Insecticide resistance detection and management in currant lettuce aphid

Dr Grant Herron
NSW Department of Industry and Investment

Project Number: VG08066

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FINAL REPORT

"Insecticide resistance detection and management in currant lettuce aphid"

VG08066

01 February 2009 to 31 May 2010

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HAL project Number: VG08066

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Other key personnel: Mrs Jeannette Rophail, Senior Technical officer and Mr Brendan Langfield, Technical Officer

Purpose of the report: Outlines initial research to maintain currant-lettuce aphid in laboratory culture for the reason of insecticide resistance bioassay development as a first step to baseline generation for the purpose of resistance monitoring.

Funding sources (levy and non levy) and collaborators: This project was facilitated by HAL in partnership with AUSVEG. It was funded using the vegetable industry levy with matched funds from the Federal Government and contributions from I & I NSW.

Date of report: May 2010

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Media Summary

The currant-lettuce aphid (CLA) *Nasonovia ribisnigri* (Mosley) is a known pest of chicory, endive radicchio and lettuce. Australia was CLA free until 2004 when it was detected in Tasmania and by 2006 had been confirmed in all Australian states. In Australia, CLA is controlled by basic Integrated Pest Management (IPM) methods using a mixture of sanitation, resistant varieties, seedling drenches and foliar sprays. The chemical control options are currently limited to imidacloprid (Confidor®), Dimethoate (Rogor®) and pymetrozine (Chess®). Imidacloprid in particular, is under enormous pressure, because it can be used as a prophylactic seedling drench. There have been anecdotal control problems reported with imidacloprid and CLA but currently no method exists in Australia to test CLA for resistance. To allow resistance in Australian populations of CLA to be detected methodology development is required to do this. Here we present our experiments to maintain field collected CLA in laboratory prior to establishing bioassay methodology for the purpose of resistance detection. The study successfully derived an Australian interim preliminary discriminating dose for imidacloprid (Confidor®) for the purpose resistance monitoring.

Technical Summary

The currant-lettuce aphid (CLA) *Nasonovia ribisnigri* (Mosley) is a known pest of chicory, endive radicchio and lettuce. Its distribution includes many European countries, the USA and Canada, South America and New Zealand. Australia was CLA free until 2004 when it was detected in Tasmania and by 2006 had been confirmed in all Australian states. In Australia, CLA is controlled by basic Integrated Pest Management (IPM) methods using a mixture of sanitation, resistant varieties, seedling drenches and foliar sprays. The chemical control options are currently limited to imidacloprid (Confidor®), Dimethoate (Rogor®) and pymetrozine (Chess®). Imidacloprid in particular, is under enormous pressure, because it can be used as a prophylactic seedling drench. There have been anecdotal control problems reported with CLA and imidacloprid but currently no method exists in Australia to test for resistance. To allow resistance to be detected in Australian populations of CLA specific bioassay methodology is required. Here we present our experiments to maintain field collected CLA in the laboratory prior to establishing bioassay methodology for the purpose of resistance detection. Study progress was slowed because many CLA strains did not survive with some strains slowly dying out with CLA numbers never sufficient for experimentation. The reasons for this are not clear and possibility relate to: 1. a mix of susceptible and CLA resistant lettuce from the wholesale suppliers; 2. the high spike temperatures in the mass culture facility that often exceeded 30°C in full sun; 3. the use of artificial light only in our temperature controlled rooms or cabinets that caused lettuce to 'bolt'. Whatever the reason it was clear that trying to fit CLA culture maintenance into existing EMAI facilities and protocols causes culturing compromise that sometimes results in strain failure. Consequently, more research into CLA culturing and strain maintenance is required. Despite this huge problem the study did establish the basic methodology required for resistance detection in CLA as a first step in the long term sustainable chemical control and management of CLA. Initial dose response data generated against imidacloprid indicated an optimum bioassay withholding period of 72 h (indicated by a higher regression slope of 2.58) but that was offset by an unacceptable control mortality (CM) of 27.8%. Initial tests suggest 48 h may be the best withholding period compromise (slope 1.80, CM 5.6%) but more testing is still required to finalise this. None-the-less there was success with the study producing an interim preliminary discriminating dose of 0.1 g imidacloprid / L with a 48 h withholding period at 25°C for the purpose of CLA resistance monitoring.

