleh and Alheji (2003)
12	(EPN) —*Rhabditis* sp., *H. indica*	Larvae	India	Banu and Rajendran (2002, 2003)
13	Fungi—*Beauveria bassiana* and *Metarhizium anisopliae*	Pupae and adults	Iran	Ghazavi and Avand-Faghih (2002)
14	Fungi—*Beauveria* sp.	Adults	India	Shaju *et al.* (2003)
15	Fly—*Sarcophaga fuscicauda*	Adults	India	Venkatasubbaiyer (1940)
16	Wasp—*Scolia erratica*	Larval parasite	N/A	Burkill (1917)

(ENP) = entomopathogenic nematodes

2.1.3. Pheromone trap

Pecan weevil

The study by Mulder *et al.* (2003) did not show any advantage of any pheromone tested. Different trapping systems have been investigated for the pecan weevil (Mulder *et al.,* 2003*;* Ree, *et al*., 2005; Mulder *et al*., 2012), but *C. caryae* tends to crawl up the tree trunk, which to date has not been reported from *Sigastus* weevil.

Red Palm weevil

In a comprehensive review, Faleiro (2006) reports on the development of pheromone traps for the red palm weevil. The model that has been successful, consists of a polyethylene bucket (5L) with a rough surface, with four windows (1.5 x 5.0cm^2), below the upper rim of the bucket (Faleiro *et al.*, 1998; Al-Dosary, 2016). The trap is baited with a pheromone and ethyl acetate lure, which is hung from the inside of the bucket lid with wire. Further 200g of kairomone releasing food bait (e.g. dates, coconut petioles and sugar cane) mixed in 1L of insecticide (0.05% Carbofuran 3G solution was added to the attractant in the bucket of the trap (Anon., 1998, Oehlschlager, 1998, Faleiro, 2006). Al-Dosary *et al.* reports in the review from 2016 that the dark coloured traps (red) were more successful in capturing weevils (Abuagla and Al-Deeb, 2012, Al-Saoud *et al*., 2010; Al-Saoud, 2013; Al-Dosary, 2016).

A number of pheromones have been tested in previous studies (Faleiro, 2006). Details are listed in Table 2.1.3.3.1. A trap density of 1 trap per ha gave successful results (Faleiro and Satarkar, 2003b; Faleiro, 2006).

Pheromone traps for the red palm beetle have been successfully integrated into IPM systems in Saudi Arabia, Israel, United Arab Emirates (UAE), Sri Lanka, India, Iran (Faleiro, 2006).

Al-Dosary *et al* (2016) reports that a new dimension to the use of semiochemicals in an area-wide program involving a push pull strategy, for the sustainable management of the red palm weevil could be the addition of insect repellents with pheromones. Alpha-pinene on its own or in combination with methyl salicylate has been identified as a potential repellent for the red palm weevil (Guanio *et al*., 2013; Al-Dosary *et al.*, 2016).

Banana weevil borer

De Graaf (2006) investigated options for trapping weevils (*Cosmopolites sordidus*) in South Africa. Pseudostem traps, pitfall traps containing a pheromone (either Cosmolure® or Cosmolure+® were compared to unbaited pitfall traps (control) over 5 weeks during all seasons along the Southeast coast of South Africa (DeGraaf, 2006). Pseudostem traps treated with an insecticide, and rhizome traps were included as additional treatments in autumn. In summer two treatments were also added: individual suspension of both pheromones above a pitfall trap either in combination with or without a pseudostem trap (DeGraaf, 2006). The adult beetles collected were sexed, and the number of internal eggs noted. Cosmolure proved to be the most effective of the different traps (DeGraaf, 2006). Grouping of the pheromones resulted in a synergistic response, while combining the pseudostem did not increase trap efficacy. The different plant material traps and the control were usually equally effective in catching weevils (DeGraaf, 2006). Plant material traps caught greater numbers of fecund females, but pheromone traps captured a higher proportion of females (DeGraaf, 2006). Treatment effects were reduced in summer, and compared to a pseudostem trap, pitfall traps were the most effective in spring (DeGraaf, 2006). Compared to conventional pseudostem trapping, Cosmolure pitfall traps should be optimally applied during spring in South Africa (DeGraaf, 2006).

Table 2.1.3.3.1: List ferruginol based pheromone lures for *Rhynochphorus ferrugineus* in coconut and date plantations (from Faleiro, 2006)

No.	Formulations tested	Country/crop/duration of trial	Superior lure	Reference
1	Chem Tica International (high release/slow release)	Saudi Arabia date palm 90 days	High release	Faleiro *et al.* (2000)
2	Agrisense (fast release/slow release) Chem Tica International (Ferrolure, Ferrolure +) Calliope	Saudi Arabia date palm 30 days	Ferrolure +	Faleiro *et al.* (2000)
3	Agrisense lures Chem Tica International (Ferrolure improved and Ferrolure +)	India coconut two trials 45 days each	Ferrolure improved	Faleiro and Chellappan (1999)
4	Chem Tica International (Ferrolure +) ISCA Technologies lure CPCRI lure Pherobank lure	India coconut two trials 30 days each	Pherobank 400mg lure	Faleiro and Satarkar (2003a)
5	CPCRI lure Chem Tica International (Ferrolure +)	India coconut 150 days	Ferrolure +	Faleiro *et al.* (2004)
6	Chem Tica International (Ferrolure +) ISCA Technologies lure	India coconut trial discontinued after lure was exhausted	ISCA technologies	Kalleshwaraswamy *et al.* (2004)
7	Agrisense lures Chem Tica International (Ferrolure +)	India coconut 30 days	Agrisense lures and Ferrolure +	Abraham *et al.* (1999)

Tinzaara *et al.* (2007a) also investigated in laboratory and field trials, the attraction of adult banana weevil borer to volatiles from banana pseudostem tissue and the synthetic pheromone Cosmolure+ either by itself or in combination. Laboratory studies showed that 50g of fermented banana pseudostem tissue was as attractive as the pheromone, but more attractive than 50g of fresh pseudostem tissue (Tinzaara *et al.*, 2007a). Volatiles from pseudostem tissue and pheromone lures showed an additive effect in attracting weevils in the laboratory, but not in the field trials (Tinzaara *et al.*, 2007a). In the field, the attractiveness to weevils was positively correlated with the amount of fermented tissue added to the pheromone (Tinzaara *et al.*, 2007a). The results indicate that even though fresh or fermented pseudostem tissue may increase pheromone trap catches, it isnot enough to warrant the wider use of the combination of pheromone and plant tissue on a commercial scale (Tinzaara *et al.*, 2007a).

Using pheromone trap (ground trap) for mass trapping has a great potential to replace inefficient insecticide treatments (Reddy *et al.*, 2009).

2.1.4. Cultural control

Sigastus weevil

Fay *et al.* (1998) consider that the sweeping of fallen nut into the interrows and solarisation to kill larvae (between mid-September and mid-December), is an important part of the management of the weevil.

