



Final Report

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Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

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Project Title: Evaluation of insecticides for control of solenopsis mealybug – Burdekin.

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Part 4 – Final Report Executive Summary

The objectives of the project were to (i) develop techniques for evaluating insecticide efficacy against solenopsis mealybug, and (ii) evaluate the efficacy of a range of insecticides that have potential to provide effective control of solenopsis mealybug in cotton and (iii) consider the place of potential insecticide options in the context of existing IPM and IRMS strategies.

Nine insecticides were evaluated. Whilst all the products have registration in cotton, none currently have registration for mealybug. In addition, some of the products were provided by the companies on a commercial-in-confidence basis. Hence this summary provides an overview of the outcomes without mentioning the products directly.

Glasshouse bioassays have been completed and a range of insecticides for control of solenopsis mealybug successfully evaluated. The results show that there is value in pursuing a number of options that will cause minimal disruption to key natural enemy species, particularly the ladybird *Cryptolaemus montrouzieri* and lacewings. The addition of organosilicone adjuvants increased the efficacy of some products significantly.

Field evaluation of the promising options now need to be undertaken to determine whether the levels of control that they can achieve is, in practice, sufficient to manage a mealybug outbreak. Importantly, this work must be conducted in the field where the contribution of natural enemies will be an important component of the outcome. Factoring in the contribution of natural enemies will be critical to developing sustainable management strategies for this pest.

Techniques for maintaining mealybug colonies in the glasshouse were developed, methodology for establishing infestations on trial plants were refined and a bioassay protocol for assessing insecticide efficacy against solenopsis mealybug in controlled glasshouse conditions was successfully implemented. The experience we have gained in conducting these trials has equipped both scientists and technical staff in the DEEDI Field Crops Entomology group with the skills necessary to conduct similar evaluations in the field. The bioassay techniques are now available for the controlled screening of biopesticide or other candidate control options.

Management of neonicotinoid resistance, particularly in cotton aphid, is a significant consideration in potentially introducing additional uses for this group of insecticides. Also worthy of consideration is the implication of pursuing more than one consecutive application of promising products.

Part 3 – Final Report

Background

An outbreak of mealybug occurred in cotton in Emerald, in January 2010. Infested fields suffered localised damage where infestations were heavy, but the outbreak was largely confined to the western side of the irrigation area. The species was identified as the solenopsis mealybug (*Phenacoccus solenopsis*). As this species was not previously recorded as being present in Australia, the outbreak was treated as an exotic incursion. Further investigation confirmed *P. solenopsis* was the species that had infested cotton in the Burdekin in the 2008-09 season, causing localised retardation of growth and where severe, plant death.

During the 2009-11 seasons, *P. solenopsis* has been identified from cotton in a number of regions in Queensland (Darling Downs, St George, Rockhampton, and Byee). Positive identifications of the species have also been made from horticultural crops in Bowen and Bundaberg, weeds in north Queensland, and garden plants in Brisbane. These positive identifications of solenopsis mealybug, from such a wide geographic distribution suggests that the species is likely to be even more widely distributed at low densities. It is likely that local outbreaks will occur when conditions are suitable.

Outbreaks of *P. solenopsis* have occurred in a number of countries over the past 5 years. Solenopsis mealybug is native to north America, and other than an outbreak in cotton in Texas in 1989-1990 ((Fuchs et al., 1991), is not recognised as a pest of cotton in the USA. Severe outbreaks have occurred in Brazil in 2005 ((Culik, 2005), Pakistan and India in 2006-07 (Hodgson et al., 2008; Nagrare et al., 2009), and China in 2008.

Mealybugs have piercing sucking mouthparts and feed directly on the phloem of plants. Damage to host plants may include direct damage from feeding with symptoms including yellowing, stunting, twisted growth, defoliation and plant death. Indirect impact may be caused by the reduction in photosynthetic capacity resulting from sooty mould growth on honeydew excreted by the mealybugs. In cotton, the excretion of honeydew and the growth of sooty mould may also impact on lint quality.

