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Prepared by: Peter James[1], April Wardhana[2], Geoff Brown[3] and Rudolf Urech[3]

[1]QAAFI, University of Queensland
[2]Indonesian Research Centre for Veterinary Science, Bogor Indonesia
[3]Dept Agriculture Forestry and Fisheries, Queensland

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Chemical containment and eradication of screw−worm incursions in Australia

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Abstract

The Old World screwworm, *Chrysomya bezziana* (OWS) is one of the most serious exotic pests threatening Australia's livestock industries. The AUSTVETPLAN Screwworm Fly Disease Strategy indicates a plan consisting of containment with chemical treatments and eradication using sterile insect release in the event of an incursion. However, there is no operational OWS sterile insect production facility anywhere in the world and institution of a program would most take at least 2 years. During that time containment of the infestation and protection of animals will be almost totally dependent on effective chemical treatments. This project tested the therapeutic and prophylactic efficacy of Australian-registered chemical formulations against OWS in a series of animal and laboratory studies.

Topical ivermectin, spinosad and chlorfenvinphos/cypermethrin combination were 100% effective in curing OWS strikes. A capsule formulation of ivermectin and spray-on formulation of dicyclanil gave complete protection against the establishment of new strikes for at least 12 weeks, significantly longer than any formulations currently available. All compounds shown to be most effective against OWS are registered for sheep treatment in Australia. Only one is currently registered for use on cattle. Action is urgently needed to enable the rapid deployment of these formulations for use on cattle in the event of an OWS incursion, particularly in light of the significant delay likely before a sterile insect eradication program could be instituted.

Executive summary

Screwworms are obligate, invasive parasites of warm-blooded animals. The female flies lay batches of eggs at the edge of wounds or other lesions. These eggs hatch to larvae or 'screwworms' which feed on affected animals for 6-7 days, burrowing deeply into subcutaneous tissues and causing severe trauma to animals, production loss and potentially death. Susceptible sites include wounds resulting from management practices such as castration, de-horning and ear tagging and lesions caused by the activities of other parasites such as buffalo flies and ticks. The navels of the new born and the vulval region of their mothers following parturition are highly susceptible and body orifices such as nose and ears are also frequent targets for ovipositing screwworm flies.

The Old World screwworm, *Chrysomya bezziana* (OWS) is considered one of the most serious exotic insect pest threatening Australia's livestock industries and is endemic in a number of our closest neighbouring countries. New World screwworm (NWS), *Cochliomyia hominivorax,* endemic to South America, has also entered Australia on at least 2 occasions. Many tropical and subtropical areas of Australia are suitable for the establishment of OWS and the potential range is expected to increase with climate change. The Australian screwworm preparedness strategy indicates a program of containment with chemical treatments followed by eradication of OWS using sterile male release and parasiticides. However, there is no longer an operational OWS sterile insect screwworm facility anywhere in the world and establishing a large scale production facility would most optimistically take at least 2 years. In the interim, containment would be almost totally dependent on the availability of effective chemical controls.

A review of chemical formulations available for potential use against OWS in Australia found that currently only one chemical, ivermectin administered by subcutaneous injection (s.c.) is registered for use against OWS and that many of the chemicals previously shown to be effective against OWS were no longer registered for animal use in Australia.[18] From this review a number of Australian-registered chemicals were recommended as a priority for testing against OWS. The Australian Pesticides and Veterinary Medicines Authority (APVMA) can issue an emergency use permit for use of pesticides if they are registered in Australia for other animal uses and shown to be effective against OWS. This project tested the therapeutic and prophylactic efficacy of chemicals with potential for use in the treatment and control of OWS.

Animal studies
Three experiments were conducted with Javanese thin tail hair sheep. These sheep have a coarse fibre coat and when clipped present a skin surface that approximates that of cattle. As such these animals were considered a good experimental model for both sheep and cattle.

Therapeutic tests: Four chemical treatments were evaluated against 2 day old and 4 day old OWS strikes. The treatments tested were topical ivermectin, a chlorfenvinphos/cypermethrin mixture, aerosol spinosad formulation and a formulation containing propetamphos and eucalyptus oil. The ivermectin, chlorfenvinphos/cypermethrin and aerosol spinosad formulations all gave 100% cure of 2 day old and 4 day old strikes. Larvae in the 4 day old strikes survived treatment with propetamphos/eucalyptus oil in a number of instances. The first three noted compounds are all considered suitable for use in treating strikes in a containment and eradication program. Spinosad has a nil withholding period and may have advantages for use where animals are destined for slaughter or on organically certified properties. The chlorfenvinphos/cypermethrin treatment is registered for application to cattle whereas the ivermectin, spinosad, and propetamphos/eucalyptus oil formulations are currently only registered for application to sheep.

Prophylactic tests: Formulations tested for prophylactic effect against OWS strikes included a s.c. long acting (LA) formulation of ivermectin, s.c. doramectin and abamectin formulations, an aqueous spinosad formulation, ivermectin controlled release capsule and a dicyclanil spray-on formulation. The longevity of protection was tested by implants with first instar OWS larvae at 3 days, 2, 4, 8 and 12 weeks post treatment.

The two formulations giving best effect were the ivermectin capsule and dicyclanil spray-on which both gave 100% protection for the full 12 weeks of the study. The dicyclanil formulation was applied in two overlapping bands along the backline of the sheep. Larvae were implanted within the treated band in one group and outside of the treated area in another group to investigate the protective effect in areas not directly covered by the band. A third group of sheep had the wool clipped before treatment to more closely approximate the skin surface of cattle and had implants made outside the treatment band. Protection was effective for the 12 week period of the study in all of these treatment groups.

At 3 days post treatment ivermectin, doramectin and abamectin s.c. all gave 100% protection. However, at 2 weeks and later times this protection had become incomplete with 50%, 25% and 58% animals struck in the 3 groups respectively at 2 weeks and 83%, 58% 75% respectively struck at 4 weeks. The spinosad dipping formulation did not give complete protection at any time with 75% struck at 3 days, 25% struck at 2 weeks and 83% struck at 4 weeks.

Ivermectin s.c. is currently recommended in the AustVetPlan strategy [1] for prophylaxis and suppression of SWF populations. The results reported here and those of previous studies suggest that ivermectin s.c. is unlikely to provide reliable protection against OWS for more than 2 weeks. The ivermectin controlled release capsule and dicyclanil spray-on both gave significantly longer protection than ivermectin s.c. or any of the other formulations tested in this study and offer significant advantages during a containment and eradication program.

Laboratory studies

The chemical actives and formulations tested in the animal studies, as well as a number of additional chemicals, were evaluated in a series of laboratory studies. These investigations were conducted with both OWS (*Chrysomya bezziana)* and a closely related species, *Chrysomya megacephala*, which is endemic to Australia. The results of the laboratory studies broadly reflected those of the animal studies.

The results of the larval dipping assays, where 3rd instar larvae were immersed in treatment formulations for varying periods of time generally reflected those for the therapeutic sheep studies. The one slight exception to this was ivermectin which gave better results in the live sheep tests than in the laboratory assays. The difference may have been because the larvae were only subject to short topical exposure in the laboratory dipping tests, whereas it is likely that larvae probably also ingested systemically active ivermectin contained in serum when the treatment was applied to sheep.

The spinosad aerosol formulation and the chlorfenvinphos/cypermethrin formulations gave complete and very rapid kill of OWS with no larvae developing to pupae, reflecting the good results seen in the sheep studies. However, the aqueous formulation of spinosad, mixed as directed for treatment of *Lucilia* flystrikes, was not as effective as the aerosol formulation with larvae pupating and developing to adult flies in all 3 time of immersion groups. The difference in efficacy of the two formulations was likely due to differences in concentration, with the aerosol formulation containing 2.8g/kg spinosad compared to only 0.125 g/L spinosad in the aqueous mixture, although differences in formulation may also have been involved.

Dicyclanil and cyromazine are growth regulator compounds and not recommended for treating strikes because live larvae can persist in wounds for extended periods after treatment. For this reason they were not tested as therapeutic agents in the animal studies. However, in the laboratory studies dicyclanil was particularly effective with only a few larvae successfully pupating and none developing to adult flies. Cyromazine was also relatively effective with only a few larvae completing development and emerging as flies in the three tests. In addition to providing good protection against new strikes, dicyclanil treatment may provide insurance against the development of a second generation of flies, an important consideration in a containment and eradication program.

In tests with first instar *C. bezziana* and *C. megacephala* larvae, the three macrocyclic lactone compounds (MLs) tested, ivermectin, doramectin and moxidectin, had LD50s of approximately equivalent magnitude. Doramectin was slightly, but significantly, more effective than ivermectin in the animal studies reported here whereas in other studies moxidectin has been shown to have poor effect against both OWS and NWS. These differences are likely due to different pharmokinetics for the three compounds and underline the importance of animal testing to support the results of laboratory tests, particularly with systemically active compounds. The LD50s of spinosad, chlorfenvinphos and cypermethrin were all significantly higher than for the MLs. Dicyclanil had lower LD50 than the three MLs, but as in studies with other Diptera was approximately 10x more toxic than cyromazine. LD50s for third instar C *megacephala* larvae in feeding tests were usually 4-10x those of first instars confirming a need for higher concentrations of chemical active to kill larger larvae, even when the chemical is incorporated into the feeding medium and larvae are exposed over a prolonged period of time.

Conclusions

Three of the formulations tested for therapeutic efficacy, topical ivermectin, spinosad aerosol and a chlorfenvinphos/cypermethrin mixture showed good effect in animal tests and should provide effective means of treating struck animals in the event of an OWS incursion. Dicyclanil was not tested for therapeutic use in the animal studies but was 100% effective in preventing 3rd instar larvae developing to flies in laboratory tests.

Ivermectin in controlled release capsules and dicyclanil spray on formulation provided protection against OWS strikes for the full 12 week period of testing. This was markedly longer than currently recommended s.c. ivermectin formulation and ~~and~~ also longer than the other s.c. ML formulations tested, which all gave less than 2 weeks protection.

Of the therapeutic and prophylactic formulations found to be most effective, only one, the chlorfenvinphos/cypermethrin mixture, is currently registered for use on cattle. All of the other formulations are currently only registered for use on sheep. A similar ivermectin capsule formulation for cattle has been tested and shown to be effective and although not registered for use in Australia, is registered overseas. This formulation could provide the basis for prophylactic cattle treatments. The use of dicyclanil in cattle will require further studies to develop a suitable application protocol. Pre-emptive actions to facilitate rapid deployment of these compounds for use in the event of a SWF incursion in Australia is urgently needed, particularly in light of the extended time likely before an SIT eradication program could be commenced.

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1 Background

The Old World screwworm, *Chrysomya bezziana* (OWS) is considered to be the most serious exotic insect pest threatening Australia's livestock industries and is endemic in a number of our nearest neighbouring countries. Establishment in Australia would be particularly damaging to the northern cattle industries which are characterised by extensive production systems and low labour inputs. The total cost of the establishment of OWS in Australia was estimated in 1993 as $775M p.a. and could be significantly higher than this at present day costs. Based on 2003 prices, bio-economic modeling indicates that direct producer losses in the northern cattle industry alone would be of the order of $400 million per year if eradication measures were not implemented.

Habitat conditions over much of tropical and subtropical Australia are considered favourable for the establishment of OWS [5,46] and the potential range of OWS is likely to increase with climate change.[46] OWS was first introduced into Iraq, a country with similar climate in many areas to that in Australia, in mid 1996 and strikes assumed epidemic proportions during the winter months of late 1996 and early 1997. Failure to eradicate the infestation has resulted in a permanent population with persisting foci limited by low temperatures during the winter and hot dry conditions in the summer and spreading out to cause major seasonal flare ups when conditions become favourable.[35] This is also the likely pattern if OWS became established in Australia and is very similar to the situation with sheep flystrike, one of the most costly production and welfare issues for sheep production in Australia.[31]

Live screwworm flies or larvae (OWS and NWS) have entered Australia on at least 3 occasions. In 1988, several adult OWS were trapped in an empty livestock vessel moored in Darwin harbour.[26] The vessel had just returned from delivering cattle to Brunei. In 1992, NWS larvae were identified in a lesion on the back of the head of a traveller who had just returned to Australia from a visit to Brazil and Argentina [32] and more recently in 2012 27 NWS larvae, the largest approximately 2 cm in length, were extracted from a lesion behind the ear of a passenger returning to Australia from south America.[48]

Dead OWS have also have been detected on live transport ships in Australia in a number of instances [26] and OWS adults have been found on two occasions in the wheel wells of aircraft travelling from Bombay and landing in Sydney.[26] Insects can survive long periods in the wheel bays of international aircraft and one study found that 93% of caged house flies survived a 9-hour flight from Singapore to Melbourne.[26] It is notable however that OWS has never been detected on Torres Strait islands within Australian territory so introduction by this means ~~of~~ may be less of a risk than it intuitively seems.[6] It is considered that the risk of an incursion has probably increased in recent years with the growing live animal export trade from northern Australia.

