Evaluating the efficacy, drug resistance and ecological impacts of sarcoptic mange treatments in wombats

Curb Wombat Mange Program
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Cover photo: Wombat with sarcoptic mange in care, and at post treatment release. Yolandi Vermaak.

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1. Summary

Sarcoptic mange is a contagious skin infestation caused by the parasitic mite *Sarcoptes scabiei*. The disease results in severe skin crusting, scratching, hair loss, and death due to malnutrition or secondary infections if left untreated. Sarcoptic mange is a serious welfare issue for bare-nosed wombats in south-eastern Australia, with high prevalence in many areas of New South Wales. There is a strong need to improve the currently limited options for treatment of mange in free-living wombats.

This project aimed to better understand why sarcoptic mange in wombats is so difficult to control and provide evidence to support improved treatment methods. We monitored treatment efficacy in wombats with severe mange being rehabilitated in the NSW and ACT, by documenting clinical improvement and mite responses.

We addressed concerns around treatment efficacy and developing drug resistance by multidisciplinary and complementary approaches. Firstly, we directly measured the mite-killing activity of scabies treatments and tested the effect of different drug delivery formulations on mites. We developed a robust drug sensitivity test that could readily be adopted by wildlife carers and in the field. We also conducted the first comprehensive genetic and functional studies on the scabies mite drug receptor targets for moxidectin and fluralaner, that have been associated with developing drug resistance. This allowed us to get a picture of overall genetic diversity of these genes and see whether genetic variation may contribute to altered treatment outcomes, now and in the future. This was complemented by the development of a functional expression system, enabling the precise identification of drug targets within the mite and investigation of how treatments such as ivermectin, moxidectin, and fluralaner exert their effects.

We also explored the environmental impacts of mange treatment by testing the toxicity of acaricides to non-target freshwater species. Given that many wombats inhabit areas near rivers and streams, there is potential for these chemicals to enter freshwater systems and pose risks to aquatic life, thus such studies are of critical importance.

Project outcomes will help inform best practice guidelines for the treatment of mange in one of our most iconic marsupials and will be relevant to the welfare of mange-impacted species worldwide.

2. Acknowledgements

This work was funded by the Curb Wombat Mange program, administered by the New South Wales Government National Parks and Wildlife Service.

Ethical approvals for the collection of mites from wombats was via the University of Tasmania Animal Ethics Committee (29540) or the University of the Sunshine Coast Animal Ethics Committee (exemption pathway ANE2399). Collection of mites from a laboratory model was under the University of Queensland Animal Ethics Committee (AE001015).

We thank the many collaborating researchers and community volunteers who supported various aspects of this project. The following specific contributions are noted:

Wombat Rescue volunteers: for treatment and care of wombats, Deb Thomas for support with collation and analysis of clinical data (Section 3).

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University of Sydney- Irina Lotsaris (Section 6).

University of Canberra- Maddy Knight and Rod Ubrihien (Section 7).

3. Monitoring the treatment efficacy of Bravecto in wombats with severe sarcoptic mange

3.1 Background and Objectives

Sarcoptic mange has devastating welfare impacts on bare-nosed wombat populations. Challenges to control include limited treatment formulations, application methods, need for repeat treatments, and hyperkeratotic crusts impeding drug penetration and accurate delivery. Our understanding of drug pharmacokinetics in wombats is scant, which limits the ability to make empirical decisions around treatment. While both Cydectin (moxidectin) and Bravecto (fluralaner) are effective, uncertainty surrounds appropriate treatment protocols, especially in wombats with severe mange (Mounsey et al., 2022, Old et al., 2021, Takano et al., 2023). While preliminary pharmacokinetic and efficacy studies showed the potential of Bravecto as an effective single dose treatment (Wilkinson et al., 2021), practical experience and more recent studies suggests that additional doses may be required (Wilkinson et al., 2024a). More evidence is required regarding optimal treatment intervals, and how wombats with severe mange respond to treatment. We will assess the efficacy of Bravecto in severe sarcoptic mange and attempt to measure concentrations of fluralaner in the skin of treated wombats.

3.2 Methods

This work was undertaken in partnership with two wombat rehabilitation organisations led by investigators Vermaak and Holme- Wombat Rescue ACT, and Cedar Creek Wombat Hospital, Hunter Valley, NSW. Our focus was monitoring wombats with severe mange entering care, but monitoring of treatment undertaken in the field also occurred. Animals generally received 1-3 doses of Bravecto, at a concentration of 45-85mg/kg (average 50mg/kg). Scoring of skin condition to monitor the clinical efficacy of Bravecto in wombats was undertaken at regular intervals. Where time permitted, skin scrapings were assessed by microscopy to measure live versus dead mites after treatment, and in-vitro assays to support Section 4 were undertaken. While the intention was to collect samples non-invasively for pharmacokinetic studies of fluralaner concentration in the skin,

this did not occur due to logistical difficulties in collection and storage, and the late withdrawal of the laboratory intended to do the pharmacokinetic research.

3.3 Outcomes

An overall summary of Bravecto doses administered by Wombat Rescue and treatment outcomes over 2024-2025 is presented in Table 1.

Table 1. Bravecto doses by outcome and care for treated wombats (2024-5). Data provided by Wombat Rescue.

	In care			Wild		
Doses	**Died**	**Survived/ Unknown**	**Total in care**	**Died**	**Survived/ Unknown**	**Total wild**
1	8	7	**15**	9	63	**72**
2				1	27	**28**
3	1		**1**	2	11	**13**
4					3	**3**
Total	**9**	**7**	**16**	**12**	**104**	**116**

For field treated wombats, treatment with one or two doses of Bravecto was successful in many cases, although many animals were also lost to follow up (Figure 1).

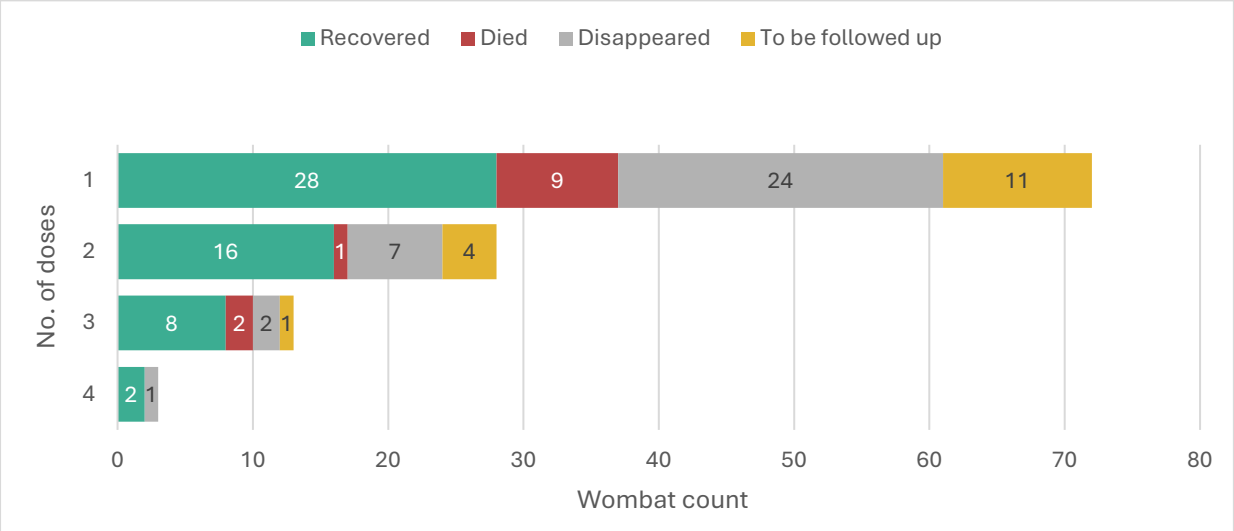

Figure 1. Bravecto doses by outcome (grouped) for field treated wombats, 2024-2025. Data from Wombat Rescue.

During the entire project period (July 2023-June 2025), approximately 22 wombats were taken into care for mange via Wombat Rescue and Cedar Creek Wombat Hospital. The majority of these were in a state of severe advanced mange, with >50% of body surface area impacted, and in a malnourished state (normal score on intake 4C or 4D). Despite receiving treatment, only around 40% of wombats survived. The decision to take a mange impacted wombat into care rather than treating in field or euthanizing is not made lightly. Carers need to consider the additional stress exerted on an already critically ill animal- it was not uncommon for wombat to make an initial recovery with death of mites, before wombats "crashed", and died 5-7 days later. A comparison of intake reports between surviving and not surviving suggests two main factors impacting outcome. Firstly, many wombats that died presented with severe secondary bacterial infection, either evident as deep skin fissures or abscesses, or as sepsis/peritonitis detected post-mortem. Secondly, animals that had better condition had an improved outcome compared to those entering care in a severely emaciated body condition.