Banana weevil borer

DeGraaf (2006) investigated cultural control options for the banana weevil borer over 2 years at an ongoing trial in the Southern KwaZulu Natal, South Africa. Treatments tested were the following: 1. harvesting at ground level and dissection of remnants; 2. covering of the mat with soil and moving debris to the inter-row; 3. a positive control that involved treatment of plants with a registered pesticide and 4. a negative control that involved harvesting at approximately 150 cm with no soil or sanitation amendments (De Graaf, 2006). Yield, weevil damage and pseudostem girth of plants were measured from August to November annually, while adult beetle densities were assessed over 4 weeks in October/November and April. Nematode samples were analysed in October/November every year. Soil cover and recession of remnants was the only effective treatment, significantly reducing the Coefficient of Infestation, but not the adult density or any other damage parameter. The former showed promise as a cultural control method because it only needs to be applied seasonally. It also reduced the percentage cross sectional damage of the central cylinder which is the damage parameter most closely related to yield, by 14.18% De Graaf, 2006).

2.1.5. IPM

Pecan weevil

An IPM strategy investigated for pecan weevil (Reid, 2002; Reid and Mulder, 2003; Ree *et al.,* 2011). The pecan weevil has proven to be an extremely difficult subject, which impeded on the development of an IPM system (Reid and Mulder, 2003). Ree *et al.* (2011) are taking a trap and spray approach, using monitoring as triggers for insecticide treatments. This is still the more current recommendation (Hudson *et al.*, 2006). They recommend at least 2 or up to 4 properly timed applications of insecticides. The initial treatment is suggested when the earliest maturing nuts are at the gel stage. A second application is recommended when adult emergence traps are collecting adult weevils or 6 days after the application. If adult emergence continues, a third application is recommended after a

further 5 days (Ree, *et al.*, 2011).

A study by Shapiro-Ilan *et al.* (2011) investigated the compatibility of the pathogen *Beauveria bassiana*, the nematode *Steinernema carpocapsae* (Weiser) and insecticides. Laboratory trials indicated a synergy effect of carbryl and antagonistic effect of cypermethrin with *B. bassiana* in control of pecan weevils (Shapiro-Ilan *et al.*, 2011). Both chemicals had a synergistic effect with *S. carpocapsae* controlling weevil larvae and an additive effect controlling of weevil adults.

Red palm weevil

In a comprehensive review, Faleiro (2006) looked at different options that have been investigated over time for control of the red palm weevil. Options included chemical control, biological control, monitoring and lure and kill, host plant resistance in different palm species and varieties and male sterile technique (Failero, 2006). Failero (2006) concludes that with the currently available management options for red palm beetle, a pheromone based strategy offers a sustainable approach. Early detection of an infestation in the field and therefore monitoring are vital for a successful management program (Faleiro, 2006). Effective biological control combined with host plant resistance was also suggested as an important part of an IPM approach for the red palm weevil (2006).

Al-Dosary *et al.* (2016) did a very recent review of management of the red palm weevil in date palms. The current control strategy for the weevil is a food-baited pheromone trapping system to locate and eliminate breeding sites, including neglected, abandoned gardens, maintaining crop sanitation, pest surveillance, mass trapping and chemical treatments (preventative and curative).

Monitoring options are visual detection (observation of damage) (Al-Shawaf *et al.*, 2012), bioacoustics detection (gnawing sound from feeding larvae) (Abraham *et al.*, 1966; Soroker *et al.*, 2004), chemical detection (trained dogs) (Nakash *et al.*, 2000*;* Soroker, *et al.*, 2013), Thermal imaging detection (larval feeding causes fermenting and increased temperature) (Soroker, *et al.*, 2013; El-Faki *et al.,* 2015) (Al-Dosary. *et al.*, 2016).

As part of cultural management, Al-Dosary *et al.* (2016) consider sanitation (Abraham *et al.*, 1998; Al-Ajlan, 2008), varietal preference or host plant resistance (varieties' sugar content are more susceptible and varieties with higher calcium content inhibit growth and development of the weevil) (Farazmand, 2002; Faleiro, 2006; Al-Ayedh, 2008; Al-Dosary *et al.*, 2016), plant density, pruning of fronds and removal of off-shoots as important part of an IPM strategy. Gene silencing or RNA interference (RNAi) are considered a potential future path to develop resistant plants (Niblett and Bailey, 2012; Al-Dosary *et al.*, 2016). Furthermore, a strict quarantine regime is very important (Al-Dosary *et al.*, 2016). Al-Dosary *et al.* (2016) gives a good summary of case studies of area wide management, which are listed in Table 2.1.3.5.1.

Banana weevil borer

Strains of *Beauveria bassiana* were selected for investigating their use in integrated pest management of the banana weevil borer (Tinzaara *et al.*, 2007b). Different delivery systems, including aggregation pheromones, were investigated to evaluate the field transmission of *B. bassiana* between banana weevil borers (Tinzaara *et al.*, 2007b). The study showed that infected weevils could transmit the fungal pathogen to healthy individuals and 52% of the weevils that died due to *B. bassiana* infection were found at the base of banana plants in the leaf sheath or in the soil near banana plants (Tinzaara *et al.*, 2007b). Mortality due to *B. bassiana* was significantly higher where the pathogen was applied in combination with the pheromone (Tinzaara *et al.*, 2007b).

Table 2.1.3.5.1: Impact of area-wide management of *R. ferrugineus* in date plantations of different countries in the Middle-East (from Al-Dosary *et al.*, 2016)

No	Country	Highlights	Reference
1	United Arab Emirates	A major study carried out in the UAE between 1994 and 1998 carried out in 1,466 farms initially containing 349,342 palms with an average infestation rate of 1.9%. Infestations were found to decrease in 1998 by 64% from 1997 levels in the farms that received insecticide treatments and pheromone traps, as compared to a decrease of 36% in the farms that received only chemical treatment.	El-Ezaby *et al.*, 1998; Oehlschlager, 2006
2	Saudi Arabia	In Al-Qatif date palm oasis (in 4,000 ha) weevil captures reduced from 4.12 weevils per trap per week in 1994 to 2.02 weevils per trap per week in 1997 Infestation levels in the date palm plantations containing traps decreased from 6.6% in 1993 to 2.5% in 1997.	Vidyasagar *et al.*, 2000
3	Saudi Arabia	Red palm weevil was effectively monitored with traps in pest free areas at a density of one trap per 100 ha and successfully controlled (in 4000 ha) between 1994-1997 by mass trapping adult weevils at 1 trap/1.5 ha.	Abraham *et al.*, 2000
4	United Arab Emirates	In another report from UAE six date plantations in which pheromone traps captured the highest numbers of weevils, exhibited the greatest reduction of infestation. In this study the average annual reduction in infestation over all six farms was 71%.	Kaakeh *et al.*, 2001; Oehlschlager, 2006
5	Sultanate of Oman	In Oman pheromone trapping of *R. ferrugineus* in date plantations is credited with reduction in eradications from 24% in 1998 to 3% in 2003.	Al- Khatri, 2004
6	Israel	In Israel mass trapping of *R. ferrugineus* in 450 ha of date plantations along with other red palm weevil-IPM tactics between 1999 and 2001 resulted in the decrease in the number of weevils trapped by the end of 2001, with no infestation being found since 2002.	Soroker *et al.*, 2005

Table 2.1.3.5.1: Impact of area-wide management of *R. ferrugineus* in date plantations of different countries in the Middle-East (from Al-Dosary *et al.*, 2016) (cont.)

