AWI PROJECT NO: ON-00491

SHEEP BLOWFLY RESISTANCE UPDATE

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SUMMARY
The impact of insecticide resistance on flystrike is usually a reduction in the protection period provided by treatments rather than complete control failure. The blowfly that initiates most flystrike in Australia is *Lucilia cuprina* which has developed widespread, high-level, stable resistances to insecticides like the organophosphates. To provide up to date information on the presence of resistance, the level of resistance and its distribution across the sheep producing areas of Australia, this project investigated six insecticides registered for flystrike control. The six insecticides included 1) diazinon[a], which is representative of the Organophosphate group; 2) ivermectin[b] a macrocyclic lactone (ML); 3) spinosad[c] a spinosyns; 4) imidacloprid[d] a noenicitinoids; 5) cyromazine[e] a triazine and 6) dicyclanil[f] a pyrimidine derivative, the final two belonging to the IGR group.

This study provides an update on previous studies which did not detect resistance to spinosad (2002) or ivermectin (2002) but did find cases of low-level resistance to dicyclanil and cyromazine (2012-14) [(1)]. Also, initial data were collected on imidacloprid, which has only recently been released for flystrike prevention. Spinosad, ivermectin and imidacloprid are also used to control the sheep biting louse and incidental exposure of the blowfly could increase selection pressure and provide additional opportunity for the development of resistance.

While individual submitters received the resistance profile for their property, this project identified the need for, and informed on the development of, an integrated resistance management plan for flystrike control across Australia.

Example products: [a] Coopers Diazinon™ [b] Coopers Blowfly and Lice™; [c] Extinosad™; [d] Avenge + Fly™; [e] Vetrazin™; [f] CLiK™

METHODOLOGY
Approximately 455 maggot collection kits were distributed, upon request, across Australia. To date 122 submissions have been received of which 101 were *Lucilia cuprina* maggots that survived transport and were reared for testing. To date, the resistance profiles of 87 viable submissions have been reported back to their submitters. These were from NSW (n=42), Western Australia (n=21), South Australia (n=12), Victoria (n=11) and Tasmania (n=1). There have not been any submissions from Queensland (see Figure 1).