The potential for solenopsis mealybug to impact on cotton production has prompted questions about effective control. Internationally, mealybug pests are controlled by biological control (predators and parasitoids) and little is published in relation to chemical control in broadacre situations. However, given the persistence of solenopsis mealybug in Emerald through the 2009-10 season and then again into the 2010-11 season, and the detrimental impact of the infestations on crop growth, evaluation of insecticide options was identified by growers as a priority research endeavour.

Objectives

The objectives of the project were to:

- develop techniques for evaluating insecticide efficacy against solenopsis mealybug, and
- evaluate the efficacy of a range of insecticides that have potential to provide effective control of solenopsis mealybug in cotton
- consider the place of potential insecticide options in the context of existing IPM and IRMS strategies.

Importantly, soft options that are minimally disruptive to natural enemies were included in the evaluation. Soft options are potentially viable options, even if they only provide suppression, because the contribution of biological control to mealybug control is anticipated to be significant. In addition, use of insecticides for the control of mealybug must be compatible with existing management strategies for other key cotton pests, particularly aphids and whitefly.

The initial intention was to evaluate the candidate insecticides in the field (in the Burdekin) so that natural enemy impacts could be evaluated alongside efficacy against mealybug. However, we were unable to establish populations in the field sufficient for an insecticide evaluation. Attempts to conduct the trials in Emerald in the 2010-11 season were also thwarted by the prolonged flooding in the region.

Consequently the evaluation of nine candidate products was undertaken in the glasshouse with potted plants infested with mealybug sourced from Emerald. Several bioassays were undertaken to test methodology, and to evaluate the efficacy of these products. Initially the nine products were tested without any additives, and subsequently a subsample of the products tested was evaluated with adjuvants (organosilicones and PSO).

The process of establishing mealybug colonies, infesting plants for the trial, developing bioassay protocols and evaluating infestations and the impact of products have all been extremely useful in learning about working with solenopsis mealybug.

Methods

Several bioassays were conducted to develop and refine the methodologies used for the trials. Here we report specifically on two of the bioassays for which we felt we had the optimal conditions (COT11-2 and COT11-4), and demonstrate the progression of testing from basic screening of products (COT11-2) to evaluating the potential benefit of additives (COT11-4).

Stock plants and establishment of mealybug for insecticide efficacy evaluation

Cotton plants (Sicot 81) were grown from seed in the glasshouse before being infested with mealybugs from the stock colony originating from Emerald. Plants were infested by placing excised leaves and terminals from heavily infested colony plants at the base of the trial plants. The infested plant material was left for 24-48 hours, allowing the mealybugs to relocate themselves to the trial plants.

Plants were infested when they were around 2-3 nodes. Earlier infestation typically resulted in plant death before the trial was completed. Plants were generally 4-5 nodes by the commencement of the trial and the application of the insecticide.

Infestations on trial plants were left until all lifestages were present (adult females, crawlers, 1st to 3rd instar nymphs) and well distributed on plants.

Plants were selected for inclusion in the trial based on consistency in growth and the density and distribution of mealybug.

Attempts to move individual mealybugs (adults and juveniles of differing ages) onto trial plants failed to establish colonies, and were extremely time consuming. Therefore we persisted with using plant material from the colony plants. The downside of using plant material from the colony plants was the resultant transfer of thrips from colony plants to trial plants. In one bioassay we suspect that the impact of thrips on the plants may have contributed to the mortality of early instar mealybug nymphs. Chemical control of thrips was not possible without disrupting the mealybug. In subsequent bioassays, thrip populations were kept as low as possible by the use of yellow sticky traps, caging trial plants and regular inspection of trial plants and manual removal of thrips.