Introduction

The currant-lettuce aphid (CLA) *Nasonovia ribisnigri* (Mosley) is a known pest of chicory, endive radicchio and lettuce. Its distribution includes many European countries, the USA and Canada, South America and New Zealand. Australia was CLA free until 2004 when it was detected in Tasmania and by 2006 had been confirmed in all Australian states (McDougall and Creek 2007).

In Australia, CLA is controlled by basic Integrated Pest Management (IPM) methods using a mixture of sanitation, resistant varieties, seedling drenches and foliar sprays (McDougall and Creek 2007). Horticulture Australia limited has previously funded an IPM focussed CLA project in Tasmania and two more recent studies, namely VG05044 and VG07076, but nothing on chemical control. The chemical control options are currently limited to imidacloprid (Confidor®), Dimethoate (Rogor®) and pymetrozine (Chess®) (Infopest 2008). Imidacloprid in particular, is under enormous pressure, because it can be used as a prophylactic seedling drench. There have been anecdotal control problems reported with imidacloprid and CLA but currently no method exists in Australia to test CLA for resistance.

Insecticide resistance in Australian populations of CLA is a real possibility because there have been numerous detections overseas (Barber et al. 1999, Kitt et al. 2004 and Rufinger et al. 1997 & 1999). Unfortunately CLA is an exotic pest to Australia so reference susceptible strains are not available. That makes baseline data for comparison problematic because the truly susceptible genotype is unknown. Under such conditions baseline data is best generated over several seasons so that normal variation from very susceptible to very tolerant can be accurately quantified. With extensive baseline data resistance can then be diagnosed with confidence with high level tolerance quickly separated from low level resistance. Such extensive baseline data is required for all chemical used against CLA including imidacloprid (Confidor®), Dimethoate (Rogor®) and pymetrozine (Chess®) plus any other chemicals that are used in the future.

Chemicals available for use against CLA include the neonicotinoid, imidacloprid (Confidor®) and the pyridine azomethine, pymetrozine (Chess®). Additionally, other new novel chemicals will likely become available for CLA control as they are made available by Industry but again no methodology is available to test them. The pyridine azomethine, pymetrozine (Chess®) in particular doesn't work like conventional insecticides but rather starves the insect to death over a prolonged period. For that reason methods to detect resistance based on older conventional chemistry are not particularly applicable. Previous personal experience by the author with cotton aphid indicates significant additional methods development will be required to successfully detect pymetrozine (Chess®) resistance. Similar method development would also be required if lipid biosynthesis inhibitor spiromesifen (Oberon®) or spirotetramat (Movento®) or the new ryanodine receptor inhibitor compounds, such as flubendiamide or rynaxypyr are made available for CLA control.

Although resistance is yet to be detected in CLA the potential clearly exists with CLA known to be pirimicarb, endosulfan and pyrethroid insecticide resistant (Barber et al. 1999 and Rufinger et al. 1997). Pirimicarb resistance would appear to be due to a modified acetylcholinesterase (Rufinger et al. 1999) but endosulfan (Rufinger et al. 1999) and pyrethroid Barber et al. 1999) resistance implies broad spectrum detoxification that can cause cross resistance to unrelated compounds.

To allow resistance in Australian populations of CLA to be detected and monitored locally developed and verified methodology is required to detect resistance. Here we present our first attempt to maintain field collected CLA in laboratory prior to establishing bioassay methodology for the purpose of resistance detection.

Materials and methods

Aphid source
CLA were sourced via established entomological colleagues that included Dr Paul Horne (IPM Technologies), Dr Sandra McDougall and Ms Sylvia Jelinek (Industry & Investment NSW), Dr Sonya Broughton (Agriculture WA) and Mr Craig Futrill (SARDI).

Lettuce maintenance
CLA susceptible lettuce for aphid culturing was sourced from wholesale seed distributors and growers as well as commercial retailers such as Bunnings Wharehouse.