No	Country	Highlights	Reference
7	Saudi Arabia	Area-wide management of the pheromone based *R. ferrugineus* –red palm weevil control program in Al-Hassa was validated in 15 operational areas comprising of more than 4000ha (>35% of the area with over 1.08 million palms) for six months from April to September 2011. This was based on mean monthly values for weevil captures in food-baited pheromone traps, infestation levels and eradication of severely infested palms. Results revealed that mean monthly weevil captures were significantly different in the 15 operational areas sampled, but were statistically on par in the three major zones (centre, north and east) of the oasis. Infestation levels in the operational areas varied significantly and were found to be well below the 1% action threshold in the east of the oasis, nearing 1% in the centre, while being well above the threshold (1% infestation) in the north. In general, the study showed that while the pheromone based IPM strategy adopted had the desired impact in the east, the strategy needed minor adjustments in the centre, but called for major reinforcement in the north of the oasis.	Al-Shawaf *et al.*, 2012
8	Saudi Arabia	Data spanning over a six year period (2007 to 2012) from Al Ahsa, Saudi Arabia in a 1,104 ha date producing region of the Al –Hassa date palm oasis, involving a 10 fold increase in the number of pheromone traps that was initiated in October2009, revealed that the total number of *R. ferrugineus* adults captured in 2012 declined by 86% when compared to total captures for 2010. Furthermore, over the same time period, insecticide application and palm eradication rates dropped by 91% and 89%, respectively.	Hoddle *et al.*, 2013

Pheromone–based methods have shown to be effective and reliable, especially in area-wide programs, therefore, future applications should be planned on a landscape level. It should be more effective if Geographic Information Systems (GIS) are incorporated with pheromone trap methods to capture, organize, and evaluate insect population data and to visualize spatial and temporal fluctuations in a regional level (Witzgall *et al*., 2010).

Elephant weevil

In order to assess IPM strategies, Murdoch (2010) investigated the compatibility of the entomophathogenic fungus *Bauveria bassiana* with a number of pesticides commonly used in blueberry production in Australia. The pesticides screened for compatibility included the fungicides captan, mancozeb and propiconazole and the insecticides malathion, dimethoate, methomyl, indoxacarb, spinosad, clorpyrifos and imidacloprid, as well as the nucleopolydedrovirus (NPV) product Vivus gold and *Bacillus thuringiensis* (Murdoch, 2010). Malathion, methomyl, indoxacarb, NPV and spinosad showed a slightly harmful effect on *B. bassiana*. Murdoch (2010) concluded that these pesticides can be used in combination with the entomopathonogenic fungus. Propiconazole was considered moderately harmful and the use of this fungicide should not overlap with *B. bassiana* applications (Murdoch, 2010). *Bacillus thuringiensis*, and imidacloprid had minimal impact on *B. bassiana* (Murdoch, 2010) but the contact of fungicides captan and macozep with *B. bassiana* has to be avoided (Murdoch, 2010). Insecticides clopyrifos and dimethoate were highly toxic to *B. bassiana* (Murdoch, 2010).

2.2. Species identification, biology and life-cycle

2.2.1. Species identification

The DNA barcodes show that there is no species difference between the samples from the sites and some haplotypes present are found in both Alstonville and Tolga (QLD). Unfortunately there was no previous record of the *Sigastus* genera on the GenBank or BOLD databases (Appendix 1, Figure 1.2.1.-3). This has been updated now, and the Juniper and Britton (2010) samples would be a worthwhile comparison.

Sigastus weevil is limited to breeding in the young expanding nut, and is capable of feeding on other macadamia tissue during periods between crops (Figure 2.2.1.-1) which means extending the cropping cycle is important in controlling this pest. The rearing of the three *Sigastus* weevil colonies confirmed the anecdotal evidence for a need to have nutlets in the pre shell hardening stage to trigger a breeding response. Weevils were fed the nuts cv. 246 from an unsprayed section of the CTH Alstonville site each week beginning in late December 2015, the switch to young nutlets from out of season crops occurred from the 4/4/2016 when sufficient nutlets were present (also from CTH Alstonville cv. A4, L64 and XXX). Survival from the initial emerged adults in December 2015 had dropped to around 50% for each colony after 100 days, and the weevils remaining had managed to produce around 200 eggs in the 5 weeks since the young nuts were introduced (Table 2.2.1.-1).

A few questions do remain: How fertile these eggs are? How long the weevils can keep breeding? Within the timeframe of this project we were unable to answer these questions.

A

B

C

D

E

F

Figure 2..2.1.-1: A) Comparison between rat damaged macadamia with incisor marks on the shell (5 nuts far left) and the "golf ball" marked husk fed by *Sigastus* weevils (centre) and emergence holes and laying marks caused by *Sigastus* weevil (8 nuts on right).B) *Sigastus* weevil feeding on the bark of macadamia. C) *Sigastus* weevil feeding on the leaf of macadamia. D) Macadamia kernel damage by *Sigastus* weevils is rarely seen because the kernel is usually mulched up in the pre harvest clean up by growers. E) Kernel damage by *Sigastus* weevil is discarded as fungal, general insect or even nutborer. F) *Sigastus* weevil failed to exit shell hole in the macadamia.

Table 2.2.1.-1: *Sigastus* weevil survival and oviposition rates after emerging from field collected nut obtained from Alstonville research station (CTH), Dunoon (DUN) and Tolga in Far North Queensland (FNQ). Note the immediate response to immature nut provision from 4/4/2016.

Date	Day #	Live weevils in colony			% survival rate			Cumulative oviposition			Diet
		CTH	DUN	FNQ	CTH	DUN	FNQ	CTH	DUN	FNQ	nut type
29/12/2015	0	56	22	47							246
4/01/2016	6	55	23	62							246
11/01/2016	13	50	22	50	100	100	100				246
18/01/2016	20	44	21	50	88	95	100				246
25/01/2016	27	43	21	50	86	95	100				246
1/02/2016	34	33	21	48	86	95	100				246
8/02/2016	41	33	21	47	86	95	98				246
15/02/2016	48	32	20	46	80	91	96				246
22/02/2016	55	32	17	42	80	77	88				246
29/02/2016	62	32	16	42	80	73	88				246
7/03/2016	69	31	15	41	78	68	85				246
14/03/2016	76	30	15	41	75	68	85				246
21/03/2016	83	27	15	21	68	68	44				246
29/03/2016	90	25	14	17	63	64	35				246
4/04/2016	96	21	12	15	53	55	31				246
11/04/2016	103	21	12	15	53	55	31				young
18/04/2016	110	21	11	15	53	50	31	13	33	41	young
26/04/2016	118	20	10	14	50	45	29	34	58	74	young
2/05/2016	124	20	9	13	50	41	27	87	89	139	young
9/05/2016	131	19	9	12	48	41	25	128	123	204	young
16/05/2016	138	18	7	12	45	32	25	176	161	252	young

2.2.2. Biology and life-cycle

Egg hatching time is 6 +/- 1 day at 25^{o} C (n=12). Emergence rate of the adult weevils from infested nut >10mm diameter usually ranges between 30-70% (Figure 2.2.2.-1) the rate is temperature dependent and the specific temperature rearing trials are yet to be done to determine minimum temperature thresholds. Under variable temperatures from a range of field sites a figure of 40 +/- 8 days for development of *Sigastus* weevil eggs to adult emergence from the nut during the October to January period (Table 2.2.2.-1). The longevity of the weevil is 100-150 days conservatively in the field with some of the sheltered laboratory individuals already over 1 year old and still going (Table 2.2.2.-2) and being capable of laying over 300 eggs during this lifespan and averaging between 10-20 eggs per week (Table 2.2.2.-2).

