Final report

Glasshouse assays to determine the role of mealybug in pasture dieback.

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Abstract

Pasture dieback causes unhealthy growth and death in a range of introduced and native grasses across Queensland and into northern NSW, resulting in large losses in beef production areas. Pasture mealybug, *Heliococcus summervillei* Brookes, has been identified as the primary cause of pasture dieback. Screening of potential controls for the mealybug, and methods to conduct those screens, are required to identify insecticide control options. Dose response bioassays are also required to support applications to APVMA for minor use permits. This requires data on efficacy and residues.

Two emergency permits (for Imidacloprid and Spirotetramat (Movento, Bayer) were obtained from APVMA. Methods to conduct standardised bioassays to determine efficacy of insecticides were developed. These were used in artificially infested screenhouse trials, and 4 replicated field trials to determine efficacy of a range of products, and application rates of Movento. Residue tests were established with Eurofins and Bayer. Data will be compiled and submitted to APVMA to obtain a minor use permit for Movento.

The impact and timing of dieback from initial infestation with mealybug to death of grass were determined in laboratory and screenhouse experiments. Symptoms appear rapidly within a week of infestation, and at very low numbers of mealybugs, possibly as low as 1 bug per plant. Death of the grass was slow to occur. Grasses tolerate high numbers of mealybugs through early summer without immediate death.

The population of mealybugs increases from December through to March, a pattern also observed in the field (B.PAS 0004). The actual death of the grass, with the appearance of 'dieback' did not occur until the wetter weather in late summer, when the peak in numbers of early and medium, foliar-feeding instars coincides with wet weather. This is when the combination of mealybugs (which disrupt the plant's immune responses) and conditions favourable to fungal infection coincide, resulting in the dramatic and apparently sudden death of grass with all the symptoms of 'pasture dieback'. Mealybug numbers declined rapidly in the screenhouses through March and April as cooler, wetter conditions continued and the amount of live grass for feeding decreased.

These findings are important for both management and conduct of research trials. Firstly, there is a very short window in which trials can be conducted, between emergence of summer populations feeding on leaves and the decline in numbers from late February. Secondly, infested grasses, while symptomatic, do not 'die back' until the late summer abundance of mealybugs and wet weather combine to create the apparently sudden 'dieback' of grass. Finally, leaves with symptoms (yellow, red or purple streaking) don't recover; grass must be actively growing to recover. These effects combine to give graziers a window to monitor and then manage mealybugs and reduce 'dieback'.

Pesticides are only one part of possible dieback management. Costs, withholding periods (subject to residue testing) and impracticality of application over large areas limit their overall use. They remain useful in spot-treatment of emerging spring populations to reduce later, more severe, summer infestation. Pesticides are, however, a useful tool for research.

Monitoring for symptoms and presence of mealybugs from spring (September) is important detect early-season populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted to disrupt the mating and feeding populations in summer. Timing of management interventions to maximise efficacy needs further investigation.

Executive summary

Background

Pasture dieback causes unhealthy growth and death in a range of introduced and native grasses across Queensland and into northern NSW, resulting in large losses in beef production areas. Pasture mealybug, *Heliococcus summervillei* Brookes, has been identified as the primary cause of pasture dieback. This mealybug was previously reported to have caused severe pasture dieback in Queensland in 1926 (Summerville 1928) and the 1930s (Brooks 1978), in New Caledonia in 1998 (Brinon et al 2004), and more recently in Puerto Rico and Barbados.

Few effective management practices been determined. Definitive tests are required to determine the progress of dieback in a range of grasses, from initial infestation to death of grass, and any association with pathogens that may lead to dieback. Screening of potential controls for the mealybug, and methods to conduct those screens, are required in order to identify insecticide control options. Dose response bioassays are also required to support applications to APVMA for minor use permits or registration of products for use by growers under minor use permits. This requires data on efficacy and residues.

This project determined the progress of pasture dieback from infestation to death of the grass. It will evaluate control treatments for the mealybug and generate data to support applications to APVMA for permits and registration of control options.

This project has developed methods to screen multiple controls in glasshouse and laboratories that will inform and focus future field trials to identify effective chemical and biological control products. This will reduce the time and cost of field work and provide high quality data to support APVMA applications for registration and minor use permits.

Objectives

The project objectives, as outlined in the research agreement, are as follows:

1. Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass
2. Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback
3. Conduct efficacy assessments (primarily in glasshouses) of control treatments for mealybug, and collect data that may assist APVMA registration processes
4. Outline at least one draft journal manuscript
5. Provide to MLA periodic information summaries suitable for general media on the role and management of mealybugs in relation to addressing pasture dieback
6. Prepare a two-page summary outlining key facts and findings of mealybugs and how to identify mealybugs causing dieback

Methodology

The impact and timing of dieback from initial infestation with mealybug to death of grass were determined in laboratory and screenhouse experiments. These included small infestations monitored over time in the laboratory, and longitudinal studies with replicated weekly sampling over 10 months in screenhouses that recorded symptoms and mealybug populations structure over time.

Two emergency permits (for Imidacloprid and Spirotetramat) were obtained from APVMA. Methods to conduct standardised bioassays to determine efficacy of insecticides were developed. Field and screenhouse assessments were conducted to generate efficacy data for control treatments for mealybugs, including Spirotetramat (Movento), new chemical products (Sivanto) and microbial products. Residue tests were established with Eurofins and Bayer. Data will be compiled and submitted to APVMA to obtain a minor use permit for Movento.

All methods and outcomes will be published. A draft paper on methods and pesticide efficacy has been prepared for submission to Journal of Economic Entomology.

MLA were provided with information and summaries suitable for general media on the role and management of mealybugs in relation to addressing pasture dieback. Workshops, media outputs, farm visits, presentations at Beef Week, a webinar, panel discussions with NABRAC and other activities were used to provide information to MLA, the MLA communications team, dieback program participants and with graziers and others in livestock industries, and to communicate findings and implications for mealybug and dieback management throughout the project.

Results/key findings

Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass

Field surveys (B.PAS 0004), grass variety experiments (B.PAS 0006), and the final report of B.PAS 0505 *(Rapid diagnosis of pasture dieback using SIFT-MS)* all show a correlation between mealybug numbers and severity of dieback symptoms (purpling and yellowing of leaf and percentage of green leaf as a proportion of the whole leaf). The severity of symptoms (proportion of grass affected) is proportional to the number of mealybugs at low numbers, but at higher numbers the visible symptoms remain at around 100% of leaf affected.

Symptoms appear rapidly within a week of infestation, and at very low numbers of mealybugs, possibly as low as 1 bug per plant. Death of the grass was slow to occur. Grasses tolerate high numbers of mealybugs through early summer without immediate death, though impacts on productivity have not been assessed. This suggests that graziers have a window of opportunity in spring and early summer to monitor and detect the mealybug and symptoms of dieback and to target management at affected areas.

The population of mealybugs increases from December through to March, a pattern also observed in the field (B.PAS 0004). The actual death of the grass, with the appearance of 'dieback' did not occur until the wetter weather in late summer, when the peak in numbers of early and medium, foliar-feeding instars coincides with wet weather (in both 2021 and especially in 2022). This is when the combination of mealybugs and conditions favourable to fungal infection coincide, resulting in the dramatic and apparently sudden death of grass with all the symptoms of 'pasture dieback'.

Mealybug numbers declined rapidly in the screenhouses through late March and April as cooler, wetter conditions continued and the amount of live grass for feeding decreased. This also coincided with the rapid decline in field populations observed in field monitoring in both 2021 and 2022 (B.PAS 0004), and in insecticide trials in both 2021 and 2022.

Three findings are particularly important for management and conduct and assessment of research trials. Firstly, there is a very short window in which research trials can be conducted, between emergence of summer populations feeding on leaves (November) and the decline in these populations from the end of February, a pattern also seen in field monitoring (B.PAS 0004). Secondly, infested grasses, while symptomatic, do not appear to die back until the late summer abundance of mealybugs and wet weather combine to give the apparently sudden 'dieback' of grass following late summer rain events. Finally, leaves with symptoms (yellow, red or purple streaking) don't recover and eventually die back: grass must be actively growing to recover.

These effects combine to give graziers a window to monitor and then manage mealybugs and reduce the effects of 'dieback'. Monitoring for symptoms and presence of mealybugs from spring (roughly September) through to December can detect early populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted at these populations to disrupt the mating and feeding populations in late summer.

Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback

Laboratory and field methods were developed to conduct and quantify assays and trials of insecticides on the mealybug and symptoms of pasture dieback.

As reported in B.PAS 0004, adult mealybugs do not feed. Assays must be conducted using small and medium (feeding) instars. Adult mealybugs also disperse, females by crawling and males by flying. Infestation must be conducted using feeding instars. The methods developed were found to work consistently, by encouraging migration from infested grasses to fresh material for successful and timely infestation by the required lifestage (feeding-stage nymphs).

Sampling methods: cut grass and dug samples, assessment for field trials.

The cut grass method was found to be more practical and reliable compared to dug sampling in field trials. The sample material is lighter to transport and quicker to sample: transporting total (dug) samples was bulky, heavy and slow to sample in the field. In addition, leaf sampling, samples the feeding instars on foliage which are the ones shown to be causing 'dieback'.

This places a constraint on field trials, with a very small window for trials during the peak leaf-feeding mealybug populations (December to February) and a rapid shut down from February onwards (in this report and in B.PAS 0004).

A 10-minute search of material from each plot was found to be effective. In subsequent trials across all 3 projects (B.PAS 0003, 0004, 0006) a comparison of 10-minute count versus full destructive sampling showed that 10' counts captured a higher proportion of total mealybugs where total counts were under 100 mealybugs, but the proportion counted of the total declined at higher numbers. Our observations across a number of assays suggest that this decrease in proportion recorded by 10' counts at very high total numbers not to be important: multiple assays across all 3 projects suggest that there is little change in indicators such as symptoms at higher numbers of mealybugs. Only a few mealybugs are required to cause symptoms and induce changes in transcriptome making plants susceptible to infection. Overall the 10' counts resulted in a higher degree of statistical significance in assays than the full counts (B.PAS 0006).

There is a narrow seasonal window in which trials can be conducted in both screenhouses and in the field. In both 2021 and 2022, mealybug numbers increased rapidly from December, and declined rapidly with the cooler conditions from the end of February, and the reduction in live grass suitable for. This was anticipated, and aligned with field observations that from April onwards mealybugs migrate underground to avoid the cool conditions.

Key factors learned from both the screenhouse trials and the field trials were the impacts of season on mealybug populations and distribution on plants and on recovery of grasses, and the impacts of sampling methods and trial design on analysis.

Seasonal effects.

All field trials were conducted on commercial properties and were affected by hot and dry conditions in early summer (up to February 2021) and wet conditions in 2022. Systemic products like Movento will not be taken up effectively if applied to dry, brown leaf) and the hot, dry weather prior to the start prevented the growth of green leaf.

It is important for assessment of trials. Leaves with symptoms (yellow, red or purple streaking) don't recover following insecticide treatment. Grasses must be actively growing to show a response to treatment. In all trials to date, glasshouse or field, the leaves that developed symptoms before treatments were applied did not recover i.e return to green. Instead, it is the new growth that increases the overall green leaf on the grass.

Screenhouse versus field assessment.

Ultimately all products must be tested in the field. However, there are significant restrictions on field trials. Similarly, screenhouse trials in pot plants are irrigated and well replicated but are still affected by cold conditions and there is again a narrow window for start of tests, approximately November to February.