Treatments and application

COT11-2_The candidate products initially screened:

1. Natra Soap @ 3 L/ha + Spraytech oil @ 500 mL/ha
2. Canopy oil @ 2% v/v concentration
3. Pulse surfactant @ 0.5% v/v concentration
4. Buprofezin 440 g/L SC (Clap) @ 1.0 L/ha
5. Sulfoxaflor @ 400 mL/ha
6. Spirotetramat 240 g/L SC (Movento) @ 400mL/ha + Hasten adjuvant @ 1 L/ha
7. Clothianidin 200 g/L SC (Shield) @ 250 mL/ha + Maxx surfactant @ 2 mL/1L water
8. Acetamiprid 225 g/L SL (Intruder) @ 100 mL/ha
9. Methidathion 400 g/L EC (Supracide) @ 1.4 L/ha
10. Untreated

COT11-4_Bioassay to evaluate the efficacy of a subsample of products with the addition of adjuvents:

1. untreated
2. Buprofezin 440 g/L SC (Clap) @ 1.0 L/ha
3. Buprofezin 440 g/L SC (Clap) @ 1.0 L/ha + Maxx surfactant @ 0.2% (2 mL/1L water)
4. Buprofezin 440 g/L SC (Clap) @ 1.0 L/ha + Maxx surfactant @ 0.2% + Canopy @ 2%
5. Sulfoxaflor @ 400 mL/ha
6. Sulfoxaflor @ 400 mL/ha + Maxx surfactant @ 0.2% (2 mL/1L water)
7. Acetamiprid 225 g/L SL (Intruder) @ 100 mL/ha
8. Acetamiprid 225 g/L SL (Intruder) @ 100 mL/ha + Maxx surfactant @ 0.02%
9. Methidathion 400 g/L EC (Supracide) @ 1.4 L/ha

Promising treatments from COT11-2 were retrialed in this bioassay with and without additives. The additives selected were based on published literature on mealybug control in horticultural crops (e.g. grapes, Lo et al. (2009)) and recommendations of the manufacturers.

Treatments applied with a hand-held boom sprayer fitted with 4 DG110015 nozzles calibrated to deliver 117.1 L/ha based on 50 cm nozzle spacing and 5 km/hr walking speed.

All 10 replicates in each treatment were sprayed together with one pass of the hand-held boom. Spraying was done in an igloo, and then plants returned to the glasshouse where they were arranged on benches and maintained for the duration of the trial.

Trial design, data collection and analysis

Ten replicates of each treatment were laid out in a randomised design. Each replicate was a single potted plant.

Assessment of mealybug densities was categorised as follows:

- i) location on the plant (bottom, mid, top, stem, new growth)
- ii) lifestage (adult, small nymph, large nymph)

In the initial bioassays counts were used. In later bioassays a rating system was used. The rating scale used was: 0 = 0, 1 = 1-5, 2 = 6-10, 3 = 11 -20, 4 = 21-50, 5 = 51-100, 6 = 100+. The rating was useful for estimating mealybug densities in locations where destruction/dissection of the structure would have been necessary to get count data e.g. terminals, squares. Rating also sped up the assessment process, but our confidence with the rating system would not have been as high if we had not first collected count data.

Assessment of mealybug densities was made at the following intervals:

COT11-2: 0 DAT, 4 DAT, 10 DAT and 14 DAT (DAT = Days after treatment) – count data
COT11-4: 0 DAT, 7 DAT, 14 DAT and 21 DAT – rating data

The relatively slow acting nature of some of the products used meant that maximum efficacy was not expected until at least 7 DAT, therefore we delayed the first post treatment assessment beyond the traditional 4 days.

Statistical analyses

COT11-2

To satisfy the linear model assumption of equal variance, the counts were transformed by the natural log of the count plus one.

A linear mixed model was fitted to the data. Random terms were Rep, Plant, Node, Days after treatment (DAT) and Stage (maturity groups). Chemical treatments, DAT, Stage and their interactions were considered as fixed terms and assessed for significance. The residual maximum likelihood (REML; Patterson and Thompson, 1971) algorithm through ASReml-R (Butler et al., 2008) was used to estimate variance components. Best linear unbiased estimates (BLUEs) and their standard errors were obtained for the fixed treatment effects.