We highlight the trajectory of recovery in representative successfully treated cases (Figure 2, Figure 3). In general, mites were killed quickly after a single dose of Bravecto, with live mites generally not detected after 7 days, at which point skin plaques begin to fall off. Condition steady improves or plateaus over next 1-2 weeks, with most animals recovering after 2 months. Depending on the original severity, amount of hair loss, and level of malnutrition, full recovery and rehabilitation may take many months.

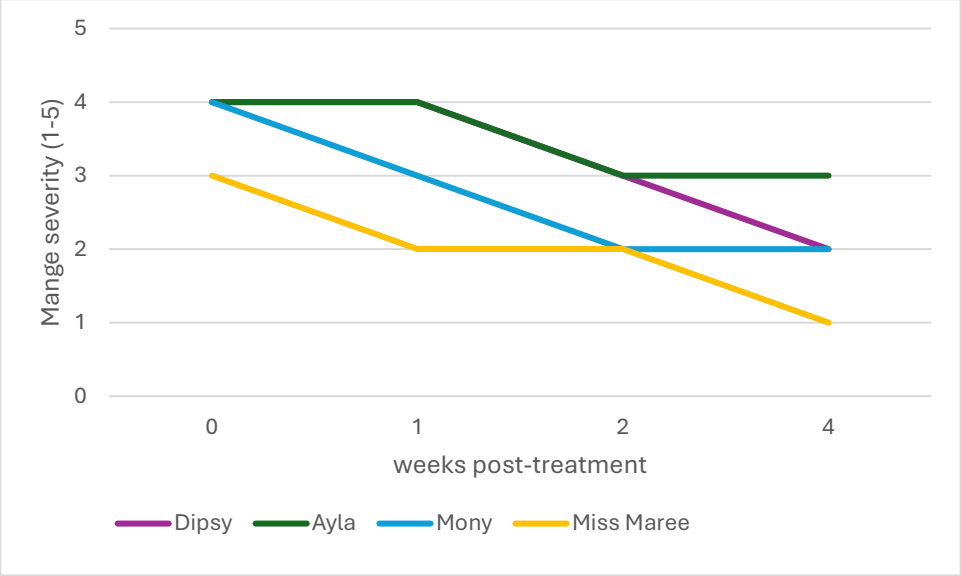

Figure 2. Reduction in mange severity following treatment with Bravecto. Mange severity is assessed on a scale of 1-5 (1= possible mange, 5= 60-100% of body surface with hair loss/mange plaques)

3.4 Discussion and Implications

These findings contribute to the growing evidence demonstrating the clinical efficacy of Bravecto for the treatment of sarcoptic mange in wombats. Initial pilot studies by Wilkinson and colleagues (2021) showed efficacy of a single dose of Bravecto containing 25mg/kg of fluralaner in wombats with mild to moderate mange. Subsequent studies of Bravecto in a larger field trial suggested that higher concentrations (45-85mg/kg), and in some cases, additional doses were required to clear mange in free-living populations (Wilkinson et al., 2024a). The data compiled by Wombat Rescue, presented herein concurs with this, with >100 wombats treated successfully with one or two doses of Bravecto, with 45-60mg/kg most frequently utilised.

Monitoring treatment efficacy in free living wombats is challenging, with a significant number of animals not sighted again after treatment (Mounsey et al., 2022, Rowe et al., 2019). For this reason, an objective of this project was to report on the progress of Bravecto treatment in wombats with severe mange in care. As has been reported for humans with severe crusted scabies (Roberts et al., 2005), without intervention, mortality for wombats with untreated sarcoptic mange is high. As such, it was not surprising that >50% of wombats died in care despite confirmation that scabies mites were killed effectively by Bravecto. It is noted that despite similar mange scores, it was wombats with obvious secondary infections that were less likely to survive. Sarcoptic mange is very commonly associated with secondary bacterial infection, which can progress to septicaemia and death without timely intervention. Furthermore, sarcoptic mange is a major cause of systemic inflammation (Wilkinson et al., 2024b) and together with bacterial infection, the pathologic immune and inflammatory responses can become overwhelming, and animals may succumb, especially where they are malnourished. Indeed, a comparison of healthy and severe mange wombats shows that tissues and organs display transcriptomic (gene expression) profiles akin to sepsis (Naesborg-Nielesen, manuscript in preparation). It is important to recognise that dead/dying mites will still contribute to these ongoing host immune responses, and thus it possible that these individual factors may have a greater impact on successful treatment rather than the treatment itself. As our understanding of mange immunopathology develops, additional adjunctive treatment to better support recovery should be considered.

Figure 3. Progress photos of wombats with severe sarcoptic mange following one dose of Bravecto (~50mg/kg fluralaner). Yolandi Vermaak, wombat rescue.

4. Phenotypic assessment of *Sarcoptes scabiei* responses to current mange treatments

4.1 Background and Objectives

Effective treatment of mange in wombat populations presents considerable challenges. Commonly used acaricides such as ivermectin and moxidectin are often ineffective as single-dose treatments due to their short plasma half-lives in wombats. Topical application of fluralaner has demonstrated prolonged systemic retention, with plasma half-lives of up to 167 days at higher dosages. Its clinical efficacy against *S. scabiei* has been documented in multiple species, including wombats. Bravecto is approved by the APVMA for direct application in the treatment of sarcoptic mange. However, the current 'spot-on' formulation presents practical limitations for non-invasive delivery methods such as pole-and-scoop or burrow flap techniques, primarily due to its low volume (<3.57 mL per pipette). In mange-affected wombats, fur density and hyperkeratotic skin lesions further complicate effective topical delivery.

To address these challenges, the use of a diluent to increase the volume and improve distribution of Bravecto has been proposed. One candidate is 'Orange Power Sticky Spot and Goo Dissolver', which contains citrus essential oils such as limonene and citral. These compounds are readily available, cost-effective, and possess solvent properties compatible with Bravecto (Wilkinson et al., 2021).

This study aimed to assess the in vitro efficacy of Bravecto, Orange Power, and its key constituents- limonene and citral- against *S. scabiei*. As no prior studies have documented the in vitro activity of Bravecto on this mite, the findings will contribute to establishing a baseline drug response profile in a previously untreated mite population. This is useful as monitoring in-vitro treatment responses of mite populations over time has identified patterns of reduced treatment sensitivity prior to the emergence of full resistance, which can be used to support management (Mounsey et al., 2009). The results will also inform the potential use of Orange Power as a delivery vehicle for Bravecto in the treatment of mange in free-living wombats.

4.2 Methods

We tested four treatments in the lab to see how quickly and effectively they kill mites-Bravecto, Orange Power, two main essential oil constituents found in Orange Power: limonene and citral. We also tested Cydectin (moxidectin) on wombat derived mites. In vitro activity has been previously characterised for moxidectin (Mounsey et al., 2017, Sharaf et al., 2020).

Mites for testing were obtained from a laboratory model (Mounsey et al., 2010), and wombats with mange. Skin scrapings were collected from the shed skin crusts and mites isolated and examined using a light microscope. Mites from wombats were tested against Bravecto, Orange Power, and moxidectin. Controls for these experiments included Benzemul lotion (25% Benzyl Benzoate, positive control), known for its consistently high potency against *S. scabiei,* and Mineral Oil (negative control), which contains no active ingredients (mites known to survive for >24 hours with no mortality).

Three different in-vitro analyses were conducted on our lab model mites. Only female mites were used in lab testing, to control for developmental differences previously demonstrated (Mounsey et al., 2017). Firstly, we measured how long it took for half the mites to die at different doses (termed LC_{50}). Secondly, we measured survival times to a fixed concentration of treatment. Survival analysis only was conducted on wombat-derived mites, using a range of different developmental stages due to lower numbers of mites available. Thirdly, pilot assays were undertaken to see if any of the compounds or their constituents had ovicidal activity (i.e. prevented egg-hatching). Eggs were collected from skin-scrapings and incubated for up to 5 days in treatment and controls, with the numbers of hatched eggs counted daily.