Trials typically require 9 weeks from first application to final sample. These seasonal factors suggest that there is a very narrow window in which the different effects of treatments – number of mealybugs, or recovery of grass – can be successfully conducted. The optimum time appears to be a window immediately following rain when there is sufficient fresh leaf to uptake insecticides, and the cold and dry conditions from April onwards.

Data from the field trials were considerably more dispersed (over-dispersed) than the glasshouse tests, which increases the difficulty in determining significance. Data from these two trials wil be used to conduct a full power analysis to determine the degree of replication required in future trials conducted under similar conditions.

Full destructive sampling in pot plant assays is very informative, resulting in detailed data on abundance and distribution of mealybugs. The field sampling is more problematic: it must assess populations with different distributions, including soil and thatch and in a timely way that can be practically conducted in a field trial. It is our conclusion that cut leaf sampling is a more effective method of sampling mealybugs in insecticide trials, since it samples the instars most affected (those feeding on leaf). This in turn narrows the window in which trials can be conducted (see above).

Information on trials and methods will be published and provided to program partners.

Conduct efficacy assessments (primarily in glasshouses) of control treatments for mealybug, and collect data that may assist APVMA registration processes

Replicated glasshouse and field trials with the systemic insecticides Imidacloprid (‘Confidor’) and Spirotetramat (‘Movento’) have demonstrated control of the mealybug, and that treated grasses recover from dieback symptoms compared to untreated plants.

Movento is effective in reducing mealybug density even when plants are in close proximity to untreated plants (in screenhouse and small-plot trials) and where mealybugs are rapidly increasing in numbers.

Secondly, trials in both screenhouse and field resulted in no significant difference between the high rate of Movento (400ml/ha) which is specified in the APVMA permit and the middle rate (approximately equivalent to 200ml/ha). This suggests that there may be scope to reduce the rate of Movento required.

Residue tests have been conducted and sample analysis is being completed. Together, these results suggest a pathway to reductions in costs withholding periods and will contribute to the conversion of the emergency permit to a minor use permit.

Pesticides are only one part of the dieback response. Costs, withholding periods (subject to residue testing) and impracticality of application over large areas limit their overall use. They remain useful in spot-treatment of emerging spring populations to reduce later, more severe, summer infestation. Pesticides are, however, a useful tool for research.

Timing of management needs investigation along with other management interventions.

Conclusions

This research has identified a window to monitor and then manage mealybugs and reduce the effects of ‘dieback’. Infested grasses, while symptomatic, do not appear to die back until the late summer abundance of mealybugs and wet weather combine to give the apparently sudden ‘dieback’ of grass following late summer rain events.

Two emergency permits for insecticides were obtained from APVMA. Insecticides were found to be effective against mealybug populations at lower rates than those in the current permit, and grasses were shown to recover. Residue tests have been conducted and sample analysis is being completed. Together, these results suggest a pathway to reductions in costs, withholding periods, and will contribute to the conversion of the emergency permit to a minor use permit.

Pesticides are only one part of the dieback response. Costs, withholding periods (subject to residue testing) and impracticality of application over large areas limit their overall use. They remain useful in spot-treatment of emerging spring populations to reduce later, more severe, summer infestation. Pesticides are, however, a useful tool for research.

Monitoring for symptoms and presence of mealybugs from spring (roughly September) through to December can detect early populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted at these populations to disrupt the mating and feeding populations in late summer. Timing of management needs investigation along with other management interventions.

Future research and recommendations

Monitoring for symptoms and presence of mealybugs from spring (roughly September) through to December can detect early populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted at these populations to disrupt the mating and feeding populations in late summer.

Detailed work on the timing of management interventions with population biology in the field are required.

1. Background

Pasture dieback causes unhealthy growth and death in a range of introduced and native grasses across Queensland and into northern NSW, resulting in large losses in beef production areas. Pasture mealybug, *Heliococcus summervillei* Brookes, is the primary cause of pasture dieback. This mealybug was previously reported to have caused severe pasture dieback in Queensland in 1926 (Summerville 1928) and the 1930s (Brooks 1978), in New Caledonia in 1998 (Brinon et al 2004), and more recently in Puerto Rico and Barbados.

Few effective management practices have been developed or tested. Definitive tests are required to determine the progress of dieback in a range of grasses, from initial infestation to death of grass, and any association with pathogens that may lead to dieback. Screening of potential controls for the mealybug are required to identify insecticide control options. Dose response bioassays are also required to support applications to APVMA for minor use permits or registration of products for use by growers under minor use permits. This requires data on efficacy and residues.

This project will deliver knowledge to determine the progress of pasture dieback from infestation to death of the grass. It will evaluate control treatments for the mealybug and generate data to support applications to APVMA for permits and registration of control options.

This project initially obtained emergency use permits for two insecticides, Imidacloprid and Spirotetramat (Movento, Bayer). It then developed protocols and use them to screen multiple controls in screenhouses and field trials. The methods and results will inform and focus future field trials to identify effective chemical and biological products, and to provide high quality data in support of applications to APVMA minor use permits.

Definitive tests are required to determine the progress of dieback in a range of grasses, from initial infestation to death of grass, and any association with pathogens that may lead to dieback. Screening of potential controls for the mealybug are required to identify insecticide control options. Dose response bioassays are also required to support applications to APVMA for minor use permits or registration of products for use by growers.

2. Objectives

In this project we aimed to:

1. Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass
2. Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback.
3. Conduct efficacy assessments (primarily in glasshouses) of control treatments for mealybug, and collect data that may assist APVMA registration processes
4. Outline at least one draft journal manuscript
5. Provide to MLA periodic information summaries suitable for general media on the role and management of mealybugs in relation to addressing pasture dieback
6. Prepare a two-page summary outlining key facts and findings of mealybugs and how to identify mealybugs causing dieback

3. Methodology

3.1 Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass.

3.1.1 Establish and manage a culture.

A culture of the mealybug, *Heliococcus summervillei*, was established. The mealybug is highly seasonal, and rearing was split into a small colony maintained through the year in the laboratory in temperature-controlled cabinets, and larger seasonal populations in the screenhouse.

3.1.1.1 Laboratory culture

American buffel seed (PGGW Seeds Ltd) are germinated by soaking for 12 hours before sowing. Seeds are sown sparingly into potting mix in standard 80mm square horticulture pots and grown in a temperature-controlled plant growth cabinet at 26°C for 4 weeks. Plants may be cut back, fertilised with a high nitrogen lawn food and grown-on if required.

Mealybugs are reared in bug dorms (Australian Entomological Supplies) inside a temperature-controlled plant growth cabinet at 26°C and 12hour light / 12 hour dark cycle. Fresh buffel plants are introduced weekly in close contact with older, infested plants and mealybugs allowed to move to the fresh material and older (dead) plants removed. Plants are watered sparingly twice a week (to avoid waterlogging).
Mealybugs are collected for experimental work by cutting leaves with feeding nymphs or removing infested pots, depending on the experiment.

3.1.1.2 Screenhouse culture

This technique is suitable for infestation of larger screenhouse trials. It is only for use September to April in SE Qld conditions. It cannot be used in colder months when grasses hay off and mealybugs disperse.
American buffel as standard, or other varieties as required, are germinated and grown in 4 litre square horticulture pots, in potting mix in glasshouses as above. At 6 to 8 weeks, grasses are transferred to insect screenhouses on horticultural benches and drip irrigation installed in all pots. Drip irrigation is turned off during large rain events to reduce waterlogging.

Pots are infested by placing infested plants between the fresh material such that the leaves are in contact, and mealybugs are allowed to move across. Old plant material is removed after one or two weeks.

3.1.2 Lab assays/time lapse

Female mealybugs had been shown to feed little or not at all (B.PAS 0004). Infestation was therefore initially conducted using neonate mealybugs up to 5 days old.

In an initial test, single plants were infested with 3 densities (5, 25 and 40) neonates per plant and reared in bugdorms in a temperature-controlled cabinet as above. Development of symptoms was observed to the death of grass.

A second experiment was conducted by infesting 5 plants with 5, 25 and 50 neonate mealybugs (up to 5 days old) per plant, and 5 uninfested controls. Time to death, percentage leaf showing symptoms and photography were used to monitor development and severity of symptoms up to complete death of infested grass plants (45 days), at which point the leaf and stem material was cut, dried and weighed.

In a final demonstration, 5 plants were each infested with a single female producing young within a single mesh and plastic cage (bug dorm) in a controlled temperature room with artificial plant growth lighting. A camera fitted with time lapse capability was used to photograph plants twice per day before and up to 45 days after infestation. A time lapse video and GIF were assembled.

3.1.3 Screenhouse longitudinal studies

Season-long and detailed experiments were established.

American buffel grass grown in 4 litre pots as above and infested with mealybugs at QUT SERF as above in a replicated design with 6 replicates in May 2021 and sampled weekly until December 2021. A second study was established by infestation in November 2021 and observations commenced as above in January 2022 through to May 2022.

Plants were assessed weekly. One plant from each replicate was sampled weekly. Symptoms of dieback were recorded by photography and by direct observation and used for visual assessment of percentage leaf area green, yellow or purple, and dead. Plants were then bagged and transported to the laboratory for destructive sampling. The number, instar, and distribution of mealybugs (on plant and plant pot), and the number of male pupae on each plant were recorded.

3.2 Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback.

Testing of controls requires standardised protocols including standardised age of test plants, insect populations, time of treatment application, monitoring intervals and sampling procedures, and recording of impact of treatments on mealybug populations and dieback symptoms.

Replicated trials to determine efficacy of a range of products were conducted in screenhouses and in the field. These included dose rate trials of Movento (Spirotetramat) and comparison of multiple products at single rates. The methods described below were critically reviewed and the most effective and identified as the protocols for use.

3.3 Conduct efficacy assessments of control treatments for mealybug, and collect data that may assist APVMA registration processes

Three field trials and two screenhouse trials were conducted to determine rates and efficacy in controlling the mealybug primarily using the two actives for which we have emergency permits in place: Movento (Spirotetramat) and Imidacloprid.

Four other products (Mycoforce, Nutritech Pty Ltd: Entofix, Novum Life Sciences; and Endofight '5 in 1', Novum Life Sciences) and a new, unregistered systemic insecticide (Sivento, from Bayer) were included in field trials and in a second screenhouse trial reported below.

3.3.1 Glasshouse assays:

American buffel (trial 1) or Rhodes grass (trial 2) was sown into 4 litre square horticultural pots initially in standard potting mix, and then in potting mix with added perlite to improve drainage, at the DAF Qld Redlands facility and transferred to insect screenhouses at the QUT Samford Environmental research facility (SERF). Plants were irrigated with a drip irrigation system on a timer and stood on free draining tables (to avoid waterlogging).

Plants were infested with mealybugs by placing infested plants between the uninfested grasses at a ratio of 4 infested plants to 18 test plants. In the first trial (December to January 2020/2021) plants were left for 4 weeks to ensure infestation. In the second trial (April – June 2021) infested plants were left for 2 weeks. In both cases presence of mealybugs on the test plants and development of symptoms was confirmed before spray application.

Table 1: Rates of products used in screenhouse tests of Movento and Sivanto (Bayer).

