SUMMER SCHOLARSHIP REPORT

1.	**Project Title**	: Can insects spread cotton plant pathogens in Australia?
2.	**Proposed Start Date** **Proposed Cease Date**	: 13 January 2020 : 29 March 2020
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4.	**Organisation & Location** **for the project**	: Australian Cotton Research Institute, Narrabri
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1. Executive Summary

Providing Australian cotton protection from biotic threats and environmental stresses requires informed surveillance and management. One such threat is the fungal disease, Verticillium wilt, caused by the pathogen *Verticillium dahliae*. Verticillium wilt remains a severe disease in Australian cotton, with more virulent strains causing significant yield losses (Chapman et al. 2016). This disease would be better managed if potential pathways for transmission of *Verticillium* could be identified. Overseas publications report other species of *Verticillium* that can be transmitted by insects (Kalb et al. 1986, Huang & Harper 1985, Price 1975). The aim of this study was to identify if any insects could digest and then transmit *V. dahliae*.

A pilot study (to optimise the methodology required) and four experiments (Experiment 1, 2 and 3 exposing insects to a pure fungal culture of *V. dahliae,* and Experiment 4 exposing insects to the diseased cotton plant tissue (leaves with symptoms of Verticillium wilt)) were undertaken to assess if insects found on or in the soil around the cotton plant could transmit *V. dahliae.* Results showed that the pathogen was transferred externally on the insects: *Corticaria subtilissima* (minute brown scavenger beetle), *Anthicus australis* (ant-like beetle), Elateridae (wireworm), *Dicranolaius bellulus* (red and blue beetle) and *Creontiades dilutes* (green mirid). In addition, the pathogen survived the digestive tract of several insects: *Dicranolaius bellulus* (red and blue beetle), *Iridomyrmex* (small meat ant), *Corticaria subtilissima* (minute brown scavenger beetle), *Anthicus australis* (ant-like beetle) and Elateridae (wireworm). The faecal matter isolations and subsequent recovery of *V. dahliae* from these insects are evidence that they are capable of transmitting the *V. dahliae* pathogen.

The findings suggest that insects are a potential source of pathogen transmission within Australian cotton crops. While insects could spread *V.dahliae*, additional research is needed to establish if they can be inoculated by Verticillium wilt plants, and to fully understand the relationship between the pathogen and these potential vectors to inform the surveillance and management of this key biotic threat.

2. Background

Critical to containing and mitigating cotton disease is knowing how pathogens spread within cotton crops. Overseas research (Kalb et al. 1986, Huang & Harper 1985, Price 1975) report that an important pathogen, *Verticillium albo-atrum* Reinke & Berthold, can be transmitted by insects.

In Australia a closely related pathogen, *Verticillium dahlia*e Kleb., has caused significant disease issues and yield losses. Recently, the defoliating strain VCG1A were reported in Australia for the first time (Chapman et al. 2016). If this disease is spread by insect transmission, then this could explain why diseased plants are often found in localised patches, and could offer another opportunity to better manage this pathogen and control its spread.

3. Aims and Objectives

The aim of this project was to determine if *Verticillium dahliae* in Australia could be transmitted by insects, both via external extremities, and internally through faecal matter.

Consequently, the objective was to undertake several experiments to test for pathogen transmission by insects as outlined below:

- Pilot Study: This was undertaken to help determine the methods for the following experiments (including insect collection, media preparation and isolation techniques).
- Experiment 1-3: Exposure to pure fungal cultures of *V. dahliae* to insects from a non-infected and infected field to determine the potential transmission via external extremities and or internally through faecal matter.
- Experiment 4: Exposing insects to cotton plant tissue showing symptoms of Verticillium wilt.

4. Methods

Plant material was sourced from ACRI and nearby farms. Processing of all plant material at ACRI was conducted using existing Biosecurity Assurance Plans. The methods used throughout this research are outlined below.

Insect Sampling

The pitfall trap consisted of one plastic cup placed within another cup but kept separate by blue tack. Moistened kangaroo manure was contained within breathable material (nappy liner) and suspended over the internal cup using wire. Between 1 and 10 pitfall traps were active on any given night. They were inserted into the ground within a cotton field so that the lip of the external cup was flush with the ground. The pitfall traps (Figure 1) were left over night and collected at 6am the following morning to avoid baking the trapped insects. Insects were taken to the laboratory for identification and processing, to avoid the insects escaping, the traps were covered with the lid of the container. All ACRI site biosecurity policies (Come Clean – GO Clean) were followed both before and after entering the field.