COT11-4

While the rating scale has unequally spaced categories, the scale is applied to the linear models framework to allow variance modelling of the stage and DAT factors. Although of lesser concern than the unequally spaced categories, there was no violation of the linear model assumption of equal variance obvious in assessment of the residual plots. No transformation of the rating scale was made.

Results and discussion

Results for COT11-2: Screening candidate products without additives

Results from the final model showed a significant three-way interaction of chemical treatment by lifestage by DAT. Results of this bioassay are presented in Figure 1.

Because there was a significant effect of lifestage, the data is presented by lifestage (adult, large nymph, small nymph). The significance of the difference in performance of the treatments against each lifestage will have implications for timing of application in a field situation.

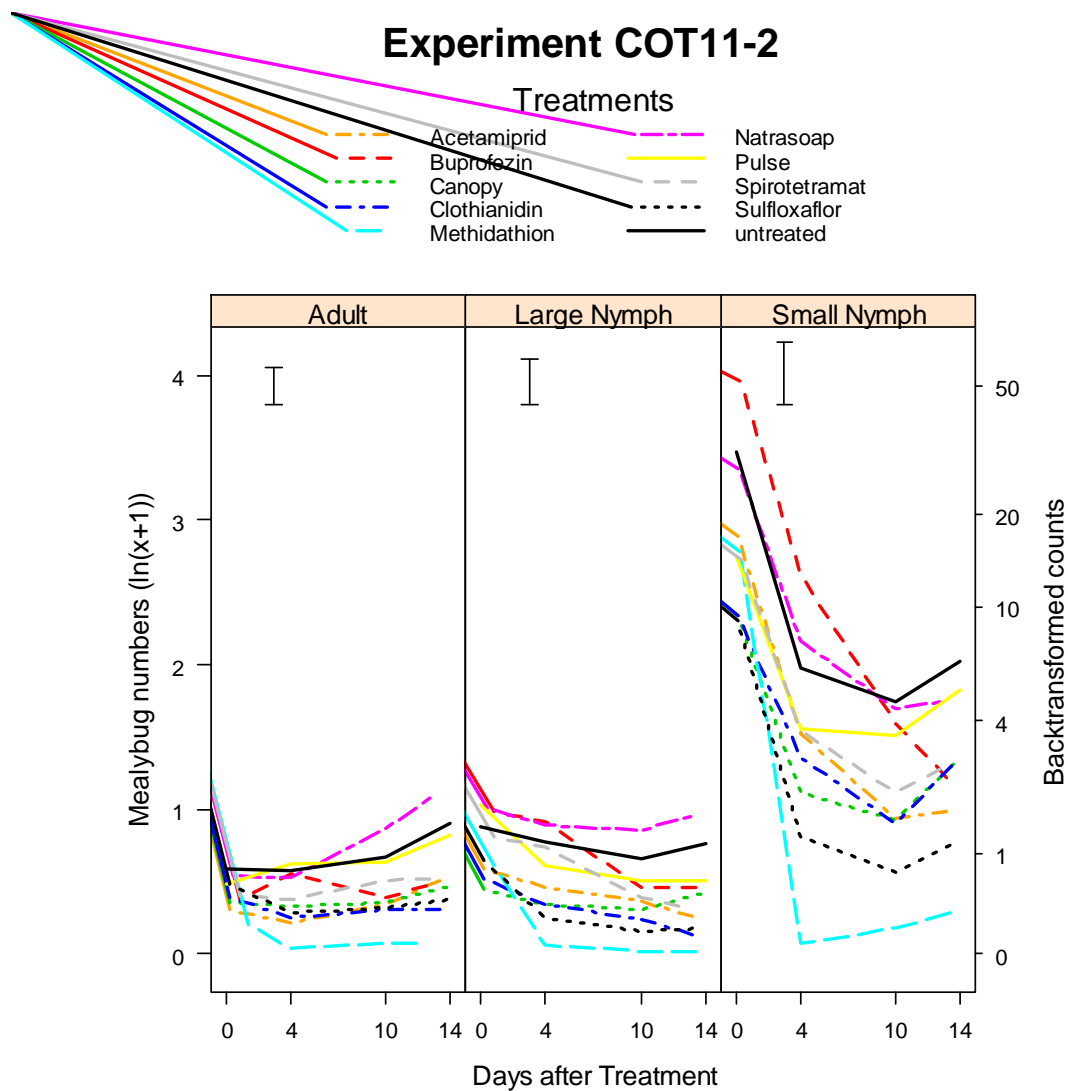


Figure 1. The impact of insecticides on the different lifestages (adults, large nymphs and small nymphs) of solenopsis mealybug, *Phenacoccus solenopsis*. LSD's represented by the bars in the top left hand corner of each graph.

The relative efficacy of all treatments at peak performance (10 DAT) is presented in table 1.

The majority of treatments tested had reasonable efficacy against all lifestages. The exceptions were Natrasoap and Pulse which did not differ significantly from the untreated in any comparison.

Natrasoap was included in the bioassay because it has been used by growers in Emerald attempting to control mealybug with least disruption to natural enemies. On the basis of these trial results, the use of Natrasoap as a knockdown for mealybug cannot be recommended. However, as with some of the other products in the trial (e.g. Canopy and buprofezin) two successive applications may have more impact than a single treatment. This strategy may warrant investigation in future research.

Pulse provided no significant control of any lifestage under these trial conditions. We know from disinfestation trials that Pulse will dehydrate and kill mealybug when applied with exceptional coverage (to runoff). However, these trial results indicate that under field application rates it has no insecticidal effect on mealybug.