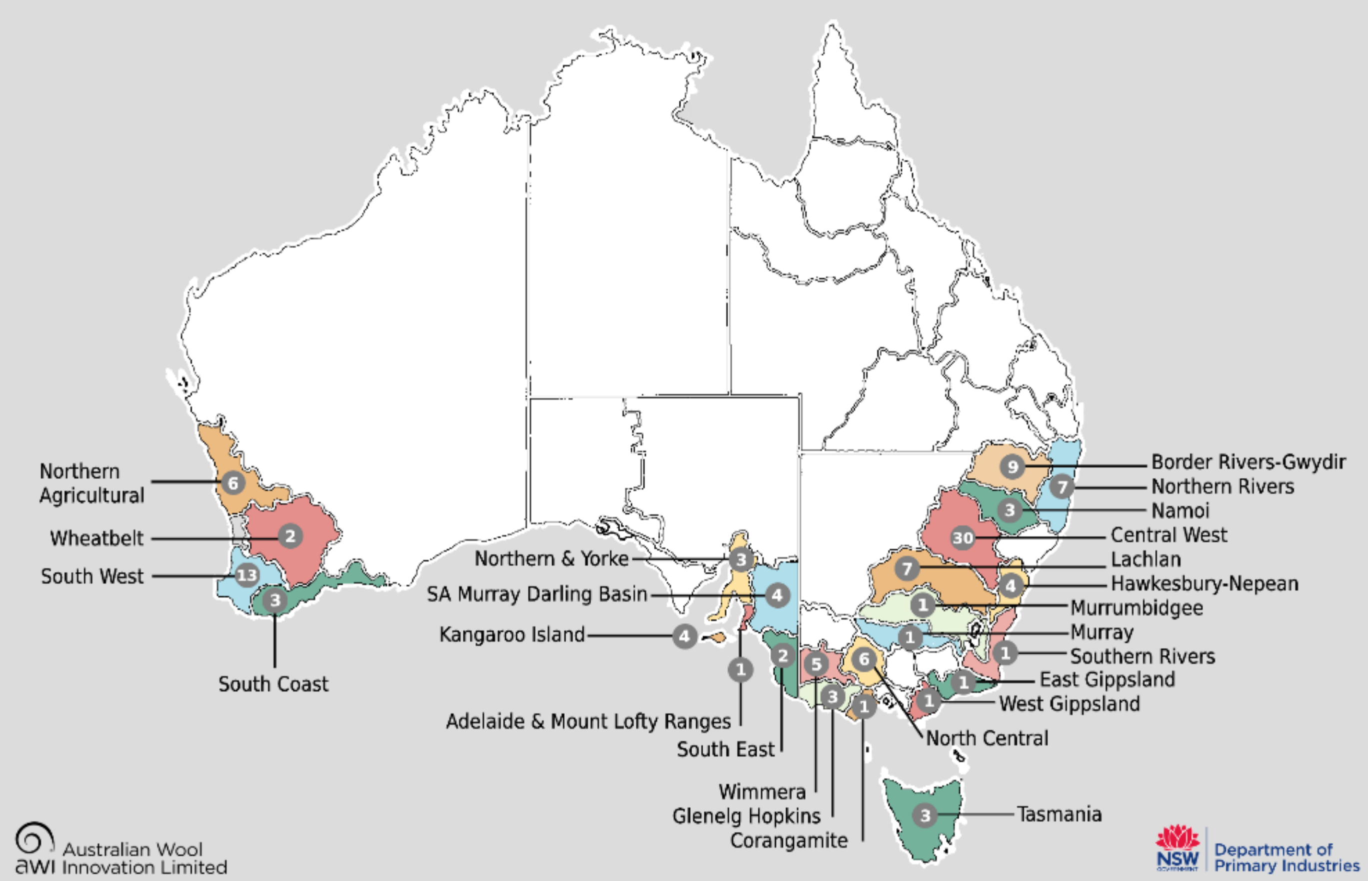

Figure 1. Number of maggot submissions by region.

FINDINGS

To enable comparison between submitted blowfly strains for each insecticide, a resistance ratio (RR) was calculated for each strain relative to a susceptible reference strain. Here we report the project findings to date on 87 completed submissions. Analysis of the final data set will be required to determine the statistical significance.

1. Regardless of the state of origin, and despite decreased use of OPs for many years, all submissions were resistant to the OP diazinon as expected. A strain from Tasmania had the minimum RR of 8-fold while the maximum RR of 51-fold was a strain from NSW. Comparison of these RR ranges with those determined in 1985 (5 to 60-fold)[(2)] provide further evidence of the stabilisation of OP resistance.
2. A significant increase in the range of response of blowfly strains to ivermectin has occurred since 2002[(3)] when RR's ranged from 0.6- to 2.8-fold (n=74) while this study determined RR's ranging from 1.3-fold to 9.2-fold.
3. The response of current NSW submissions to spinosad (n=42) with those reported in 2002[(4)] show the range of RR's has also increased from 0.2- to 2.6-fold in 2002 (n=31) to 0.4- to 4.6-fold in 2020.
4. For many years imidacloprid has been used for lice control but only since 2019 for flystrike. Base line data is usually collected prior to extensive use of an insecticide. In the absence of base line data on imidacloprid, this study reports the current range of response which cannot be assumed to be a susceptible range. The range of RRs is large, being from 3.2-fold (Tas) to 42.5-fold (Vic). Further categorization is required but this data will act as a benchmark for the future.
5. Low level resistance to cyromazine (without dicyclanil resistance) was found in 48% of strains from WA (n=21), 33% from South Australia (n=12) and 9% from Victoria (n=11). The percentage of cyromazine only resistant maggots ranged from "present but below 1%" to 73%.
6. To date 100% of submissions from NSW have concurrent dicyclanil and cyromazine resistance (n=42). This is not the case for other states with only 29% from WA (n=21), 25% from South Australia (n=12) and 82% from Victoria (n=11). In individual strains the percentage of dicyclanil resistant maggots ranged from 2% to 93%.

The high prevalence of resistance to dicyclanil and cyromazine in NSW submissions (100%) prompted a trial which determined the % reduction in protection provided by currently marketed products against strike by highly dicyclanil resistant maggots. A 50mg/L dicyclanil spray-on product had protection reduced by 78% while the 12.5g/l and 65g/l dicyclanil based spray-on products had 73% and 69% reductions respectively. Jetting fluids with cyromazine and ivermectin as actives had protection periods reduced by 50% and 33% respectively. Against maggots which were susceptible to dicyclanil all of the products protected for the periods listed on the product labels.

The ability of dressing products to kill full grown, dicyclanil resistant and dicyclanil susceptible maggots was determined in vitro. The ranking of products from most effective to least effective are as follows:

a) Dicyclanil susceptible: Flystrike Powder > Spinosad aerosol > ivermectin jetting fluid > cyromazine jetting fluid > Diazinon > Propetamphos > Spinosad jetting fluid.
b) Dicyclanil resistant: Spinosad aerosol > Ivermectin jetting fluid > Cyromazine jetting fluid > Flystrike powder > Spinosad jetting fluid > Propetamphos > Diazinon.