Figure 1. A pitfall trap set up in the soil within a non- infected cotton field.

Pilot study
The pilot study trialled various insect sampling techniques before the most effective (as suggested by Mr Ethan Towns) was adopted. Inoculation of two small ground dwelling detritus feeding beetles, *Crytophillius integer (n=2)* and *Corticaria subtilissima* (n=3) were undertaken to develop isolation techniques and to identify the most effective media in which to cultivate V. dahlia spores.

The pilot study demonstrated that the best semi-selective media for insect survival was PDA + S. Based on this finding PDA + S was the semi-selective media used across the four experiments. The 90 mm petri plates used in the pilot study were later substituted to vials in Experiment 1, 2, 3 and 4 to reduce the surface area available to individual insects, which reduces the chance of the insect escaping, the likelihood of contamination and conserves laboratory material (Experiment 2).

Isolation
To ensure the insects (hereafter "insect" includes spiders that were also collected) digestive tract and external extremities were clean of any potential pathogens, individuals were transferred to 90 cm petri plates containing semi-selective media. Three different semi-selective media (Table 1) were assessed to determine the optimum medium for isolating and culturing *V. dahliae* from the insects. Insects in the control treatment were placed directly onto petri plates containing Potato Dextrose Agar (PDA) with Streptomycin (S), with no fungal culture food source. Aseptic techniques were implemented for transferring insects, fungal isolations and culturing. The petri plates with insects were kept at room temperature in the dark for 24 hours, whilst the fungal culture plates were placed into the incubator at 23°C for 24 hours.

Table 1. List of semi-selective media used for culturing

Media
Potato Dextrose Agar + Novobiocin
Potato Dextrose Agar + Streptomycin
Sorensen's NPX

Fungal exposure and fungal isolation (Experiments 1-3)
Verticillium dahliae culture plugs (4 mm) were obtained from a mother culture provided by Dr Karen Kirkby from the NSW DPI long-term culture collection. Fungal plugs were taken from the actively growing edge of the mother culture using a sterilised 4mm cork borer and used for fungal exposure Experiments (1-3). Each infected culture plug was placed into an individual well of a Linbro 24 well cell culture plate.

Insects used in these experiments are listed in Table 2. They were placed individually in the middle of the well close to the food source (*V. dahliae* culture plugs) for the feeding experiment. Small insects were transferred using a sterile paintbrush and larger insects were transferred using thin sterilised tweezers. After 24 hours each insect was transferred into a 90cm clean petri plate to collect any faecal matter. After another 24 hours any faecal matter was collected, and placed on to 90 cm petri plates containing PDA + S. The insects were each transferred to another 90 cm petri plate containing PDA + S. All plates were incubated for 2 weeks prior to assessment for the presence of *V. dahliae.* Verticillium found on petri dishes without faecal matter indicated external transmission via the insect's surface area, such as its legs; Verticillium found on petri dishes with transferred faecal matter indicated internal transmission, via consumption of the fungi. Photographs were taken of cultures of interest using an Olympus SZX9 dissecting microscope.

Experiment 1. *Fungal exposure to a fungal culture as feed source*
Insects were collected across two nights using baited pitfall traps. Insects were placed into two Linbro 24 well plates containing the control feed source (PDA + S). These insects were therefore tested for any pathogens obtained from the field prior to exposure to fungal plugs. One insect was placed into each well. Parafilm was placed over the top of each well plate and a small breathing hole was punched in the middle of each well using a very fine needle secured in a cork. Well plates were labelled and incubated at room temperature (23°C) for 2-5 days in the dark. The plates were observed under the dissecting microscope Olympus SZX9 for the presence of any pathogen from the field. All surviving insects were removed and retained for Experiment 2. Those plates that had the insects removed were placed in the incubator at 23°C for up to 2 weeks to culture potential pathogens.

Experiment 2. *Fungal exposure to a fungal culture as feed source, with insects that have a known infection status*
The insects used in this experiment were collected from an un-infected field. Insects were exposed to four rounds of fungal exposure and PDA + S exposure (Figure 2). Insects were transferred into 2ml clean plastic cryogenic vials containing a 3mm fungal plug for 24 hours and repeated up to 4 times. In between the fungal exposure assays (rounds 1 – 4), insects were placed into 2ml plastic cryogenic vials containing PDA + S for 48 hours to eliminate potential carry over of pathogen and to identify the presence of *V. dahliae* isolated. Any faecal pellets found on the PDA + S vials were transferred to separate PDA + S vials for culturing. The PDA + S vials were incubated for 2 weeks to culture potential pathogens isolated.