4.3 Outcomes

All the tested treatments were effective at killing mites. After 1 hour, the dose needed to kill half the mites was (Figure 4):

- Bravecto - 14.61 mg/mL
- Orange Power - 4.5%
- Limonene - 26.53%
- Citral - 0.76%

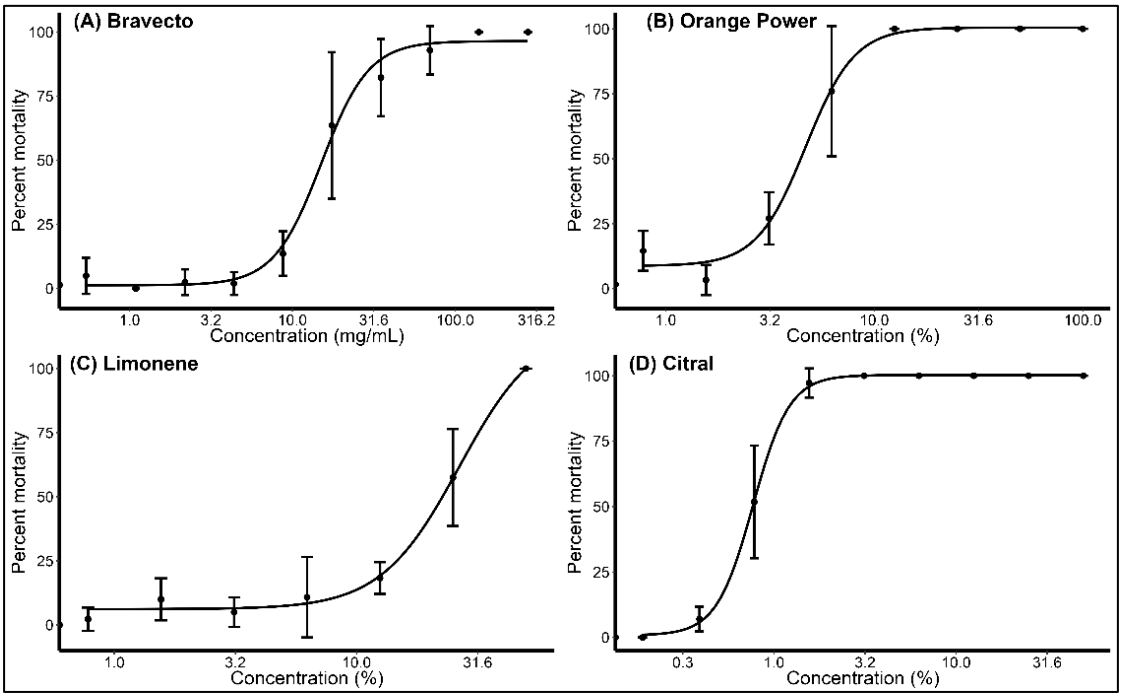

Figure 4. Dose response curves of *S. scabiei* mortality to Bravecto, Orange Power, limonene and citral. Mites were exposed for 1 hour to serial dilutions of compounds. Points show median mortality, bars show standard deviations.

Survival assays results supported the findings of the LC_{50} assays, confirming the high acaricidal activity of the treatments tested. Mites died within 5 minutes of exposure to Orange Power, 15 minutes of exposure to Bravecto, and 10 minutes when exposed to a mixture of both (1:20 Bravecto to Orange Power). In comparison, mites stayed alive for greater than 24 hours when exposed to the negative control (mineral oil) and died within 15-30 minutes of exposure to the positive control scabicide (Benzemul, 25% Benzyl Benzoate) (Figure 5).

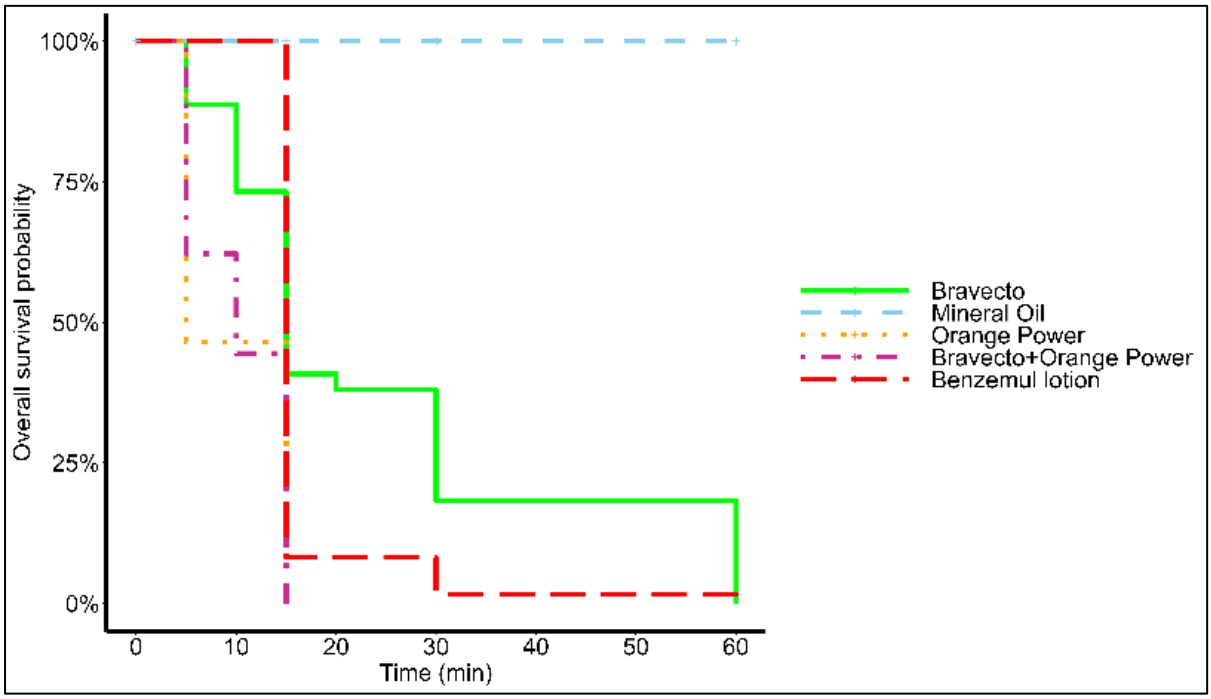

Figure 5. Kaplan-Meier survival curves of *S. scabiei* exposed to Bravecto, Orange Power, or Bravecto/Orange Power combination. Benzemul lotion (25% Benzyl Benzoate) and Mineral Oil were used as positive and negative controls respectively.

Survival assays of mites collected from mange infested wombats showed similar results to the laboratory model, with survival of <10 minutes when exposed to Bravecto and Orange Power, and 20 minutes when exposed to moxidectin (Table 2).

Table 2. Survival assay on *S. scabiei* collected from wombats

Test solution	Concentration	n	Median survival time (mins)
Orange Power	10%	10	5
Bravecto (fluralaner)	20 mg/mL	25	10
Cydectin (moxidectin)	50 µg/mL	100	20
Mineral Oil (negative control)		20	>120

Tests to measure ovicidal activity of these treatments were also piloted, but variable results were obtained, and this would require further development.

4.4 Discussion and Implications

These results confirm the acaricidal properties of Bravecto, demonstrate acaricidal properties of Orange Power and support the potential suitability of Orange Power and its active constituents as a diluent for Bravecto. As well as killing mites via direct exposure, Orange Power could potentially enhance the topical delivery of Bravecto to wombats by increasing drug penetration in hyperkeratotic crusts. Further research evaluating the physiochemical properties, and modes of action of Orange Power and its constituents as a formulation vehicle would be of value. We confirmed that both currently utilised treatments have high in-vitro efficacy in wombats- with no one treatment “better” than the other from this perspective. Wombat derived mites had comparable drug response profiles to laboratory mites with no previous acaricide exposure, showing that resistance was a not a problem in the population tested. This provides important baseline data in the case of future resistance being suspected as Bravecto becomes used more frequently in wombats.