1.1 Screwworm biology

Screwworms are obligate parasites of warm-blooded animals, the females laying batches of eggs at the edge of wounds caused accidentally, or through management practices such as castration, de-horning and ear tagging. The navels of the new born and the vulval region of their mothers following parturition are highly susceptible. Body orifices such as nose and ears and lesions made by the activities of other parasites such as buffalo flies, ticks and sheep blowflies are also prime targets for ovipositing screwworm flies (SWF). During the 6-7 days of feeding, the larvae burrow deeply into subcutaneous tissues causing severe trauma to the animal, loss of production, and potentially death.

Australian native fauna have also been shown to be susceptible and humans are occasional hosts.[45] OWS has been found in red kangaroo and agile wallabies in the Malaysian Zoo on wallabies and tree kangaroos in New Guinea and in a number of species of deer.[45] Dogs are

also frequent hosts in Hong Kong.[22] However the degree of susceptibility of most Australian native species is unknown and the welfare and diversity impacts on Australian wildlife is uncertain. The importance of native wildlife and feral animal species as reservoir hosts in any eradication program is also unclear.

New World screwworm (*Cochliomyia hominivorax*) (NWS) and Palaearctic screwworm (*Wohlfahrtia magnifica*) (PSW) are other potential invaders with a similar life habit. These species are distributed mainly in the Americas and northern hemisphere (southern Europe, Russia, the Middle East, North Africa and China) respectively and considered less of a threat. PSW has never been recorded in Australia, but seems to be expanding its range and increasing in importance in a number of countries.[37] As noted earlier live NWS have entered Australia on at least two occasions, both times in human infestations.[32,48] Therefore the possibility of an incursion of other myiasis species should not be discounted.

1.2 Screwworm preparedness strategy

The AUSVETPLAN screwworm preparedness plan indicates a two stage strategy in the event of a screwworm incursion into Australia consisting of (i) containment with chemical treatments and (ii) eradication using sterile male release (SIT) and complementary insecticide applications.[1] However, there is currently no operational OWS SIT production facility anywhere in the world and commissioning of a rearing facility would take at least two years and substantial capital investment. The existence of sibling species within the OWS population[15,49] presents an added potential difficulty as such variation could impair the effectiveness of an SIT response if the production colony was derived from populations incompatible with the invading strain.

The strategic use of insecticides was pivotal to the successful SIT eradication of NWS from the United States, Central America and Libya and, particularly in the absence of an SIT production facility, will be a critical component of any response action in Australia. Effective chemicals will be necessary to treat struck animals and limit livestock losses and to protect stock against new strikes. They will also be critical to the establishment of quarantine barriers to contain an incursion, to enable the movement of livestock to slaughter facilities and to reduce the numbers of sterile males required to effect eradication.

In the event of a screwworm fly incursion into Australia, chemical control products registered for animal application and with known efficacy against SWF but without a specific claim can be approved for use at short notice following application for an Emergency Use (Category 43, off-label permit) from the Australian Pesticides and Veterinary Medicines Authority. However, most chemicals previously shown to be effective against OWS are no longer registered for animal use in Australia [17] and without good efficacy data a treatment would need to be based on “best bet” choices and “learning on the run”. This is a far from satisfactory situation with a major exotic pest

1.3 Chemical controls

A wide range of chemicals has been used to treat screwworm infestations, particularly for NWS in the USA and Central and South America (reviewed in part by Graham 1979[13]; Drummond *et al.* 1988[11]; Spradbery 1994[39]). Studies on chemical control of OWS have been less intense but a range of insecticides has been evaluated [41,44], acaricides[43] the salicylanilide, closantel *per os*[40] and macrocyclic lactones (MLs) [25,42,51]. Many of these products are now not available in Australia and many chemical products studied and found to be effective for control of other screwworm fly species have not been registered for such use in Australia. In particular, coumaphos, which has been described as the ‘work horse’ of insecticide for NWS control and the standard for any new treatments, is no longer registered for animal use in Australia. The only products currently registered for control of screwworm

are based on one chemical, ivermectin and this is only registered for such use in cattle. There are currently no products registered for control of OWS in other animal species.

Insecticide formulations with demonstrated or potential efficacy against SWF can be divided into those that are primarily therapeutic in their action and those that provide extended protection and may be able to fulfil a prophylactic role.

Treatment of strikes: Insecticides that could be used for the treatment of animals with SWF infestations and which are currently registered for use on food animals in Australia include the organophosphates diazinon, chlorfenvinphos and fenthion, macrocyclic lactones, applied topically or systemically, spinosad and possibly some synthetic pyrethroids. Ivermectin administered subcutaneously has been found to be effective against early OWS larvae but may not reliably kill older larvae [42]. Topical application of ivermectin may be more effective against late stage larvae but hasn't been previously tested. Spinosad is a relatively newly registered compound for animal application in Australia that has the attraction of a nil withholding period and which is approved for use on organic properties by a number of certifying bodies. It could also be used to provide short term protection for animals during transport to market.

Prevention of strikes: In many extensive areas re-mustering to monitor and retreat animals will be impractical. Therefore insecticide formulations that can provide extended periods of protection will be required to be practically useful. In addition, chemicals providing extended periods of protection will be important in establishing barriers around eradication areas and minimising the numbers of sterile flies required for eradication.

The list of chemicals shown to provide significant prophylactic effect against OWS is limited to MLs, closantel and zeta-cypermethrin formulated in ear tags. Studies with the currently registered injectable formulations of ivermectin indicated protection periods of approximately 2 weeks[25,42], a pour-on formulation of doramectin protected for 7 days but failed at 14 days, eprinomectin pouron protected for 3 days but failed at 7 days [51]. Zeta-cypermethrin ear tags provided up to four months protection although low level strike was recorded during the later part of this period [47].However, the advisability of using products with a primarily repellent effect during an eradication program and the likely efficacy of the tags in protecting more severe predisposing lesions such as castration wounds has been questioned.[50] These tags could play a significant role in integrated approaches and may be of particular use on dairy enterprises as they have a nil milk withholding period. The dose of closantel required for extended protection against OWS is higher than presently registered for use in sheep and there is a risk of inducing optic neuropathy, particularly in young animals, at these higher rates. [7,12]

A number of more recently registered ML products have shown encouraging results against other myiasis species but are yet to be tested against OWS. These include s.c. doramectin, which gave superior protection to ivermectin in studies against NWS [3,9,23,24,] s.c. abamectin[4,21,23] and the insect growth regulator dicyclanil which provides up to 6 months protection against wool myiasis.[8] Spinosad is also registered for protection against sheep flystrike and presents a useful low residue option. Long acting capsule formulations of ivermectin for cattle, not registered for use in Australia, have given extended periods of protection against screwworm myiasis [19,51] but a similar formulation registered for sheep use in Australia [27] has not been tested. This project tested the prophylactic and therapeutic efficacy of these formulations and makes recommendations about their suitability for use in the event of a SWF incursion.

2 Project objectives

i. Assess the relative efficacy of Australian registered chemicals for potential use in containment and eradication of OWS incursions
ii. Provide data for potential control compounds on attributes such as protection period and efficacy as therapeutic agents, to enable recommendations on the design of optimal programs for the containment and eradication of an incursion prior to, or together with, a sterile male eradication program
iii. Provide efficacy data for chemicals already registered for other animal use in Australia suitable for APVMA to grant emergency use permits for OWS treatment and prevention in the event of an incursion.

3 Methodology

3.1 Preliminary studies

Most of the previous OWS larval implants done by Bbalitvet staff in Bogor had been as part of studies towards a potential vaccine for OWS. The methodology was relatively intensive and included use of a metal ring, glued to the shaved skin of the sheep, with the implant made within the ring. A moistened foam rubber disk was then placed above the implant area within the ring and all covered by a 15cm x 8cm plastic box padded with moistened foam rubber and attached to the sheep using packing tape encircling the sheep's trunk.
This technique was designed for trials where retrieval of all larvae was required. We felt that this method was too artificial to provide a good assessment of likely field efficacy, subjected the sheep to unnecessary stress and that a simpler method was required. With the small numbers of sheep being used (due to animal ethics considerations), it was important to ensure high establishment of screwworm strikes. It was also important to ensure that the local anaesthetic that we planned to use, lignocaine, had no adverse effects on establishment or progress of the myiasis. As the technique was critical to success of the studies we conducted a preliminary study to compare the effectiveness of different implant methods, in particular to determine the importance of covering the implant with a moistened pad in comparison to no covering and to identify any effect of lignocaine on larval survival.

The wool was clipped from the left and right side flank of 6 sheep using small animal clippers. To establish the implant, a small crossed incision (10mm x10mm) was made and approximately 100 newly hatched *C. bezziana* larvae were applied to the centre of the cross. Two implants, one on each side, were made on 4 sheep and 4 implants, 2 on each side, on 2 sheep (Table 1).

As it was considered that failure of implants was most likely to result from dehydration of the implant sites, half of the wounds were covered with a plastic box (approximately 150 x 80 mm) lined with wetted sponge as described for the standard Bbalitvet technique. The boxes were removed after 24 h. The other half of the implants were left uncovered. In addition, two 0.2 ml injections of lignocaine were applied to the incision area in 50% of the wounds. No anaesthetic was used with the other implants (Table 1).

Two treatments were tested, one representative from each of the proposed application methods, aerosol application (spinosad) and spray (chlorfenvinphos/cypermethrin combination). The sprayer was a 1L hand held, hand pump pressure sprayer. Blockade® (chlorfenvinphos/cypermethrin mixture) Extinosad® (aerosol formulation of spinosad) was applied on day 2 to one half of the wounds on 4 sheep and, for animal welfare reasons, (see next section) to all wounds on 2 sheep. The other wounds were treated on day 4. See Tables 1 and 2 below for more detail on apportionment of treatments in this study.

All animals were checked once or twice daily until day five. At the end of the study the wounds were treated with iodine and all sheep given a vitamin supplement (Biosalamine).

3.2 Therapeutic effect

3.2.1 Products tested

Compounds to be tested were drawn from the recommendations of James et al. (2006) on the basis of a number of criteria and included:

- Spinosad: (Extinosad® Aerosol for Wounds (2.8 g/kg spinosad, 0.39 g/kg Chorhexidine digluconate, Elanco Animal Health, West Ryde NSW)
- Ivermectin topical (Coopers Paramax® Multi-purpose Concentrate for Sheep – (0.032 g/L ivermectin, Coopers Animal Health, Bendigo East Vic) 1:500 dilution in water
- Propetamphos and Eucalyptus oil (Mules and Mark® II Blowfly Dressing; 0.5 g/L propetamphos, 150 g/L, Eucalyptus oil, 5 g/L cresol, (Bayer Animal Health Ltd, Pymble NSW)
- Chlorfenvinphos/cypermethin combination (Coopers Blockade® 'S' Cattle Dip and Spray; 1:250 dilution in water (0.1 g/L cypermethrin, 0.552 g/L chlorfenvinphos, Coopers Animal Health, Bendigo East Vic)
- Controls (water spray)

Table 1. Location of implants, lidocaine treatment, plastic box placement and wound observations (day 1) in a preliminary study

Sheep No.	Wound [1] location	Lignocaine	Plastic box	Observation (Day 1)
641	RF	*	*	Many larvae
	LF		*	Many larvae, eggs
643	RF	*	*	Many larvae, light-moderate inflammation
	LF		*	Many larvae, light-moderate inflammation
640	RF	*	*	Many larvae, light-moderate inflammation
	RB		*	Many larvae, large wound
644	RF		*	Many larvae, less inflammation
	RB	*	*	Many larvae, inflammation, larger wound
639	RF	*		Larvae, medium inflammation; sheep not happy
	RB			Many larvae, medium inflammation
	LF			Many larvae
	LB	*		Many larvae
642	RF	*		Many larvae, medium inflammation; sheep not happy
	RB			Many larvae, medium inflammation
	LF			Many larvae, medium inflammation
	LB	*		Many larvae, medium inflammation

[1] R=right; L=left; F=flank; B=back

3.2.2 Sheep and establishment of screwworm strikes

The study was conducted with Javanese thin tail sheep which have a coarse fibre coat that more resembles hair than wool. When clipped, the skin surface resembles that of cattle. As such these sheep were considered a good experimental model for both sheep and cattle. The sheep were purchased at least 3 weeks before the test to allow them to adapt to their new environment and frequent human handling, penned indoors in groups of 6 in 3m x 4m pens and fed a diet of fresh elephant grass with a commercial concentrate supplement.