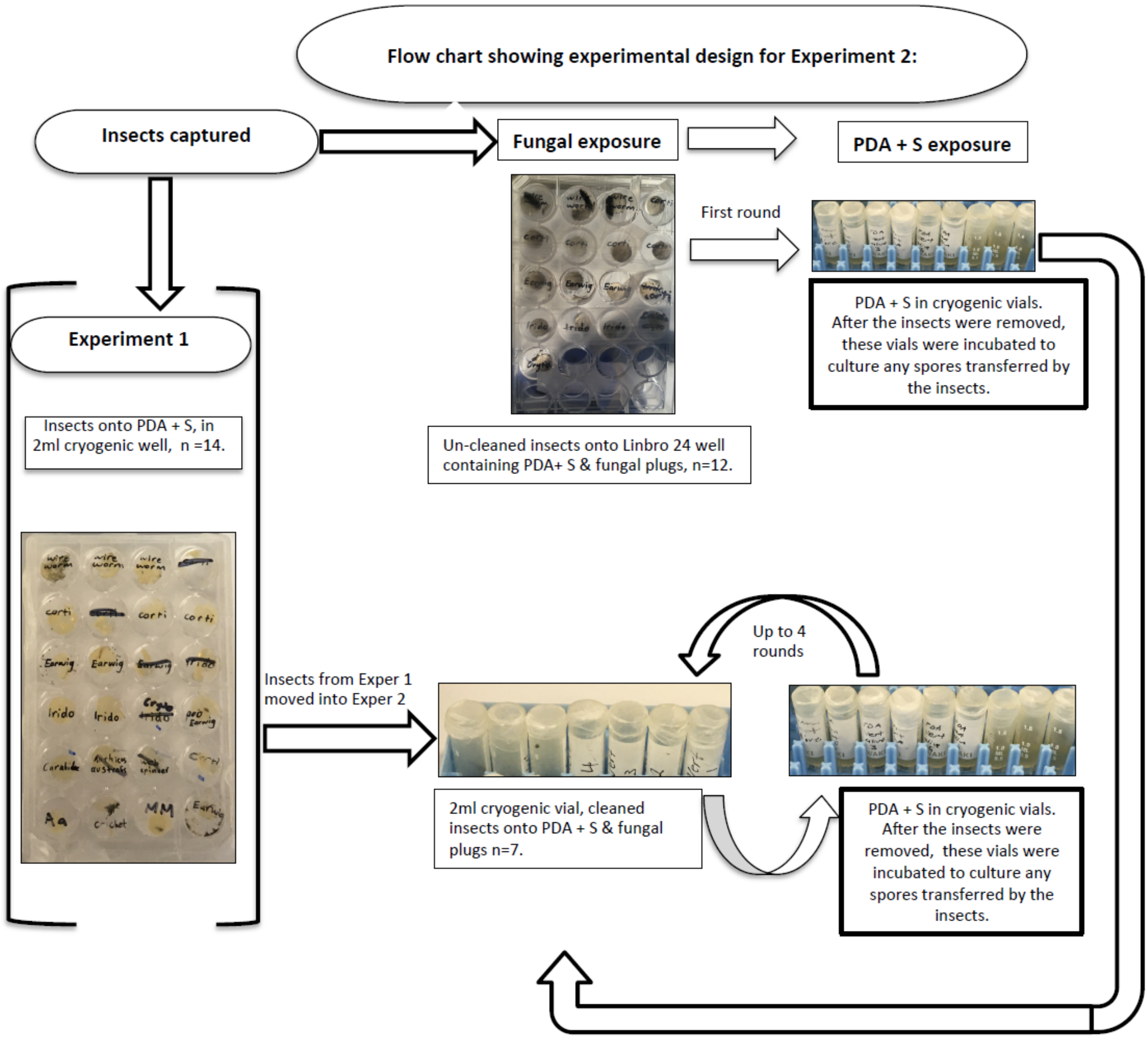

Figure 2. Experiment 2 flow diagram. The insects captured were separated into Experiment 1 (n=14) and Experiment 2 (n= 7). Those that had been part of Experiment 1 (and therefore had clear alimentary canals) were placed into 2ml plastic cryogenic vials containing a 3mm fungal plug (round 1 of fungal exposure). The uncleaned insects (that were not part of Experiment 1) were exposed to their first round of fungal spores in a Linbro 24 well plate containing the PDA + S and a *V. dahliae* fungal plug. After a period of 5 nights in the Linbro 24 well plate, the uncleaned insects were transferred to cryogenic vials for 48 hours, before transfer to cryogenic vials containing fungal cultures for another 3 rounds.