Lettuce as tube stock was initially transferred to 150 mm diameter pots with premium potting mix (Yeates Premium Potting mix) and left under constant fluorescent light in CT cabinets to develop to a size where they could be used for insect culturing. Natural light was also trialed in a small home greenhouse but abandoned due to the risk of contamination (Figure 1). A mass culture insectary was used but also abandoned when the CLA slowly died out (also see Appendix 1) (Figure 2)

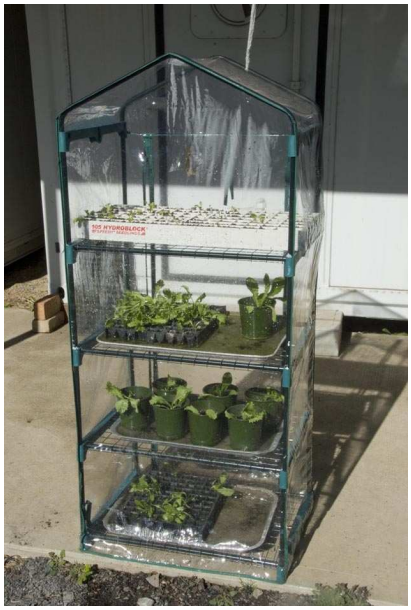

Figure 1. An early attempt to maintain lettuce in full sun with the protection of a small home green house

Finally, lettuce was germinated from seed and maintained under sodium lights and when some 20 mm tall transferred to bigger pots as above. Subsequently all lettuce maintained prior to CLA infestation was maintained under sodium light (Figure 3).

Suitability of different lettuce varieties for CLA maintenance
Lettuce sourced from commercial wholesalers were returned to the laboratory at EMAI and transferred into 150 mm pots described above and left to develop until ready for use. Lettuce were then transferred to individual aphid proof cages measuring 450x450x450 mm (Figure 2) using a conventional randomised complete block trial with each cage considered a block (Herron *et al*. 2004). CLA were then added to the individual plants using methods described in Langfield (2007) but five rather than three aphids were used per treatment replicate. The experiment evaluated Cos, Iceberg and Butter variety of lettuce for use with CLA. Discussion with the biometrician concluded eight or more replicates were required to achieve statistical significance.

Figure 2. EMAI mass culture insectary maintained at 24°C (but can spike >30°C) showing various insect species contained in insect proof cages including CLA

Figure 3. Lettuce seed in germination trays directly under a sodium light source surrounded by older previously germinated lettuce

CLA Bioassay

Bioassay methodology was adapted from that previously used for melon aphid, *Aphis gossypii* Glover (Herron *et al*. 2001). Briefly, the method utilised 35 mm Petri dishes into which an excised Cos lettuce leaf disc was placed onto 3 mL of cooling liquid agar. When the agar had set, batches of about 20 adult aphids were transferred onto the leaf discs. Leaf disc and aphids were then sprayed by a Potter spray tower (Burkard Scientific, Uxbridge, Middlesex, UK), producing an aqueous deposit of 1.6 ± 0.07 mg cm^{-2} with a 2 mL aliquot. Each dose-response assay was replicated once and included a water only sprayed control. After spraying, Petri dishes were covered with finely perforated clear plastic film that maintained high humidity but prevented condensation. Tests were maintained as per *A. gossypii* at 25 ± 0.1 ^{o}C in constant light for 24, 48 and 72 h with mortality assessed after each time interval.

Data analysis

All bioassay tests were control mortality corrected (Abbott 1925) and probit regressions were calculated (Finney 1971) for imidacloprid. Control corrected LC_{50} or $LC_{99.9}$ values were calculated from the dose response regressions using Genstat computer software (Barchia 2001).

Results

Lettuce maintenance

Figure 4. Lettuce with CLA in adequate light showing normal plant growth

Figure 5. Lettuce with CLA in poor light causing spindly plant growth and plant collapse

Small differences in light within the greenhouse insectary (Figure 2) or fluorescent light caused lettuce growth to deviate from optimum (Figure 4) and caused lettuce collapse (Figure 5) that severely hindered aphid production. Additionally, less that optimal light for lettuce in the germination rooms (they are currently designed for beans and cotton) further stifled lettuce production until the sodium light was obtained (Figure 3).