Treatment	Volume (ml) trial 1	equivalent ml per hectare	Volume (ml) trial 2	equivalent ml per hectare
Control	50ml		50ml	
Hasten	0.5	1%	0.5	1%
Movento	0.06	400	0.08	400
Movento	0.02	133	0.04	200
Movento	0.01	67	0.02	100
Sivanto			0.08	400

Treatments (Table 1) were applied twice at 3-week intervals using a knapsack sprayer. Each treatment was replicated 6 times with 3 plants in each replicate (one for each timepoint sampled). Before the first and second applications, one plant from each treatment by replicate was assessed for proportion of leaf affected by symptoms or green, bagged and returned to the laboratory for full destructive sampling to quantify mealybugs. The final sample was assessed in the same way 3 weeks after the second application. The total trial time is thus 9 weeks.

3.3.2 Field trials

Four trials were conducted, initially to test two concepts:

- That systemic insecticides would control the mealybugs *H. summervillei*
- That reducing the mealybug population would result in a reduction in dieback symptoms

Subsequent trials were conducted to develop quantitative methods, to test efficacy of a range of products against mealybugs, and to compare rates of Movento towards support of a minor use permit for that product.

3.3.2.1 Preliminary trial: proof of concept

A preliminary trial was conducted at Birkdale, Queensland to demonstrate the potential impact of systemic insecticides on *Heliococcus summervillei* and infested pasture grasses.

Imidacloprid and Spirotetramat were applied at label rates with the spray adjuvant 'Hasten' (BASF) and an unsprayed control. Two varieties of *Urocholoa* were treated in a fully replicated design with 4 replicates each containing every treatment in each variety (4 replicate blocks per variety, 8 blocks in total).

Plants were checked prior to the first application to confirm mealybug presence and location (i.e. soil, foliage). Grass samples were cut from the test plots immediately prior to the first and second applications application and at 3 weeks following the final application. Three samples of leaf and stem per plot were cut at the base of the plants, coiled into a ziplock bag (1 bag per plot) and returned to the laboratory. In the laboratory, a 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number and size recorded.

3.3.2.2 Movento dose range and product tests

Field trials were conducted in 2021/22 on two commercial beef production properties (Banana and Biggenden) using two sampling methods (dug samples and leaf sampling) to quantify mealybug numbers and treatment impacts of a range of doses of Movento (Spirotetramat) and a selection of potential products (Table 2). Both trials were affected by hot and dry seasonal conditions which delayed the start date until later in the summer. Both trials were conducted using four replicates of every treatment in a randomised block design.

In 2022 a final trial was conducted on rates of Movento at Crow's Nest (Toowoomba region).

Table 2: products, rates and volume per hectare applied in 3 field trials

Treatment	Banana rates per hectare	Biggenden rates per hectare	Crows Nest rates per hectare
Control (water)	Untreated	200L	250/L
Hasten	1%	200ml/200L	
Designer		500ml/400L	
Movento	400 ml/200L	400ml/200L	400ml/250L
Movento	200 ml/200L		200ml/250L
Movento	100 ml/200L		100ml/250L
Sivanto		400ml / 200L	
Mycoforce	1kg / 200L	1kg / 400 L	
Entofix		500ml / 400L	
Endofight		500ml / 400L	

Field trial 2: Dose rate trial, Movento (Spirotetramat), Banana field station, 25th March 2021, in buffel grass.

Aim: to determine dose rates based on the emergency permit 88482 for Movento with recommended spray adjuvant 'Hasten', and comparison with a microbial product (Mycoforce) that has been reportedly used by graziers.

All treatments were applied using a small tractor spray rig by NVD Consulting in a randomised block design with 4 replicates.

Plants were checked prior to the first application to confirm mealybug presence and location (i.e. soil, foliage). Grass samples were cut from the test plots immediately prior to the first and second applications application and at 3 weeks following the final application. Three samples of leaf and stem per plot were cut at the base of the plants, coiled into a ziplock bag (1 bag per plot) and returned to the laboratory. In the laboratory, a 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number and size recorded.

Field trial 3: product trial, Biggenden, start 26th March 2021, in Bisset bluegrass

Aim: to compare efficacy of a range of products at a single (label) rate against mealybugs.

Two chemical insecticides provided by Bayer Pty Ltd, Movento (Spirotetramat) and a new product pending registration ('Sivanto') were tested at a single rate equivalent to 400ml/ha with the recommended adjuvant 'Hasten' (see emergency permit 88482).

Three microbial insecticides were tested at the manufacturers' recommended rates: Mycoforce (Nutritech Pty Ltd), Entofix (Novum Life Sciences) and Endofight 5 in 1 (Novum Life Sciences)), together with the recommended wetting agent 'Designer'.

All treatments were applied with a knapsack sprayer in a stratified randomised block layout with 4 replicates x 8 treatments per block.

Plants were initially checked to confirm mealybug presence and distribution on plants (i.e. soil, foliage). All plots were assessed before both applications and 3 weeks after the final application for grass cover and symptoms (percentage green, symptomatic or dead) using a standard assessment sheet that has been provided to MLA and all project participants.

Soil and grass samples were then collected immediately prior to the first and second applications application and at 3 weeks following the final application. Samples were collected by digging a square 30cm^2 and approx. 10cm deep from all plots using a post hole shovel. Samples were immediately bagged and returned to the laboratory.

In the laboratory, a 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number and size recorded.

Data was analysed in R studio using general linear modelling and Poisson errors: Glm (formula = MB ~ Timepoint * Movento, family = poisson, data = varieties).

Field trial 4: Movento Rates trial, Crows Nest (Toowoomba region) 1st February- 14th March 2022 in Bluegrass

Aim: to determine dose rates based on the emergency permit 88482 for Movento with anionic surfactant Hasten

All treatments were applied with a knapsack sprayer in a stratified randomised block layout with 4 replicates

Plants were checked prior to the first application to confirm mealybug presence and location (i.e. soil, foliage). Grass samples were cut from the test plots immediately prior to the first and second applications application and at 2 weeks following the final application. Three samples of leaf and stem per plot were cut at the base of the plants, coiled into a ziplock bag (1 bag per plot) and returned to the laboratory. In the laboratory, a 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number and size recorded.

3.3. Progress on preparation of residue trials data package for APVMA renewal of Emergency Permit for Spirotetramat.

QUT contracted Eurofins to conduct residue analysis in partnership with Bayer to support conversion of the emergency permit for Movento to a minor use permit. Trials were applied at 3 rates at 2 sites at Calliope and Biggenden. Analysis of residues is ongoing and to be completed.

QUT are working with Bayer to present a package to APVMA including trial data and residue testing towards conversion of the emergency permit to a minor use permit for Movento.

3.4 Outline at least one draft journal manuscript

A publication on the impacts of insecticides on mealybug and grass recovery is drafted. As second paper on the seasonal biology in screenhouses and impact on grasses will be prepared shortly.

3.5 Provide to MLA periodic information summaries suitable for general media on the role and management of mealybugs in relation to addressing pasture dieback

Communications were provided frequently to MLA program leaders and comms team through reports, emails, a webinar, on-line participant meetings, material for specific media outputs, and a national science panel review in April 2022. A summary has been provided separately for all 3 QUT projects.

3.6 Prepare a two-page summary outlining key facts and findings of mealybugs and how to identify mealybugs causing dieback

A summary of key findings is provided in results. An article on QUT research and management strategies for graziers in in preparation with MLA comms team.

4. Results

4.1 Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass

4.1.1 Establish and manage a culture

A culture was successfully established and methods (as described above) demonstrated to manage cultures seasonal variation by combining laboratory rearing with larger seasonal colonies in screen houses through spring/summer.

4.1.2 Lab assays/time lapse

Tests showed that mealybugs alone are sufficient to cause symptoms of dieback and death of grass in approx. 40 days from infestation. Laboratory infestation of American buffel grass with the mealybug resulted in rapid development of symptoms of dieback (5 days) and at low numbers of mealybug (5 or less). Time lapse compilation was shared with MLA and program participants in the science review in 2022.

Measure of dry weight showed there was a decrease in dry weight of grass between in infested grass relative to uninfested control, but no significant differences between treatments. The highly significant difference between uninfested controls and infested plants was that all plants infested with mealybugs at all 3 densities developed symptoms consistent with dieback (yellow and red streaking of leaves) and all died, but all the control plants survived without symptoms. All infested plants showed rapid development of symptoms and death of grass by 45 days post infestation.

The results appear to suggest that even very low numbers of neonate mealybugs can kill buffel grass by 45 days post infestation under laboratory conditions, even with good plant nutrition.

4.1.3 Screenhouse longitudinal studies

Longitudinal observation of mealybug infested plants in screen houses have shown that symptoms of dieback – yellowing and purple or red streaks on leaves – appear very quickly after infestation, typically within a week (fig 1).

Figure 1: Mealybug-induced dieback symptoms on buffel grass in screenhouses, Samford Environmental Research Facility.

The numbers of all instars and dieback symptoms decreased into winter (May to June 2021) (Fig. 2). Numbers were steady through to August, when there was a short increase in the number of small instars, a few male pupae, and a single adult male was observed.

Numbers of small instars remained low, and the population was mostly adult females until early September when a sharp increase in numbers of early instars began that marked the start of the 2021/22 season. Symptoms of dieback increased from mid-September into October as populations of feeding stage nymphs increased.

In early October the first adult males were observed, and the numbers of small, medium, and adult female instars all increased. The proportion of grass canopy affected by symptoms stayed relatively constant as the grasses also grew in the warm conditions.

From December the numbers of all instars increased dramatically, peaking in mid-February (Fig. 2). At its peak there were around 2,500 mealybugs per plant, a value of 8 on the natural logarithmic scale used in figure 2.

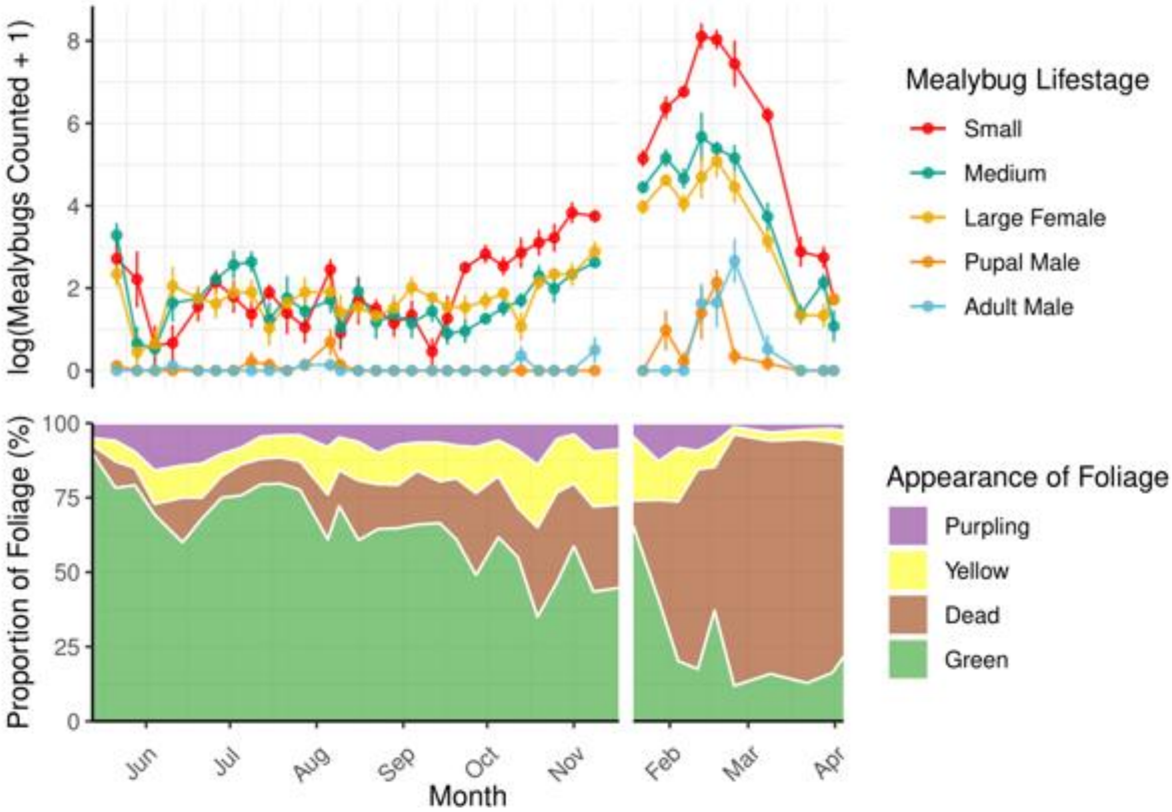

Figure 2: Weekly monitoring of longitudinal observation of mealybugs and symptoms of dieback in screenhouses showing rapid development of symptoms after infestation and subsequent seasonal variation. Numbers of small and medium nymphs and dieback symptoms decrease through winter. Symptoms of dieback increase from mid-September as populations of feeding stage nymphs increase. Death of grass in summer coincides with the rapid summer increase in foliar-feeding instars and warm, wet weather favourable to fungal infections.