Sheep were randomly allocated to 5 groups of 5 sheep each for each experiment.
Animals were penned in their treatment groups enabling social interaction, extremely important for calmness in animals with strong flocking instincts. Sheep pellets (Comfeed GT 03), and a vitamin supplement (Biosalamine) were provided daily and the sheep were provided *ad lib* with freshly cut elephant grass (*Pennistum purpureum*).

The day before the administration of larval implants, individual sheep were held in a race by animal handlers and 10 cm x 10 cm areas clipped on each sheep in the intended implant areas using small animal clippers. Two implants were made on each sheep at two day

intervals with the first implant on the left side of each sheep and the second on the right side. On the day of each implant a local anaesthetic (Lignocaine HCL 2%,0.4 ml) was administered subcutaneously at the site of the implant. Sheep were left for 5 minutes or until the area was suitably anaesthetised and then a small crossed incision (10 mm in length for each crossed arm) was made. Approximately 100 newly hatched *C. bezziana* larvae were then carefully implanted into each incision using a camel hair brush. Sheep were carefully monitored and larvae allowed to develop for two days following the second implant to provide strikes containing 2 and 4 day old larvae on the day of treatment. Strikes were treated according to label instructions for the treatment of *L. cuprina* myiasis.

All treatments were sprayed directly into the implant area and to wet an area approximately 25 mm on all sides of the implant. Spinosad aerosol formulation was applied directly from the can by a number of short sprays. The other treatments were applied from a hand held pressure sprayer set to deliver a coarse droplet spray with an average of 40 ml per sheep for the ivermectin and chlorfenvinphos/cypermethrin formulations and 54 ml per sheep for the propetamphos/eucalyptus oil formulation.

The implants on all sheep were examined at 4 h, 24 h, 3 days and 5 days post treatment. At each examination, the implant was photographed, a score from 0 to 3 given for larval survival (0 = none dead; 1= some dead; 2 = most dead; and 3 = all dead) and a score from 1 to 3 assigned for appearance of the wound (1- No healing apparent; 2 some healing, wound still exuding; 3 – wound dry, healing commencing)

As all strikes were healthy and developing well, all sheep in the control group were treated by the application of Gusanex® (a screwworm treatment product containing 1% dichlofenthion) and application of an antibiotic powder at 24 hours after implantation.

3.3 Prophylactic efficacy

3.3.1 Products tested

Experiment 1 tested the following treatments:

– Doramectin injection - Dectomax ® Injectable endectocide (10mg/ml doramectin, Pfizer Animal Health Group, East Ryde NSW) administered at 0.1mL/ 5 kg bodyweight delivered by s.c. injection into the shoulder/upper neck.

– Ivermectin - Virbamec® LA Injection Endectocide for Cattle; (10mg/ml ivermectin, Virbac S.A. France) administered at 0.1mL/ 5 kg bodyweight delivered by subcutaneous injection into the shoulder/upper neck.

– Dicyclanil spray - CLiK®; 50g/L dicyclanil, Novartis Animal Health Australasia, North Ryde NSW) delivered as two overlapping 8 mL bands along the backline of each sheep using the recommended CLiK applicator gun; implants made within treated area.

– Dicyclanil - As above, but implants made outside of the treated area.

– Control - untreated

Experiment 2 included:

– Abamectin injection - Genesis™ Injection abamectin antiparasitic for cattle and sheep; (10mg/ml abamectin, Ancare Australia Pty Ltd, Kingsgrove NSW) administered at 0.1mL/ 5 kg bodyweight delivered subcutaneous injection into the shoulder/upper neck

– Spinosad dipping - Extinosad® Lice Fly and Maggot Eliminator (25g/L spinosad, Elanco Animal Health, West Ryde NSW), applied by immersion dipping at the blowfly strike rate of 25 ppm). Sheep were manually immersed in a bath containing the test mixture and

the dipping fluid manually massage into the fleece until each sheep was completely wetted.

– Dicyclanil – CLiK® applied as above, but after all wool on the back had been clipped short.
– Ivermectin slow release capsule - Ivomec® Maximiser Controlled Release Capsules for Weaner sheep (80 mg ivermectin/capsule, Merial Australia Ltd, Parramatta NSW) delivered intraruminally
– Control - untreated

3.3.2 Sheep and establishment of screwworm strikes

The tests were conducted with Javanese thin tail sheep. The sheep were purchased at least 3 weeks before each experiment and housing and management was as described for the therapeutic study.

Six sheep were used per group as recommended by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for assessing the efficacy of ectoparasiticides against myiasis.[17] For logistic and management reasons two separate experiments were conducted, each with four treatment groups and a control (untreated) group. Sheep were allocated to groups balanced for bodyweight for each experiment. This was done by ranking sheep for bodyweight on weights, dividing them to groups on the basis of ranking and then assigning them randomly to treatment within each weight group. The means (range) in body weight for Experiments 1 and 2 were 21.6 (16.0 to 27.6) kg and 16.2 (13.8 to 20.4) kg respectively. The animals were penned in their treatment groups throughout the study.

Two larval implants were conducted on each sheep, one on the left side and one on the right side, by the methods outlined above at 3 days, 2, 4, 8 and 12 weeks after the treatments were applied. All implants were made at sites that were freshly clipped and not previously used for an implant.

Implant sites were inspected and scored for larval viability 24 and 48 hours after the larvae were applied. After the 48 h inspection, in most cases the implant sites were treated with Extinosad to protect against possible new ovipositions and then the sheep checked twice daily until it was considered that the strikes were healed and the sheep were not at risk of further strikes or infection. In a number of cases, particularly in the dicyclanil treated sheep, a few small larvae were still alive at 48 h and treatment was delayed until 72 h to determine if the strike would persist. If the strikes in the control sheep were viable and vigorous (100% of cases) these sheep had larvae physically removed and were treated at 24 h on animal welfare grounds. At each scoring larvae were assessed as 1. No live larvae; 2. Most larvae dead, but a few still alive or, 3. Larvae healthy and strike developed. Healing of the implant area was scored as 1. Healing not apparent; 2. Healing commenced, wound still exuding or, 3. Healing underway, wound dry.

3.4 Laboratory assays

3.4.1 First instar feeding assays

Chemical solutions were made up in acetone (or appropriate solvent) containing 0.2% Triton X-100. Batches of larval rearing liquid (LRL) comprising 300 g whole cattle blood (with sodium citrate 4.5 g/L and kanamycin sulphate 60 mg/L), 30 g low fat milk powder, 30 g whole egg powder, 1 ml formalin and 980 ml water were prepared. 100 µl of the solvent solution was then added to 10 ml LRL in 50 mm x 25 mm diameter glass tubes. These were then held overnight (18 hours) without a lid at 23^{o}C. After 18 hours the

volume of LRL was measured, percent loss calculated and the concentrations of test chemical adjusted for evaporative loss.

Test containers consisted of 15 mm diameter glass tubes (Samco, 50 mm high**,** flat bottom) containing chromatography paper (120 mm x 30 mm, Filtech, 1803C) folded to form a 'concertina' shape. 1.5 ml of the test mixture was added to each 15 mm diameter test tube, with at least three replicates for each concentration. Twenty *C. megacephala* larvae from eggs collected from a laboratory colony and allowed to hatch overnight at 25^{o}C and 70% RH were then added to the paper in each container and the tubes covered with fine mesh gauze held in place by a plastic cap with a 6 mm hole in the centre. Controls with and without solvent were used.

After 24 hours the tubes were inspected, the numbers of live and dead larvae recorded and larval size scored using the scale:

0 = No stunting
1 = Slight stunting
2 = Moderate stunting
3= Severe stunting

3.4.2 Third instar dipping assays

This assay was designed to approximate the therapeutic effectiveness of formulated products against third instar larvae when applied to treat a strike. All formulations tested were mixed according to manufacturer's instructions. The products tested were:

- Spinosad: (Extinosad® Aerosol for Wounds (2.8 g/kg spinosad, 0.39 g/kg Chorhexidine digluconate, Elanco Animal Health, West Ryde NSW); undiluted
- Ivermectin topical (Coopers Paramax® Multi-purpose Concentrate for Sheep – Coopers Animal Health, Bendigo East Vic); 1:500 dilution in water (0.032 g/L ivermectin).
- Propetamphos and Eucalyptus oil (Mules and Mark® II Blowfly Dressing; 0.5 g/L propetamphos, 150g/L, Eucalyptus oil, 5g/L cresol, (Bayer Animal Health Ltd, Pymble NSW); undiluted
- Chlorfenvinphos/cypermethrin combination (Coopers Blockade® 'S' Cattle Dip and Spray; Coopers Animal Health, Bendigo East Vic 1:250 dilution in water (0.1 g/L cypermethrin, 0.552 g/L chlorfenvinphos)
- Dicyclanil (CLiK®; 50g/L dicyclanil, Novartis Animal Health Australasia, North Ryde NSW); undiluted
- Cyromazine – (Vetrazin Liquid Sheep Blowfly Treatment, Novartis Animal Health Australasia, North Ryde NSW); 1:500 dilution in water (1g/L cyromazine)

Batches of 20, 4 or 5 day old *C. megacephala* larvae were collected from a laboratory culture, dried on paper towelling and immersed in test formulation in a 20 ml vial for 10 seconds, 60 seconds or 3 minutes. Extinosad aerosol was collected for this assay by gently spraying 10 ml into a 50 ml beaker to provide enough formulation to completely immerse the larvae. The larvae were then removed and placed onto paper towel for 10 seconds to removes excess chemical before transfer into 520 ml plastic containers (110 mm diameter, 75 mm high) with gauze lids, containing vermiculite to10mm depth in the bottom. There were 3 replicates for each treatment. The containers were then held at 28^{o}C and 70% RH until adult emergence. Observations of larval behaviour were made immediately after treatment and numbers of pupae and ecloded adult flies recorded after 15 days.

3.4.3 Third instar feeding assays

Chemical concentrations and LRL were prepared as described for 1st instar assays with the components mixed by gentle stirring until all were dissolved, except that larval media had 3 g cat litter (Breeders Choice™ Fibre Cycle Pty Ltd) (Recycled paper pellets) added per 15 ml LRL(98). Solvent solution (500 µl) was added to 50 ml LRL(98) and held at 23°C in 70 ml plastic containers (Techno Plas, 40 mm diameter, 53 mm high) overnight (approximately 18 hours). After 18 hours the volume of LRL was measured, percent loss determined and the concentrations of test chemical recalculated to adjust for this loss. Control mixtures with and without solvent were used.

Third instar larvae were obtained from eggs collected from a laboratory colony and reared for four days at 25°C and 70% RH for on cattle liver/heart. Twenty larvae were collected with soft forceps from the rearing container and added to each container. After 24 hours the containers were inspected and all live larvae transferred to new containers of fresh LRL cat litter and the same concentration of test insecticide. These containers were placed uncovered into 500 ml containers with vermiculite covering the base for pupation and sealed with a gauze lid. They were held at 28°C and 70% RH and numbers of pupae and ecloded flies in each container recorded after 15 days.

3.4.4 Egg dipping assays

Egg dipping assays were conducted to test the ovicidal effects of the same formulations assessed in the larval dipping assays (see above).

Freshly deposited *C. megacephala* eggs were collected over 2 hour periods, placed into a 15 ml centrifuge tube, covered with 0.1N NaOH, and gently shaken to loosen eggs from the egg masses. Eggs that settled to the bottom of the tube were collected with a pipette and transferred to a container of clean water. Floating eggs were found to have low viability and were not used in assays.

Batches of 100-200 eggs were pipetted into 15 ml centrifuge tubes, excess water removed by pipette and the eggs dried with paper towelling. The eggs were covered with the test solution for 10 seconds, 1 minute, or 3 minutes and then placed onto a piece of dry filter paper to remove excess chemical. Using a fine brush, 30-40 eggs were gently transferred onto clean black filter paper moistened with 1 ml deionised water The filter paper and eggs were then placed on a layer of moistened Wettex® sponge approximately 6 mm in thickness inside 90 mm x 12 mm height Petri dishes sealed with Parafilm®, and held at 28°C and 70% RH. Numbers of hatched and unhatched eggs were recorded after 24 hours.