Suitability of different lettuce varieties for CLA maintenance
After eight days Iceberg produced 56, Cos 53 and Butter 11 CLA total (Table 1). Iceberg appeared the superior variety for CLA production but replicate numbers were not sufficient to support a statistical analysis. Although Iceberg appeared the better of the three for strain maintenance Cos was used for bioassay as it had a large leaf and midrib that aphids preferred. For this reason Cos was used for CLA culturing as well as bioassay.

Table 1. Randomised complete block evaluation of lettuce varieties Iceberg, Cos and Butter for CLA strain maintenance

		Day 1			Day 2			Day 8		
Replicate	Variety	Alate	Aptera	Nymph	Alate	Aptera	Nymph	Alate	Aptera	Nymph
1	Ice		5			5	2		12	15
1	Cos		5		1	4			9	21
1	Butter		5		1	3	3		3	1
2	Ice		5			5	1		14	15
2	Cos		5			3			14	9
2	Butter		5		1	3			6	1

CLA bioassay

Table 2. Replicated control corrected dose response probit regression summary for CLA strain 'Horne' tested against imidacloprid with post test withholding (WHP) period of 24, 48 and 72 h.

WHP	CM%	Chi-square (DF)	Slope (SE)	*LC_{50} (95% FL)	*$LC_{99.9}$ (95% FL)	*DD
24 h	5.6	29.64 (14)	1.46 (0.344)	0.0028 (0.00066-0.0057)	0.37 (0.095- 18.47)	0.4
48 h	5.6	16.07 (8)	1.80 (0.583)	0.0014 (0.00021-0.0030)	0.071 (0.011- 29.93)	0.1
72 h	27.8	15.55 (6)	2.58 (1.211)	0.0010 (0.00011-0.0035)	0.016 (0.0012-3.28)	0.02

* = g/L
CM = Control Mortality (see Abbott 1925)
DF = Degrees of Freedom
SE = Standard Error
LC = Lethal Concentration
FL = Fiducial limit
DD = Discriminating Dose. ffrench-Constant and Roush (1990) note that such a dose to delineate resistance should cause >99.9% mortality (i.e. >$LC_{99.9}$) on what is considered a susceptible reference population.

The best response was achieved after 72 h (indicated by the higher regression slope of 2.58) but that was offset by unacceptable control mortality (CM) (27.8%) with 48 h being the best withholding period compromise (slope 1.80, CM 5.6%).

Data was sufficient to interpolate a discriminating dose for the purpose of resistance monitoring at each withholding period tested. These were based on the $LC_{99.9}$ level response and were 0.4, 0.1 and 0.02 g/L with withholding periods of 24, 48 and 72 h respectively. Un-replicated bioassay data indicates that discriminating doses may be further reduced with more replicated baseline data that would make them more sensitive at detecting low level resistance (Appendix 2).

Discussion

The currant-lettuce aphid (CLA) is widely distributed internationally but a relatively new pest to Australia where it was first seen in 2004 but has since spread to all Australian states (McDougall and Creek 2007). CLA is controlled by basic IPM plus prophylactic chemical drenches, the most popular being imidacloprid (Confidor®)((Infopest 2008). This puts enormous pressure on CLA to develop resistance but no Australian methodology is available to manage or even detect resistance. Here we present that basic methodology for resistance detection in CLA as a first step in the long term sustainable chemical control and management of CLA. Initial dose response data generated against imidacloprid indicated an optimum withholding period for the bioassay of 72 h (indicated by the higher regression slope of 2.58) but that was offset by unacceptable control mortality (27.8%). Initial tests suggest 48 h may be the best withholding period compromise (slope 1.80, CM 5.6%) but more testing is required. Bioassay of field collected CLA strains should continue and repeat the initial bioassay to try and reduce control mortality at 72h to <10%. It may be possible because one un-replicated bioassay did achieve a 96 h withholding period without control mortality (see Appendix 2). A small drop in bioassay temperature from 25°C to 23°C would be a good first step to try and reduce control mortality to acceptable levels. If bioassay control mortality can not be reduced then a 48 h withholding period can be used. ffrench-Constant and Roush (1990) note that a dose to delineate resistance should cause >99.9% mortality on what is considered a susceptible reference population. Using that dose criterion we propose an interim preliminary discriminating dose of 0.1 g imidacloprid / L for the purpose of CLA resistance monitoring with a 48 h withholding period at 25°C.