The proportion of grass with symptoms diminishes into February, but the proportion of dead grass increases dramatically into February and March, with the grass showing all the signs of 'pasture dieback' observed in the field.

Conditions in Brisbane from December to March were very wet, with a major rain event at the end of February. From early March the number of all instars declines, but the proportion of dead grass remains high through to the end of April 2022. By April the population of mealybugs appeared to enter the winter phase, as cool and wet conditions continued into winter.

Actual death of the grass, with the appearance of 'dieback' did not occur until the heavy rains of February and April 2022, when high numbers of late summer mealybugs coincide with rain. We believe that this infestation with a heavy load of feeding early and medium instars combines with infection of the plant by fungi in the wet conditions, resulting in death of the grass with the characteristic grey, ashy appearance seen in 'pasture dieback'.

Symptoms appear rapidly after infestation, within a week, but death of the grass was slow to occur. This suggests that graziers have a window of opportunity in spring and early summer to monitor and detect the mealybug and symptoms of dieback and to target management at affected areas.

Mealybug numbers declined rapidly in the screenhouses through late March and April as cooler, wetter conditions continued and the amount of live grass for feeding decreased. This also coincided with the rapid decline in field populations (B.PAS 0004) and the decline in mealybug numbers in the field trials observed in 2021 and 2022.

Two observations are important for conduct and assessment of research trials. Firstly, there is a very short window in which research trials can be conducted, between emergence of summer populations (November) and the decline in numbers from the end of February, a pattern also seen in field monitoring (B.PAS 0004). Secondly, leaves with symptoms (yellow, red or purple streaking) don't recover and eventually die back. Management needs to target early populations before the 'dieback' of grass in late summer.

4.2 Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback.

Infestation

Methods to conduct screenhouse evaluation of pasture varieties with efficient sampling methods were developed. As reported in B.PAS 0004, adult mealybugs do not feed. Assays must be conducted using small and medium (feeding) instars. Adult mealybugs also disperse, females by crawling and males by flying. Infestation must be conducted using feeding instars. The methods described in section 3 above were found to work consistently, by encouraging migration from infested grasses to fresh material.

Sampling methods: cut grass and dug samples, assessment.

In the Birkdale, Banana and Crows Nest field spray trials, grass samples were cut from the test plots prior to application and at 2-week intervals after application: 3 samples per plot of leaf and stem were cut at the base of the plants and coiled into a bag and returned to the laboratory. In the Biggenden trial the population was found to be mostly large instars and the trial was sampled by digging up soil, thatch and grass material and returning that to the laboratory for assessment.

The cut grass method was found to be more practical and reliable. It is lighter to transport and quicker to sample: transporting total (dug) samples was bulky, heavy and slow to sample in the field. In addition, leaf sampling samples the feeding instars on foliage which are the ones shown to be causing 'dieback'.

This places a constraint on field trials, with a very small window for trials during the peak leaf-feeding mealybug populations (December to February) and a rapid shut down from February onwards (in this report and in B.PAS 0004).

A 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number recorded. This demonstrated that 10-minute searches can find 90% of all mealybugs in 10 minutes and will find 100% of the mealybugs 75% of the time.
In subsequent trials across all 3 projects (B.PAS 0003, 0004, 0006) a comparison of 10 minute count versus full destructive sampling showed that 10' counts captured a higher proportion of total mealybugs where total counts were under 100 mealybugs, but the proportion counted of the total declined at higher numbers.

Our observations across a number of assays suggest that this decrease in proportion recorded by 10' counts at very high total numbers not to be important. Overall, the 10' counts resulted in a higher degree of statistical significance in assays than the full counts (B.PAS 0006).

Furthermore, multiple assays across all 3 projects suggest that there is little change in indicators such as symptoms at higher numbers of mealybugs. Only a few mealybugs are required to cause symptoms and induce changes in transcriptome making plants susceptible to infection.

There is a narrow seasonal window in which trials can be conducted in screenhouses and in the field. In both 2021 and 2022, mealybug numbers increased rapidly from December, and declined rapidly with the cooler conditions from the end of February, and the reduction in live grass suitable for sampling. This was anticipated and aligned with field observations that from April onwards mealybugs migrate underground to avoid the cool conditions.

Methods will be included in publications from the project.

4.3 Conduct efficacy assessments (primarily in glasshouses) of control treatments for mealybug, and collect data that may assist APVMA registration processes

4.3.1 Screenhouse assays

Trial 1:

Movento (Spirotetramat) with anionic surfactant Hasten resulted in a highly significant ($P=<2 \times 10^{16}$) reduction in mealybug numbers compared to both controls (water and Hasten) (Fig 3, Table 3).

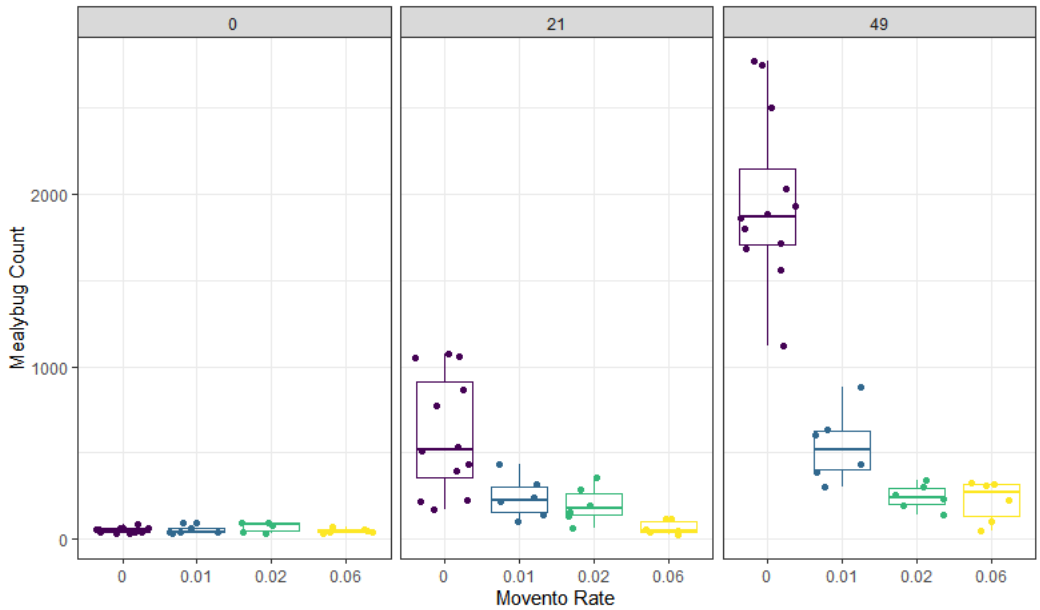

Figure 3: Change in mean number of mealybugs per treatment over 3 time points (0, 21 and 49 days after first application) between December 2020 and January 2021 in screenhouse pot plant assay of 3 rates of Movento (0.01ml, 0.02ml and 0.06 ml).

Table 3: significance values from the statistical model.

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	4.88E+00	1.58E-02	309.39	<2e-16	***
Timepoint	5.30E-02	3.64E-04	145.52	<2e-16	***
Movento	-2.84E-03	1.35E-04	-21	<2e-16	***
Timepoint:Movento	-1.22E-04	3.61E-06	-33.63	<2e-16	***

The impact on mealybugs was greatest at the two higher rates of Movento, approximately equivalent to 400ml/ha, the rate in the APVMA emergency permit, and 133ml/ha. The effect of these two rates was not significantly different. The lowest rate, approx. equivalent to 65 ml/ha was less effective than the top two rates, but still significantly reduced mealybug numbers compared to the control. The effect of higher rates becomes more pronounced over time. All p = <2e-16.

Trial 2: Movento rates test on Rhodes grass, April-June 2021

Movento (Spirotetramat) with anionic surfactant Hasten resulted in a highly significant ($P=<2 \times 10^{16}$) reduction in mealybug numbers compared to both of the controls (water and Hasten) after both the first and second applications (Fig. 4, Tables 4). Impacts on mean mealybug numbers using Sivanto were less significant after the first application but improved after the second application.

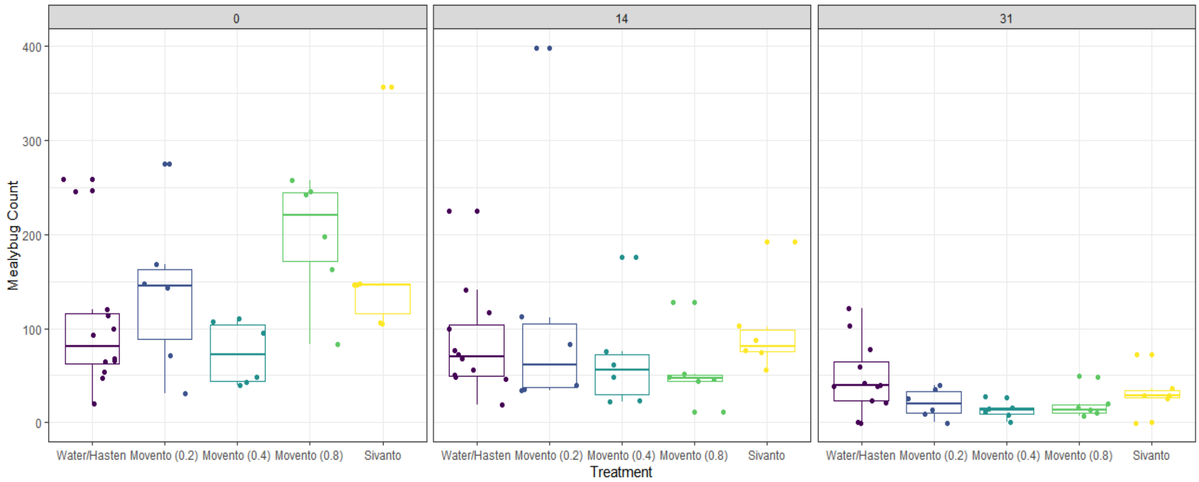

Figure 4: Mean number of mealybugs per treatment (3 rates of Movento and one of Sivanto) in screenhouse pot plant assays prior to treatment (0 days), and at 14 days after 1st and 31 days after 2nd applications.