KEY MESSAGES

The widespread drought conditions determined the number and location of origin of the maggot submissions we received. The most submissions were received from NSW which all displayed resistance to both cyromazine and dicyclanil. We determined that dicyclanil resistance greatly reduced protection, allowing maggots to form strikes many weeks earlier than claimed by the five products tested. Producers should note that the labels of dicyclanil based products have been modified to claim protection from flystrike “caused by dicyclanil-susceptible strains of blowflies.”. These products performed according to the label claims against dicyclanil susceptible maggots. Under favourable flystrike conditions dicyclanil resistance may govern management practices across the production year. Alternatives to a single treatment with a dicyclanil based product may be required to protect sheep across the spring through to autumn fly seasons. If dicyclanil resistance is on your property, rotate to other insecticide groups. In WA, SA, and Victoria, where there appears to be lower levels and lower frequencies of dicyclanil resistance, producers still have the opportunity to decrease selection pressure. Producers should adopt an integrated resistance management plan based on rotation between insecticide groups to reduce the selection of resistance and protect the effective life of flystrike products.

Spinosad, imidacloprid and ivermectin are also registered to prevent flystrike and belong to different insecticide groups. Products based on these actives provide shorter periods of protection than dicyclanil but can still be used effectively, for example, late in the fly season or prior to crutching or shearing. Use a dressing product from a different insecticide group to the one used to prevent flystrike and also rotate groups if you treat twice in the year. Weigh sheep and follow the label instructions as under-dosing and overdosing provide the opportunity to select for, or increase the levels of, resistance.

There are non-insecticidal flystrike tools like breeding sheep that are less prone to flystrike. Until this process is successful some form of breech modification may be required. By knowing the highest flystrike risk periods on your property, you can strategically time drenching, crutching and shearing so that minimum wool length and/or clean breeches occur at those times. A flystrike calendar can be developed for your property using the FlyBoss Tools which uses data from your closest weather station. An integrated flystrike management plan will provide protection for your flocks when needed, allow other activities such as cropping to be undertaken and ultimately be cost effective.

FURTHER INFORMATION

1) “Resistance Management Strategy for the Australian Sheep Blowfly (*Lucilia cuprina*)” (April 2019) FlyBoss. http://www.flyboss.com.au/sheep-goats/files/pages/treatment/insecticide-resistance/resistance-management-strategies/190415-SHEEP-BLOWFLY-RESISTANCE-MANAGEMENT-STRATEGY-FINAL-GD3349.pdf

2) "A Fly in the ointment" (December 2019) FlyBoss. http://www.flyboss.com.au/sheep-goats/files/pages/treatment/insecticide-resistance/resistance-management-strategies/A-Fly-in-the-Ointment-Managing-Chemical-Resistance-to-Blowflies-20191129.pdf
3) Sales, Suann and Koeford (2020) "Dicyclanil Resistance in the Australian Sheep Blowfly, *Lucilia cuprina*, Substantially Reduces Flystrike Protection by Dicyclanil and Cyromazine Based Products." International Journal for Parasitology: Drugs and Drug Resistance. (Accepted: in press).

ACKNOWLEDGEMENTS
Monica Suann, Blake Brangwin and Kim Koeford.

REFERENCES
(1) Levot G., Resistance to flystrike preventative treatments. AWI Breech Strike R&D Technical Update 2014.
(2) Hughes and Levot (1985) Final Report WRTF Project K/1/1103.
(3) Levot and Sales (2002) Australian Journal of Entomology 41, 75-78.
(4) Levot and Sales (2002) Australian Journal of Entomology 41, 79-81.

Published by Australian Wool Innovation Limited, Level 6, 68 Harrington Street, THE ROCKS, NSW, 2000

ADDENDUM TO AWI PROJECT NO: ON-00491 Sheep Blowfly Resistance Update

AUTHOR

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COMMENT

AWI has funded a series of blowfly resistance research with NSWDPI which is described in past published papers. Points about the NSWDPI methodology covering a larval implant technique, and the production and use of pure-breeding blowfly strains, as quoted directly from these published papers, is presented in this addendum. It is hoped these quotes provide some context to assist the reader in drawing conclusions from the current work. The preceding work, including the "Nimmitabel strain" and the "Nimmitabel Selected (NS) strain" was published as:

- Levot GW. Response to laboratory selection with cyromazine and susceptibility to alternative insecticides in sheep blowfly larvae from the New South Wales Monaro. *Aust Vet J* 2013; 91:61-64.