Although VG08066 did have a delayed start once underway it progressed well with a CLA culture established and initial culturing issues seemingly solved. However, following that initial success CLA strains did not survive with many strains just slowly dying out with numbers never sufficient for experimentation (see Appendix 1). The reasons for this are not clear and possibility relate to a mix of susceptible and CLA resistant lettuce from the wholesale suppliers (we tried a few different ones) that slowly kill the CLA. It is clear from the literature that resistant varieties will slowly kill CLA (Liu and McCreight 2006). Alternatively, there could have been a problem with the culturing methodology (possible as it is still being developed) or its application by staff. The established literature is not particularly useful when it comes to CLA culturing with no problems highlighted. Liu and McCreight (2006) noted that CLA will breed on all susceptible cultivars. Liu (2004) successfully caged and evaluated CLA in the field without problem. Rufingier *et al*. (1997) maintained CLA on lettuce in cages approximately the same as ours measuring 500x500x500 mm but at a lower 20°C. The life history study of Diaz and Fereres (2005) found CLA performed best at 20-24°C. They noted survival of CLA was more affected by high temperatures (26-28°C) than lower temperatures with the optimum temperature 20°C. The mass culture insectary used in this study was set to 24°C as a compromise for the many insect species maintained within. However, temperatures in the facility often spikes to >30°C in full sun and such temperatures are not conducive to optimum CLA production. Conversely, the light in the mass culture facility is good at stopping lettuce ‘shooting’ or ‘bolting’ but the temperatures may be too high in full sun to maintain CLA long term. Alternatively, temperature can be fully controlled under artificial light but lettuce ‘shooting’ or ‘bolting’ again hinders optimal CLA production (Figure 6). More work is required to define the optimal conditions for CLA culture maintenance for the purpose of resistance testing and baseline data generation. Clearly, trying to fit CLA maintenance into existing EMAI facilities and

Figure 6. CLA infested plants maintained in insect proof cages at 21°C under fluorescent light

protocols is a compromise that sometimes results in strain failure.

Liu and McCreight (2006) noted small but significant differences between CLA susceptible lettuce varieties in their ability to maintain CLA. For that reason we investigated Cos, Iceberg and Butter lettuce varieties to determine which might be most appropriate for maintaining CLA prior to bioassay. CLA numbers available for this experiment were not sufficient to achieve statistical significance but two replicates were completed but advice from a biometrician suggested eight more are required. Gross aphid totals suggest Iceberg or Cos varieties would be the better choice for CLA culturing but work continues to be confounded by small differences in light within the greenhouse and insectary container where CLA are currently maintained. Although Iceberg produced the greater number of aphids it was found that the Cos leaf had the most desirable properties for bioassay (i.e. a broad flat strong leaf with big mid vein) so for that reason Cos is the variety of choice for CLA culturing and subsequent bioassay.

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Rijk Zwaan Australia Pty Ltd supplied certified CLA susceptible seed. Dr Markus Reigler, UWS Hawkesbury, supplied the Sodium grow lights to germinate the certified seed provided. Our entomological colleagues including Dr Paul Horne (IPM Technologies), Dr Sandra McDougall and Ms Sylvia Jelinek (Industry & Investment NSW), Dr Sonya Broughton Agriculture WA) and Mr Craig Futrill (SARDI) supplied live CLA for culturing. We thank Leppington Speedy Seedlings, Choice Seedlings and Austral Seedlings for supplying tube stock lettuce for culture maintenance. This project was facilitated by HAL in partnership with AUSVEG. It was funded using the vegetable industry levy with matched funds from the Federal Government and in kind contributions from I & I NSW

References

Abbott WS. (1925) A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.

InfoPest (2008) InfoPest MSDS/AGFET build 21/01/2008. Queensland Government

Barber, MD., Moores, GD., Tatchell, GN., Vice, WE. and Denholm, I. (1999) Insecticide resistance in the currant-lettuce aphid, *Nasonovia ribusnigri* (Hemiptera, Aphididae) in the UK. *Bulletin of Entomological Research*, **89**: 17-23.