4 Results

4.1 Animal studies

4.1.1 Preliminary studies

All screwworm implants established and developed to screwworm strikes, regardless of whether boxes with wetted foam padding were used (8/8 implants developed to strikes) or no boxes were used (8/8 strikes). As there was no apparent advantage from attachment of covering boxes to maintain humidity, uncovered implants were used in the main study. Fly eggs were deposited on one uncovered wound during the initial 48 h of the test. The egg mass was whitish in colour and had the ‘shingle’ configuration characteristic of OWS. These eggs were removed and placed onto rearing media. No eggs hatched and a definitive diagnosis of the species responsible was not possible.

Anaesthesia with lignocaine reduced the reaction of the sheep when making incisions for implants, although there was little reaction from most sheep even when the anaesthetic was not used. As there was no apparent difference in establishment or progression of strikes in the implants made with and without lignocaine, local anaesthesia was used in all instances on animal welfare grounds.

Calibrating the amount of chemical delivered by the aerosol formulation proved difficult because, with the pressure from the spray can and prior removal of wool from near the wound, there was often some fluid splash, particularly from prolonged sprays. Product instructions suggest a 6 second spray to treat a 200 cm^2 area for flystrike. However, screwworm burrow deeply forming wound "pockets" rather than across the surface of the skin as with most *Lucilia* strikes. Therefore the spray was directed as deeply as possible into the strike pockets and crevices in the lesion and onto the surrounding skin. This was usually best achieved by a series of instantaneous sprays. The hand sprayer, with the nozzle set to deliver a coarse droplet spray, proved a satisfactory method of application for the topically delivered aqueous formulation. The spray was directed to wet the larvae within strike pockets, the strike surface and the surrounding wooled area. The average amount of compound used per sheep was estimated from the total volume of product used for all sheep in the group.

Insecticide treatments: Twenty-four hours after treatment the larvae in untreated wounds had grown markedly and inflammation around the wounds was obvious. No live larvae were detected in any of the wounds treated with the spinosad or chlorfenvinphos/cypermethrin formulations.

The 4 day old larvae in treated strikes appeared to move closer to the surface of the wound within minutes of spraying, presumably stimulated by the presence of insecticide. Whether this was due to directed movement away from the insecticide or simply to nervous excitation and random movement of the larvae caused by the treatments is uncertain. On one sheep some larvae exited from the chlorfenvinphos/cypermethrin treated wound and fell to the ground. However, with other similarly treated sheep few larvae exited the wound. Cypermethrin is known to have repellent effects against some insects and may have been responsible for larvae exiting the wound. The exiting larvae were collected and transferred to containers with vermiculite. None of these larvae survived to pupation.

Larvae remained in the spinosad-treated wounds but their behaviour also appeared to change. On day 5, no live larvae were present in any of the treated wounds. At the first inspection the wounds treated with spinosad appeared to have healed better than the chlorfenvinphos/cypermethrin treated wounds on one sheep, but not the other. However, by the following day and at subsequent inspections this difference was no longer noticeable.

Sheep health: Sheep with two implants appeared to be active and feeding normally 24 h after the larval implant. Larvae were active in all wounds and there was some inflammation around the incisions. The two sheep that had four implants were less active than the other sheep, frequently lying down and feeding less often. All strikes were treated at this time and by day 3 post treatment strikes were resolving and all sheep were active and feeding normally. At day 5 all sheep were healing well and their behaviour appeared normal. As a result of these observations two implants were used per sheep in subsequent experiments.

Table 2. Product and day of insecticide treatment and subsequent wound observations on sheep in preliminary studies

Sheep Number	Wound location	Treatment Product	Larvae age	Observation (Day 1 after treatment)	Observation (Day 5 after treatment)
641	RF	C	2	No live larvae	No larvae in wound; healing
	LF	C	4	No live larvae	No larvae in wound; puss
643	RF	S	2	No live larvae	
	LF	E	4	No live larvae	Some dead larvae in wounds; moist
640	RF	C	4	No live larvae	Some dead larvae in wound; no puss
	RB	C	2	No live larvae	
644	RF	S	4	No live larvae	No obvious larvae in wound; some puss
	RB	S	2	No live larvae	
639	RF	C	2	No live larvae	No difference; all wounds healing
	RB	C	2	No live larvae	
	LF	S	2	No live larvae	Bigger wounds
	LB	S	2	No live larvae	Bigger wounds
642	RF	S	2	No live larvae	No obvious differences in healing; dry
	RB	S	2	No live larvae	
	LF	C	2	No live larvae	
	LB	C	2	No live larvae	

C = Chlorfenvinphos/cypermethrin; S = Spinosad

4.2 Therapeutic efficacy

All implants on control sheep developed to viable strikes indicating a consistent larval challenge across treatments. All test treatments were effective in resolving strikes containing 2 day old larvae. No surviving larvae were found on any treated sheep at 24 h after treatment or subsequent inspections and lesions healed rapidly on all animals (Table 3). Fly eggs were found at the 24 hour inspection on one strike in the ivermectin-treated group. These eggs were white in colour and deposited in an oval mass in a ‘shingle’ type formation characteristic of screwworm fly. This animal was closely monitored, but no strike established and no live larvae were seen at any of the subsequent inspections.

Three of the 4 treatments (ivermectin, chlorfenvinphos/cypermethrin and spinosad) gave total resolution of strikes containing 4 day old larvae (Table 4). The barest movement was detected in 2 larvae on one of the spinosad treated sheep. These larvae were removed from the strike to test viability. On closer inspection they were found to be blackened at the posterior end, suggesting septicaemia and when placed onto vermiculite did not show any movement away from light or attempt to burrow or pupate. Two hours later they were confirmed dead, still on the surface of the vermiculite.

Table 3. Two day old strike: Number of live larvae and mean larval score (± s.e.) at different times after treatment

Treat	24 h		3d		5 d	
	Number with live larvae	Mean Larval score	Number with live larvae	Mean Larval score	Number with live larvae	Mean Larval score
Control	5	0	NA	NA	NA	NA
Spinosad	0	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)
Propetamphos/ eucalyptus	0	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)
Chlorfenvinphos/ cypermethrin	0	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)
Ivermectin	0*	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)

Two sheep in the group treated with the propetamphos/eucalyptus oil formulation still had live larvae present at 24 h after treatment in the 4 day old strikes. As these larvae were 5 days old at this inspection and therefore approaching the age at which they could evacuate the wound to pupate at further inspections it would not have been possible to determine whether larvae had left the strike and pupated successfully or had been killed by the treatment, the surviving larvae were removed from the wound and placed on vermiculite to test their viability. The larvae burrowed into the vermiculite and pupated successfully.

Table 4. Four day old strikes: Number of live larvae and mean larval score (± s.e.) at different times after treatment

Treat	24 h		3 d		5 d	
	Number with live larvae	Mean Larval score (±s.e.)	Number with live larvae	Mean Larval score (±s.e.)	Number with live larvae	Mean Larval score (±s.e.)
Control	5	0 (0.0)	NA	NA	0	NA
Spinosad	0[a]	3.00 (0.2)	0	3.00 (0.0)	0	3.00 (0.0)
Propetamphos/ eucalyptus	2	2.40 (0.4)	0 [b]	3.00 (0.0)	1 [b,c]	2.60 (0.4)
Chlorfenvinphos/ cypermethrin	0	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)
Ivermectin	0	3.00 (0.0)	0	3.00 (0.0)	0	3.00 (0.0)

[a] 2 morbid larvae (very slight movement observed) extracted from the wound and found to be partially blackened; soon died, did not pupate.
[b] Eggs deposited on wound
[c] Two egg masses and reinfested with 3rd instar *C. bezziana* larvae

Strikes on the propetamphos/eucalyptus oil treated sheep appeared to heal more slowly than in the other treatments (Figure 2) and eggs with appearance and formation characteristic of *C. bezziana,* were seen on one sheep at the inspection on day 3. These eggs hatched and established a restrike. Third instar larvae were collected from the restruck sheep at day 5 inspection and confirmed as *C. bezziana.*

Healing of strike lesions: The pattern of healing following treatment of 2 day old myiases was similar in all groups and there was little variability between sheep within groups (Figure 1). The healing score for the controls may have been slightly lower than for the propetamphos/eucalyptus oil and spinosad treatments and this was no doubt due to treatment 24 hours later than the other groups. However, there was no significant difference between products in their effect on healing or between the controls and treatment groups at any inspection (P>0.05)

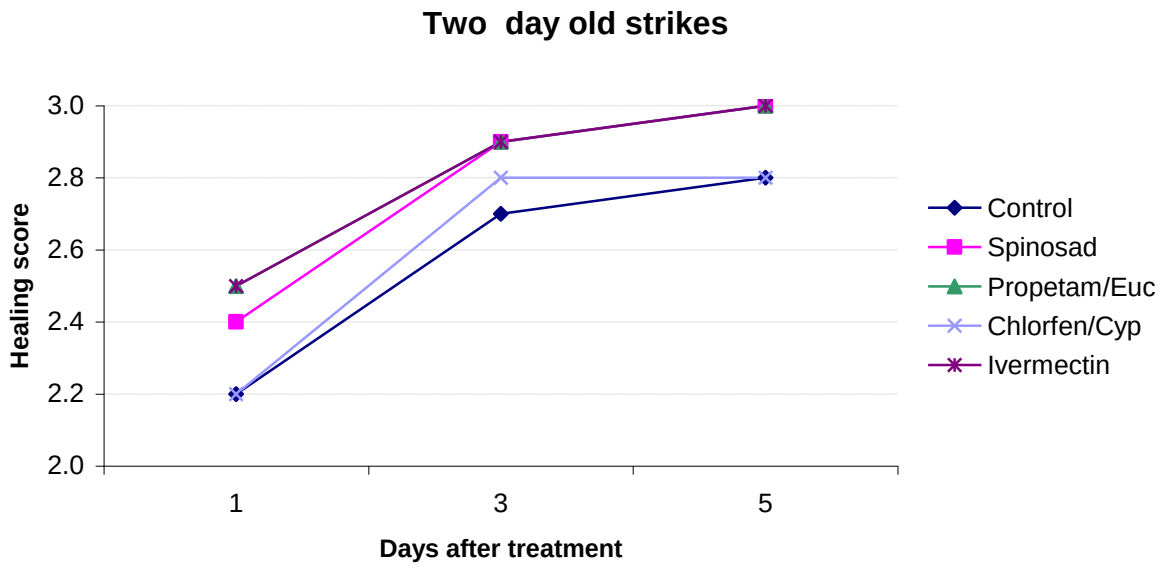


Figure 1. Healing scores for strikes treated with 2 day old larvae

In the four day old strikes, healing was generally quickest in the ivermectin treated group and poorest in the sheep treated with propetamphos/eucalyptus oil formulation (Figure 2). There was no significant difference between any of the treatments for healing scores on day 1 after treatment (P>0.05), but on day 3 the healing score for the ivermectin treated wounds was significantly higher than for all other treatments (P<0.05). There were no other significant differences between treatment groups on day 3. In order to fully assess the effectiveness of the therapeutic formulations we did not physically remove larvae from wounds before treatment. As a result, in a number of instances strikes healed with dead larvae caught in the strike. This did not seem to interfere with resolution of the wound and infection did not occur in any instance. At day 5 there was no significant difference between groups treated with ivermectin, chlorfenvinphos and spinosad, but the healing scores for all of these 3 treatments were significantly higher than for the propetamphos/eucalyptus oil formulation (P<0.05).