Experiment 3. *Insects from an infected field exposed to fungal culture*
Above ground insects were collected from a field with a known history of Verticillium wilt using the beat sheet technique, where plants are beaten onto a yellow sheet. To determine the infection status on insects immediately after collection they were placed in vials with PDA + S for 5 days. After this time insects were transferred into the 2ml plastic cryogenic vials containing either a 3mm fungal plug or a control PDA + S plug for fungal exposure (round 1, 2, 3 and 4).

Experiment 4. *Insects fed on cotton leaf tissue*
The following insects were targeted *Iridomyrmex*, and Elateridae because they had positive results for transmission of *V. dahliae* from fungal culture exposure experiments. The insects to be used in the tissue-feeding assay had an unknown feeding history. The methods were as described in Experiment 1 except fungal plugs were replaced with un-infected and infected cotton leaf tissue. The infected leaf material identified by symptoms and un-infected identified by no disease symptoms was collected and stored in the cool room. The insects stomachs were cleansed on two rounds of PDA + S. The 3mm plugs were taken from non-infected and infected leaf tissue using a sterilised cork borer. Half of the insects were fed un-infected leaf tissue (control) and the other half were fed on *Verticillium* infected leaf tissue.

Statistical analysis. *Comparing insects from infected and non-infected fields.*
We used a Chi-square test of independence using the statistical program Genstat® 19th edition to compare insects from infected and non-infected fields.

5. Results

Pilot study

Of the five insects on the various semi-selective media listed in Table 1, only two survived which were cultured on PDA + S. Only one insect was successfully transferred (*Corticaria subtilissima)* and the other (*Crytophilus integer*) escaped. No *V. dahliae* was recovered on any media, however other fungi for example *Rhizopus* species on PDA + S (Figure 3), and *Aspergillus niger* on PDA + N (Figure 4), were isolated and identified.

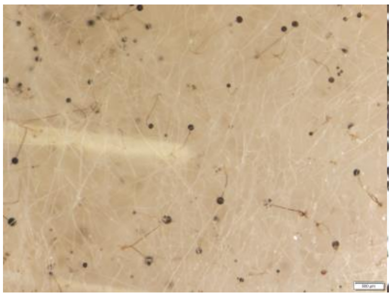

Figure 3. *Rhizopus* species (9 day culture), isolated from *Crytophilus integer*.

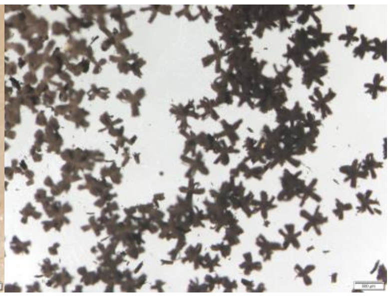

Figure 4. *Aspergillus niger* (9 day culture), isolated from *Corticaria subtillissima.*

Experiment 1, 2, 3 and 4

In Experiment 1 the insects were not exposed to the fungal plugs. Other fungi (not *V. dahliae*) was detected from insects isolated immediately following collection from a cotton field with no known history of Verticillium wilt, and therefore identified as other fungi only were isolated from the insects (n=14).

In Experiment 2 after exposing insects (n=60) to fungal culture plugs, *V. dahliae* was isolated from 7 insects. *V. dahliae* was isolated from *Corticaria subtilissima*, *Anthicus australis,* Elateridae: external and internal (faecal pellets) and from *Iridomyrmex* faecal pellets only.

In Experiment 3 no *V. dahliae* was detected from insects (n=17) isolated immediately following collection from a cotton plant with external symptoms. However, *V. dahliae* was isolated from these insects following exposure to fungal plugs. *V. dahliae* was isolated from *Achaearanea wau*, *Austroasca viridigrisea*, and *Creontiades dilutes* via external transmission only and from *Dicranolaius bellulus* via both external and internal transmission (Figure 5).