5. Genetic analysis of acaricide target genes in *Sarcoptes scabiei*

5.1 Background and Objectives

Several studies have reported prolonged survival of *S. scabiei* mites following intensive acaricide use, such as ivermectin treatment for crusted scabies in humans (Currie et al., 2004, Mounsey et al., 2009). Similar concerns have been raised regarding the high concentrations of moxidectin (Cydectin) used to treat mange in wombats, with suggestions that mites may be developing resistance (Old et al., 2021). As resistance is often linked to changes in drug target sites, investigating the genetic diversity of these target drug receptors in *S. scabiei* is a critical first step. Glutamate-gated (GluCl) and GABA-gated (Rdl) chloride channels are the proposed targets of both moxidectin and fluralaner. In this study, we analysed the DNA sequences of these GABA and glutamate receptor genes in scabies mites and compared them with historical samples to assess whether mange treatment programs are influencing genetic variation in mite populations.

5.2 Methods

Three Rdl and two GluCl genes were sequenced from mite samples collected from different geographic regions where mange is prevalent in wombats (Figure 6). Skin scrapings were collected from infested wombats and stored until analysis. DNA was extracted and PCR used to amplify exons of each of the genes. We focused on exons forming the transmembrane domains of the channels, as these are commonly associated with altered channel properties and resistance. Successfully amplified PCR products were cloned into sequencing plasmids, and plasmid DNA sequenced. Sequences were assembled and analysed using the SnapGene software package. Multiple sequence alignments were generated using the MUSCLE method, which highlighted any sequence differences between samples and compared to the published *S. scabiei* reference sequences (Korhonen et al., 2020). These alignments were then visualised by phylogenetic trees constructed using the Neighbour-Joining method (Madeira et al., 2024). Changes in nucleotide sequences that corresponded to a change in the amino-acid sequence (non-synonymous variation) were further analysed to assess their possible significance. The likelihood of damaging or modifying effects of non-synonymous variations identified in the sequences on receptor function was

predicted using online bioinformatic tools, Sorting Intolerant from Tolerant (SIFT) (Sim et al., 2012) and Variant Phenotyping v2 (Polyphen-2) (Adzhubei et al., 2013).

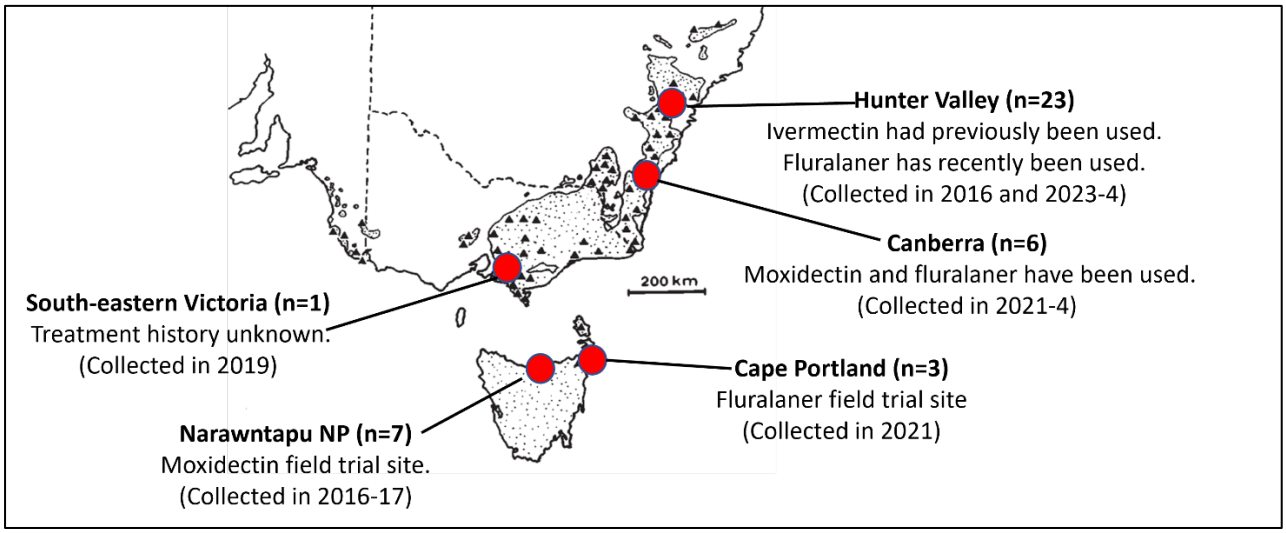

Figure 6. The source locations and sample sizes of *Sarcoptes scabiei* collected from bare-nosed wombats (*Vombatus ursinus*).

5.3 Outcomes

PCR for each of the genes was successful for most samples, although there were differences in PCR efficiency and template concentrations that influenced successful amplification in some cases. Overall, Rdl1 and GluCl2 genes showed the most sequence variation (Table 3). Notably however, most sequence variation was found in non-coding "intron" regions of genes, and therefore will not cause any changes. There was comparatively a much lower number of non-synonymous variations in coding regions (exons), which may potentially alter the receptor structure. Of these, **three variants were identified in Rdl1, and one in Rdl2.** No non-synonymous variants were found in the GluCl genes.

Table 3. Summary of sequence results of the three Rdl and two GluCl genes of *S. scabiei* collected from mange infested wombats.

Gene	Number of total variations	Number of intronic variations	Number of exonic variations	Number of non-synonymous variations
Rdl1 Exons 1-3 and 6-7	34	6	3	2
Rdl1 Exon 4-5	24	22	2	1
Rdl2 Exons 6-7	6	5	1	1

Rdl2 Exons 8-9	0	0	0	0
Rdl3 Exons 3-7	13	12	1	0
Rdl3 Exons 8-9	9	6	3	0
GluCl1 Exon 6	4	4	0	0
GluCl2 Exons 4-7	21	19	2	0

In Rdl1, a nucleotide substitution of G to A in exon 5 (forming part of the N-terminal extracellular domain) resulted in an amino acid change E220K. This variant was detected across the geographic range in wombat mites isolated from Narawntapu national park (3/4 samples), Cape Portland (1/3 samples), Victoria (1/1 samples) and the Hunter Valley (2/9 samples) (Figure 7). The bioinformatic tools SIFT and Polyphen predicted that this variation would have no functional impact on the receptor structure.

Another common variant was the substitution of A to C in exon 6 (forming part of the TM 3-4 intracellular loop), resulting in an amino acid change L327M. This variant was detected in all but three samples collected from wombats in the Hunter Valley but was absent elsewhere (Figure 8). For the L327M substitution of Rdl1, while the SIFT score was 0.06 indicating this variant was tolerated, the Polyphen-2 score was 0.784 suggesting it was possibly damaging.

Finally, a third but rare variant was identified in exon 7 (forming part of TM4) – G468V. This was detected in one sample from Canberra, and one from the Hunter Valley. The substitution at G468V was predicted as a likely damaging variant, with a SIFT score of 0.0 and a Polyphen-2 score of 0.78.

For Rdl2, a substitution in Exon 7 (forming part of the TM 3-4 intracellular loop) resulted in the amino acid change G455E. This variant was found in all samples amplified from Narawntapu national park but was absent from all other samples. While the SIFT program predicted this would affect protein function, Polyphen-2 was unable to predict the functional effect of this substitution, which may be due to some stretches of repeating amino acid sequences in this region.

An overall comparison of the genes examined showed very low genetic diversity, with pairwise genetic distances (Fst values) ranging from 0-0.008. In the phylogenetic tree of Rdl1, there was a weak trend of geographical and to a lesser-extent, time-based clustering. Samples from Tasmania, the ACT and Victoria appeared to be more similar to the reference sequence (SAR368), while the samples from the Hunter Valley tended to cluster separately and closer together (Figure 9). For Rdl2, pairwise differences were extremely low, indicating this gene

is highly conserved. Like Rdl1, there was some weak geographical clustering of Rdl3, GluCl1, and GluCl2.