Table 4: Significance values from the statistical model 3 rates of Movento, Sivanto, and the non-ionic surfactant Hasten relative to water (control) in screenhouse pot plant assays 2 weeks after 2nd application (4 weeks after 1st application).

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	4.281285	0.048002	89.191	< 2e-16	***
Movento	-0.00425	0.000282	-15.079	< 2e-16	***
Hasten	-0.05713	0.06449	-0.886	3.76E-01	
Sivanto	-0.48648	0.076309	-6.375	1.83E-10	***

Effects of treatment with Movento increased 2 weeks after a second application (4 weeks from first treatment) compared to 2 weeks after 1st treatment (Fig 5). The treatment with Sivanto was not as effective in reducing mealybug numbers, with a mean mealybug count of 42 compared to the three means for Movento (19, 22 and 33 mealybugs) and the highest, medium and lowest rates (Figs 5). Hasten alone again resulted in an increase in mealybug numbers compared to water only, possibly due to its effects on the leaf lamina, which increases trans-laminar uptake of the active but can increase susceptibility to some pathogens and pests.

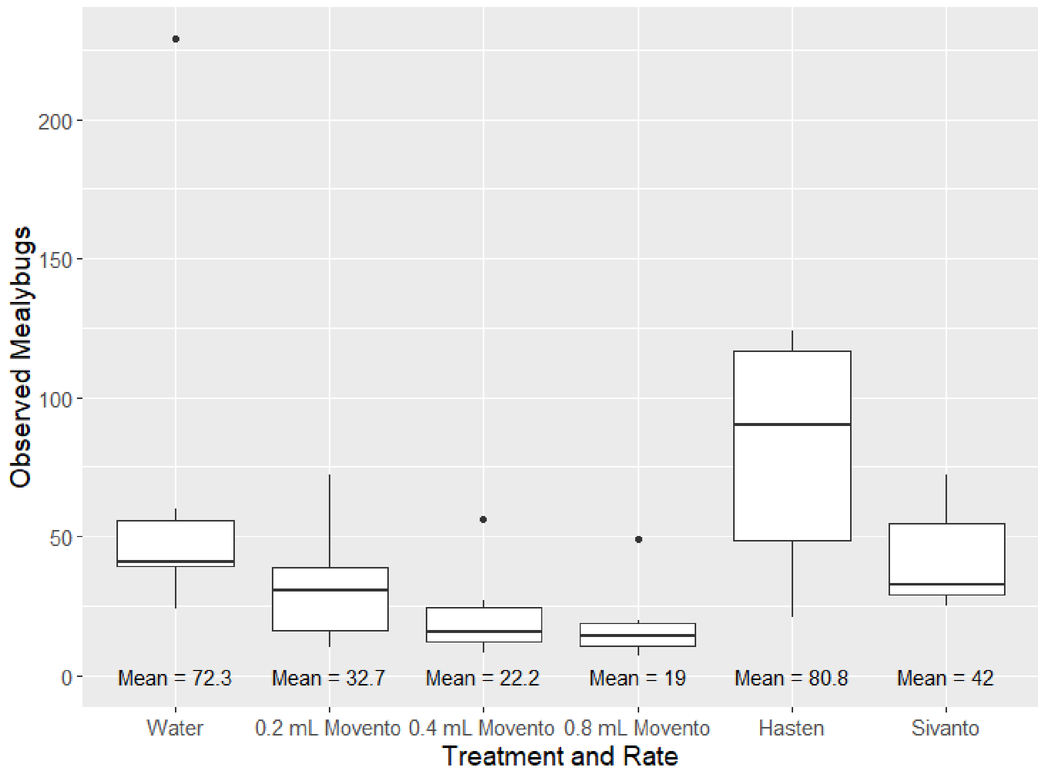

Figure 5: Mean number of mealybugs per treatment (including 3 rates of Movento) in screenhouse pot plant assays 2 weeks after 2nd application.

4.3.2 Field trials

4.3.2.1 Preliminary trial: proof of concept

There was a significant decrease in mealybug numbers in both insecticide treatments (Imidacloprid and Spirotetramat) but a significant increase in numbers in the control and carrier-only plots (Fig 6). Plants in all insecticide treatments recovered during the period of the trial but unsprayed controls developed severe symptoms of dieback and were close to death by 100 days.

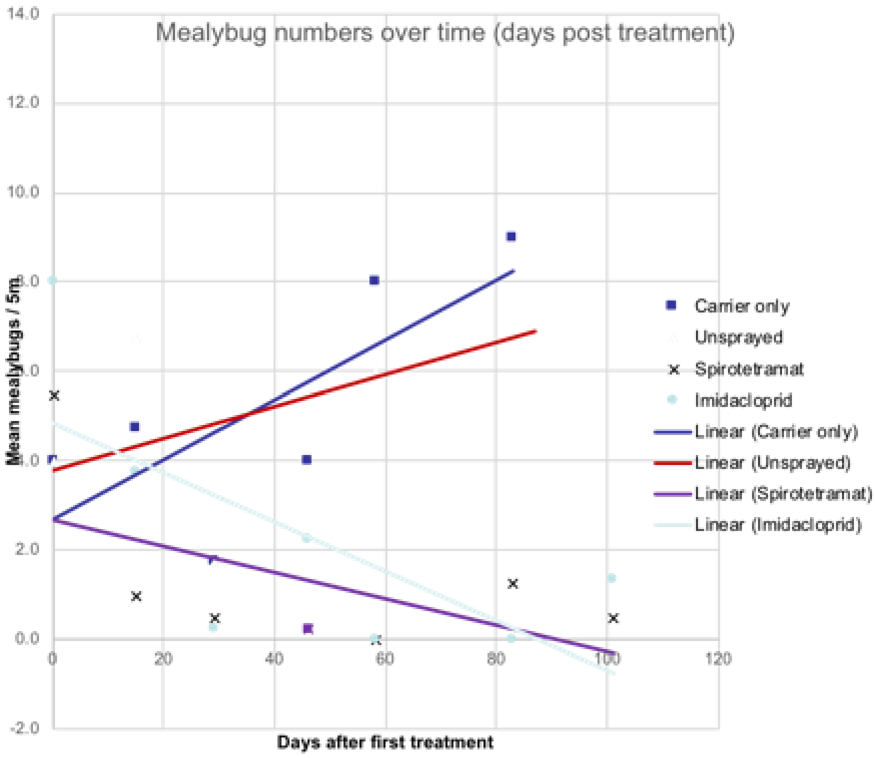

Figure 6: Results of the initial trial of two systemic insecticides, Imidacloprid and Spirotetramat, at label rates against the mealybug *H. summervillei* in *Urochloa*.

The effect of insecticides on foliage was pronounced. Grass (*Urochloa*) in insecticide treated plots recovered much of the green foliage within 100 days of first treatment. In contrast, grass in untreated plots was close to death after 100 days (Fig 7).

Leaves that were already damaged or showing symptoms prior to treatment did not recover. Instead, the grass plant grew new leaves. Furthermore, symptoms began to reappear in new foliage as the mealybugs reinvaded the plots at low numbers as residue levels decreased after treatment.

Interestingly, there was an apparent difference in response on foliage in the two varieties. Impacts on foliage were less clear on CPI 47122 than on CPI 60123, where untreated patches died back. It is possible that CPI 47122 is a little more resistant to the mealybug.

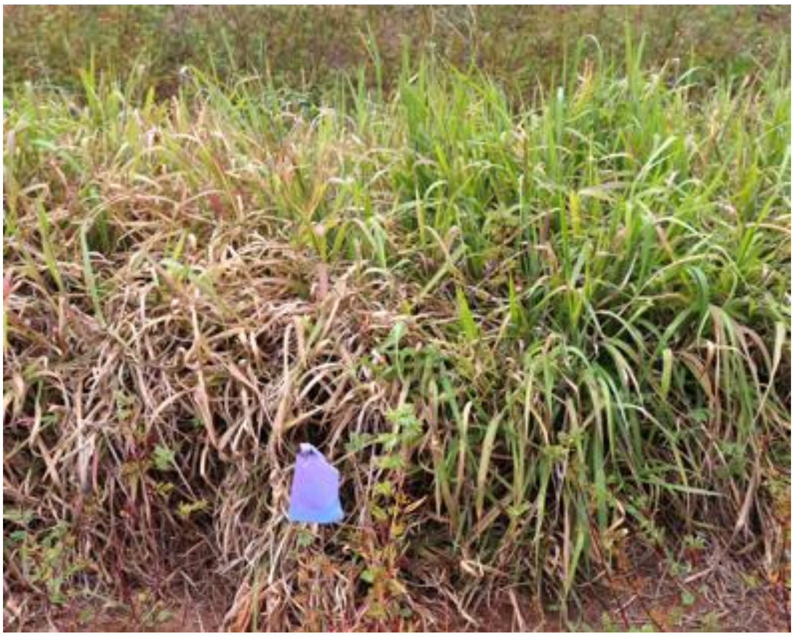

Figure 7: Effect of insecticide treatment on recovery of *Urochloa* following infestation by the mealybug *H. summervillei*.
Left of purple marker: Untreated
Right: Treated with insecticide.

4.3.2.2 Banana and Biggenden

Both trials were conducted from late March to mid-May 2021. Both field trials were affected by hot and dry seasonal conditions through summer which delayed the start date until after rains later in the summer when grass flushed to produce leaf suitable for application of insecticides. The trials take 8 to 9 weeks, and thus completion was in mid-May, after what we now know to be the peak breeding season of the mealybugs. A cold snap and dry conditions in May 2021 had a significant impact on the third and final sample data: mealybugs move off leaf and into the crown and thatch in cold and dry conditions. These seasonal impacts reduced the number of mealybugs in the final samples.

Both trials were conducted using four replicates of every treatment in a randomised block design and using different sampling methods. The impact of the effect of season, trial design and sampling on the conduct of trials were important lessons for future field trials with mealybugs and dieback and are discussed below.

Trial 1: Banana

This trial was conducted following rains in March 2021 and completed mid-May 2021. A cold snap and dry conditions in May 2021 had a significant impact on the third and final sample data: mealybugs move off leaf and into the crown and thatch in cold and dry conditions. This has a significant impact on data from the final sample.

Results from the second sample (Table 5) show a significant effect of Movento on mealybug numbers but not from Hasten alone or Mycoforce.

Table 5: significance values from the statistical model.

Treatment	Cooeficient	Std error	Pr	Significance
Intercept	2.66	0.13	<2 x10^{16}	***
Movento	-0.01	0.001	2.5 x10^{16}	***
Hasten	0.36	0.17	0.03	*
Mycoforce	0.10	0.18	0.58	Ns

The correlation between Movento rate and number of mealybugs was weak, with no significant difference between rates (Fig 8).

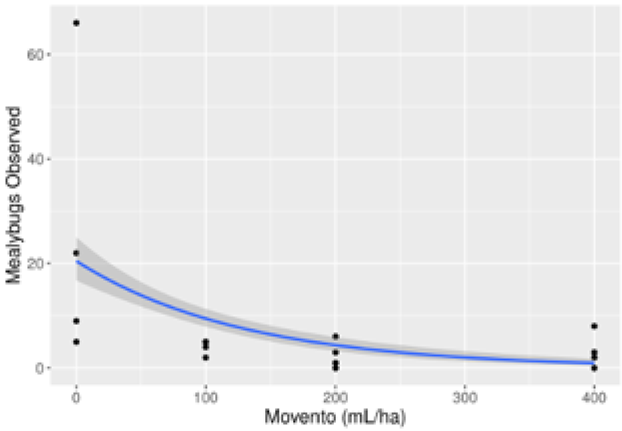

Figure 8: plot of dose rate of movento against mealybug number, Banana 2021.