From the field monitoring and nut drop experiment in the CTH "Sink block", it is evident that the numbers of adults emerging weekly, rises to 50-60 per day during November, drops back through December, then rises again in January as the second generation begins to emerge (Figure 2.2.2-2). Nut on the normal development cycle at this stage of the crop will be too hard to support larval development. *Sigastus* weevil adults at this point stop breeding and begin feeding on husk, leaf, bark of macadamia and may even feed on other plant foliage nearby. The actual emergence rate from the field nut collected is averaging 50% when the size is >10mm and is not different to that of the laboratory colony or the FNQ sample acquired for the DNA comparison (Figure 2.2.2-2).

The impact on the crop is determined by how many weevils are present in the October to January period. If the orchard has no young nutlets around, the population is limited to what has flown in at flowering, waiting to breed. That population would normally be confronted with two to three insecticide sprays to target lace bug and FSB, and would normally be controlled if the sprayer equipment is capable of covering the tree height of the orchard.

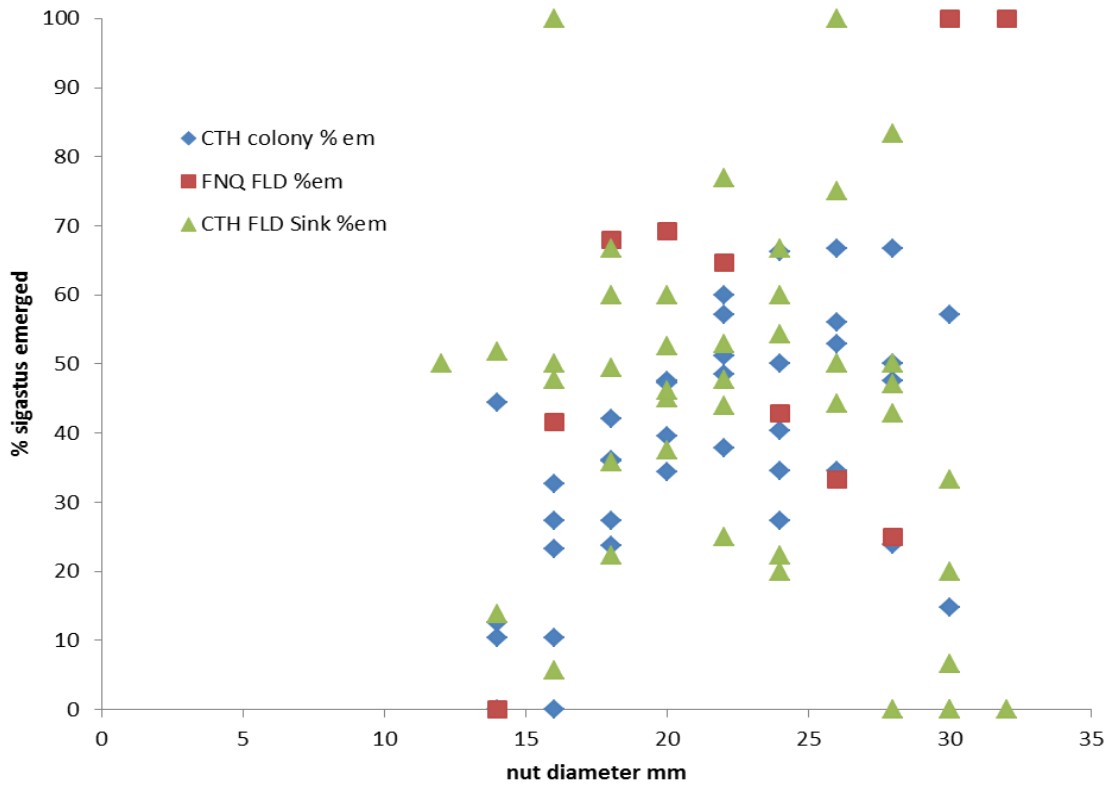
Trees with out-of-season nut can enhance the *Sigastus* weevil egg production within a plot by a factor of 4 (68/tree vs 270+/tree between July and January). This resulted in a virtually endless supply of fresh young weevils, 1450 extra off 16 trees emerged before the main crop began to fall in November (Table 2.2.2.-4). This occurred onto a continually fruiting macadamia crop, giving plenty of breeding opportunities (Tables 2.2.2.-3 and 2.2.2.-4). The weekly collection of fallen nuts from under the trees, prevented most of the second generation from establishing. It was not possible to account for nut that remained in the tree with *Sigastus* weevil eggs inside, but we could see how much crop was consumed by the first generation. This reached over 30% of the nut drop under some trees but only surpassed the losses due to FSB on 4 trees within the block (Table 2.2.2.-5). *Sigastus* weevil did spread to almost every tree within the block, but losses were heavier in the northern trees (trees 1-8 down each row) and the damage to kernel was only present at harvest on one tree and far less than the impact of FSB on the final crop quality (Tables 2.2.2.-5 and 2.2.2.-6). The total crop lost to the weevil cannot be determined until the final crop is harvested and that will not be until spring 2016. The initial harvest figures are positive for the nuts of cv. 246 trees (the trees running on the normal cropping cycle) with over 20kgs of nut in husk (NIH) under most trees and kernel losses to FSB generally below 10% and plenty still to drop. The harvest for the other trap tree varieties is poor (1-10kgs NIH) (trees with continual flowering and out-of-season crops) and the FSB damage to the kernel is much higher 20- 50% (Table 2.2.2.-6).

The take home message is that the number of infected nut on the ground in late winter/spring is crucial in the build up and damage caused by the Sigastus weevil population. Where possible we should be removing that infected nut in spring and trying to limit how much of that crop is available to Sigastus weevil by maintaining a good nut set in spring.