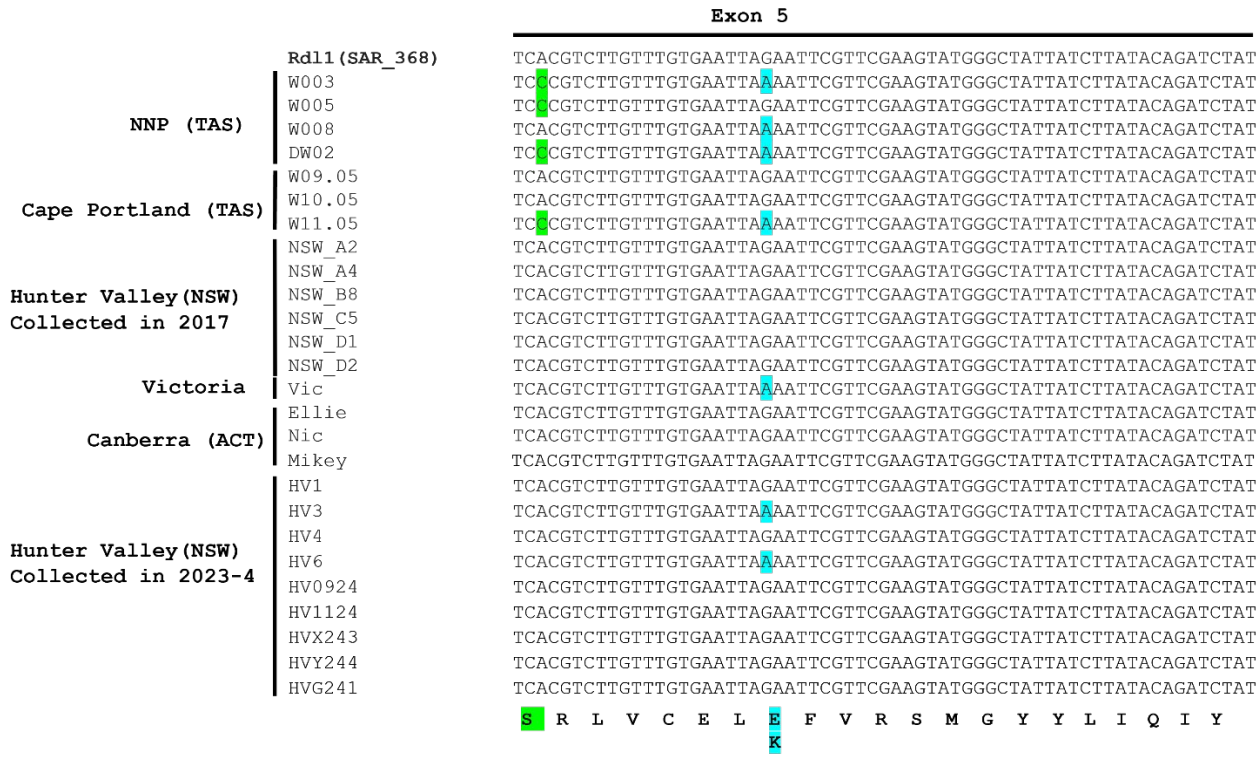

Figure 7. Nucleotide substitution (blue highlight) resulting in the amino acid change E220K in the N-terminal extracellular domain of Rdl1 gene of *S. scabiei*. A synonymous substitution is highlighted in green, which does not change the amino acid sequence.

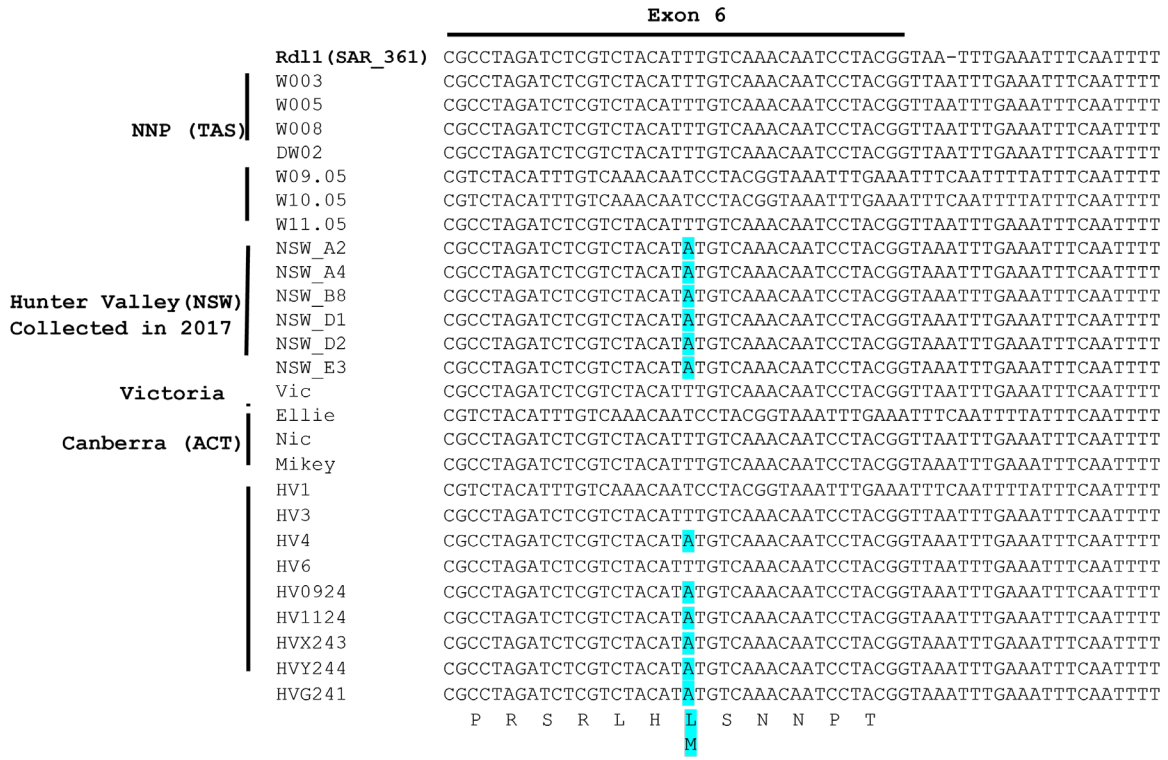

Figure 8. Nucleotide substitution (blue highlight) resulting in the amino acid change L327M in the M3-M4 intracellular loop of Rdl1 gene of *S. scabiei* mites collected from the Hunter Valley region, NSW.

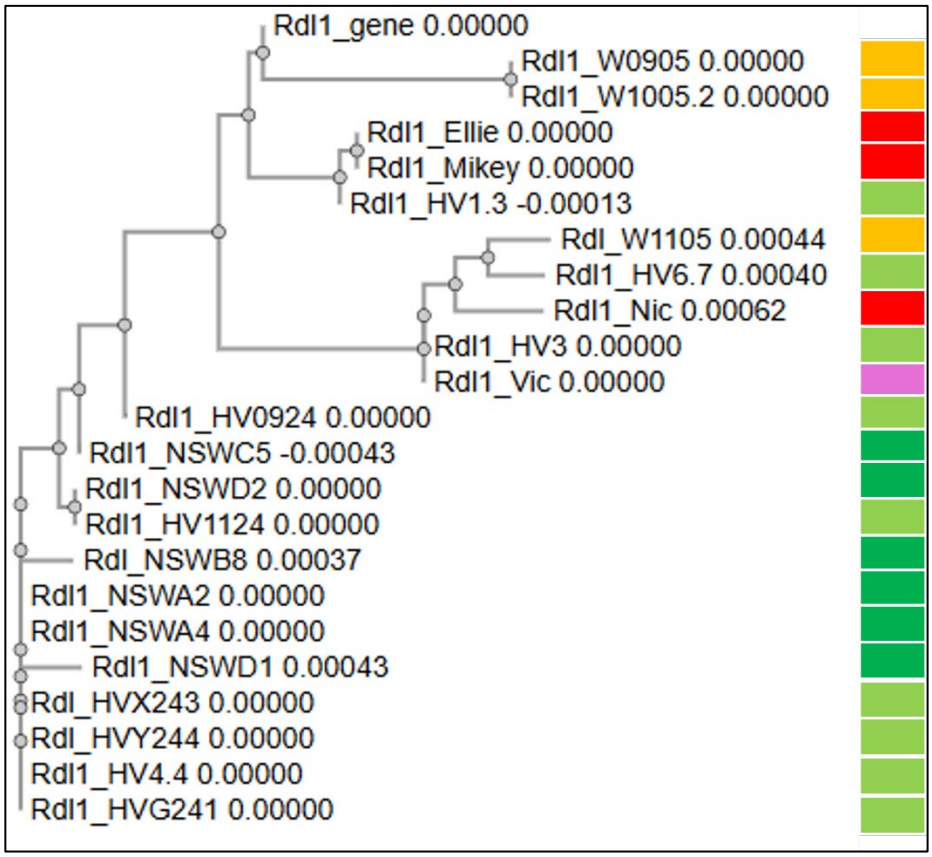

Figure 9. Phylogram showing genetic distances of Sarcoptes scabiei sequences isolated from mange wombats, relative to the reference S. scabiei reference Rdl1 sequence (Rdl1_gene). Geographic information of the sample source locations is indicated by colour key (light green, Hunter Valley NSW, collected in 2023-4; dark green, Hunter Valley, NSW, collected in 2017; orange, Cape Portland, Tasmania; pink, South-East Victoria; red, Canberra). Genetic distances shown as Fst values, with low to moderate considered 0.02 - 0.4 (Hamadeh et al., 2018). The phylogenetic tree was constructed via the Neighbour joining method from multiple sequence alignment performed using the MUSCLE method.