Field Trial 2: Biggenden

This trial was conducted following rains in March 2021 and completed mid-May 2021. A cold snap and dry conditions in May 2021 had a significant impact on the third and final sample data, when mealybugs move off leaf and into the crown and thatch in cold and dry conditions, but sampling was less affected than at Banana by the use of dug samples that also collect mealybugs in thatch (Fig 9).

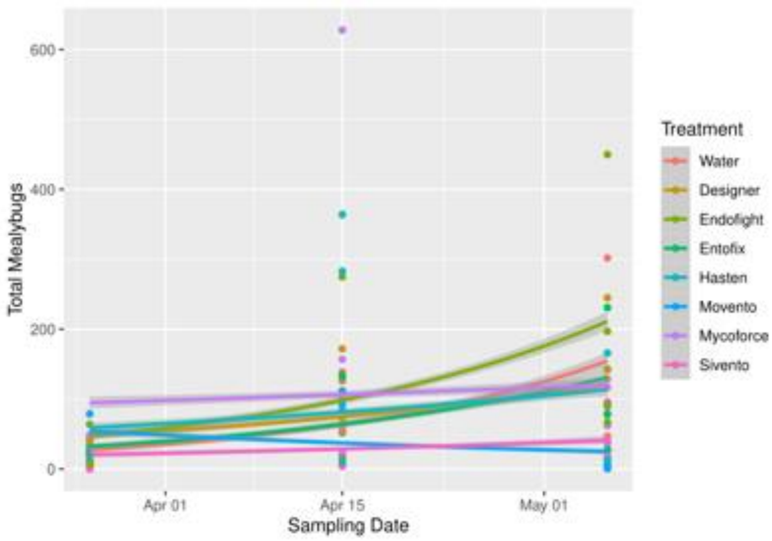

Figure 9: Change in mean number of mealybugs per treatment over 3 time points between March and May 2021 in field trial of insecticide products at Biggenden

The modelled data shows a significant effect of Movento (400ml/ha) and a smaller but still significant effect of Sivanto (400ml/ha), a new, unregistered insecticide provided by Bayer in comparison to the 3 controls (water, and the adjuvants Hasten and Designer) (Fig 10). Mycoforce showed a weak but significant impact on mealybugs compared to water and Designer. The other 2 microbial products had no impact on mealybug numbers.

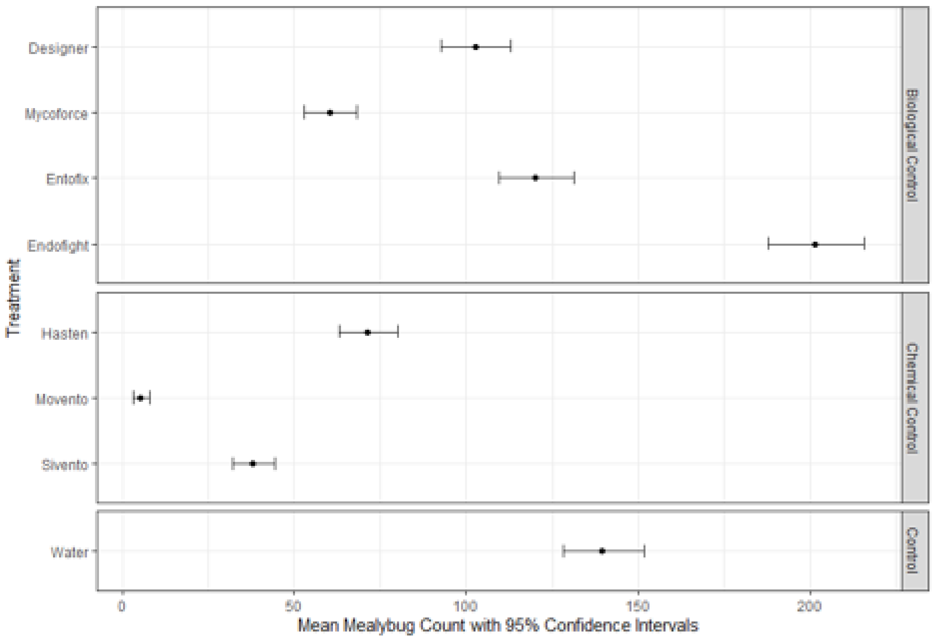

Figure 10: Effect of insecticides on total mealybugs in bluegrass. Biggenden, May 2021.

Crows Nest 2022

The mealybug infestation at Crow's nest expanded rapidly but unevenly across the sites. Variation between plots was such that significant results could not be obtained (Fig 11).

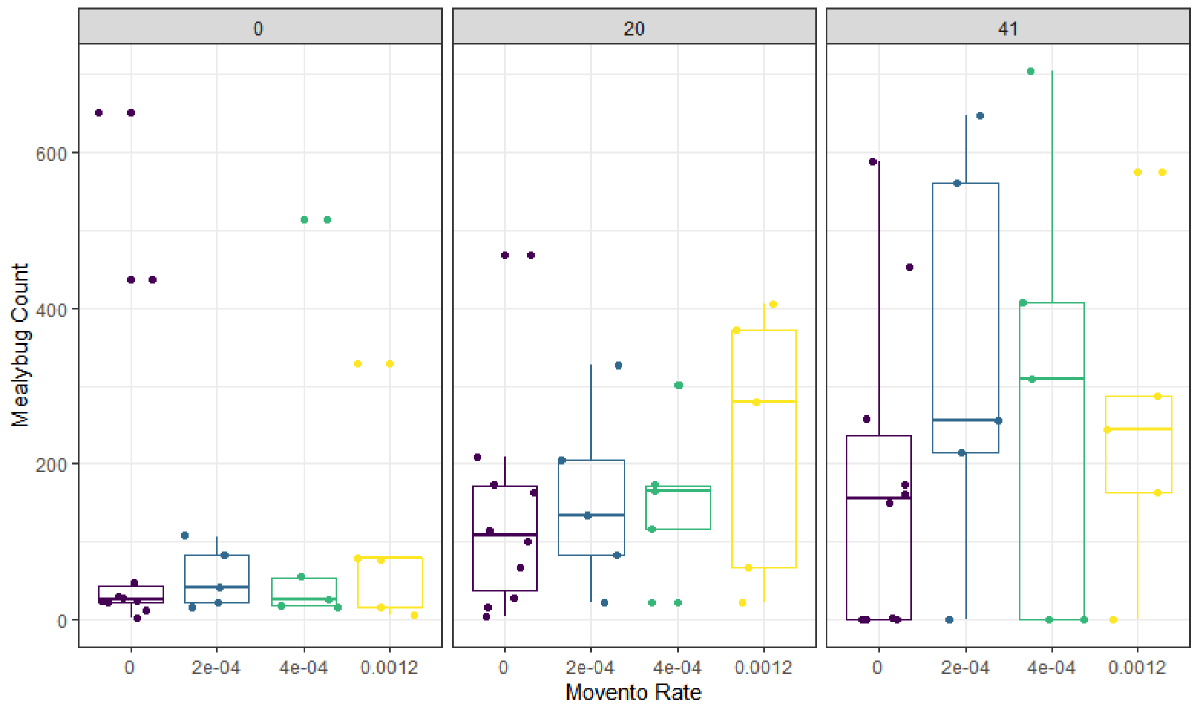

Figure 11: Mean mealybug counts at three timepoints (before first application (0 days) and at 2 weeks after first (20 days) and second (41 days) applications) in a trial of 3 rates of Movento in bluegrass, Crows Nest, 1st February to 14th March 2022.

Summary of screenhouse and pesticide trials

Laboratory and field methods were developed to conduct and quantify assays and trials of insecticides on the mealybug and symptoms of pasture dieback.

Replicated glasshouse and field trials with the systemic insecticides Imidacloprid ('Confidor') and Spirotetramat ('Movento') have demonstrated control of the mealybug, and that treated grasses recover from dieback symptoms compared to untreated plants.

Movento is effective in reducing mealybug density even when plants are in close proximity to untreated plants (in screenhouse and small-plot trials) and where mealybugs that are rapidly increasing in numbers. The correlation between Movento rate and number of mealybugs was highly significant.

Secondly, the trials resulted in no significant difference between the high rate of Movento (400ml/ha) which is specified in the APVMA permit and the middle rate (approximately equivalent to 200ml/ha). This suggests that there may be scope to reduce the rate of Movento required. If this is confirmed in field trials it would be a significant cost saving to graziers.

Lessons learned: Impacts of season, sampling method and trial design on future trials and tests.

Key factors learned from both the screenhouse trials and the field trials were the impacts of season on mealybug populations and distribution on plants and on recovery of grasses, and the impacts of sampling methods and trial design on analysis.

Seasonal effects.

All field trials were conducted on commercial properties and were affected by hot and dry conditions in early summer (up to February 2021) and wet conditions in 2022. Systemic products like

Movento and other trans-laminar products will not be taken up effectively if applied to dry, brown leaf, and hot, dry weather will prevent the flush of green leaf. The pesticides can only be applied after seasonal rain when the grasses flush. However, wet conditions also impact populations.

Secondly, hot dry conditions mean that the mealybugs are found mainly in the crown and thatch layer at both sites, where they would not be exposed to the insecticide. Even after rain, field sites may have a majority of larger female mealybugs (which don't feed and thus are not exposed to systemic insecticides) in the crown and thatch at the proposed start of the trial. The Biggenden trial was sampled by digging and bagging samples and returning them to the laboratory for destructive sampling, but this was found to be difficult.

A final observation is important for assessment of trials. Leaves with symptoms (yellow, red or purple streaking) don't recover following insecticide treatment. Grasses must be actively growing to show a response to treatment. In all trials to date, glasshouse or field, the leaves that developed symptoms before treatments were applied did not recover i.e. return to green. Instead, it is the new growth that increases the overall green leaf on the grass.

It is our conclusion that cut leaf sampling is a more effective method of sampling mealybugs in insecticide trials, since it samples the instars most affected (those feeding on leaf). This in turn narrows the window in which trials can be conducted.

Overall, trials need to be rapid, seasonal, manageable and robust (in terms of sampling and assessment) and well-replicated. This project has provided the founding information for this for all researchers and industry.

Information on trials and methods will be published and provided to program partners.

4.4. Progress on preparation of residue trials data package for APVMA renewal of Emergency Permit for Spirotetramat.

QUT obtained emergency permits from APVMA for both Imidacloprid and Movento for use against the mealybug *H. summervillei* in pastures.

Residue testing was conducted by Eurofins at two sites in Queensland: Calliope, and Biggenden. Material for the tests was supplied directly by Bayer. Eurofins and Bayer are completing laboratory analysis.

We are engaging with Bayer to conduct a full review of trial design and data in conjunction with residue data trials towards inclusion of mealybugs in a minor use permit Movento in pastures.

4.6. Provide timely communication on research activities for use in industry briefings at least biannually throughout the project.

Communications were provided frequently to MLA program leaders and comms team through reports, emails, a webinar, a presentation at an MLA panel event at Beef Week 2021, on-line participant meetings, material for specific media outputs, and a national science panel review in April 2022.

A summary of media outputs and extension activities has been provided separately for all 3 QUT projects.

4.7. A 2-page summary outlining key facts and findings of tolerant and susceptible grass species and management created in consultation with MLA's communications team.