There appeared to be a higher incidence of *V. dahliae* transfer (n=9) from insects collected on a field with a known history of infection (n=17, Experiment 3) relative to the insects (n=7) collected on an un-infected field (n=60, Experiment 2). That is, insects collected from a field with a known history of Verticillium wilt were significantly more likely to transfer *V. dahliae* when exposed to the pathogen in the laboratory (chisq=10.58, df=1, P=0.001).

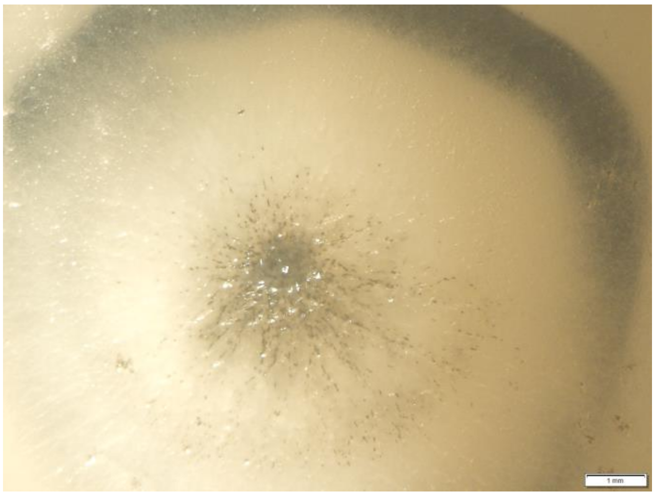

Figure 5. *Verticillium dahliae* (12 day culture) isolated from *Dicranolaius bellulus* via external transmission.

In Experiment 4 several other fungal species were isolated from the insects, but no *V. dahliae* was isolated (n=23).

Table 2. Insect and isolation results of all experiments

Insects and spiders (scientific name)	Order: family	Common name	# samples collected	Collected from		Transmission	
				V. dahliae infected field	*V. dahliae* un-infected field	External*	Internal faecal
Achaearanea wau	Araneae: Theridiidae	Tangle web spider	1	yes	no	**yes (n = 1)**	no
Dicranolaius bellulus	Coleoptera: Melyridae	Red and blue beetle	4	yes	no	**yes (n = 2)**	**yes (n = 1)**
Austroasca viridigrisea	Hemiptera: Cicadellidae	Jassid	1	yes	no	**yes (n = 1)**	no
Creontiades dilutus	Hemiptera: Miridae	Adult green mirid	2	yes	no	**yes (n = 1)**	no
Corticaria subtilissima	Coleoptera: *Latridiidae*	Minute mould beetles	23	no	yes	**yes (n = 1)**	no
Anthicus australis	Coleoptera: Anthicidae	Ant-like beetle	2	yes (n=1)	yes (n=1)	**yes (n =1)**	**yes (n = 1)**
Iridomyrmex sp	Hymenoptera: Formicidae	Meat ant	25	no	yes	no	**yes (n = 1)**
Unknown	Coleoptera: Elateridae	unknown wireworm	8	no	yes	**yes (n = 1)**	**yes (n = 1)**
Labidura truncata	Dermaptera: Labiduridae	Common brown earwig	6	no	yes	no	no
Teleogryllus commodus	Orthoptera: Gryllidae	Black field cricket	1	no	yes	no	no
Microlestes macleayicsiki	Coleoptera: Carabidae	Ground beetle	1	no	yes	no	no
Cryptophilus integer	Coleoptera: Erotylidae	Pleasing fungus beetle	5	yes (n=1), no (n=4)	yes (n=4), no(n=1)	no	no
Campylomma liebknechti	Hemiptera: Miridae	Apple dimpling bugs	10	no	yes	no	no
Oxyopes molaris	Araneae: Oxyopidae	Lynx spider	1	yes	no	no	no
Linyphiidae	Araneae: Linyphiidae	unknown sheet web spider	1	yes	no	no	no
Coccinella transversalis	Coleoptera: Coccinellidae	Transverse lady beetle	2	yes	no	no	no
Paratrechina sp	Hymenoptera: Formicidae	Crazy ant	1	yes	no	no	no
Anthicus sp. D	Coleoptera: Anthicidae	Ant-like beetle	1	yes	no	no	no

* All insects were identified by Mr Ethan Towns and confirmed by Dr Mary Whitehouse (CSIRO). *Verticillium dahliae* cultures were identified and confirmed by Dr Karen Kirkby (NSW DPI). Other fungi were not identified to genus or species level and recorded as other fungi.