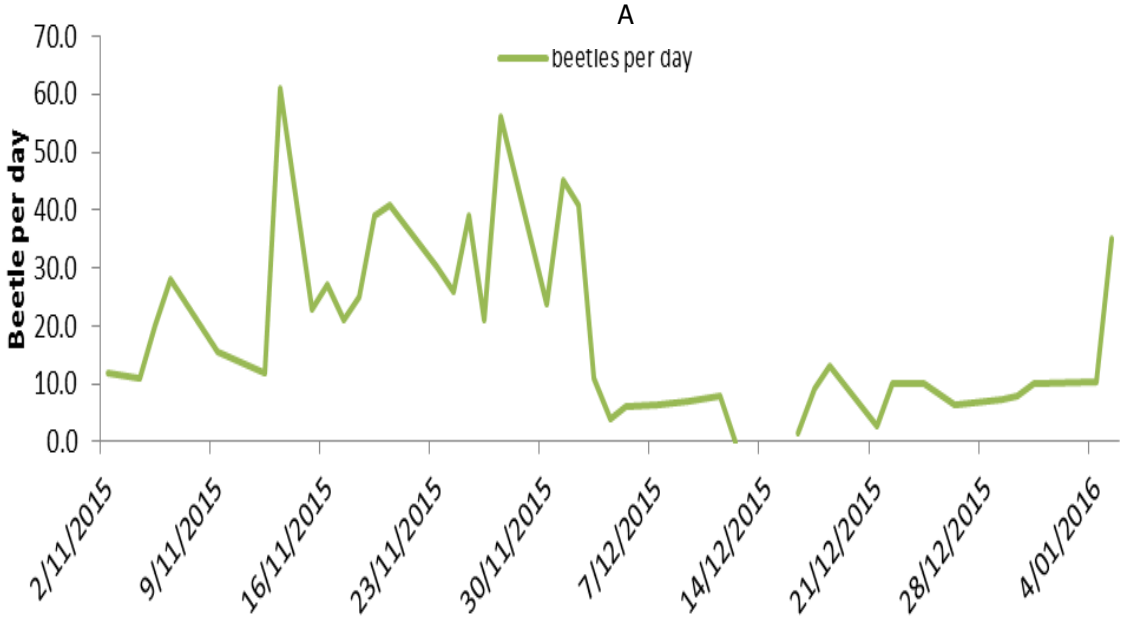
The method used to calculate the initial breeding *Sigastus* weevil population was the following: We removed all the dropped nuts, preventing the F1 mingling as best we can. For this purpose of the calculation, we assumed that all nut with eggs had dropped. FSB was active in the plot and caused complete nut drop on most varieties in the crop, up until mid November (NSW DPI data 1995-1999). We observed numerous nutlets with both FSB feeding and *Sigastus* weevil oviposition (Appendix 1, Figure 1.4.3.-1). Average weekly oviposition rate is around 10 eggs/female per week (maximum is 40 from Table 2.2.2.-2), the warmer it gets probably the more active they become. Over the 18 week period shown in Table 2.2.2.-3, 2900 eggs were laid. Therefore the initial population of females is approximately 2900/(10 X 18)=16weevils , even less if the laying rate is higher, and half of those will die out naturally by 100 days if unsprayed (Table 2.2.1.-1). This turns into 1500 quickly, if dropped nut is not removed and the original 16 weevils are insignificant then. Spraying for macadamia lace bug (*Ulonemia decoris*) in August/September, and then for FSB in October to December needs to be effective, and good spray coverage is critical for *Sigastus* weevil and FSB management (Table 2.4.2.-1 and 2015/2016 spray trial data in MT 10049).

A B

Figure 2.2.2.-1: A) Comparison between *Sigastus* weevils (far right) and other weevils found commonly on macadamia in NSW and QLD. On the left are the wattle pigs *Leptopius,* which feed on the bark, leaf and grafts of trees, and in the centre is the husk feeding Anthribid *Araecerus palmaris* (emergence holes in husk of background nuts identification courtesy Justin Bartlett and Eddy Dunn). B) The plastic rearing trays used to follow *Sigastus* infested nut populations, which are checked daily and ventilated metal trays for larger samples with the nut size and emergence levels measured from each tray after 8 weeks. When separate emergence populations are needed, the tray is surrounded with a gauze bag and weevils are collected from inside each week.



A



B

Figure 2.2.2.-2: A) Comparison between the emergence rate of laboratory reared *Sigastus* weevil and field collected nut from Alstonville CTH sink block and far north Queensland (FNQ FLD) plotted across the nut diameter they emerged from. Weevils tended to lay into nut >10mm diameter and overall average emergence was 50%. If shell hardening was too advanced, the weevil larva would not penetrate to the kernel and failed to develop. B) Plot of the *Sigastus* weevil emergence rate by date collected, from field infested nut in the untreated Centre for Tropical Horticulture Alstonville (CTH) "Sink block" and stored in shaded ambient temperature. Much lower recruitment is noted during December 2015, but rises again in January 2016 as the 2nd generation of the season begins to emerge.

Table 2.2.2.-1: Adult *Sigastus* weevil emergence times from field collected nut samples (n>25nuts freshly laid eggs <4 days old) stored at ambient temperature in the shade at the Wollongbar Primary Industries Institute (WPII).

Collection area	Collection Date	First emerged	Days	Last emerged	Days	Average emergence (Days)
Eureka Martin	7/10/2013	6/11/2013	30	9/12/2013	63	48
Alstonville HWNS	11/10/2013	25/11/2013	45	28/11/2013	48	46
Alstonville CTH	25/10/2013	27/11/2013	33	21/12/2013	56	45
Alstonville Silver	21/10/2013	29/11/2013	39	20/12/2013	60	47
Clunes Heesom	6/11/2013	10/12/2013	34	2/01/2014	57	42
Durroughby	5/11/2013	10/12/2013	35	2/01/2014	58	42
Corndale	25/11/2013	24/12/2013	29			29
Clunes Gough	25/11/2013	6/1/2014	11*	16/01/2014	55	37
Federal Madras	25/11/2013	6/1/2014	42			42
Nashua	26/11/2013	10/1/2014	44			44
Alstonville CTH 2	26/11/2013	2/1/2014	37	3/02/2014	68	43
Overall average					Egg–Adult	40 +/-8 days

Table 2.2.2.-2: Adult *Sigastus* weevil longevity estimates and total oviposition rates when fed fresh macadamia nut weekly in 750ml Vacola jars with gauze lids stored at room temperature (t=23^{o} C) at the Wollongbar Primary Industries Institute (WPII). (+) denotes individual insect is still alive and the numbers will increase. (*) rate is very much determined by the age of the nut they are fed. The laying rate drops sharply when shell hardening commences.

Life stage	Sample size (Beetles)	Average lifespan (Days)	Maximum Lifespan (Days)	Mean weekly Oviposition (Eggs)	Maximum weekly Oviposition (Eggs)	Maximum Lifespan Oviposition (Eggs)
Kept single after 2 months						
Female adults	15	120 +/-30	320+	10-20*	40	130
Male adults	5	80 +/-20	370+			
Permanent pairing of female						
Female adult	10		340			294+

Table 2.2.2.-3: Fortnightly nut collection data from July 2015- January 2016 showing *Sigastus* weevil oviposition levels as part of the mean nut drop per tree by variety, (mean ND and standard deviation SD ND) at the unsprayed CTH Alstonville macadamia "Sink block" (planted 2007). This shows the impact of out of season fruiting on the L64 and XXX varieties, supporting early and heavy oviposition by the *Sigastus* weevil females as the new crop is about to set. The impact on the 246 variety, which was flowering normally, was minimal, except for the November/December period, when all varieties are susceptible. This is an underestimate, as not all nut with *Sigastus* weevil eggs will fall to the ground.