Table 1. COT11-2. Comparison of treatment impact on mean solenopsis mealybug numbers at 10 DAT (ln(x+1) transformed means). Means followed by the same letter are not significantly different (p>0.01).

Adults			Large nymphs			Small nymphs		
Natrasoap	0.87	a	Natrasoap	0.85	a	untreated	1.75	a
untreated	0.67	ab	untreated	0.66	ab	Natrasoap	1.70	a
Pulse	0.63	abc	Pulse	0.50	bc	Buprofezin	1.59	a
Spirotetramat	0.51	bcd	Buprofezin	0.45	bcd	Pulse	1.51	ab
Buprofezin	0.38	cd	Spirotetramat	0.38	bcd	Spirotetramat	1.11	bc
Canopy	0.36	d	Acetamiprid	0.36	bcde	Acetamiprid	0.94	cd
Acetamiprid	0.34	de	Canopy	0.30	cde	Canopy	0.93	cde
Sulfloxaflor	0.32	de	Clothianidin	0.24	cde	Clothianidin	0.90	cdef
Clothianidin	0.30	de	Sulfloxaflor	0.15	de	Sulfloxaflor	0.56	def
Methidathion	0.07	e	Methidathion	0.02	e	Methidathion	0.18	f
LSD	0.26			0.31			0.43	

All other treatments significantly reduced the numbers of adults and large nymphs, and there was little difference in the efficacy of the products. Acetamiprid, clothianidin, Canopy and sulfloxaflor were effective in reducing populations of adults and nymphs significantly. These treatments were included in the subsequent testing with adjuvants.

Spirotetramat was not as effective as the group of products mentioned above and was not included in further testing.

Buprofezin had little impact on adults and large nymphs. Impact on numbers of small nymphs was not significant until 14 DAT when the effect of the other treatments was in decline and small nymph numbers rebounding (Figure 1). Buprofezin may have a useful

place in mealybug control because of its suppressive effect on small nymphs and its 'soft' profile against key mealybug predators e.g lacewings and ladybirds, and parasitoids (Smith & Papacek 1990, Liu & Chen 2000, Cloyd & Dickinson, 2006). In grapes buprofezin gives improved control when applied as two consecutive applications (Lo et al, 2009).

Maximum mortality was achieved by 10 DAT and the resurgence of small nymphs by 14 DAT suggests that there is no residual effect of these products, and that there is no ovicidal effect constraining the small nymph numbers. The exception to this trend was for buprofezin where numbers of small nymphs continued to decline to 14 DAT (the end of the trial). Close examination of ovisacs in the buprofezin treatments showed some egg mortality (browning and collapse of eggs) in the outer layer of the ovisacs where they were exposed to the insecticide (Figure 3).