6. Discussion and Conclusions:

Insects from fields without a known history of contamination by Verticillium wilt did not carry *V. dahliae* (Experiment 1, 2 and 3). However, if insects were exposed to fungal cultures of *V. dahliae*, some insects could transfer the pathogen either externally and/or internally in faecal matter (Experiments 1, 2 and 3). That is, when provided with fungal plugs, *V. dahliae* was isolated from insects via both external and internal transmission. Our results suggest that insects from infected fields had a significantly higher recovery of the pathogen from fungal plugs, but we have not controlled for the insects species tested, this requires more investigation. Experiment four was conducted in response to the positive results for transmission of *V. dahliae* from culture force-feeding experiments. When the insects *Iridomyrmex*, and Elateridae were exposed to a diseased leaf there were no positive transfers of *V. dahliae*.

In summary *Anthicus australis, Austroasca viridigrisea, Achaearanea wau, Corticaria subtilissima, Creontiades dilutes, Dicranolaius bellulus and* Elateridae transferred the pathogen externally, and *Dicranolaius bellulus, Iridomyrmex, Anthicus australis,* and Elateridae transferred the pathogen internally. That is, as *V. dahliae* was found on petri dishes containing only insect faecal matter, the pathogen was transferred and survived the insects' digestive tract. Therefore, if *Dicranolaius bellulus*, *Iridomyrmex*, *Corticaria subtilissima*, *Anthicus australis*, and Elateridae feed on the pathogen, they could transfer it into a new cotton plant.

Dicranolaius bellulus are usually regarded as being beneficial to cotton protection, consuming pest eggs and slow moving pests (Mansfield 2003, Pyke & Brown 1996), some feed on pollen however they aren't known for attacking the plant (Horne, Edward, Kourmouzis 2001, Mansfield 2003). *Dicranolaius bellulus* are also active on the soil surface, (Horne, Edward, Kourmouzis 2001) sheltering in soil cracks on hot afternoons, the eggs, larvae and pupae stages all exist in the soil, the eggs are layed in clusters on soil debris, and the larvae feed on small worms and other soil organisms (Queensland Government 2009). *Dicranolaius bellulus* travel long distances (Ingrid 2007, Horne, Edward, Kourmouzis 2001) and have the capacity to consume *V. dahliae* (potentially through detritus or as free living microsclerotia in the soil) and for the pathogen to remain viable following digestion. The consequence of these factors may lead to the *Dicranolaius bellulus* adult spreading the pathogen to new sites through defecating infected faecal matter in cotton soils (adding to the soil inoculum in fields). This is significant as the nature of this pathogen means microsclerotia can lay dormant and germinate when conditions are favourable, thus starting a new site of infection that was previously free of disease.

Anthicus australis are omnivorous and known to eat fungi (Chandler 2002, Horvath 2003, Majka 2011) but could transfer the fungi if they fed on cotton infected with the pathogen, as could Elateridae, which are already a well known pest in cotton (Gerritsen et.al 2016; Toepfer et al. 2014, Pyke & Brown 1996, Fitt 1994). Elateridae are generalist feeders, including roots and other plant and decaying material, and are likely candidates for spreading *V. dahliae*. These insects have been connected with enabling secondary crop damage by pathogens in other crops (Keiser, Häberli & Stamp 2012, Parker & Howard 2002).

Iridomyrmex nests are in the soil (Greenslade 1973), so it could pick up *V. dahliae* by consuming detritus (Vander Meer 2019). *Iridomyrmex* are problematic in cotton because they tend pest species such as *Aphis gossypii* (cotton aphids) and *Phenacoccus solenopsis* (cotton mealybugs) by protecting them from predators (Lester et al. 2003, Buckley & Gullan 1991). *Aphis gossypii* can be detrimental to cotton production, being a regular late season pest responsible for producing sticky cotton and enabling moulds to grow on honeydew secretions (Wilson, Whitehouse & Herron 2018, Fitt 1994). *Phenacoccus solenopsis* can spread rapidly, feeding on the growing part of the plant. *Phenacoccus solenopsis* can excrete honeydew, and when population levels are high, similarly the cotton will grow sooty-mould fungi, reducing photosynthesis in the plant. These insects generate major economic and aesthetic damage to cotton, with potential for heavily infested plants to die (Pellizzari & Porcelli 2013, Miller, Miller, Watson 2002, Vincenzo & Gerson 2012, Charleston et al. 2010).