Date sampled	246 (n=10)			A4 (n=8)			L64 (n=8)			XXX (n=8)		
"Sink" BLK CTH	mean ND	SD ND	*Sigastus* egg/tree	mean ND	SD ND	*Sigastus* egg/tree	mean ND	SD ND	*Sigastus* egg/tree	mean ND	SD ND	*Sigastus* egg/tree
6/07/2015							7	6	6	7	6	4
15/07/2015	0	0		2	2		75	79	45	56	68	18
28/07/2015	0	0		0	0		16	24	15	7	9	1
12/08/2015	0	0		0	0		33	43	20	36	54	10
24/08/2015	0	0		0	0		44	36	22	29	31	20
4/09/2015	0	0		7	21	6	166	106	45	55	63	38
16/09/2015	0	0		1	4	0	78	69	27	54	56	42
29/09/2015	0	0		0	0	0	67	60	27	92	78	82
13/10/2015	0	0		0	0	0	26	19	11	36	29	30
27/10/2015	0	0		0	0	0	18	11	8	15	16	5
9/11/2015	413	164	17	0	0	0	33	14	4	17	10	5
25/11/2015	310	144	28	66	51	8	410	149	13	11	5	0
15/12/2015	175	96	11	68	40	5	216	95	17	20	5	1
5/01/2016	18	11	2	19	20	5	46	33	10	5	2	0
Total egg/tree		**total**	**68**		**total**	**24**		**total**	**270**		**total**	**256**
fortnightly means	78	154	14	14	32	5	90	123	19	34	47	19

Table 2.2.2.-4: Fortnightly nut collection data from July - October 2015 showing the nut size changes and frequency of egg laying by *Sigastus* weevil at the unsprayed CTH Alstonville macadamia "Sink block" (planted 2007) pooled across varieties XXX, and L64. This is the egg laying collected under 16 trees with out of season nut set in May/June 2015. Assuming 50% survival rate, this could mean an extra 1450 *Sigastus* weevils emerging before the real crop is coming through in November.

nut size	15/07/2015	28/07/2015	12/08/2015	24/08/2015	4/09/2015	16/09/2015	29/09/2015	13/10/2015	27/10/2015	Total
8-10mm	6									6
10-12mm	26									26
12-14mm	59	8	29	8	9	2	0	2	0	117
14-16mm	75	27	68	43	52	33	10	10	2	320
16-18mm	48	26	55	66	128	88	47	29	5	492
18-20mm	15	13	38	78	162	93	93	39	11	542
20-22mm	3	5	5	42	123	95	140	41	16	470
22-24mm		1	2	22	67	65	159	64	20	400
24-26mm			1	12	34	41	139	42	16	285
26-28mm				4	18	21	92	28	19	182
28-30mm						7	27	10	13	57
30-32mm							0	0	0	0
Total eggs	**232**	**80**	**198**	**275**	**593**	**445**	**707**	**265**	**102**	**2897**

Table 2.2.2.-5: Spatial representation of nut drop/tree and *Sigastus* weevil ovipositon and *Amblypelta nitida* (FSB) feeding damage within that nut drop from November 2015 to January 2016 at the unsprayed CTH Alstonville macadamia sink block (planted 2007). This shows the weevil is present on nearly all trees during the main nut drop period for the crop. It is causing more nut loss than FSB on only 4 of those trees (highlighted in yellow for the November/ December period when all varieties are susceptible. No pesticides have ever been applied in this area and parasitoids for fruitspotting bug (FSB) and macadamia nutborer (MNB) were used at the first sign of activity in September (10 *Anastatus* sp. cards were used from October 2015 in this block) and *Trichogrammatoidea cryptophlebiae* were also released fortnightly to manage MNB (much less than *Sigastus* weevil).

Site map "Sink"	Row			Nut drop / tree Nov Dec 2015			% *Sigastus* egg in nut			%FSB damage in nut		
BLK	1	2	3	1	2	3	1	2	3	1	2	3
1	246	246	246	1072	1403	719	14.8	8.5	15.0	22.4	28.8	40.8
2	L64	A4	XXX	283	54	75	2.3	10.3	10.0	27.7	51.3	8.3
3	XXX	L64	A4	49	535	120	1.4	22.9	17.9	15.3	30.3	47.2
4	A4	XXX	L64	243	32	947	30.3	4.8	20.8	22.4	16.7	27.5
5	246	246	246			466			6.7			35.8
6	XXX	L64	A4			343			33.3			37.8
7	A4	XXX	L64	99	71	714	3.3	7.3	22.9	56.1	16.3	29.2
8	L64	A4	XXX	780	89		4.5	4.2		31.9	43.3	
9	246	246	246	1185	225	1138	11.8	36.7	27.0	43.9	26.7	40.4
10	A4	XXX	L64	159	40	910	4.4	3.0	17.0	55.0	13.1	20.8
11	L64	A4	XXX	584	125	46	10.2	0.0	26.6	31.2	63.6	15.6
12	XXX	L64	H2	33	881	254	0.0	5.3	0.8	11.5	25.0	43.3
13	246	246	246	1064	960	935	0.0	5.0	7.8	45.0	42.1	37.7

Dead Tree

Table 2.2.2.-6: Spatial representation of the initial harvest of nuts / tree, *Sigastus* weevil damage in nut and *Amblypelta nitida* (FSB) feeding damage within that nut to May 2016 for the unsprayed CTH Alstonville macadamia "Sink block" (planted 2007). This shows that very little weevil damage makes it to the processor, and FSB is the most significant pest of the crop when the *Sigastus* weevil infested nut is removed during the winter/spring period. Significant nut was collected during the first harvest (>25kgs NIH with more remaining) on the 246cv with minimal FSB damage and only the brief period of *Sigastus* weevil attack November/December. No pesticides have been applied in this area and parasitoids for FSB and MNB were used at the first sign of activity in September (10 *Anastatus* sp. cards were used from October 2015 in this block) and *Trichogrammatoidea cryptophlebiae* were also released fortnightly to manage MNB (higher than *Sigastus* weevil this time).

Site map "Sink" BLK	Row			Harvest in May 2016 nuts/ tree			% damage in nut			%FSB damage in nut		
	1	2	3	1	2	3	1	2	3	1	2	3
1	246	246	246	570	1182	460	0	0	0	1.7	11.7	3.3
2	L64	A4	XXX	158	75	47	0	0	0	35.7	16.7	7.4
3	XXX	L64	A4	47	213	71	0	0	0	10.0	11.7	23.3
4	A4	XXX	L64	171	80	163	0	0	0	21.7	9.3	23.3
5	246	246	246			240			0			6.7
6	XXX	L64	A4	17		110	0		0	0.0		34.5
7	A4	XXX	L64	136	68	210	0	0	0	13.8	3.2	27.6
8	L64	A4	XXX	384	167		0	0		56.7	1.7	
9	246	246	246	1296	324	1088	0	0	0	16.7	1.7	10.0
10	A4	XXX	L64	185	50	471	0	0	0	12.1	6.7	46.7
11	L64	A4	XXX	545	159	36	0	0	10	30.0	38.3	18.3
12	XXX	L64	H2	57	546	120	0	0	0	21.4	20.0	33.3
13	246	246	246	1788	1250	1146	0	0	0	3.3	5.0	11.7

Dead Tree

2.3. Current distribution

The distribution of *Sigastus* weevil was established using an annual survey as part of the AMS Benchmarking program. Figure 2.3.1 shows the expanding distribution of the pest over time.

The red area represents the initial outbreak. The orange represents the distribution 1 year later and green area represents the currently known distribution area.