Barchia I. (2001) Probit analysis and fiducial limits in Genstat. In: *Genstat 2001 Program and Abstracts* (eds V Doogan, D Mayer & T Swain) p. 3. Mecure Resort, Surfers Pardise, Gold Coast, 31 January - 2 February 2001, Australia.

Diaz, B.M. and fereres, A. (2005) Life table and population parameters of *Nasonovia ribisnigri* (Homoptera: aphididae) at different constant temperatures. *Environmental Entomology* **34**: 527-534.

Finney, D.J. (1971) *Probit Analysis*, Third Edition. University Press, Cambridge.

ffrench-Constant, R.H. and Roush, R.T. (1990) Resistance detection and documentation: The relative roles of pesticidal and biochemical assays. *In*: (Roush, R.T. and Tabashnik, B.E. Eds) Pesticide resistance in Arthropods. Chapman and Hall, New York.

Herron, G.A., Gullick, G., and Holloway, J. (2004) Amparo®: a new cotton seed-dressing mixture of imidacloprid and thiodicarb. *General and Applied Entomology*, **33**: 25-28.

Herron, G.A., Powis, K. Rophail, J. (2001) Insecticide resistance in *Aphis gossypii* Glover (Hemiptera: Aphididae), a serious threat to Australian cotton. *Australian Journal of Entomology*, **40** (1): 85-89.

Kitt, NB., Mead, A., Reynolds, K., Sime, S., Barber, MD., Denholm, I. and Tatchell, GM. (2004) The impact of insecticide resistance in the currant-lettuce aphid, *Nasonovia ribusnigri*, on pest management in lettuce. *Agricultural and Forest Entomology*, **6**: 295-309.

Langfield B.J. (2007) Population dynamics of currant-lettuce aphid (*Nasonovia ribisnigri*) on potential alternate hosts. Honours Thesis, University of Sydney.

Liu, Y.B. (2004) Distribution and population development of *Nasonovia ribisnigri* (Homoptera, Aphididae) in iceberg lettuce. *Journal of Economic Entomology*, **97**: 883-890.

Liu, Y.B. and McGreight, J.D. (2006) Responses of *Nasonovia ribisnigri* (Homoptera, Aphididae) to susceptible and resistant lettuce. *Journal of economic Entomology*, **99**: 972-978.

Rufinger, C., Schoen, L., Martin, C. and Pasteur, N. (1997) Resistance of *Nasonovia ribusnigri* (Homoptera, Aphididae) to five insecticides. *Journal of Economic Entomology*, **90**: 1445-1449.

Rufinger, C., Pasteur, N., Lagnel, J., Martin, C. and Navajas, M. (1999) Mechanisms of insecticide resistance in the aphid *Nasonovia ribusnigri* (Mosley)(Homoptera, Aphididae) from France. *Insect Biochemistry and Molecular Biology*, **29**: 385-391.

McDougall, S and Creek A (2007) Currant lettuce aphid *Nasanovia ribusnigri* (Mosely). Primefact 155, NSW DPI.

Appendix 1 – Chronology of CLA culturing

April 2009

Cos lettuce seedlings were bought from a local supplier and re-potted with native potting mix and placed under fluorescent grow-tube in a growth cabinet (Figure 7).

Figure 7. EMAI insectary growth cabinet room showing a range of different CT cabinets available for use with CLA

May 2009

Cultures were received form Dr Paul Horne of IPM Technologies, Mr Craig Futrill South Australia and Dr Sandra McDougall, I&I NSW Yanco. Aphids were put into culture in cages in the glasshouse insectary (see Figure 2).

June 2009

A randomised complete blocking experiment was done to determine the best lettuce variety for CLA culture culturing. Trial plants including Cos, Ice-burg and Butter varieties purchased from Bunnings Warehouse that were infested using the 'McDougall' CLA strain. CLA were placed under no-choice conditions and monitored at daily intervals over a 10 day period with aphid numbers (alate, aptera and nymphs) recorded. Initial results suggested Cos and Iceberg were the more suitable varieties for CLA culturing but additional replication was required to achieve a statistical robust result. It was additionally noted that Cos also possessed other desirable traits in regard to culturing, such as hardiness and ease of aphid collection over Iceberg. Cos was chosen to rear CLA.