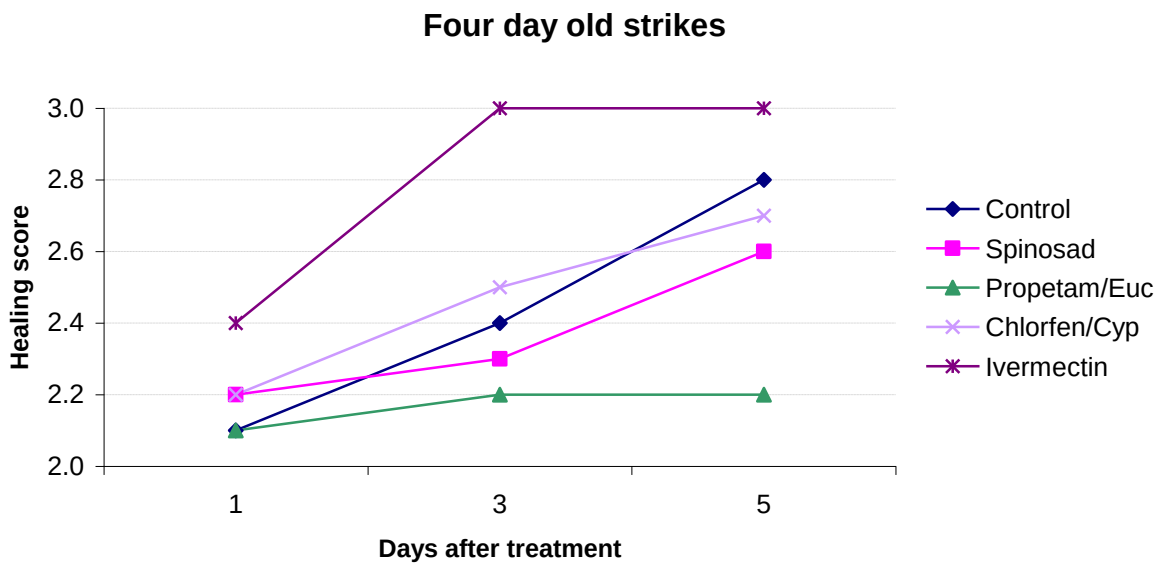


Figure 2. Healing scores for strikes treated with 4 day old larvae

4.3 Prophylactic efficacy

As the number of animals that could be tested was limited by available space, two separate experiments were conducted. All implants developed viable and persisting strikes on all control sheep at each implant date in both experiments suggesting a reliable challenge in both studies.

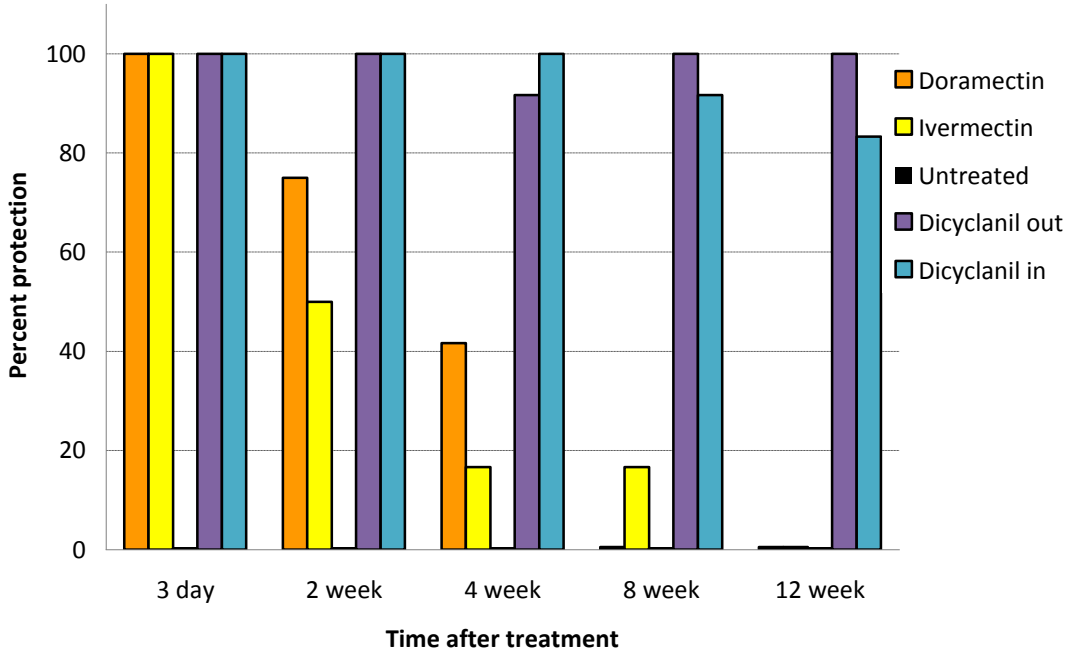
The LA formulation of ivermectin gave complete protection when tested 3 days after application with all larvae dead at the first inspection 24 hours after implantation (Figure 3). However viable strikes developed in 50% of implants at 2 weeks after treatment and by 4 weeks protection had waned even further with strikes developing in 75% of implants. Results for the other two injectable MLs were much the same, with both doramectin and abamectin providing 100% protection at 3 days, but less than 100% protection at later implants. Doramectin appeared to give slightly better protection than ivermectin and abamectin at implants at 2 and 4 weeks, although the difference was small and probably not of practical significance. It is notable that although 100% of strikes occurred in the untreated animals, strike was less than 100% in sheep in the injected ML treatments up to week 8 suggesting that a low level effect against development of strikes may have extended until this time.

In comparison, the ivermectin capsule formulation provided complete protection against development of OWS strikes at all times except for the first implant (Figure 3). When these implants were examined at 24 h after implantation at the 3 day test, most implants contained live larvae. However, by 48 h all larvae were dead in all but one implant. In this study the capsules were administered the day before implants were applied. It is likely that plasma concentrations of ivermectin were still building at this time and were insufficient at 3 days after administration to kill all larvae within 24 h. It is notable that at the last challenge in this group at 12 weeks, when examined at 24 h all implants also contained live larvae. However by 48 h larvae were dead in 11/12 implants and by 72 hours all larvae were dead. This may indicate that the plasma concentrations of ivermectin provided by the capsule were beginning to fall at 12 weeks.

Almost complete protection was also provided in all three dicyclanil-treated groups for the 12 week period of the trial. Live larvae were often present in strikes at 24 hour inspections but most were dead at 48 or 72 h. Even though Figure 3 shows some surviving larvae in

implants inside the dicyclanil treated area in Experiment 1, the surviving larvae in these implants (1 implant at 8 weeks and 2 implants at 12 weeks) were all assessed as score 2 and had moderate stunting. In this experiment follow up inspections were not conducted at 72 h to determine whether or not the larvae would have survived. However, in Experiment 2 when the implants containing score 2 larvae were not treated, but were left and reinspected at 72 h, all larvae were found dead, wounds had dried and healing had commenced. It is likely that if the implants with surviving larvae in Experiment 1 had been left untreated they would have similarly resolved.

(a) Experiment 1



(b) Experiment 2

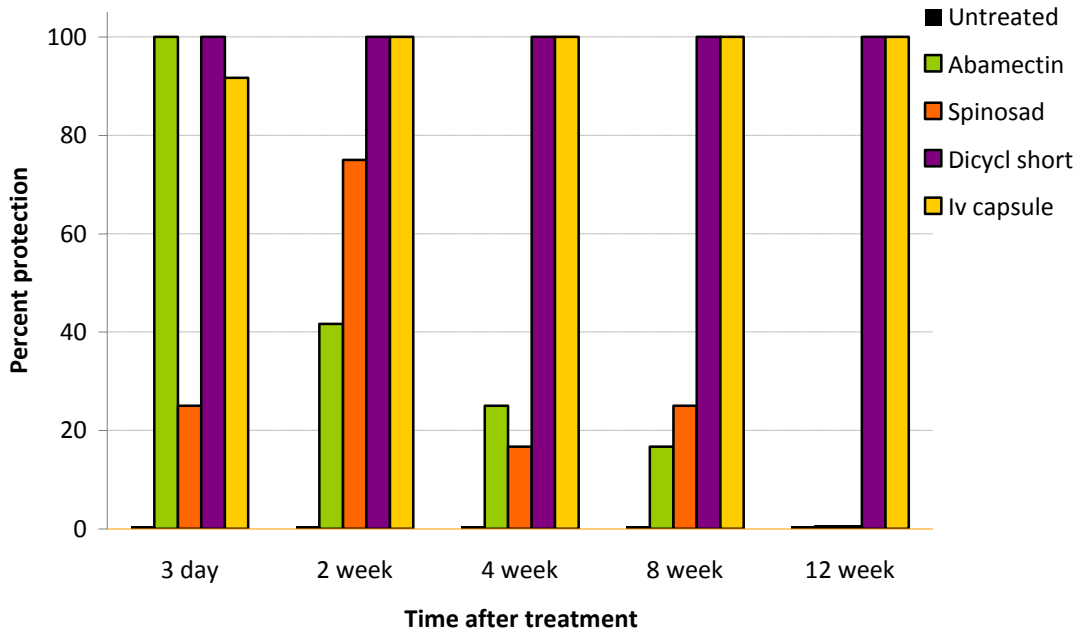


Figure 3. Percent protection against the development of strikes following implantation with 1st instar *C. bezziana* larvae at different times after treatment with a range of prophylactic products

Footnote: In Experiment 1 live larvae in the dicyclanil treatments were score 2 at 48 h and in light of later information are considered unlikely to have survived. In Experiment 2 observations were continued longer after the implant and larvae that were score 2 at 48 h were all dead at 72 h.

Spinosad did not completely prevent the establishment of strikes at any challenge (Figure 3). Even when tested 3 d after treatment only 3/12 implants resolved without further treatment. Interestingly, the effect from spinosad was better at the 2 week test than at 3 days with 9/12 implants failing to establish. In a number of instances where larvae survived in these challenges they appeared to be deep inside the wounds where they were probably able to avoid contact with lethal concentrations of chemical on the skin surface. In one instance the wound was almost closed above the larval mass. Efficacy was lower at subsequent inspections with all larvae dead in only 2/12 and 3/12 implants at 4 and 8 weeks respectively. As it was considered that protection had broken down, no implant was conducted in this group after 8 weeks and the sheep were removed from the study on animal welfare grounds. Where effective spinosad acted rapidly and results did not change between the 24 and 48 h inspections. In all cases, if larvae were not killed within 24 h, they were still present at 48 h and the strike persisted.

4.4 Laboratory studies

4.4.1 First instar assays

Tables 5 and 6 provide estimates of LD values of compounds tested in the study for *C. bezziana* and *C. megacephala* respectively. A number of repeat assays were conducted with most of these compounds. Where model assumptions were met, these data sets were combined and re-analysed to provide the estimates presented.

There was a very close correlation between results achieved with the same compounds in assays against the two fly species ($r=0.98$, $p<0.001$), although values estimated for *C bezziana* were generally slightly lower than for *C. megacephala*. This is likely due, at least in part, to differences in larval sizes, with *C megacephala* larvae generally slightly larger than *C. bezziana*. Degree of stress in the assay system may also have been involved as *C. bezziana* is adapted to the very specific and constant environment within mammalian tissues whereas *C megacephala* larvae breed in a much more variable range of larval habitats.

There was also relatively close association between the results achieved in the laboratory assays and the protection studies with animals, which also used first instar larvae. This not withstanding, many other factors affect efficacy on animals, in particular formulation, application method and host pharmokinetics so that animal studies are also critical to assessing efficacy. This is underlined by results with moxidectin. In assays conducted against *C megacephala* we estimated LD50 as 0.011 which was comparable with or slightly lower than ivermectin and doramectin. However, a previous animal study with a moxidectin pouron formulation indicated little or no protection against OWS.[51]

Although cyromazine was recommended for testing in the review of James et al 2006 it was not included in the animal studies because it has a similar mode of action, but lower toxicity, than dicyclanil.[13] We did however include it in the first instar assays and results are presented in Table 6 for *C. megacephala*. Despite attempts on six occasions, and a number of experiments to improve methodology, assays testing cyromazine against *C bezziana* gave inconsistent results and no reliable estimate of LD values could be obtained for this species. Similar difficulties in estimating reliable LD values for growth regulator compounds in serum based systems have been experienced with *L. cuprina*.[33]

Table 5. Toxicity of insecticides to first instar *C. bezziana*

	LD50 (95% CI)	**LD95 (95% CI)**
Chlorfenvinphos	0.089 (0.084-0.093)	0.14 (0.13-0.15)
Cypermethrin	0.125 (0.092-0.177)	1.70 (0.95-3.98)
Dicyclanil	0.0074 (0.0062-0.0085)	0.02 (0.02-0.04)
doramectin	0.012 (0.010-0.014)	0.03 (0.02-0.04)
Ivermectin	0.019 (0.016-0.023)	0.05 (0.04-0.08)
Spinosad	0.199 (0.151-0.27)	0.86 (0.56-1.73)

Table 6. Toxicity of insecticides to first instar C. megacephala (95% CI in parentheses)

Chemical	**LD50 (95% CI)**	**LD95 (95% CI)**
Chlorfenvinphos	0.125 (0.11-0.16)	0.25 (0.21-0.32)
Cypermethrin	0.17 (0.52-1.0)	0.34 (0.25-0.60)
Dicyclanil	0.014 (0.013-0.016)	0.019 (0.018-0.020)
Doramectin	0.037 (0.031-0.046)	0.071 (0.060-0.092)
Ivermectin	0.024 (0.019-0.035)	0.047 (037-0.074)
Spinosad	0.37 (0.030-0.046)	0.76 (0.63-1.01)
Cyromazine	0.155 (0.13-0.18)	0.245 (0.20-0.33)
Moxidectin	0.011 (0.006-0.018)	0.026 (0.018-0.053)

4.4.2 Third instar dipping assays

This assay approximated treatment of strikes on animals and enabled testing of a wider range of chemicals than was possible in the sheep studies Results for 5 day old *C bezziana and* 4 and 5 day old *C megacephala* larvae exposed to chemical for 10 s, 60 s and 3 min are shown in Figures 3 to 5. The results presented for 4 and 5 day old *C megacephala* larvae are from separate experiments and are not the same larvae assessed at the 2 ages.