- Levot GW, Langfield BJ, Aiken DJ. Survival advantage of cyromazine-resistant sheep blowfly larvae on dicyclanil- and cyromazine-treated Merinos. *Aust Vet J* 2014; 92:421-426.

<u>The larval implant technique</u>

This research technique for indicating resistance is when larvae, from the pure-breeding blowfly strains being studied, were implanted on the skin of research sheep, in 7 months wool, which were treated with chemical products.

"The larval implant technique aims to control all variables other than the effect of the insecticide treatment, but is not perfect."

"Whereas hand-jetting places insecticide onto the skin where blowfly larvae feed, spray-on products that are deposited onto the tip of the wool staple rely on formulation characteristics and rainfall to move the chemical to the skin level. Until this translocation of insecticide occurs, the larval implant technique, which places larvae at skin level, could produce erroneous results because the larvae would not contact the insecticide."

"Similarly, there is a greater likelihood of sub-optimal application with low-dose spray-on products than with the high volumes of jetting solutions."

"These factors may explain the anomalous strikes recorded on single sheep in the spray-on cyromazine and dicyclanil groups at 5 and 8 weeks, respectively. The larval implant technique aims to control all variables other than the effect of the insecticide treatment but is not perfect. Probably because of inadequate wetting of the fleeces, the F2011 controls failed at week 11 and although unfortunate, this single-point anomaly does not detract from the overall trend of the NS strain forming strikes sooner after treatment than the F2011 strain."

"With regards to the larval implant model, Hughes and Shanahan considered an implant to be positive if even a single feeding larva was present at the 24 hour post treatment inspection and a treatment to have failed when two sheep of the five sheep in a treatment group sustained strikes. This seems a very conservative approach, but by these criteria, the spray-on and liquid cyromazine formulations failed to protect sheep from strike by the NS (Cyromazine resistant) strain by 8 weeks after treatment. Protection for <8weeks is considerably shorter than the 11 (Vetrazin Spray-on) and 14 weeks (Vetrazin Liquid) claimed on the label of these and similar generic products

and demonstrates how sustained selection pressure on low-level resistant field populations could curtail the protection offered by cyromazine based products".

Pure-breeding blowfly strains

The larvae from the pure-breeding blowfly strains for the larval implant technique were bred by the researcher from the suspected resistant maggots which made up 4% of maggots originally collected by the farmer. The researcher reared each of generation of the maggots on known doses of chemical and bred them through 13 lifecycles to ensure they are "pure-bred" for resistance.

"By rearing the original Nimmitabel larvae on homogenized liver containing the susceptible discriminating concentration of cyromazine, a pure-breeding strain comprising only the 4% resistant individuals from the original population was created in the laboratory."

"The original cyromazine-resistant 'Nimmitabel' strain was reared for 13 generations on homogenized liver containing cyromazine at a concentration lethal to susceptible larvae."

"The 'Nimmitabel' strain responded to laboratory selection by becoming more resistant to cyromazine (8x) and to dicyclanil (3x)."

"The blowflies used for the implants were a composite strain called 'F2011' comprising the descendents of field-collected blowflies that were susceptible to cyromazine and the pure-breeding, cyromazine-resistant NS strain."

CONCLUSIONS

Inferences from findings in a research setting were the foundation of general messages to sheep producers on the potential for resistance emergence and development and its management.

"Resistance, even in the pure-breeding resistant strain, was not so severe as to cause treatment failure with cyromazine or dicyclanil, but was sufficient to reduce the protection period provided. It is recommended that producers adopt management practices that minimize the development of resistance to these and other compounds."

"As far as we know, populations as resistant as the NS strain do not currently exist in the field. However, such populations must be considered a possible consequence of prolonged or frequent exposure of low-level resistant larvae to concentrations of cyromazine or dicyclanil that differentially favour survival of resistant phenotypes over susceptible types."

"Whether the survival advantage possessed by the NS strain poses a real risk on-farm, however, depends on whether resistant blowflies and flystrike-susceptible sheep are likely to coexist during the critical intervals when resistant larvae can survive on treated sheep but susceptible types cannot."

"Considering the demonstrated reduction in flystrike protection provided by these insecticides against the NS strain, it would be prudent to reduce further selection for resistance by adopting practices that preserve the current levels of susceptibility to these and other compounds. Where possible, these should include fewer insecticide applications, no second treatments with the same or a related compound in the same wool growing cycle, strategic use of non-chemical management strategies such as shearing and crutching, and a longer term move towards plainer bodied sheep that are less susceptible to flystrike and require fewer insecticide treatments."

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