An article on QUT research and management strategies for graziers in in preparation with MLA comms team. See draft, Appendix 1.

5. Conclusion

5.1 Key findings

5.1.1 Determine and quantify the impact and timing of dieback from initial infestation with mealybug to death of grass

Field surveys (B.PAS 0004), grass variety experiments (B.PAS 0006), and the final report of B.PAS 0505 all show a correlation between mealybug numbers and severity of dieback symptoms (purpling and yellowing of leaf and percentage of green leaf as a proportion of the whole leaf). The severity of symptoms (proportion of grass affected) is proportional to the number of mealybugs at low numbers, but at higher numbers the visible symptoms remain at around 100% of leaf affected.

Symptoms appear rapidly after infestation, within a week, but death of the grass was slow to occur. Grasses seem to be able to tolerate high numbers of mealybugs through early summer without immediate death, though impacts on productivity have not been assessed. This suggests that graziers have a window of opportunity in spring and early summer to monitor and detect the mealybug and symptoms of dieback and to target management at affected areas.

The population of mealybugs increases from December through to March, a pattern also observed in the field (B.PAS 0004). Actual death of the grass, with the appearance of 'dieback' did not occur until the wetter weather in late summer, when the peak in numbers of early and medium, foliar-feeding instars in coincides with wet weather (in both 2021 and especially in 2022). This is when the combination of mealybugs and conditions favourable to fungal infection coincide, resulting in the dramatic and apparently sudden death of grass with all the symptoms of 'pasture dieback'.

Mealybug numbers declined rapidly in the screenhouses through late March and April as cooler, wetter conditions continued and the amount of live grass for feeding decreased. This also coincided with the rapid decline in field populations observed in field monitoring in both 2021 and 2022 (B.PAS 0004), and in insecticide trials in both 2021 and 2022.

Three findings are particularly important for management and conduct and assessment of research trials. Firstly, there is a very short window in which research trials can be conducted, between emergence of summer populations feeding on leaves (November) and the decline in these populations from the end of February, a pattern also seen in field monitoring (B.PAS 0004). Secondly, infested grasses, while symptomatic, do not appear to die back until the late summer abundance of mealybugs and wet weather combine to give the apparently sudden 'dieback' of grass following late summer rain events. Finally, leaves with symptoms (yellow, red or purple streaking) don't recover and eventually die back: grass must be actively growing to recover.

These effects combine to give graziers a window to monitor and then manage mealybugs and reduce the effects of 'dieback'. Monitoring for symptoms and presence of mealybugs from spring (roughly September) through to December can detect early populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted at these populations to disrupt the mating and feeding populations in late summer.

5.1.2 Develop a standard bioassay protocol for efficacy assessments of control treatments allowing effective assessment of possible chemical control measures for pasture dieback

Laboratory and field methods were developed to conduct and quantify assays and trials of insecticides on the mealybug and symptoms of pasture dieback.

As reported in B.PAS 0004, adult mealybugs do not feed. Assays must be conducted using small and medium (feeding) instars. Adult mealybugs also disperse, females by crawling and males by flying. Infestation must be conducted using feeding instars. The methods described in section 3 above were found to work consistently, by encouraging migration from infested grasses to fresh material for successful and timely infestation by the required lifestage (feeding-stage nymphs).

Sampling methods: cut grass and dug samples, assessment.

In the Birkdale, Banana and Crows Nest field spray trials, grass samples were cut from the test plots prior to application and at 2-week intervals after application: 3 samples per plot of leaf and stem were cut at the base of the plants and coiled into a bag and returned to the laboratory. In the Biggenden trial the population was found to be mostly large instars and the trial was sampled by digging up soil, thatch and grass material and returning that to the laboratory for assessment.

The cut grass method was found to be more practical and reliable. It is lighter to transport and quicker to sample: transporting total (dug) samples was bulky, heavy and slow to sample in the field. In addition, leaf sampling samples the feeding instars on foliage which are the ones shown to be causing 'dieback'.

This places a constraint on field trials, with a very small window for trials during the peak leaf-feeding mealybug populations (December to February) and a rapid shut down from February onwards (in this report and in B.PAS 0004).

A 10-minute search of material from each plot was conducted followed by a full destructive sample of all material from the plot, and mealybug number recorded. This demonstrated that 10-minute searches can find 90% of all mealybugs in 10 minutes and will find 100% of the mealybugs 75% of the time.

In subsequent trials across all 3 projects (B.PAS 0003, 0004, 0006) a comparison of 10 minute count versus full destructive sampling showed that 10' counts captured a higher proportion of total mealybugs where total counts were under 100 mealybugs, but the proportion counted of the total declined at higher numbers.

Our observations across all assays suggest that this decrease in proportion recorded by 10' counts at very high total numbers not be important: multiple assays across all 3 projects suggest that there is little change in indicators such as symptoms at higher numbers of mealybugs. Only a few mealybugs are required to cause symptoms and induce changes in transcriptome making plants susceptible to infection.

Overall, the 10’ counts resulted in a higher degree of statistical significance in assays than the full counts (B.PAS 0006).

There is a narrow seasonal window in which trials can be conducted in both screenhouses and in the field. In both 2021 and 2022, mealybug numbers increased rapidly from December, and declined rapidly with the cooler conditions from the end of February, and the reduction in live grass suitable for feeding. This was anticipated and aligned with field observations that from April onwards mealybugs migrate underground to avoid the cool conditions.

Key factors learned from both the screenhouse trials and the field trials were the impacts of season on mealybug populations and distribution on plants and on recovery of grasses, and the impacts of sampling methods and trial design on analysis.

Seasonal effects.

All field trials were conducted on commercial properties and were affected by hot and dry conditions in early summer up to February 2021 and wet conditions in 2022. Systemic, translaminar products like Movento will not be taken up effectively if applied to dry, brown leaf. Hot, dry weather prior to the start of trials in 2021 prevented the growth of green leaf to which products could be applied and reduced the number of feeding instars on leaf. The pesticides could only be applied after rain when the grass began to flush. However, wet conditions in late February 2022 also impacted populations, which rapidly increased after the start of the trial at Crows Nest but were very unevenly dispersed over the test site. This led to a high level of variation between plots and meant that any treatment effects could not be determined statisitcally.

Secondly, hot dry conditions meant that the mealybugs were found mainly in the crown and thatch layer at both sites, where they would not be exposed to the insecticide. The 2021 trial at Banana was delayed until after rain, when significant numbers of mealybug were on the leaf and the leaf sampling method first applied at the 2019 Birkdale trial could be used but was then affected by the shut-down in populations with the early onset of cool weather in March 2021.

Even after rain, the field site at Biggenden was found to have a majority of larger female mealybugs (which don’t feed and thus are not exposed to systemic insecticides) in the crown and thatch at the start of the trial. The trial was thus sampled by digging and bagging samples and returning them to the laboratory for destructive sampling.

A final observation is important for assessment of trials. Leaves with symptoms (yellow, red or purple streaking) don’t recover following insecticide treatment. Grasses must be actively growing to show a response to treatment. In all trials to date, glasshouse or field, the leaves that developed symptoms before treatments were applied did not recover i.e return to green. Instead, it is the new growth that increases the overall green leaf on the grass.

Glasshouse trials in pot plants are irrigated and well replicated (8 replicates of each treatment in randomised block design). These tests quantify significant treatment effects even though mealybugs are able to move between plants. They are, however, still affected by cold conditions and there is again a narrow window for start of tests, approximately November to February.

Trials typically require 9 weeks from first application to final sample. These seasonal factors suggest that there is a very narrow window in which the different effects of treatments – number of mealybugs, or recovery of grass – can be successfully conducted. The optimum time appears to be a narrow window immediately following rain when there is sufficient fresh leaf to uptake insecticides, and the cold and dry conditions from April onwards.

Screenhouse versus field tests

Screenhouse trials in pot plants are well replicated (8 replicates of each treatment in randomised block design), artificially infested under controlled conditions. Small plot field trials are more labour intensive, typically contain only 4 replicates of each treatment (at least in initial trials, to manage workloads), and are more dependent on seasonal effects on grasses and mealybug populations.

Data from the field trials was considerably more dispersed (over-dispersed) than the glasshouse tests, which increases the difficulty in determining significance. Data from these two trials will be used to conduct a full power analysis to determine the degree of replication required in future trials conducted under similar conditions.

Sampling used differs between the field trials and pot plant tests. Screenhouse tests use full destructive sampling. The Birkdale, Banana and Biggenden trials used two different sampling methods (dug samples and leaf sampling) to quantify mealybug numbers and treatment impacts.

Full destructive sampling in pot plant assays is very informative, resulting in detailed data on abundance and distribution of mealybugs. The field sampling is more problematic: it must assess populations with different distributions, including soil and thatch and in a timely way that can be practically conducted in a field trial.
It is our conclusion that cut leaf sampling is a more effective method of sampling mealybugs in insecticide trials, since it samples the instars most affected (those feeding on leaf). This in turn narrows the window in which trials can be conducted (see above).

Information on trials and methods will be published and provided to program partners.

5.1.3 Conduct efficacy assessments (primarily in glasshouses) of control treatments for mealybug, and collect data that may assist APVMA registration processes

Replicated glasshouse and field trials with the systemic insecticides Imidacloprid (‘Confidor’) and Spirotetramat (‘Movento’) have demonstrated control of the mealybug, and that treated grasses recover from dieback symptoms compared to untreated plants.

Movento is effective in reducing mealybug density even when plants are in close proximity to untreated plants (in screenhouse and small-plot trials) and where mealybugs are rapidly increasing in numbers.

Secondly, trials in both screenhouse and field resulted in no significant difference between the high rate of Movento (400ml/ha) which is specified in the APVMA permit and the middle rate (approximately equivalent to 200ml/ha). This suggests that there may be scope to reduce the rate of Movento required in any future minor use permit.

Residue tests have been conducted and sample analysis is being completed. Together, these results suggest a pathway to reductions in costs and withholding periods and will contribute to the conversion of the emergency permit to a minor use permit.

Pesticides are only one part of the dieback response. Costs, withholding periods (subject to residue testing) and impracticality of application over large areas limit their overall use. They remain useful in spot-treatment of emerging spring populations to reduce later, more severe, summer infestation. Pesticides are, however, a useful tool for research.

Timing of management needs investigation along with other management interventions.

Outline at least one draft journal manuscript

A paper for submission to the journal of Economic Entomology has been drafted. It will describe the methods for conduct and assessment of screenhouse and field trials, and the data on efficacy. *Working title: Insecticides for management of the pasture mealybug and 'Pasture dieback' in Australia: methods and impacts in screenhouse and field. Hauxwell et al (TBC). J. Econ Ent.*

5.2 Benefits to industry

This research has identified a window to monitor and then manage mealybugs and reduce the effects of 'dieback'. Infested grasses, while symptomatic, do not appear to die back until the late summer abundance of mealybugs and wet weather combine to give the apparently sudden 'dieback' of grass following late summer rain events.

Two emergency permits for insecticides were obtained from APVMA. Insecticides were found to be effective against mealybug populations at lower rates than those in the current permit, and grasses were shown to recover. Residue tests have been conducted and sample analysis is being completed. Together, these results suggest a pathway to reductions in costs withholding periods and will contribute to the conversion of the emergency permit to a minor use permit.

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Monitoring for symptoms and presence of mealybugs from spring (roughly September) through to December can detect early populations. Management such as crash grazing, slashing, or insecticides (if appropriate) can then be targeted at these populations to disrupt the mating and feeding populations in late summer. Timing of management needs investigation along with other management interventions.