Results in these assays generally reflected those for animal studies. The one slight exception to this was for ivermectin which gave better results in the animal tests than in the laboratory assays. The spinosad aerosol formulation and the chlorfenvinophos/cypermethrin formulations gave complete and very rapid kill of OWS with no larvae developing to pupae. The aqueous formulation of spinosad was not as effective as the aerosol formulation with larvae pupating and developing to adult flies in all 3 time of immersion groups. This was likely due to differences in concentration with the aerosol formulation containing 2.8g/kg spinosad compared to only 0.125 g/L in the aqueous formulation. Differences in formulation may also have been involved.

Dicyclanil and cyromazine are growth regulator compounds and were not included in the animal studies of therapeutic activity as they are recommended for their prophylactic effect, not for treatment of strikes. During an eradication campaign it is extremely important that treatment prevents any larvae that exit the wound from pupating and developing to contribute to a new generation of flies. Despite taking a longer time to act and not preventing all larvae from pupating dicyclanil was completely effective in preventing the emergence of flies.

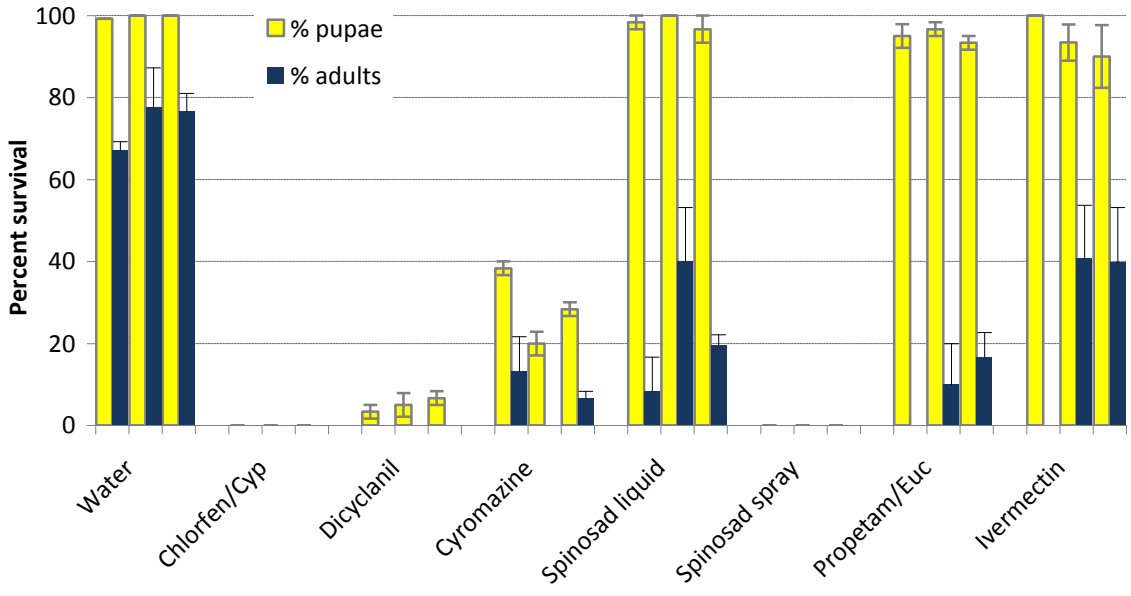
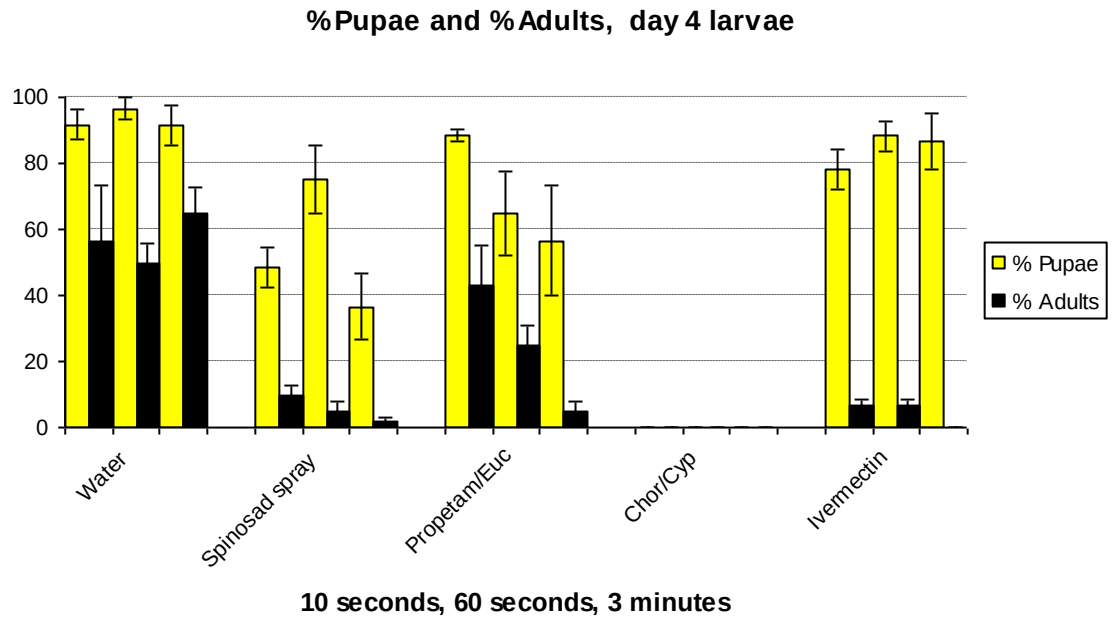


Figure 4. Percent *C. bezziana* larvae developing to pupae and adult flies following immersion in test formulations for different periods of time in laboratory studies. Results for 10 sec, 60 sec and 3 min treatment are given in order within the tick marks for each compound listed on the X axis.)

(a)



(b)

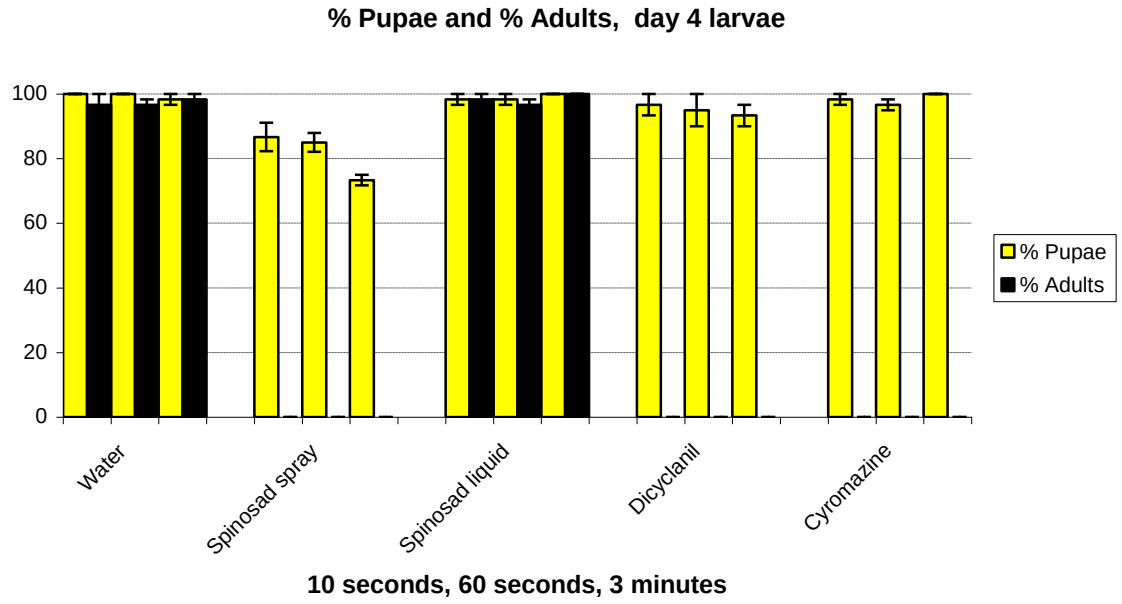
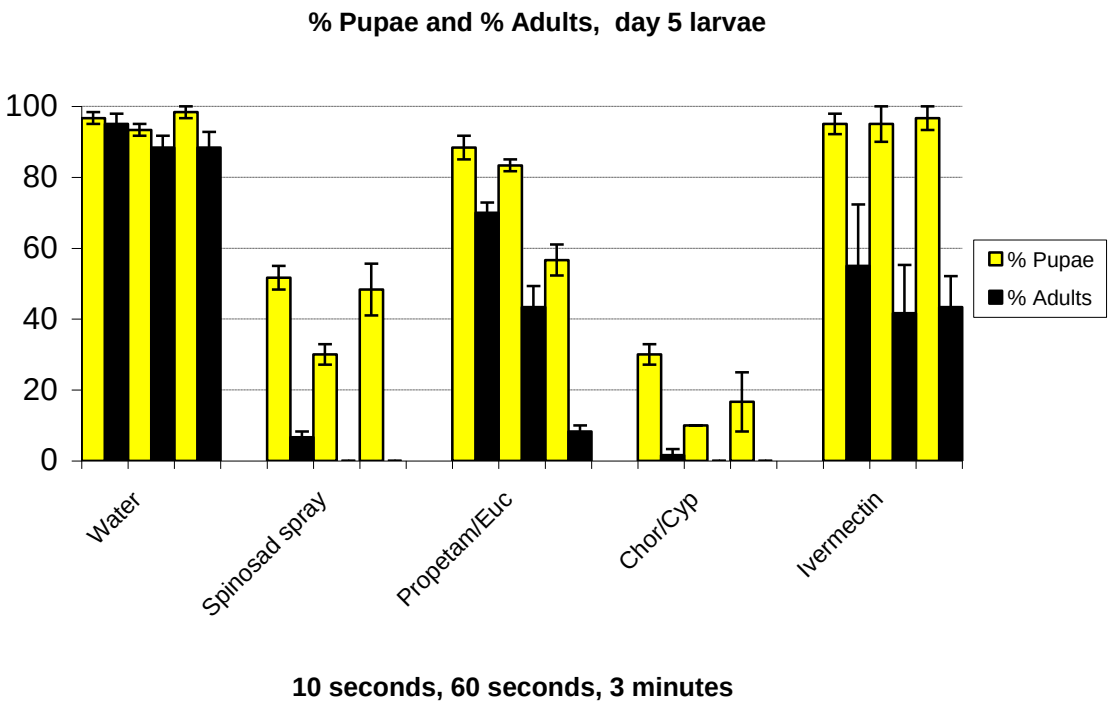


Figure 5. Percent 4 day old *C. megacephala* larvae developing to pupae and adult flies following immersion in test compounds for different periods of time. (Results for 10 sec, 60 sec and 3 min treatment are given in order within the tick marks for each compound listed on the X axis.)