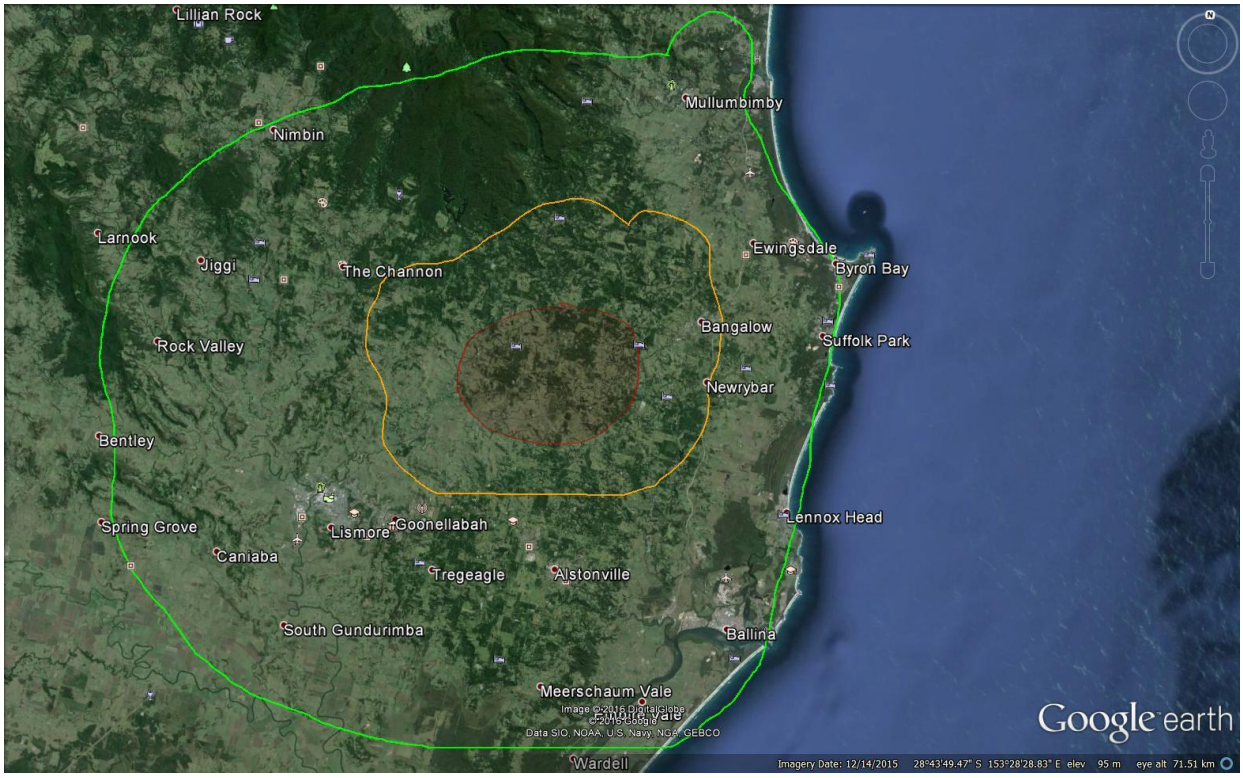


Figure 2.3.1: Expanding distribution of *Sigastus* weevil; red = initially infected area, orange = distribution after 1 year and green = current distribution.

2.4. Chemical control

2.4.1. Screening of chemicals

Topical 1µl dorsal application – pesticide knockdown (Table 1.4.4.1):

This result was concerning, as it confirmed the grower perception of a poor knockdown effect, where nothing applied gave a complete kill at the registered rates. The order of efficacy (90% mortality maximum) of the currently registered products is beta-cyfluthrin 0.1ml/L (2x rate), carbaryl 1.3ml/L, acephate 0.8gm/L, methidathion 1.25ml/L, then diazinon 1.3ml/L and trichlorfon 2ml/L. Of the soon to be registered FSB compounds sulfoxaflor 0.4ml/L was more toxic to *Sigastus* weevil than the acetamaprid mixture 0.8ml/L. The unregistered foliar compounds tested so far show methomyl 2ml/L and bifenthrin 0.5ml/L were the most noteable (Table 1.4.4.1). The only treatment that was 100% effective when topically applied was the suspension of *Beauvaria bassiana* spores when applied in the plastic containers, but this did not work as well in the vented glass jars with lower

humidity (Table 1.4.4.1). *Sigastus* weevil is harder to knockdown than FSB, our pesticide assay data presented in FSB project report MT10049 in press show 100% efficacy was achieved with trichlorfon 2ml/L and beta-cyfluthrin at 0.5ml/L in most topical application assays.

Dipped nuts - ingested potency assay

Better mortality results were achieved with this method, showing how important coverage is for *Sigastus* weevil. All registered options gave mortality figures between 85-100%. Sulfoxaflor and the acetamaprid mix also gave 100% mortality. Of the new and unregistered options tested, methomyl 2ml/L, bifenthrin 0.5ml/L, were 100% effective, and carboxamide, chlorantranilliprole and cyantranilliprole, and DC143 could all work if coverage is optimal and allowable rates adjusted, the product pricing will be a factor here (Table 2.4.2.-1).

2.4.2. Evaluation of entomopathogens

2.4.2.1. Laboratory culturing of *Beauveria* and *Metarhizium*

Four different isolates of entomopathogenic fungi were investigated. These included one strain of *Metarhizium anisopliae* (M16) known to have a wide host range and very good spore production characteristics, and three other isolates all identified as different strains of *Beauveria bassiana*, (B24, B27 and Bbsig) including the fungus infecting the *Sigastus* weevil.

All isolates showed growth responses to temperature typical for entomopathogenic fungi (Figure 2.4.2.1.-1), with optimum growth between 25°C and 30°C. The *M. anisopliae* isolate showed a slightly high optimum than the *Beauveria* isolates. Two of the *Beauveria* isolates did not growth at 35°C, although they remained alive at this temperature. The *M. anisopliae* isolate and *B. bassiana* isolate from the *Sigastus* weevil showed some growth at 35°C. Understanding growth response to temperature is important for entomopathogenic fungi, for producing spores under the best conditions and knowing the best ambient conditions for deploying the fungi for biological control.

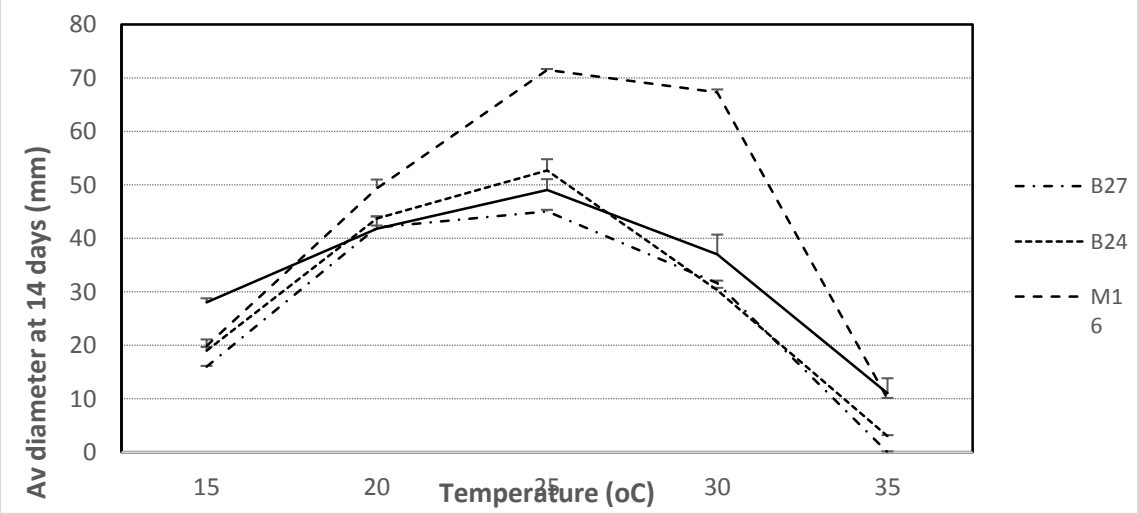


Figure 2.4.2.1.-1: Mean radial growth (mm) of four different entomopathogenic fungi (M16, B24, B27, Bbsig) over 14 days at different temperatures.