Finally, there is a very short window in which research trials can be conducted, between emergence of summer populations feeding on leaves (November) and the decline in these populations from the end of February, a pattern also seen in field monitoring (B.PAS 0004).

6. Future research and recommendations

6.1 Further research

Detailed work on the timing of management interventions with population biology in the field are required.

6.2 Recommendations to industry

This research has identified a window to monitor and then manage mealybugs and reduce the effects of 'dieback'. Infested grasses, while symptomatic, do not appear to die back until the late summer abundance of mealybugs and wet weather combine to give the apparently sudden 'dieback' of grass following late summer rain events.

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7. References

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Appendix 1: draft article for publication by MLA, Spring 2022.

RESEARCH UPDATE	
What's the project about?	
What's it about:	*Heliococcus summervillei mealybug now confirmed as vector for pasture dieback.*
Why it matters:	*Producers can now have confidence in treating pasture dieback by identifying and targeting mealybugs with a range of strategies.*
Where's it up to:	*Finalised*
Who's involved:	*Queensland University of Technology, MLA*
More information:	For general enquiries or if you would like to get involved in future R&D, please email: pasturedieback@mla.com.au or Felice Driver fdriver@mla.com.au
Seasonal Action Plan	*Include three clear actions with supporting tools: do/inspire/plan* • Inspect soil for mealybugs hidden in thatch and underground in order to get the upper-hand ahead of summer • Recognise early symptoms of pasture dieback, obtain a copy of the pasture dieback identification guide: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0009/1333692/16876-PastureDiebackGuide2021.pdf • Download the *Pasture Dieback Survey* app to report pasture die-back from your paddock at the App store or Google Play • Visit MLA's Pasture Dieback hub for resources and information: mla.com.au/dieback • View MLA's Legumes hub: mla.com.au/legumes

Mealybug confirmed as vector for pasture dieback

Pasture dieback has significant implications for QLD and Northern NSW producers with its characteristic yellow to purple tinge in most affected plants heralding a major risk to productivity and pasture health, however a wide range of colours can be seen in various affected grass species. In a boon for producers, MLA-supported research by Queensland University of Technology (QUT) with corroborating evidence from field trials by the Department of Agriculture and Fisheries, Queensland (DAFQ), has now determined that the *Heliococcus summervillei* mealybug is the primary vector of pasture dieback, with records showing its links with dieback in Cooroy, QLD, as early as 1926[1].

Caroline Hauxwell, Associate Professor at QUT has done extensive research into the causes of pasture dieback. "There is a clear correlation between the severity of symptoms and mealybug density," Caroline said.[2]

[1] Presentation Pasture dieback: research update with Caroline Hauxwell, September 2021

[2] Presentation Pasture dieback: research update with Caroline Hauxwell, September 2021

This tiny mealybug, barely visible to the naked eye, affects all commonly sown tropical and subtropical improved pastures with creeping blue-grass (cultivar Bisset), Buffel grasses, Rhodes grasses, pangola, setaria and broad-leaved paspalum[3] being particularly susceptible. Sapsuckers, they kill grass cells and cause necrotic lesions, with the weakened plant becoming vulnerable to multiple secondary infections. Highly mobile, mealybugs can be carried by wind, water, vehicles, cattle, clothes and hay.

A quick moving insect, they commonly evade detection by burrowing down to 900mm depth over winter and in dry conditions.[4] They may also be discovered under cow pats, thatch and debris.

Early detection key

Recognising early symptoms of dieback is crucial for timely use of appropriate management strategies. The symptoms can be variable in different pasture grasses or locations and can include a faint purpling along the edge of the leaf which can progress to further colour changes including purple, yellow or red, with streaks sometimes emerging. Once dieback has taken hold, grasses turn brown and become brittle and ashen. If caught early enough however, grasses can be saved.

Understanding biology and behaviour crucial

Coming into the spring-summer period means mealybugs are more active – necessitating vigilance by producers in monitoring pasture health and signs of mealybug activity. Understanding the biology and seasonal behaviour of mealybugs is a key of part effectively managing this pest. Producers should monitor for the presence of mealybugs as they emerge in warmer weather, especially after rain. It is the young mealybug in the juvenile life stage that feeds on fresh pasture growth and causes damage to productive pastures. Pink adult females and males do not feed.

New Paragraph

Highlight some of the work on suppression of plant immune system, endophytes, and resistance assays.

Strategies to combat pasture die-back

- Regularly inspect pastures for mealybugs and early signs of dieback, particularly in spring/summer when mealybug numbers start to increase
- Update and document your farm biosecurity plan i.e., conduct a risk assessment, communicate with visitors about entry and exit protocols, reduce the risks of introducing diseases, weeds and pests

[3] Presentation Pasture dieback: research update with Caroline Hauxwell, September 2021

[4] Presentation Pasture dieback: research update with Caroline Hauxwell, September 2021

- Consider sowing affected paddocks with legumes i.e., butterfly pea, stylos, Desmanthus, burgundy bean and lablab
- Grasses that exhibit more tolerance to mealybugs include panic grasses, particularly green panic
- Target mealybug-affected areas with insecticide in spring to stop the summer spread when they reproduce – spot treat only to limit unnecessary use of sprays (efficacy trials indicate half rate of MOVEVENTO is effective (permit numbers)
- Consider slashing and grazing pastures in summer to prevent adult mealybugs mating in the dense crown of plants
- Burning is less beneficial and can reduce beneficial microorganisms that improve resilience and recovery and remove valuable phosphate
- Comment on soil health and chronic PD (fusarium)?

New app joins fight against mealybugs

A new Pasture Dieback Survey app funded by MLA and developed by the Department of Agriculture and Fisheries QLD allows producers to report pasture dieback directly from their paddocks. Install the app and help researchers:

- determine pasture dieback locations and whether its spreading over time
- discover what pastures are being affected on what soil types
- if pasture dieback is linked to specific pasture management practices.

Case study:

Writer's name	Clare Le	**Feedback edition**	Spring 2022
Topic	*Mealybugs*	**Word count**	500 words plus break-out content
Category *(As per the brief)*	*Northern Cattle*	**Tag** *(As per the brief)*	Biosecurity Feedbase Invasive species
Seasonal relevance	*Summer* *Spring*	**Summary for social/digital: one snappy sentence**	*Pasture health, timed grazing vital in fight against pasture dieback*

MORE INFORMATION	
Interviewees	*Cameron and Kristy Gibson* *Coonabar* *coonabar07@bigpond.com*
MLA program manager OR relevant MLA contact	*Felice Driver* *Project Manager, Sustainable Feedbase Resources* *fdriver@mla.com.au*
RESOURCES	

SPECIFIC STORY ELEMENTS:

1.

PRODUCER CASE STUDY	
On-farm snapshot	
Name/s	Gibson Family, 'Coonabar Cattle Trust'
Location	15 km North of Rolleston, 320km west of Rockhampton, Central Queensland
Area in hectares	6781ha
Enterprise	Beef cattle trading
Livestock	2000-3500 head cattle
Pastures	Improved pastures, Buffel, Rhodes grass, green panic, and native species
Soils	Red sandy loams
Rainfall	600-650mm
Producer contact email	cameron@coonabar.com.au (07) 4984 3119
Lessons learned	1. Adjust stocking rate according to available feed: match stocking rate to carrying capacity 2. Maintain ground cover, trees, and grass health for resilience 3. Talk to other producers and organisations for support and advice
Sustainability Snapshot	**Top tip for other producers:** • Do your homework to find the right types of grass species. Not every pasture is suited to every climate. **Seasonal action plan:** • Visit MLA's Pasture Dieback page and download our pasture dieback management guide: mla.com.au/pasture-dieback-hub • Get your pastures in shape for the warmer months: mla.com.au/persistent-pastures • Access tools and information to improve soil fertility at MLA's healthy soils hub: mla.com.au/healthy-soils

Pasture health, timed grazing vital in fight against pasture dieback

'Coonabar' has been a family operated enterprise since 1988, breeding beef cattle until 2007, after which it transitioned to cattle trading. It has flourished through the strategic trading of livestock and time-controlled cell and rotational grazing systems, informed by over 22 years of grazing chart data. Careful consideration of feed availability and paddock size as well as a raft of sustainable practices have led to increased pasture health and biodiversity, with a recent Birdlife Capricornia survey verifying up to 110 bird species present at the property. The Gibson family believes these strategies have been key contributors to grass health and the property's resilience against pasture dieback, first spotted five years ago at Coonabar.

Time controlled grazing ensures pasture resilience

Located north of Rolleston, Central Queensland, Murray and Wendy Gibson, along with their son and daughter-in law Cameron and Kristy, manage the 6781-hectare property. The Gibsons have progressively converted the two original paddocks into 150 smaller ones after recognising the potential to improve both pasture utilisation and grass health. 2110 hectares are under cells with cattle moving daily between 80 paddocks. The other 4381 hectares are rotationally grazed with grazing periods adjusted depending on the paddock and mob size.

"With time-controlled grazing, our paddocks are more evenly grazed and have a large amount of manure deposited and hoof action over a 24-hour period and then given time to rest. Each paddock on Coonabar will be rested for an average of 360 days out of every year and never grazed down to stubble," Kristy said.

On a typical day, 1000 LSU graze a 25-hectare paddock for 24 hours before being moved calmly to the paddock next door. Used over the past 24 years, the results speak for themselves with carrying capacity skyrocketing from around 600 breeders in the 1980s to a maximum carrying capacity of 3500 trade heifers today.

Maintaining 40% tree cover and high ground cover has provided shade and shelter for livestock, habitat for wildlife and limited run-off into the Great Barrier Reef. Strategic cattle trading has been key to balancing stocking rate to carrying capacity. Lower cattle numbers during dry periods have limited pasture deterioration and secured future feedbase at Coonabar.

Caring for pastures key

Under this system, grasses have plenty of time to rejuvenate and the property has become highly productive, with detailed grazing charts regularly consulted to maintain sustainable stocking rates. Panic grasses and native pasture species such as Queensland Bluegrass have further contributed to pasture resilience against stressors such as the mealybug, now confirmed as the vector for pasture dieback. Legumes, which are entirely resistant to mealybugs, also flourish at Coonabar. It is this strong foundation which Cameron believes has improved the property's resilience to pasture dieback, first identified on the property in 2017. Despite being concerned about its spread, the largest patch of dieback they have seen develop has been the size of a football field.

Well-timed grazing effective against pasture dieback

In 2018-19 they saw small white insects moving on the leaves which they assumed were mealybugs. Cameron took photos with his phone and enlarged them to try to identify the pest. In December 2020, he identified Buffel grass with yellow tips across a good portion of one of his paddocks and fortuitously decided to graze it hard. He used a ratio of 40 head of cattle per hectare and grazed it for 24 hours. This timely decision effectively disrupted the young mealybug leaf-feeding life stages and breeding habitat for mature females, which restored the paddock back to health.

"Within three to four weeks of the mob coming out of the paddock there were six-inch, beautiful dark green leaves and the yellow tinge had gone. You've got to see it to believe it. People came through and they couldn't believe the grass density and grass strength," Cameron said.

In another part of the property near an internal farm road, Cameron saw a patch of dieback creeping up a hill. After cattle grazed the area for four to five days, the dieback subsided. He reflects on the random nature of its spread.

"We have a lot of tree cover, sometimes it would go through tree cover and sometimes it wouldn't. It went away by itself. Look after your grass, if it's strong and healthy with good roots and resilience, it looks after itself," Cameron said.