(a)



(b)

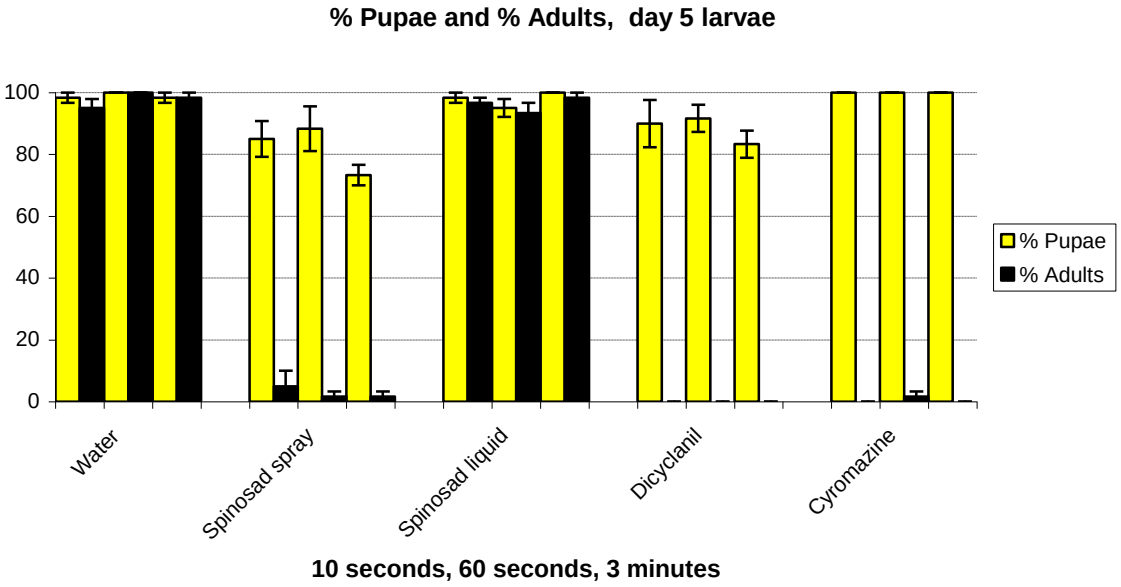


Figure 6. Percent 5 day old *C. megacephala* larvae developing to pupae and flies following immersion in test compounds for different periods of time. (Results for 10 sec, 60 sec and 3 min treatment are given in order within the tick marks for each compound listed on the X axis.)

4.4.3 Third instar feeding assays

These assays measured the relative toxicity of different chemical actives to third instar larvae when delivered in the feeding medium of older larvae. This method allows for both oral and topical exposure and more closely approximates the activity of a systemically delivered chemical than dipping assays. These studies indicated a very low LD50 (high toxicity) for dicyclanil in comparison to the other chemicals, perhaps reflecting the results in

the animal studies (Table 7). The LD values for ivermectin, doramectin and cyromazine, all known to have systemic activity, were lower than for chlorfenvinphos and cypermethrin that have mainly topical action. Even though moxidectin has systemic activity, toxicity against third instar larvae was significantly lower than for the other MLs, reflecting the results obtained against OWS and some other animal ectoparasites.[34,51]

Table 7. Toxicity of insecticides to third instar *C. megacephala* in feeding assays. LD values are based on the number of flies emerging

Chemical	LD50 (95% CI)	LD95 (95% CI)	LD99 (95% CI)
Chlorfenvinphos	2.5 (2.1-3.2)	4.8 (3.9-6.9)	5.8 (4.6-8.5)
Cypermethrin	1.9 (0.87-4.2	5.5 (3.5-15.9	7.0 4.5-20.9
Dicyclanil	0.051 (0.044-0.058)	0.10 0.092-1.12)	0.13 (0.11-0.15)
Doramectin	0.7 (0.50-0.94)	1.5 (1.25-2.70)	1.9 (1.5-3.5)
Ivermectin	0.8 (0.22-1.54)	2.7 (1.89-6.61)	3.5 (2.4-9.1)
Spinosad	2.1 (1.79-2.57)	4.1 (3.46-5.27)	9.8 (4.1-6.4)
Cyromazine	0.67 (0.55-0.80)	1.34 1.14-(1.78)	1.6 (1.3-2.2)
Moxidectin	2.4 (1.37-5.20)	5.7 (3.77-14.96)	7.1 (4.6-19.1)

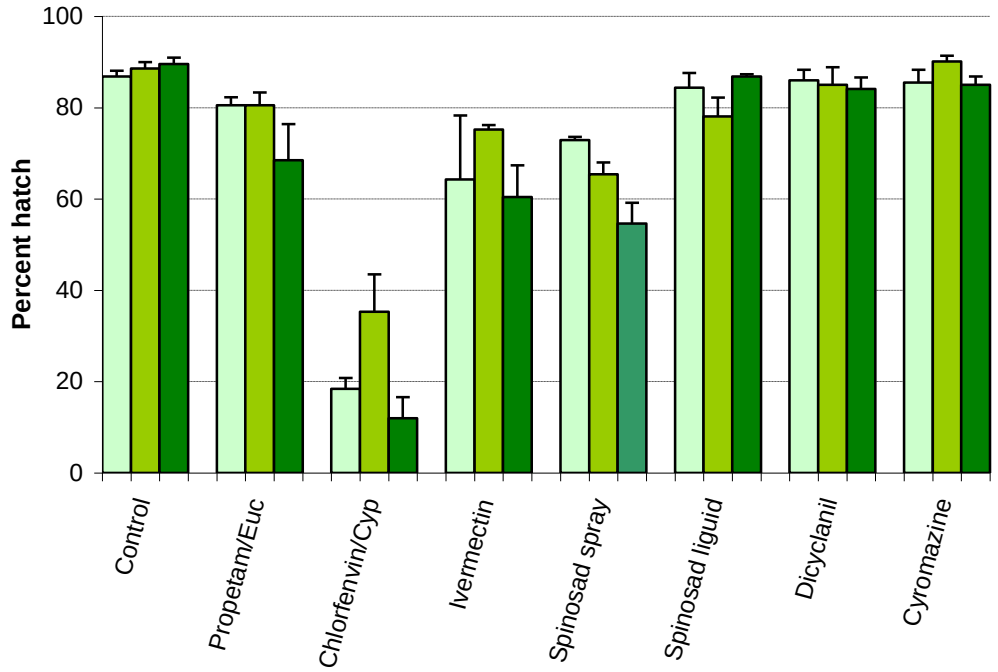


Figure 7. Percent hatch of *Chrysomya megacephala* eggs following dipping in treatment formulations for 10 s (left bar in each group of three), 60 s and 3 min

4.4.4 Egg dipping studies

Prevention of new strikes developing from eggs is important to effective resolution of strikes. None of the compounds tested showed complete kill of eggs in this assay and there was no effect of time of dipping on the level of effectiveness achieved (Figure 7). By far the most effective was the chlorfenvinphos/cypermethrin combination with an average of only 22% of eggs hatching across the three treatment times in comparison to 88% in the control treatment. Ivermectin and the spinosad spray formulation both killed some eggs (67% and 70% hatching respectively compared to 88% in controls), but none of the other formulations showed any ovicidal effect. Eggs can be prevented from starting new strikes through either direct ovotoxicity, as measured here, or by toxic effects on new larvae as they hatch. Even though this experiment indicated that none of the compounds tested was completely effective in killing eggs, given that most eggs hatch within 24 h it is likely that the treatment products tested would persist long enough to kill hatching larvae.

5 Discussion

A number of species of myiasis flies that attack cattle occur overseas, including the OWS, and NWS, but also including other economically important species such as warble fly (*Hypoderma spp.*), distributed through much of north America and northern Europe, and the torsalo or human bot fly (*Dermatobia hominis*) which infests cattle in central and southern America. The Australian cattle industries have to date remained free from any forms of myiasis fly but the sheep blowfly, *Lucilia cuprina*, itself an exotic species, is a major production and welfare issue for the sheep industries costing many millions of dollars each year in direct costs and labour for management. It is interesting to note that in Libya following introduction of OWS in 1996 a seasonal pattern of strike incidence has developed with strike generally limited by low temperatures and low humidity, but peaking, sometimes to epidemic levels, during periods of warm moist weather.[35] This is similar to current patterns of sheep blowfly strike in Australia and is a likely scenario in cattle should OWS establish in Australia.

Currently, in the absence of an OWS SIT production facility anywhere in the world, successful containment of an OWS incursion will depend almost totally on the availability of effective prophylactic and therapeutic chemical treatments. The use of insecticides will also be a critical element of programs for the eradication of NWS incursions. Effective treatment of struck animals during an incursion will be essential to prevent animal trauma and death and to limit production loss. It will be important to the success of an eradication program that any SWF treatment is completely effective in eliminating all live larvae and in preventing them leaving the wound, pupating and contributing to further generations of flies.

A range of chemicals have been tested against OWS in the past. However, most chemicals previously shown to be effective are no longer registered for animal use in Australia.[18] Since these studies a range of new chemical actives and formulations have come onto the market. Chemical formulations that may be of use in an OWS containment and eradication program were tested in this project.

5.1 Effectiveness in treating OWS strikes

Procedures for treating struck animals are outlined in the OWS preparedness strategy. These include physical removal and destruction of larvae and treatment with insecticides conducted in enclosures with concrete or sealed floors to minimise the chance of escaping larvae. Only one compound, ivermectin delivered by s.c. injection, is currently registered for treatment of OWS strikes.

Efficacy of four formulations based on ivermectin, spinosad, chlorfenvinphos/ cypermethrin and propetamphos/eucalyptus oil mixture was tested in sheep studies against strikes containing 2nd stage larvae (2 day old) and larger 3rd stage (4 day old) larvae that were closer to leaving the wound for pupation. Laboratory tests designed to approximate live sheep treatment were also conducted with 3rd instar larvae dipped in test formulations for 10 sec, 60 sec and 3 min. The laboratory assay results were generally in accord with the results of the sheep studies.

Most of the formulations tested gave good effect when used to treat strikes. All products gave 100% kill of larvae within 24 h when applied to two day old strikes and there was no obvious difference amongst treatments in the pattern or rate of healing. When the products were used against 4 day old strikes the results were slightly more variable. However three of the four formulations tested gave complete resolution of infections.

Topically applied ivermectin was one of the most effective compounds assessed for therapeutic efficacy in the animal studies and had a low LD50 value in the laboratory tests. It is notable that ivermectin gave better results in the live sheep tests than in the laboratory assays. No larvae immersed for 3 min developed to adult OWS flies but with both 10 sec and 60 sec immersion, approximately 40% of treated larvae developed through to adult flies. Similarly, in the tests with *C. megacephala* larvae, significant numbers of adult flies developed from both 4 day old and 5 day old larvae. On sheep, larvae are subject to both topical and systemic exposure, but in the laboratory dipping assays, exposure is predominantly topical. In previous studies s.c. ivermectin administered at 200 µg/kg was completely effective against 2 day old larvae, but when strikes containing 3 and 4 day old larvae were treated 15% and 18% of larvae survived.[42] Our results suggest that topical ivermectin is likely to be a better option than s.c ivermectin for treating struck animals.

The chlorfenvinphos/cypermethrin formulation gave rapid resolution of strikes on live sheep and quick kill of OWS larvae in the laboratory studies. It was clearly the most effective formulation in the laboratory assays with no larvae surviving to pupation in either the OWS study or tests with the *C. megacephala* 4 day old larvae. Although a number of other compounds were as effective when assessed on the basis of numbers of adult flies ecloding, the chlorfenvinphos/cypermethrin formulation was the only product to achieve 100% kill before any larvae pupated. Although some 5 day old *C. megacephala* larvae managed to pupate (<30% at all dipping times) and one adult fly emerged from a pupa, results for the chlorfenvinphos/cypermethrin formulation were also significantly better than any of the other formulations tested in this assay. Spradbery et al.[43] observed a high level of efficacy from a similar chlorfenvinphos/cypermethrin formulation in laboratory studies with all larvae killed in 100% of cases. However, it should be noted that when strikes on cattle containing 2 day, 4 day and 6 day old larvae were treated, although heavy mortality of larvae was induced 100% kill was not achieved in any instance. On the basis of our results, and particularly when used together with physical removal of larvae as specified in the AustVetPlan Screwworm Fly Disease Strategy[1] this formulation could be strongly recommended for treatment of OWS strikes in an incursion. In addition, it is the only formulation of those tested with current registration for application to cattle.

The spinosad aerosol formulation was also very effective in treating strikes in the animal studies, where it gave 100% cure of 2 day and 4 day old strikes. In addition, only the spinosad aerosol and chlorfenvinphos/cypermethrin formulations completely prevented any OWS larvae from pupating in the larval dipping study. The spinosad aerosol formulation was also one of the most effective compounds tested against *C. megacephala*. An antiseptic, chlorhexidine digluconate, is also included in the spinosad aerosol formulation and although no clear benefit was seen in terms of wound healing this ingredient would help minimise any possibility of secondary infection. A high level of efficacy was also reported against NWS in animal studies with spinosad aerosol formulations where 2 mg/g and 4mg/g formulations

both gave 100% cure of strikes and against OWS in Malaysia where the cure rate was 89% and 92% respectively for the two formulations.[36] An aerosol formulation of spinosad is now registered for cattle use in Brazil, Mexico and Venezuela. Spinosad has the attraction that it has a nil meat withholding period and export slaughter interval meaning that it would be useful tool for treating animals destined for slaughter. Its use is also permitted under a number of organic accreditation systems meaning that it could be used for treating struck animals on organic properties without jeopardising a property's organic status.