Figure 3. (Left) *Phenacoccus solenopsis* (solenopsis mealybug) females with ovisacs showing evidence of egg mortality (browning) following treatment with buprofezin. (Centre) Close up of *P. solenopsis* female with dead eggs in ovisac. (Right) Dissected ovisac post treatment showing dead eggs and live eggs/crawlers that were protected by the female at the time of treatment. M Miles, DEEDI.

When “node” (cotyledon, low leaf, stem, high leaf, terminal) was included as an additional fixed effect in the analysis it also showed a significant high-order interaction. The population data was partitioned in the assessment in order to examine whether there was a pattern of movement between plant parts following treatment, perhaps as avoidance behaviour in response to the treatment. However, there is no clear trend of movement around the plant following treatment (Figure 2).

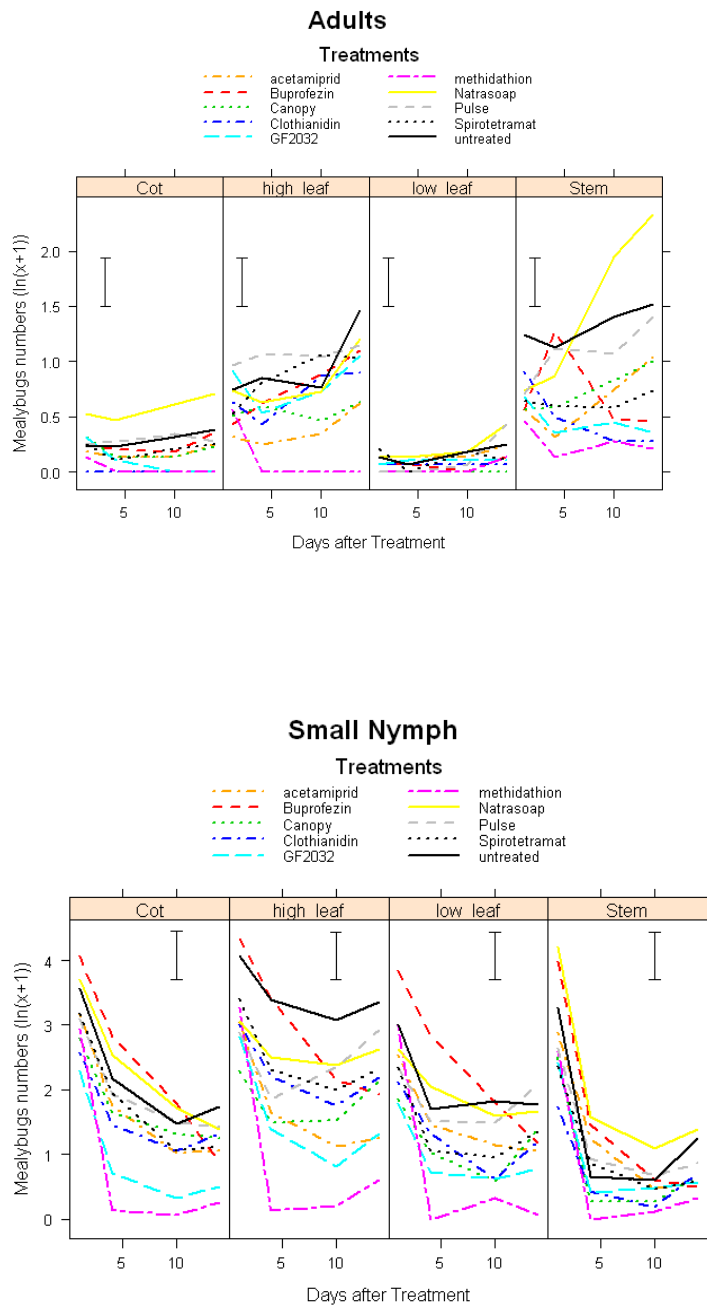


Figure 2. Partitioning of the *Solenopsis* mealybug adult (left) and small nymph (right) populations by position on the plant does not indicate a trend in movement between particular plant parts following treatment with a range of insecticides.