5.4 Discussion and Implications

This study provides new insights into genetic variation in the Rdl and GluCl genes of *S. scabiei* mites collected from bare-nosed wombats. Most observed variations were in non-coding intronic regions; however, four non-synonymous mutations were identified, including a commonly observed L327M substitution in the TM3–TM4 intracellular loop of the Rdl1 gene, and a G468V variant in TM4. Additionally, a G455E substitution in the same region of the Rdl2 gene was detected. The TM3–TM4 intracellular loop of the arthropod Rdl gene has been identified as a key site for channel gating and acaricide sensitivity (Nakao and Banba, 2021). As such, these non-synonymous mutations will be prioritised for molecular modelling and functional analysis to assess their potential impact on GABA receptor function and

acaricide binding. This work will form part of a future study, and an extension of the work presented in Section 6.

Overall, the study found weak geographical trends and low genetic diversity in LGCC genes among wombat-derived *S. scabiei* populations, suggesting these genes are highly conserved and currently subject to minimal selective pressure. An exception may be mites from the Hunter Valley region of New South Wales, which showed some divergence.

These findings enhance our understanding of potential mechanisms of drug resistance, regional variant prevalence, and allele frequency in wombat-sourced *S. scabiei*. They underscore the importance of proactive acaricide management to improve treatment outcomes for sarcoptic mange. Ongoing genetic monitoring of LGCC genes, expanded sampling across diverse regions, and integration of treatment history data are recommended to identify and track potential resistance-associated variants which may emerge with ongoing and expanded population treatment.

6. Establishment of a functional expression system for characterisation of scabies mite ligand gaged chloride channel drug targets

6.1 Background and Objectives

We characterised the functional properties of a series of ligand-gated ion channels identified in *S. scabiei* that are key targets of currently used sarcoptic mange treatments. Development of this functional expression system allows to identify which parts of the mite the drugs are targeting, and how the drugs work. This will also allow us to study the impact of genetic variants identified, to see whether they confer altered treatment responses or drug resistance. This system will also enable the testing of new treatments to define their mode of action in the scabies mite.

6.2 Methods

We focused on two specific ligand gated chloride channels from the scabies mite: **GluCl1** and **Rdl1**. These channels are very similar to glutamate and GABA receptors found in other well-studied arthropods and in other organisms, are known to be targets for drugs used in mange treatments including ivermectin, moxidectin and fluralaner. However, these have not been studied in scabies mites before. They were the main targets for genetic analysis Section 5.

Plasmid DNA constructs were prepared at UniSC for two versions of GluCl1 ('splice variants', called GluCl1.9 and GluCl1.16) and one version of Rdl1 (Rdl1.4). These were inserted into a plasmid (pOTV) that allows the genes to be expressed in cells. We then sent these plasmids to the University of Sydney for further testing. We are also working on three other channels-GluCl2, Rdl2, and Rdl3, which have been successfully cloned and sent for future testing.

To study how these channels work, we created RNA from the plasmids and injected it into frog eggs (from *Xenopus laevis*). This causes the cells to produce the ion channels on their surface. We then used a technique called two-electrode voltage clamp (TEVC) electrophysiology to measure how the channels respond to different chemicals. We tested how the channels reacted to their predicted

natural neurotransmitters - glutamate or GABA, as well as to drugs predicted to either **activate** (*agonists*, like ivermectin or moxidectin) or **block** them (*antagonists*, like fluralaner or fipronil).

If the channels are working and have been expressed correctly, adding the neurotransmitter ligand should cause a flow of chloride ions into the cell, which we can detect as an electrical current. Activators open the channels, while blockers prevent them from opening. Thus, both classes of drugs can inhibit the normal activity of channels, but by different mechanisms.

6.3 Outcomes

Application of GABA to ssRdl 1.4 did not generate any inward currents, indicating that the channel was not correctly assembled. Further analysis of the sequence suggests that a portion may have been incorrectly truncated- this has now been re-cloned (ssRdl 1.4L) and shipped for later analysis.

Functional expression for ssGluCl 1 was successful. GluCl 1.16 was found to have higher sensitivity to Glutamate (EC50 6.4 ± 1.7 µM) compared to GluCl 1.9 (EC50 36 ± 1.9 µM), showing that splice variation does have an impact on channel activity (Figure 10A). As predicted, fluralaner **inhibited the glutamate gated ion currents** mediated by GluCl1.9 with an IC50 of 0.95 ± 0.31 µM and a Hill co-efficient of -2.3 ± 0.62 (Figure 10B). It was not possible to reliably measure the inhibition of GluCl1.16 by fluralaner because of the rapidly desensitizing currents generated by glutamate. Further analysis of the activation of GluCl1.16 is required to better understand its sensitivity to fluralaner.

The sensitivity of GluCl1.9 to moxidectin was then investigated by comparing the amplitude of the glutamate activated current to that generated by a single dose of moxidectin. Moxidectin generated a **rapidly activated** current, that desensitized to a steady state but did not wash out after stopping the perfusion of moxidectin from the bath, **indicating that moxidectin is an irreversible inhibitor of GluCl1.9** (Figure 10C). This corresponds with previous studies on the related macrocyclic lactone ivermectin, which is well-known to be an irreversible inhibitor of glutamate gated chloride channels (Cully et al., 1994). Further characterization of concentration dependence of moxidectin stimulation of GluCl1.9 is required to estimate the relative potency of this inhibitor in comparison to other inhibitors.