Initial Bio-assay dose range tests were performed against both stains to determine optimal aphid test numbers, aphid age and test with-holding period with initial tests and cultures going OK. It was determined 12 – 15 aphids per 25 mm 'Cos' lettuce leaf disk held for 48 hrs produced consistent results (Figure 8).

Figure 8. Jeannette Rophail scores a bioassay

July 2009

Difficulties were noted with lettuce plants under the fluorescent grow tubes that caused 'bolting'. Light and lack of nutrients were hypothesised as potential sources. As space was at premium in the glasshouse insectary a small inexpensive external glasshouse structure was purchased to provide additional lettuce culturing space so natural light could be utilised (see Figure 1). Cos lettuce was purchased from the original supplier to use in the new structure with lettuce growing well. However, when the new lettuce was used for subsequent CLA culturing there was a slow reduction in CLA numbers. Initial controls to fix the CLA decline focussed on temperature and light and CLA cultures were moved within the glasshouse insectary. The possibility of a contaminant CLA resistant variety was raised with the supplier but the supplier did not consider it likely. However, we noted that *Nasonovia* sp. resistant lettuce was becoming common and the potential for seed to possess the resistant genetic trait had to be considered.

Cultures were now in poor condition so lettuce was bought from Bunnings Warehouse and Coles to stabilise CLA numbers but unfortunately **all cultures were lost**.

August 2009

A new lettuce supplier was approached to supply CLA susceptible lettuce for insect culturing. A range of lettuce varieties were supplied with some actually being CLA contaminated. Those CLA provided the nucleus for a new strain. CLA numbers quickly increased on the new lettuce and CLA testing was able to recommence.

September 2009

Bio-assay continued, however, difficulties arose when an additional field strain 'Syliva' arrived that contained a parasitic wasp. Much of the lettuce stock was used during the parasite isolation.

October 2009

Again more lettuce was sought from the second supplier. Aphids were placed on the new lettuce stock as required but this time it was apparent that CLA numbers were falling and not increasing. Again there was a late switch to Bunnings Warehouse sourced lettuce to try and save the CLA but **all strains were again lost**.

November 2009

The focus then turned to sourcing seed direct from a registered supplier and preparing a setup for germinating lettuce from seed.

December 2009

A Sodium grow light from the UWS was setup in a controlled climate room. CLA susceptible seed was sourced and received from Rijk Zwaan and maintained under the Sodium grow lights (Figure 9).

Figure 9. Initial sodium light set up in the insectary container to germinate lettuce seed.

January 2009

Seed germinated but lettuce not yet big enough to support CLA

February 2010

Field collected CLA were sourced via Dr Paul Horne (IPM Technologies) and put into culture on lettuce germinated from Rijk Zwaan seed and maintained under fluorescent grow lights at 21°C in individual cages 450x450x450 mm (see Figure 6). Potting mix changed to a Premium variety.

March-April-May 2010

Small numbers of CLA continuously present under fluorescent light at 21°C on Cos, Iceberg and Oak leaf varieties but CLA numbers were not enough for experimental work to recommence (see Figure 6).