It is notable that the aqueous formulation of spinosad was not as effective as the aerosol formulation when tested in the laboratory assay with significant numbers *C. bezziana* larvae pupating and developing to adult flies. The results with *C megacephala* were more stark with almost all the 4 day old and 5 day old larvae treated with aqueous spinosad developing to adult flies whereas with the aerosol formulation, few flies emerged. The difference in performance of the two formulations was likely due to differences in concentration (2.8g/kg spinosad as compared to 0.125 g/L in the aqueous mix). The 0.125 g/L concentration used in the aqueous formulation is the recommended rate for treatment of *L cuprina* strikes and is the highest concentration of this formulation of spinosad registered for animal application. Differences in formulation may also have been involved as the aerosol formulation produced a foam which appeared to penetrate into wounds better than the aqueous mixture. On the basis of the laboratory studies, as well as the incomplete protection provided by this formulation in the animal protection studies, the aerosol, but not the aqueous formulation is recommended for the treatment of OWS strikes.

Dicyclanil and cyromazine are growth regulator compounds and not recommended for treating strikes because larvae can persist in wounds for extended periods after treatment. For this reason these compounds were not tested as therapeutic agents in the animal studies. However, dicyclanil gave very good effect in the laboratory dipping assays with only a few larvae successfully pupating and none developing to adult flies. Dicyclanil treatment, in addition to providing good protection against new strikes, may assist in providing insurance against the development of new flies from strikes not detected at the time of treatment.

The propetamphos/eucalyptus oil formulation did not give 100% kill of 4 day old larvae and live larvae were found in implants on two sheep following treatment. It also appeared that the wounds from 4 day old strikes healed more slowly in the implants treated with this formulation. This may have been due in part to the longer persistence of active larvae in the wound, but as healing was also slower in sheep where no live larvae were found, may also be due to a direct effect of the oily formulation in slowing healing. This was also suggested with oil-based formulations used for mulesing wound treatment on sheep.[20] Slower healing meant that the strikes remained attractive for re-strike longer and a new screwworm egg mass was found on one of the wounds that was still suppurating 3 days after treatment. These eggs hatched and established a restrike, suggesting little persistent protection from this formulation. Third instar larvae were collected from the restruck sheep at the 5 day inspection and subsequently confirmed as *C. bezziana.* Two new egg masses were also seen on this strike at the 5 day inspection. This formulation could not be recommended for treating OWS strikes during incursions.

5.2 Protective effect

Chemical treatments that can give extended protection against OWS attack will be vital for preventing new infestations and containing the spread of SWF. This will be particularly so in the more extensive areas of livestock production where frequent mustering of animals for monitoring and/or treatment is impractical. Effective prevention of new infestations will be needed following management practices such as castration, dehorning or even relatively minor invasive techniques such as ear tagging that render animals vulnerable to attack. Protection may also be needed for newly born calves that are susceptible to umbilical

strikes, their mothers following parturition, in situations where buffalo fly lesions or ticks make animals susceptible, for the establishment of quarantine barriers and to facilitate stock movements.

Protecting stock against strikes for extended periods is problematic with currently available formulations. Ivermectin, administered by subcutaneous injection is the only compound currently registered for the treatment of OWS in Australia and remains a key component of Australia's screwworm preparedness strategy. Although it is indicated in the OWS strategy that s.c ivermectin can give 16-20 days protection[1], results from a number of studies suggest that reliable protection is unlikely to extend much longer than two weeks and may be less than this in some instances. Ivermectin injected at 200 µg/kg live weight gave 14 days protection against OWS in pen trials[42] and Perkins[25] found that new-born calves were protected against navel strike by similar treatment for 10-11 days. With NWS reported protection periods have been very variable.

Other options previously identified for potential prophylactic use were closantel and slow-release ear tag formulations of zeta-cypermethrin.[1] The concentration of closantel required for good control is higher than the currently registered dose rate and high levels of this compound have been associated with blindness in sheep and goats.[7,12] Closantel is not registered for use in cattle. Zeta-cypermethrin ear tags, currently registered for buffalo fly control in cattle, have been shown to provide extended protection against screwworm infestations[47] and may have a role in reducing strike incidence. However, the protection provided was incomplete and probably mainly achieved through repellent effect.[50] Repelling OWS from potential strike sites may result in SWF dispersing more widely and extending the infested area. Although the tags may have an important complementary role in protecting animals and keeping strike incidence low, particularly on dairy farms where a nil milk withholding period is important, alternative options for prophylactic treatments during a containment and eradication program are critically needed.

Three injectable macrocyclic lactone (ML) formulations not previously tested against OWS were tested in this project to determine the possibility that they could provide extended protection. These were a LA formulation of ivermectin injected sub-cutaneously, s.c. doramectin and s.c abamectin, all administered at 200 µg/kg. In our trial, LA ivermectin protected against strike at 3 days, but at 2 weeks protection had broken down in many sheep with strikes developing in 50% of the implants. Some protection was also provided at later implants but at levels well below that desirable during an eradication program. This suggests that the LA formulation offers no advantage over conventional injectable formulations of ivermectin.

Injectable doramectin has not previously been tested against OWS, but when used against NWS was shown to be superior to ivermectin in a number of instances. Moya Borja et al.[24] in a study with implants made at 7 days post treatment found that doramectin was 100% effective whereas larvae survived in 29% of the ivermectin treated animals. Doramectin was 100% protective in preventing development of strikes from implants up to 21 days and showed partial activity at 28 days. In a large study of 2,689 castrated cattle examined 13 and 17 days post treatment on farms in Brazil, the mean efficacy for doramectin was 94.6% (range 53.3%-100% across farms) compared to 43.7% for ivermectin (range 0%- 100%).[9] Anziani et al.[3] found that s.c. doramectin gave 90.9% and 83.3% protection against larval implants with NWS at 12 and 15 days post treatment respectively. This compared to 36% and 17% respectively with the most effective formulation of ivermectin. In field studies with castrated cattle, doramectin and ivermectin were both 100% effective until 7days post treatment, but at 10 days doramectin provided 92% protection compared to 69% for ivermectin.[23] In a study with OWS, pouron formulations of doramectin, moxidectin and eprinomectin were tested.[51] Doramectin was also found to be the most efficacious of the pouron formulations, preventing the induction of myiases for 7 to 14 days after treatment

whereas eprinomectin exhibited larvicidal activity for 3 to 7 days, and moxidectin was ineffective.

The results of our studies were broadly in agreement with those obtained with NWS larvae with doramectin providing slightly superior protection to ivermectin. At 3 days both doramectin and ivermectin gave 100% protection but at 2 weeks doramectin gave 75% protection, compared to 50% for ivermectin and at 4 weeks protection from doramectin was 42% compared to 16% for ivermectin. However, in practical terms there was little difference between the two formulations with protection from both breaking down at 2 weeks.

Abamectin has also not been previously tested against OWS. However, with NWS it was found that at 10 days after treatment the percentage reduction in strike in abamectin treated cattle was 85% compared to 69% for ivermectin.[23] Anziani et al.[4] showed that abamectin protected against NWS for 5 days, but that protection had probably broken down at 10 days (one treated animal struck) whereas Lopes et al.[21] concluded that neither abamectin or ivermectin were effective in preventing scrotal myiasis following castration. Our results suggested little difference between the ivermectin and abamectin treatments with only moderate protection from both at the 2 week implant (43% and 50% protection respectively) and little protection at times after this.

Two formulations tested in this project, dicyclanil spray-on and ivermectin capsules, gave clearly better effect than the others tested. Both of these gave complete protection against the establishment of strikes for the 12 week duration of the test experiments. Period of protection was significantly longer than the currently recommended s.c.ivermectin and also longer than the LA s.c. formulation of ivermectin tested in this project.

Ivermectin capsules are registered for use against internal and external parasites of sheep and claimed to provide protection for 100 days (c.14 weeks). It might be reasonably expected that protection against OWS would also extend to this time. It is notable that studies with *L. cuprina* found that although similar capsules provided good protection against breech strike, attributed to excretion of ivermectin in the faeces, only moderate protection was provided against bodystrike.[30] This underlines the importance of caution in extrapolating between parasite species, even to those with seemingly like habit, without thorough testing.

Dicyclanil was applied according to label instructions for the treatment of bodystrike in sheep. That is, two overlapping bands were sprayed along the backline from poll to tail with dose rate determined on the basis of bodyweight. Three different challenge circumstances were tested. Implants were made within the treated band, outside of the direct treatment area (in both cases sheep were not shorn) and in the third group the ‘fleece’ was clipped before application to provide a situation more similar to that with cattle and implants again made outside of the treated area. In all instances protection was provided for the full 12 week period of the study. Dicyclanil similarly protected sheep against field challenge by Palaearctic SWF for at least 12 weeks[38] and has provided protection of cattle against NWS in field studies where newly castrated calves were treated.[2] In the latter study application of 20 ml 5% dicyclanil to castration wounds with a paint brush protected calves against strike for up to 16 days post treatment after which time one animal was struck. Protection may have extended longer than this. However, no strikes were recorded in untreated control animals after day 16, which suggests that challenge had declined by this time, probably because wounds had healed and ceased to be susceptible.

The possibility of resistance developing because of heavy long term reliance on ivermectin needs to be kept in mind and an additional effective prophylactic chemical for use in the event of an incursion is required. Dicyclanil could fill this role. In addition, Wardhaugh et al.[51] caution against intensive use of ivermectin capsules because of damaging effects on dung beetle populations. Dicyclanil also has significant toxic effects against dung beetles when

artificially seeded into dung, but whether there is any effect when dicyclanil is applied topically to animals does not appear to have been tested. Dicyclanil is currently only registered for use in sheep. Consideration needs to be given to what data would be required to enable use of dicyclanil in other animal species, in particular cattle, in the event of an incursion.

The aqueous formulation of spinosad, applied to the sheep by immersion dipping at 125 ppm (label indication for blowfly strike protection), which is the highest registered rate for this formulation, provided poor protection against artificial OWS challenge. Although spinosad can provide protection against fleas when applied orally to dogs at relatively high dose rate[29], it is unlikely that systemically active concentrations developed following topical application of the aqueous formulation by dipping. Label indications are that this formulation provides 4-6 weeks protection against the sheep blowfly *Lucilia cuprina,* which feeds much more superficially than OWS. The deeply invasive habit of OWS may allow the larvae to avoid active concentrations of spinosad administered to the skin surface by immersion dipping and compromise the level of protection provided.

As noted earlier the aerosol formulation of spinosad tested in the present study was very effective in resolving OWS strikes. Similar formulations containing 2 mg/g and 4 mg/g spinosad were also effective in preventing OWS strikes on the navels of new born calves in Malaysia and the two formulations were 94% and 100% in protecting against NWS strikes in castration wounds in South America.[36] Even though the aqueous spinosad formulation could not be recommended for whole body protection against SWF, the aerosol formulation may provide a useful option for protecting wounds caused by management procedures such as dehorning, castration or ear tagging against OWS strikes.

6 Conclusions

Three of the formulations tested for treatment of OWS strikes gave results that could recommend their use in the event of an Australian incursion. These were the chlorfenvinphos/cypermethrin formulation, ivermectin topical formulation and spinosad aerosol formulation all of which were 100% effective in treating strikes. It should be noted however, that in some other studies some of these compounds were not completely effective. Physical removal and destruction of as many larvae as possible and treatment on a sealed surface to prevent any escaping larvae from pupating, as detailed in the AUSTVETPLAN screwworm strategy, together with monitoring following treatment to confirm complete resolution of the wound is essential.

For the protection of animals against new strikes, a critical element of any containment program, two formulations were shown to give significantly longer periods of protection than the currently recommended s.c. formulations of ivermectin. Even though it is noted in the OWS containment strategy that s.c ivermectin can give 16-20 days protection, there appears to be little data to support this period and it is unlikely that protection will extend longer than 2 weeks. Both the capsule formulation of ivermectin and the dicyclanil spray on formulation gave protection for the full 12 weeks of the protection studies and should be considered as prophylactic options in the event of an incursion.

It is noted that although all the therapeutic treatments tested are registered for use in sheep, only one, the chlorfenvinphos/cypermethrin formulation is registered for use in cattle. Similarly, both preventative treatments shown to give extended periods of protection are registered for use in sheep, but neither for cattle. A similar capsule formulation for cattle has been tested and shown to be effective and although not registered for use in Australia, this formulation could provide the basis for prophylactic cattle treatment. Further studies will be necessary to develop an application protocol to enable the use of dicyclanil on cattle. Pre-

emptive action to facilitate rapid deployment of these compounds for use in the event of an OWS incursion is urgently needed, particularly in light of the extended delay likely before a sterile insect eradication program could be instituted.

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