In Experiment 4 the leaf tissue, which was in an empty vial, had an unknown status of inoculum source. Whilst the fungal cultures in Experiments 1, 2 and 3 offered the insects access to the conidia, hyphae, microsclerotia and PDA + S, (which is an un-natural feeding method), the inoculum (*V. dahliae*), could have been on the insects or in their gut when they were collected. However this is unlikely as none of the 58 controls were found to be carrying *V. dahliae*, indicating that the likely source of the pathogen was the fungal plugs provided.

In Experiment 3 the difference in the incidence of transfer from insects collected from a known infected field compared to an un-infected field may have been because of the weather (high incidence of rainfall) as recorded in the Appendix, or the infection status of the plants. The un-precented wet weather during this period may have slowed down the movement of the insects with 214mm of rainfall, the average maximum and minimum temperature being 33.59 and 21.60°C (relatively) from the 13th of January to the 21st of February (Figure 6). The day of and prior to collection of insects on the infected field when there was little captured the maximum temperature was 40.5°C, and the average rainfall in the previous six days was 37.9ml. In Experiment 4 there were few insects captured. This may have been a result of the weather conditions the week leading up to collection with 38.2ml of rainfall, and cooler temperatures (Figure 6).

The nature of this research was time consuming with the collection of insects and plants, preparation, period of incubation (was up to two weeks) for *Verticillium dahliae* and identification; this restricted the capacity to include further feeding cycles. Although this project focussed on the transmission of *V. dahliae*, other fungal species were isolated and identified. *Rhizopus* species, *Penicillium* species (CRDC p.3) and *Aspergillus* species (Klich 2002; Mensah 2012 and Perrone et al, 2007) were fungal colonies readily transferred by the insects. These fungal colonies are ubiquitous in the field particularly in periods of weathering such as wet weather (CRDC p.3, CRDC 2016, p.16), which was prominent during the period of research (Figure 6).

The transmission of the pathogen from the external surfaces of the insect did not distinguish between external and internal transmission of *V. dahliae*. This is because the scope of this research was just to determine whether the pathogen survived in the insect's digestive tract. Any faecal pellets were transferred to separate PDA + S vials for culturing however during the culturing of the insect's external surfaces there may have been culturing of faecal pellets, particularly if there were microscopic or non-visible faecal pellets.

Overall some insects could potentially spread the pathogen *V. dahliae* externally and internally. This means that these insects could feed on, or come into contact with the pathogen at one site, move to another site, defecate or interact with the soil, and start a new site of infection. Further research into this interaction between the pathogen and these insects as well as extending the range of species samples could be undertaken in order to further understand the relationship between the ecology of the species, their environment and their ability to spread the pathogen.

7. Highlights

The pathogen survived the insect tract of *Dicranolaius bellulus, Iridomyrmex, Corticaria subtilissima, Anthicus australis*, Elateridae transferring *V. dahliae* internally, evidenced by isolations from insect's faecal matter. This was an important finding as it demonstrated the potential for insects to spread *V. dahliae*.

There were individual insects (*Anthicus Australis, and Dicranolaius bellulus*) that repeatedly transmitted *V. dahliae*.

The method of cleansing of the insects digestive tract was effective, and could be adapted to a wider ecological study.

The opportunity to collaborate and carry out research and be mentored by three wonderful supervisors from NSW DPI and CSIRO. Working in plant pathology and entomology (two passions of mine) and developing my research skills was really exciting. I felt very supported within this work place and the community of Narrabri.

8. Future Research

In this research soil dwelling insects were chosen, based on recent overseas research report (Kalb & and Millar 1986) where fungus gnats were spreading *V. albo-atrum*. Through a wider ecological screening with more insects captured, the range of species sampled could be extended from ground dwelling to plant dwelling insects, and the methodology from this research could be adapted to screen the insects that were found to positively transmit *V. dahliae*. This requires a preferred diet for the insect chosen for this assay to ensure the infected plant tissue is at the right age and with the right inoculum culture containing microsclerotia and conidia. Changing the feeding length of the assays using the same methodology as in Experiment 4 would be beneficial, with a slight modification of incorporating young and old leaf material and isolating from leaves to confirm the status of the inoculum source. Future investigation would warrant targeting the decaying stem tissue found on the soil profile of infected and un-infected plants. Artificially inoculating cotton seedlings prior to any experiment would also be beneficial, providing a known inoculated feed material from which young and old tissue could be fed. Alternative seals should be investigated such as small cheesecloth or gauze, to prevent the insects piercing the parafilm and escaping (*Corticaria subtilissima, Crytophilus integer Dicranolaius bellulus* and Elateridae ate the parafilm).