Results for COT11-4: Evaluating efficacy of the best performing treatments with additives

The addition of Maxx (an organosilicone additive) and Canopy to a range of insecticides, for the most part did not improve efficacy significantly. There was one standout improvement, the efficacy of sulfloxaflor with the addition of Maxx (Table 2, Figure 4). This result suggests that further work with additives may be warranted in developing effective, and less disruptive control options for mealybug.

Table 2. COT11-4. Comparison of treatment impact on mean solenopsis mealybug numbers at 14 DAT (ln(x+1) transformed means). Means followed by the same letter are not significantly different (p>0.01).

Adults			Large nymphs			Small nymphs		
Untreated	3.43	a	Untreated	2.43	a	Untreated	4.64	a
Sulfloxaflor	2.91	ab	Buprofezin	1.74	ab	Buprofezin + Maxx	4.48	a
Acetamiprid + Maxx	2.87	b	Sulfloxaflor	1.65	b	Buprofezin	4.44	a
Buprofezin	2.61	b	Acetamiprid + Maxx	1.50	bc	Buprofezin + Maxx + Canopy	4.21	ab
Buprofezin + Maxx + Canopy	2.57	b	Buprofezin + Maxx + Canopy	1.29	bc	Acetamiprid	3.70	abc
Acetamiprid	2.38	bc	Buprofezin + Maxx	1.17	bcd	Sulfloxaflor	3.18	bc
Buprofezin + Maxx	2.34	bcd	Acetamiprid	1.13	bcd	Acetamiprid + Maxx	3.16	cd
Sulfloxaflor + Maxx	1.41	d	Sulfloxaflor + Maxx	0.67	cd	Sulfloxaflor + Maxx	1.72	e
Methidathion	0.47	d	Methidathion	0.49	d	Methidathion	1.01	e
LSD	0.78			0.73			1.05	