When grown on different growth media all isolates had the highest growth rate on Sabouraud's Dextrose agar but sporulated best on oatmeal agar. Oatmeal was this used for producing spores for small scale test against the *Sigastu*s weevil and for inoculum for the investigations into mass production of spores.

The investigations into the mass production of spores showed that M16 and *B. bassiana from Sigastus* weevil (Bbsig) both gave reasonable yields although M16 performed best on solid rice media while Bbsig performed best on the oat solid media. However further work needs to be carried out into quantitating the scale up of mass production of these fungi, as well as investigations into increasing yield and maintaining the sporulation for the Bbsig isolate. Many entomopathogenic fungi, especially *B. bassiana* have a tendency to sporulation attenuation when kept under artificial culture. However there are protocols that can be investigated for maintaining a high level of sporulation.

2.4.2.2. Screening of entomopathogens

2ml mist spraying – combination of knockdown and ingested

The high control mortality rates in this assay make it hard to adopt, but it does show why the fungal options will continue to be pursued at this stage, particularly the local *Beauvaria bassiana*. Once this organism entered the *Sigastus* weevil colonies, the experiments needed to be restarted because the background kill was so high (Table 2.4.2.-1). After this assay all others have been done in open gauze topped glass Vacola jars to reduce this effect.

Dipped nut with Sigastus weevil eggs laid – compare emergence of adults 35 days later

Despite the limited replication at this stage we have a very promising result. The background mortality rate is normally 50% and compared to the test treatments with the fungal isolates of *Isaria* sp., *Metarhizium* sp. and *Beauvaria bassiana* (BB) as a suspension from crushed weevils or cultured, all gave good results when used with some of the adjuvants. The cultured BB from *Sigastus* weevil (Sig BB culture) was the most effective control averaging close to 100% mortality for each mixture (Table 2.4.2.-2) and the Pulse® and Synertrol® additions appeared to give the highest mortality of the developing weevil larva in nut when added to all the fungal cultures (Table 2.4.2.-2)

2.4.3. Field assessment

Data is still being collected. Within the timeframe of this project we were not able to complete field assessment of chemicals and entomopahtogens.

Table 2.4.2.-1: Mortality rates for *Sigastus* weevil when assayed using topical application 1µl dorsally applied to the pronotum, dipped nutlets allowed to dry, before adding weevils, and a 2 ml misting of weevil and nutlets. All formulations supplied were made up to 100ml volume in A grade Blau® volumetric flasks using demineralised water which was also used as the control background for each assay. Normally 2 replicates of 10 weevils were used to cover each dose tested unless weevil numbers were very short. Average mortality after 7 days is the figure quoted. More replication is needed.

Formulation		**Dipped food**		**Topically applied 1µl**	
	Rate mls/L	**% mortality**	**adults**	**% mortality**	**adults**
Abamectin 18g/L	1	65	28	10	10
	2	40	20		
	4	55	20		
Acephate 970g/kg	0.2	80	20		
	0.4	95	20		
	0.8	100	53	67	30
	1.6	98	40		
Carboxamide	0.5	85	20		
	1	100	20		
	2	100	20		
***Beauvaria* 1 weevil/100ml +1% oil**	10	100	10	100	10
Beta-cyfluthrin 25g/L	0.25	100	20		
	0.5	95	83	70	30
	1	87	100	90	10
Bifenthrin 80g/L	0.5	100	10	90	10
Carbaryl 800g/L	1.3	60	5	80	10
Chlorantranilliprole 350g/L	0.12			80	10
	1	66	18	0	10
Cyantranilliprole 100g/L	1	100	8	70	10
Diazinon 800g/L	1.25	100	15	35	20
	1.3	100	8		

Table 2.4.2.-1: Mortality rates for *Sigastus* weevil (cont.)

Formulation	Rate mls/L	Dipped food		Topically applied 1µl	
		% mortality	adults	% mortality	adults
Endosulfan 350g/L	1.5	100	5	30	10
Flonicamid 500g WP	0.2	48	40		
	0.4	35	20		
	0.8	30	20		
Flupyradifurone 200g/L	1	90	25	60	10
Methidathion400	1.25	100	5	50	10
Methomyl 400gm/L	2	99	65	50	10
Pymetrozine 500WG	0.4	20	20		
	0.8	10	10		
Pyrethrins 13g/L	2	20	5	20	10
Pyriproxyfen 124 +Acetamiprid 186	0.4	90	20		
	0.75	100	10	50	10
	0.8	90	60		
	1			40	10
SeroX	10	10	10		
	20	20	20		
Spinetoram 120	2	85	20		
Sulfoxaflor 240g/L	0.2	100	10		
	0.4	77	50	90	10
	1	100	8	80	10
Tau fluvalinate 240	0.5	40	10	30	10
Tolfenpyrad 150g/L	2	83	50	25	20
Trichlorfon 500g/L	2	98	33	50	20
Water	**0**	**20**	**183**	**30**	**30**

Table 2.4.2.-1: Mortality rates for *Sigastus* weevil (cont.)

Formulation	**Rate mls/L**	**Dipped food**		**2ml mist spray**	
		% mortality	**adults**	**% mortality**	**adults**
Water	0			70	10
***Isaria** spore**	1gm			100	10
***Isaria** spore +synertrol 1ml**	1gm			100	10
***Metarhizium** spore**	1gm			100	10
***Metarhizium** spore + synertrol 1ml**	1gm			100	10
Sig BB* spore + 100% canola 1ml	1gm			100	10
Sig BB* spore + synetrol 1ml	1gm	30	20	100	10
Sig BB* spore + water	1gm			100	10

*spores used in these assays from cultures generated by Diana Leemon DAFQ, Ecosciences Precinct.

Table 2.4.2.-2: Percentage mortality rate from cell trays of freshly laid *Sigastus* weevil egg infested macadamia nuts dipped in various solutions. The control trays are duplicates (2 X 12 nuts), the treatment trays are single 12 nut replicates. The treatment options were mixed first (100ml volume) then added to stock solutions of the various adjuvants. Further repetition is needed, as more alternate cultures and *Sigastus* weevils become available.

Treatment	**Rate g/L**	**Agridex® 1ml/L**	**Designer® 1ml/L**	**Pulse® 1ml/L**	**Synetrol® 1ml/L**	**Water**	**Overall mean**
Control		54.2	41.7	50.0	62.5	54.2	**52.5**
Sig BB 40 weevils	** 400 infested weevil	58.3	58.3	91.7	91.7	83.3	**76.7**
Sig BB culture	1g	100.0	100.0	100.0	91.7	100.0	**98.3**
Mycaforce ®	4g	66.7	83.3	91.7	75.0	66.7	**76.7**
Metarhizium culture	1g	91.7	91.7	66.7	91.7	83.3	**85.0**
Isaria culture	1g	58.3	83.3	83.3	100.0	75.0	**80.0**
Overall mean		**69.0**	**71.4**	**76.2**	**82.1**	**73.8**	**74.5**