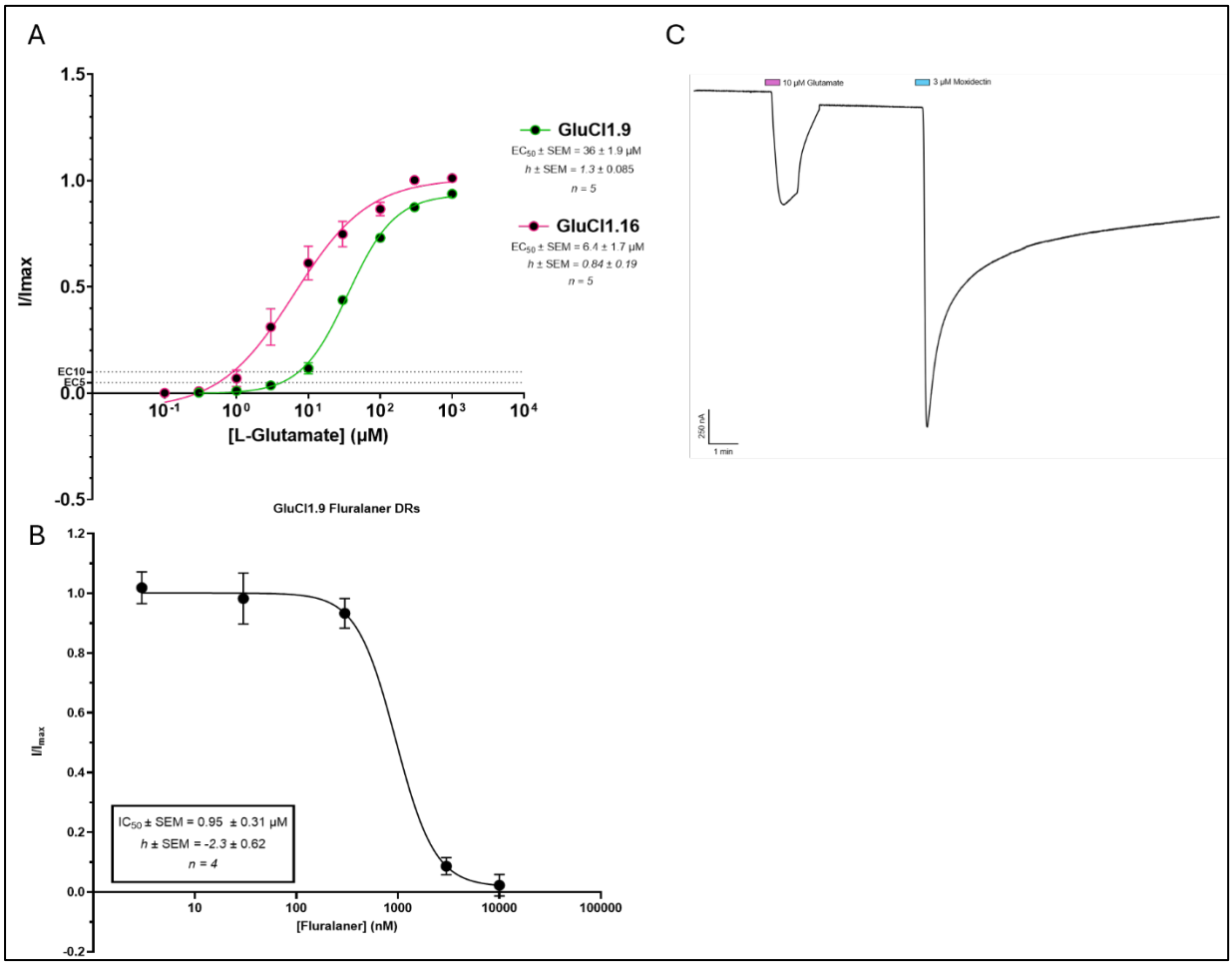

Figure 10. Functional characterisation of ssGluCl1. A) Glutamate concentration dependent ion currents mediated by GluCl 1.9 (green) and GluCl 1.16 (pink). B) Fluralaner concentration-dependent inhibition of glutamate gated ion currents mediated by GluCl 1.9. C) Sample current trace comparing the activity of glutamate (pink) and moxidectin (blue) at GluCl1.9. Moxidectin generates a rapid response, followed by desensitization and very slow if any washout from the oocyte.

6.4 Discussion and Implications

We have provided a preliminary characterization of two of the scabies mite LGICs, GluCl1.9 and GluCl1.16. Further characterization of GluCl1.16 is required to better understand the rapid desensitization process and how best to study the inhibitory actions of potential toxins. Additional variants of the Rdl LGICs have been identified in Aim 3A, and these will be tested in the very near future. We will test related inhibitors and also a series of irreversible agonists such as ivermectin and selamectin.

This work is highly relevant to scabies mite biology, representing only the second scabies mite ligand gated chloride channels to be characterised (Mounsey et al., 2007). Glutamate gated chloride channels have long been recognised as the key targets of many important insecticides and miticides and are a priority area for investigations of drug resistance, as ivermectin and moxidectin resistance is associated with mutations in GluCl genes. Researchers have been working for 20 years to complete this work, so these outcomes, while still preliminary, are highly

significant. Furthermore, although moxidectin is known to act on GluCls, its mode of action and key differences with ivermectin remain poorly characterised (Prichard et al., 2012), so these results are of broad significance to ion-channel biology.

7. Ecotoxicity of moxidectin and fluralaner to relevant freshwater macroinvertebrates

7.1 Background and objectives

Moxidectin and fluralaner are effective treatments for sarcoptic mange in wombats; however, both compounds are known to be toxic to aquatic organisms (Brumley, 2024). Given that many wombats inhabit areas near rivers and streams, there is potential for these chemicals to enter freshwater systems and pose risks to aquatic life. At present, the concentrations of moxidectin and fluralaner in waterways adjacent to treatment zones are unknown. Furthermore, existing ecotoxicological studies have not assessed their effects on stream macroinvertebrates- organisms that are predominantly insect species and are particularly sensitive to a wide range of anthropogenic stressors. The potential impacts of these chemicals on Australian freshwater species have not been evaluated. Treatment formulations typically involve combining the active ingredients (moxidectin or fluralaner) with various excipients or carriers, which differ in volume and concentration. These additional substances may influence the toxicity profile of the active ingredients or exert independent biological effects. In some cases, wildlife carers administering fluralaner (Bravecto) to wombats use supplementary carrier formulations to increase the volume of application (Wilkinson et al., 2021). One such carrier is Orange Power, a commercial product whose ecological effects, including potential interactions with the active ingredients, have not been scientifically assessed.

In this component of the project, we assess the relative toxicity of two chemicals used to control scabies mite in wombats to non-target freshwater macroinvertebrates.

7.2 Methods

We tested the toxicity to stream macroinvertebrate species formulations of moxidectin (Cydectin) and fluralaner (Bravecto), commonly used to treat wombat mange. These species were collected from two streams (Figure 11): the Cotter River

at Vanities Crossing (35°20'46.4"S 148°53'23.7"E) or Tidbinbilla Creek at Paddy River Road (35°25'37"S 148°56'39"E) in the upper Murrumbidgee River Catchment. These streams have healthy macroinvertebrate communities and are without obvious sources of pollution. Thus, the organisms collected are expected to have little, or no, prior exposure or evolved tolerance to moxidectin, fluralaner or similar chemicals. For each taxa studied, we estimated the lethal concentrations of Cydectin or Bravecto dissolved in water with 0.02% (v/v) Orange Power for 50% of the test population (LC50). While Orange Power is generally only used with Bravecto, we used it to dissolve both formulations so that toxicity results from each were comparable.

To summarise the 72-hour LC50 values estimated for Cydectin we produced a species sensitivity distribution (SSD). SSDs allow extrapolation beyond the species tested, assuming that the statistical distribution of toxicity estimates of untested species is the same as tested species. SSDs allow estimates of the concentration of a chemical which will not exceed concentrations resulting in toxicity of a given percentage of species, e.g. 95%, referred to as the protective concentration for 95% of species (PC95). Due to the presence of both right

censored (e.g. > 10 µg/L) and interval censored (between 1-10 µg/L) LC50 values, we used MOSICSSD (https://lbbe-shiny.univ-lyon1.fr/mosaic-ssd/) to produce Cydectin's SSD as MOSICSSD incorporates information from censored data.

Figure 11. Collecting stream macroinvertebrates for toxicity testing. Photo by Maddy Knight.

7.3 Outcomes

As some taxa had poor survival, even in control conditions, beyond 72 hours, we estimated these LC50 values over 72 hours of exposure, to have a consistent exposure period across all taxa. We do, however, have data to calculate LC50 values at 96 hours and 122 hours of exposure for some taxa studied. LC50 values were estimated using logistical regression using either untransformed or log10 transformation concentrations (whatever had the best model fit). The exception to this was in three cases, where no model could be fitted. In two cases there was >50% survival at the highest concentration tested, so the LC50 was regarded as greater than the highest concentrations tested, referred to as "right censored". In the remaining case there was high survival at the second highest concentration tested and < 50% survival in the highest concentration, the LC50 was regarded as being between these two concentrations, referred to as "interval censored".

Values of 72h LC50 ranged from 0.18 to 12 µg moxidectin/L for Cydectin and 2.8 to >100 µg fluralaner/L for Bravecto (Table 4). Where the same taxa were tested to both formulations, expressed in terms of the active ingredient, **Bravecto had a higher 72h LC50 implying lower toxicity compared to Cydectin**. However, as only three taxa were tested to both formulations, this implication should be treated as preliminary.

Table 4. Lethal concentrations of Cydectin or Bravecto for 50% of the test population (LC50) over 72-hours of exposure, expressed in µg active ingredient /L.

Chemical	Taxa	Order	72h LC50 (µg active ingredient /L)
Cydectin	Baetidae	Ephemeroptera	0.189
	Calocidae	Trichoptera	1-10
	Crambidae	Lepidoptera	1.326
	Gomphidae	Odonata	9.999
	Leptoceridae	Trichoptera	1.645
	Leptophlebiidae	Ephemeroptera	0.182
	Notonectidae	Hemiptera	12.261
	Spercheidae	Coleoptera	>10
Bravecto	Baetidae & Leptophlebiidae	Ephemeroptera	2.857
	Gomphidae	Odondata	>100

SSDs of Cydectin fitted with both log-normal and log-logistic distributions were very similar (Figure 12) and produced very similar protective concentrations (PC, Table 5). The median estimate of the PC that will not exceed the 72-hour LC50 of 95% of species or PC95 was about **0.1 µg moxidectin /L** (Table 5). A concentration of about **2.3-2.4 µg moxidectin /L** of and associated Orange Power would **exceed the 72-hour LC50 of 50% of species**.