Fungal cultures were identified based on morphological features. However if there was only hyphae or the fungi was not easily identifiable or there was *Rhizopus* sp. in the culture there was no capacity for further identification due to the short scope of the project and no budget for molecular testing. *Verticillium* is easily identified using a microscope and that was the focus of this work. All other fungi isolated were recorded as "other fungi". Future research should include identification of all fungi isolated from insects.

The scope of this research was to determine whether there was any transfer of *V. dahliae* from insects. It was a short study that showed some insects can transmit *V. dahliae* warranting further research to include foliar and ground dwelling insects and testing the faecal colonies after ingestion of *Verticillium*. Dissection of the insects could also be beneficial to confirm any faecal colonies in the insects' digestive tract.

9. Presentations and Public Relations:

Presentation to the Myall Vale site (CSIRO and NSW DPI) on the 5th of March 2020

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Appendix

Appendix 1. Weather Data

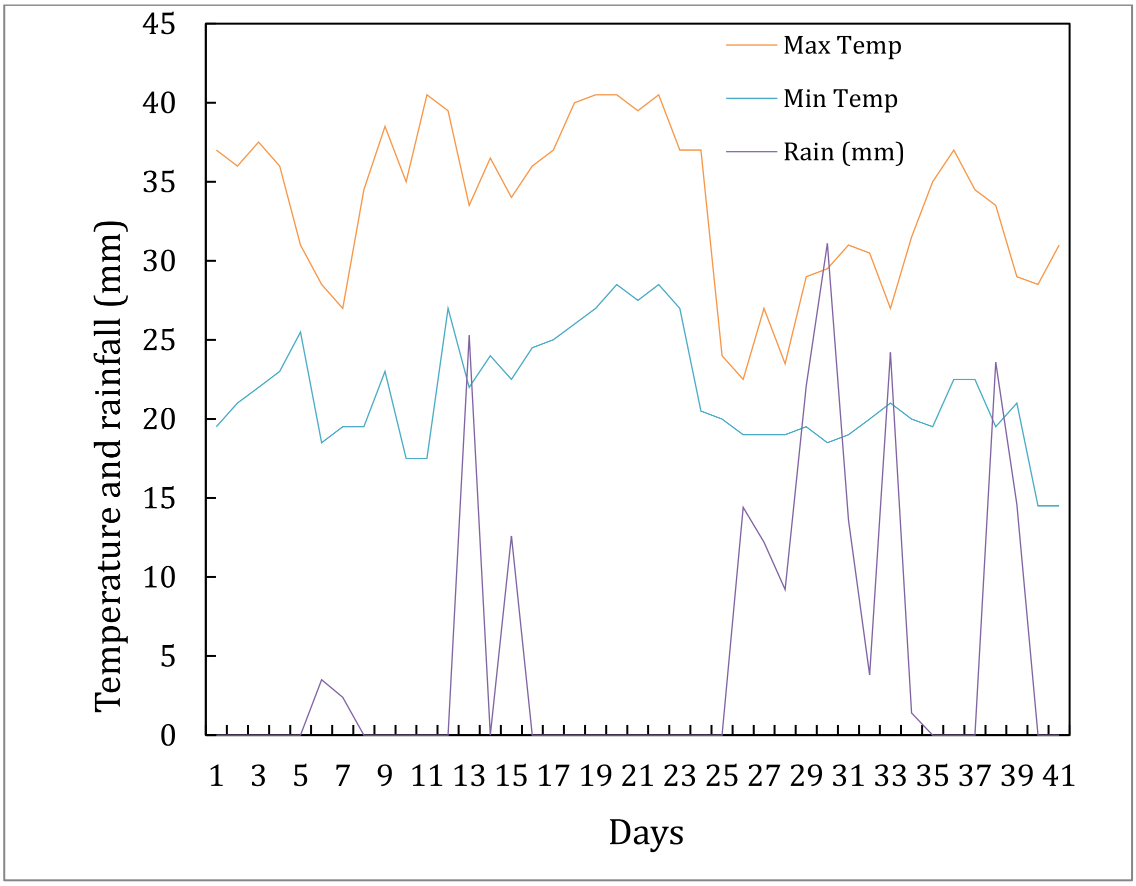

Figure 6. Real time climate data recorded at station 53044, Wee Waa recording: the rainfall in mm, the maximum and minimum temperatures from the 12th of January to the 21st of February 2020.