Appendix 2 – Dose responses for CLA against imidacloprid

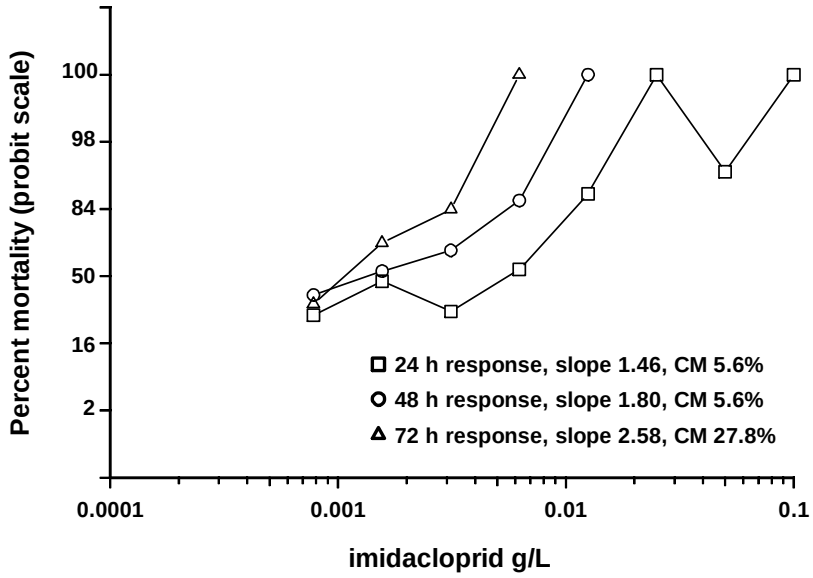

Figure 10. Replicated dose response for CLA strain 'Horne' against imidacloprid with 24, 48 and 72 h withholding periods

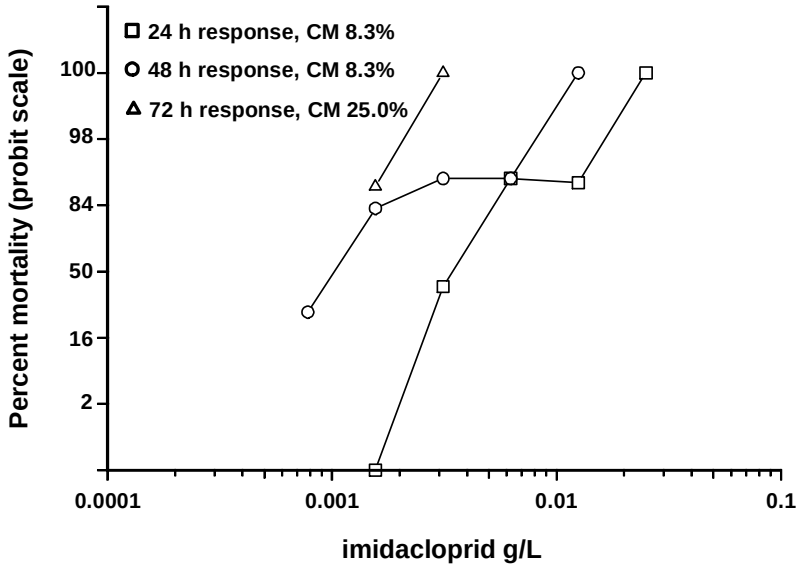

Figure 11. Un-replicated dose response for CLA strain 'South Australia' against imidacloprid with 24, 48 and 72 h withholding periods

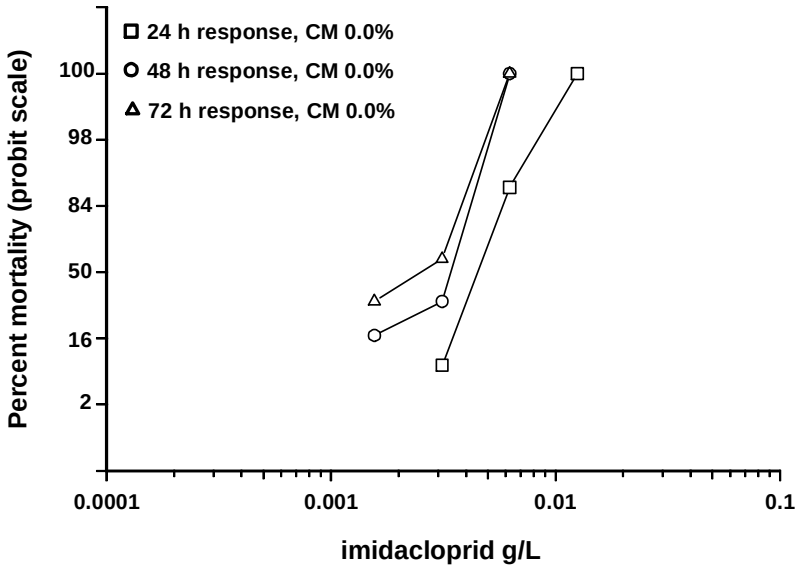

Figure 12. Un-replicated dose response for CLA strain ‘Leppington’ against imidacloprid with 24, 48 and 72 h withholding periods