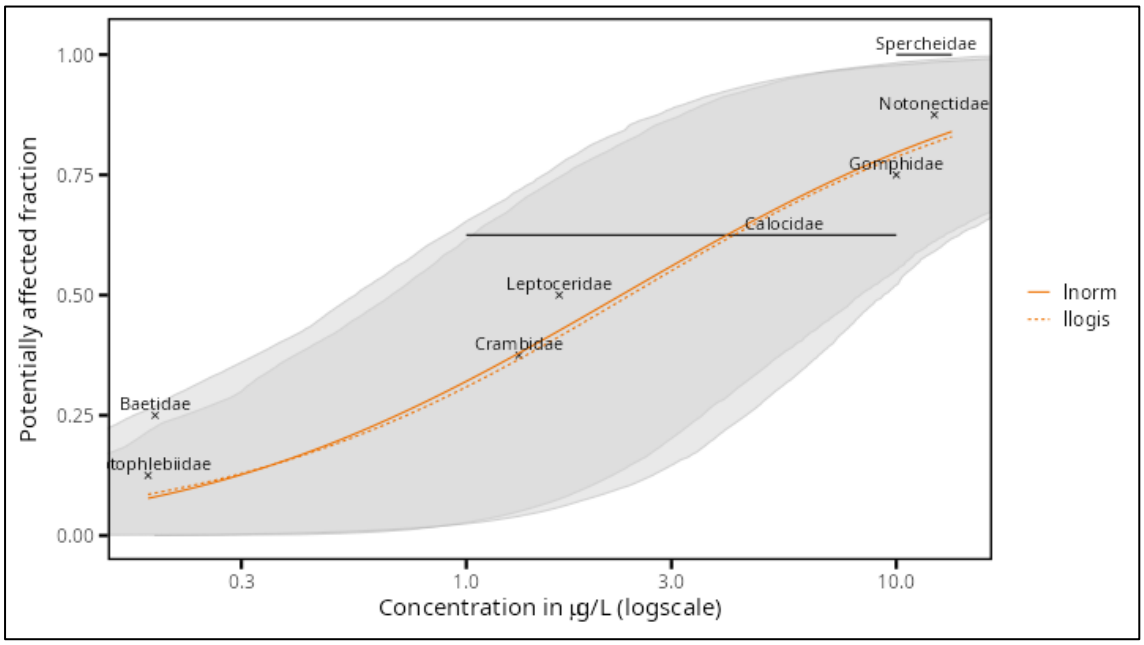

Figure 12. Species sensitivity distribution (SSD) of stream macroinvertebrates of 72-hour LC50 values for Cydectin dissolved in water with 0.02% (v/v) Orange Power) estimated using MOSICSSD (https://lbbe-shiny.univ-lyon1.fr/mosaic-ssd/). Concentration is in µg moxidectin /L. Solid red line is the SSD fitted with a log-normal distribution (lnorm) and the dotted red line the SSD with a log-logistic distribution (llogis), the horizonal black lines represent taxa with censored LC50 values. The grey shadings show the 95% confidence intervals (CI) of the SSDs fitted with the two distributions.

Table 5. Protective concentrations (PC, µg moxidectin /L) of Cydectin dissolved in water with 0.02% (v/v) Orange Power for 99%, 95%, 90% and 50% of species estimated from the species sensitivity distribution using both the log-normal and log-logistic distributions (SSD, Figure 12).

	Distribution			
	Log-normal		Log-logistic	
	Median (µg/L)	95% CI	Median (µg/L)	95% CI
PC_{99}	0.04	0.01-0.68	0.02	0.00-0.59
PC_{95}	0.12	0.04-1.32	0.10	0.02-1.50
PC_{90}	0.23	0.07-1.91	0.22	0.05-2.33
PC_{80}	0.51	0.17-2.98	0.53	0.13-3.76
PC_{50}	2.29	0.67-8.24	2.4	0.55-9.33

7.4 Discussion and implications

This study presents the first known data on the toxicity of fluralaner to aquatic invertebrates. Preliminary results suggest that fluralaner is less toxic to stream macroinvertebrates than moxidectin. However, toxicity alone is not sufficient to determine the preferred treatment option. Environmental persistence is also a critical factor. A desktop review by Wildlife Health Australia (WHA) found that moxidectin degrades more rapidly in soil and water than fluralaner and has a shorter persistence in wombats (Brumley, 2024). This suggests that, overall, environmental concentrations of moxidectin may be lower. However, Cydectin is often applied in volumes exceeding 100 mL, compared to less than 10 mL for Bravecto. As a result, higher runoff volumes may offset the lower persistence of moxidectin, meaning Bravecto may not necessarily pose less risk to aquatic invertebrates in real-world conditions.

To protect freshwater ecosystems, the Australian and New Zealand Guidelines for Fresh and Marine Water Quality recommend using PC99 and PC95 values for high conservation and slightly-to-moderately disturbed systems, respectively (Warne et al., 2018). These values should be based on chronic toxicity data or converted acute data. Due to the absence of chronic data, guideline values for moxidectin cannot be formally derived. However, based on available evidence and the greater impact of chronic exposure, provisional thresholds are proposed:

High conservation areas: <0.02–0.04 µg moxidectin/L

Slightly-to-moderately disturbed areas: <0.1 µg moxidectin/L

It is important to interpret these thresholds cautiously. There is strong evidence that chemical pollutants can affect natural ecosystems at concentrations lower than those detected in standard toxicity tests. Therefore, actual ecological impacts may occur at lower concentrations than those reported here.

Recommended Next Steps

To better understand the environmental risks of mange treatments, two key actions are recommended:

1. **Field Monitoring**

Measure concentrations of moxidectin and fluralaner (and their breakdown products) in streams and freshwater habitats within catchments where these treatments are heavily used. Due to the episodic nature of runoff, especially

following rainfall, both passive and event-triggered sampling methods should be employed to capture peak concentrations.

2. **Mesocosm Experiments**

Conduct controlled experiments in semi-natural environments (mesocosms) to assess the full ecological effects of Cydectin and Bravecto. These studies should evaluate both acute and chronic toxicity, as well as indirect effects mediated through ecological interactions (e.g. predation, competition). Mesocosms provide a more realistic assessment of chemical impacts than laboratory tests, accounting for environmental variability and additional stressors.

8. Key findings and future directions

This report evaluates the effectiveness, potential drug resistance, and environmental impacts of treatments for sarcoptic mange in bare-nosed wombats.

Key Findings

- **Bravecto is effective**: A single dose of Bravecto (45–85 mg/kg) successfully treated mange in many wombats. Wombats with severe mange often showed improvement after just one treatment, others required multiple doses. High mortality rates were still observed due to secondary infections and poor body condition, despite successful elimination of mites.
- **Orange Power shows promise**: This citrus-based product, used to dilute Bravecto for easier application, was found to kill mites effectively and may improve drug delivery through thick skin crusts. More work would be required to determine safety and pharmacokinetics before recommending its more widespread use and regulatory approvals.
- **Bravecto and Cydectin were both highly effective in vitro**. Laboratory tests confirmed that both treatments rapidly kill *S. scabiei* mites. These results were consistent across mites collected from both laboratory models and wild wombats. Functional characterisation of the glutamate gated chloride channel from *S. scabiei* showed it to be a target for both fluralaner and moxidectin.
- **Drug resistance not yet evident**: Genetic analysis of mange mites showed low variation in drug target genes. Notably however, some mutations were found that may affect receptor function or treatment effectiveness, which will be studied further in our functional expression system.
- **Environmental risks exist**: Both Bravecto and Cydectin are toxic to aquatic insects. Cydectin was more toxic in lab tests, but Bravecto may persist longer in the environment. The use of large volumes of Cydectin increases the risk of runoff into waterways.

Recommendations

1. **Adjunctive care**: Severely affected wombats may benefit from additional treatments to manage infections and inflammation. Improved diagnostic methods to monitor for systemic inflammation/sepsis like presentation may help carers decide whether a wombat may recover, or if euthanasia is a more humane alternative.

2. **Environmental monitoring**: Measure drug levels in streams near treatment areas to assess real-world impacts. Conduct controlled outdoor experiments to better understand ecological effects of mange treatments.
3. **Further genetic and functional analysis**: Continue genetic monitoring of mites and test new drug formulations and sequence variants via functional expression.

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