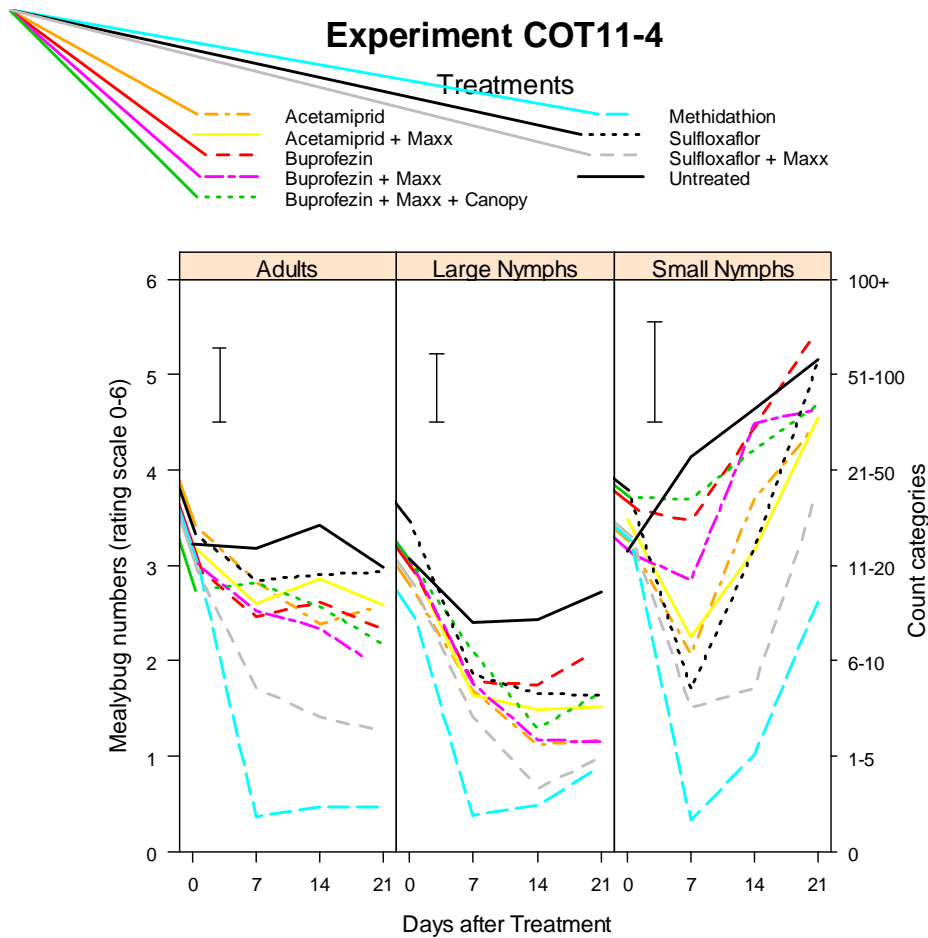


Figure 4. . The impact of insecticides, with and without additives, on the different lifestages (adults, large nymphs and small nymphs) of solenopsis mealybug, *Phenacoccus solenopsis* . LSD's represented by the bars in the top left hand corner of each graph.

Outcomes

The project has achieved the objective of developing techniques for assessing insecticide efficacy against solenopsis mealybug in controlled glasshouse conditions.

Glasshouse bioassays have been successfully conducted and enabled the evaluation of a range of insecticides for efficacy in controlling solenopsis mealybug. The results show that there is value in pursuing a range of options that are potentially less disruptive to natural enemies than the current industry standard, methidathion. The performance of Canopy is also promising, and worth further investigation. Importantly, the value of Natrasoap is questioned, but may still warrant further testing, perhaps in a sequential spray strategy.

The experience we have gained in maintaining stock colonies of mealybug, establishing infestations on plants for trials and assessment techniques have equipped both scientists and technical staff in the DEEDI Field Crops Entomology group with the skills required to conduct similar evaluations in the field.

The techniques are also now available for the evaluation of biopesticide or other candidate control options.

Conclusion

The results of this initial screening of candidate insecticides for the control of solenopsis mealybug has demonstrated that there is a range of products that show promise in terms of either control or suppression of mealybug populations.

However, we have demonstrated that Natrasoap is likely to be ineffective for the control of mealybug, particularly in a single application scenario.

There remain challenges in relation to understanding when, and against which lifestages, insecticides may best targeted to appropriately manage this pest.

Field evaluation of clothianidin, sulfloxaflor, Canopy, and acetamiprid now need to be undertaken to determine whether the levels of control that they can achieve is, in practice, sufficient to manage a mealybug outbreak. Importantly, this work must be conducted in the field where the contribution of natural enemies will be an important component of the outcome. In this testing environment the magnitude of the impact of methidathion on natural enemy populations may change the relative efficacy of the insecticide options.

Management of neonicotinoid resistance, particularly in cotton aphid, is a significant consideration in potentially introducing additional uses for this group of insecticides. Also worthy of consideration is the implication of pursuing more than one consecutive application of promising products.

Extension Opportunities

Currently, none of the insecticides evaluated for solenopsis mealybug control in cotton are registered for this purpose. Therefore, discussing the relative merit of the options is difficult.

However, the data generated from our trials will be discussed with the relevant registrant companies to determine whether there can be any progress with permit or registration. Initial discussion with Dow AgroSciences have indicated that they are prepared to include this trial data in the registration package for sulfloxaflor. There will be a requirement for field trial data in addition to the glasshouse bioassay data. OzSpray have also maintained an interest in the trial work with buprofezin. The results of these trials will be discussed with them.

Data will be made available to Dr Moazzem Khan for consideration in determining the inclusion of promising products in his trial work on solenopsis mealybug management (CRDC-funded project 2011-2014).

Results will also be discussed with key industry extension personnel, specifically Susan Maas in Emerald.

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