# final report

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# Evaluation of the Sterile Insect Technique for Sheep Blowfly Control

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# Abstract

The Australian Sheep blowfly (SBF, *Lucilia cuprina*) causes flystrike in sheep. Damage through direct losses in meat and wool production and management expenses costs the industry $125-280M annually.  Control methods (chemical, docking, mulesing) are not always sufficiently effective and suffer drawbacks from chemical resistance development, residues, and animal welfare concerns. The industry is examining more efficient and sustainable control methods while maintaining product quality.

The ecology of SBF makes it a very good target for long-term control through the Sterile Insect technique (SIT), whereby irradiated sterile males are mass-released and mate with wild females, suppressing reproduction of wild populations. SIT is used successfully against SBF in Bangladesh. Previous projects investigating SIT for SBF in Australia have failed for diverse reasons that we review to improve success of future attempts.

Australia has the capacity for upscaling of mass rearing of sterile males in 1-2 years (using existing knowledge and facilities) and for areas of 2000-5000 km$^2$ (e.g., Kangaroo Island). After upscaling SBF could be eradicated in 3-4 years for an estimated 5-6 million dollars (including facility establishment that could later be repurposed).

Further research into the ecology of SBF and optimisation of rearing and release techniques, will make it possible to plan for application of SIT on larger areas. Expansion for other pests such as buffalo fly and for biosecurity preparedness (e.g., for screwworm fly) are also advantages for developing SIT for SBF.

# Executive summary

Meat and livestock are important agricultural commodities for Australia, estimated up to $4.3 billion in lamb and mutton production (ABARES Agricultural Commodities June 2018) and includes $260 million of live export of sheep.

Sheep meat production generates considerable export tonnage (270,000 tonnes of lamb and 180,000 tonnes of mutton in 2018- Commonwealth Department of Agriculture official statistics) to over fifty countries, making Australia the largest sheep exporter in the world and returning significant profit for industry (over $3.3 billion). Australia has a reputation for meat and animals of excellent quality, achieved through industry initiatives such as red meat integrity programs and food safety, animal welfare, biosecurity and meat traceability actions. These systems ensure that meat and live export sheep meet market access standards, customer expectations and quality safe red meat standards.

Flystrike in sheep (ovine cutaneous myiasis) is a disease caused by a parasitic infection with a live insect. In Australia the primary insect responsible is *Lucilia cuprina*—the Australian Sheep Blowfly (SBF). Adult female SBF lay eggs in soiled wool or open wounds and after hatching the larvae (maggots) burrow into the wool of the sheep, under the skin and feed off the live flesh of the animal. Food intake, wool growth and quality (Walkden Brown et al 2000) and meat production are directly affected in infested sheep (Colditz et al 1995, Lihou and Wall 2019). Flystrike affects up to 80% of merino wool-producing sheep flocks in Australia. Infestations can cost the industry up to $173 million annually in stock loss, productivity loss, and control measures (AWI 2019).

Flystrike prevention is mainly based on strategic chemical control and/or mulesing. Chemical control (jetting/dipping/wound dressing) is efficient but is costly due to use of chemicals and flock handling and is under discussion for environmental impacts (Beynon 2012). The rational basis for choice and appropriate timing of chemical application is often not robust (Lihou and Wall 2019). Mild resistance of SBF to organophosphates has been recorded and is progressing (Arnold and Whitten 1976, McKenzie and O'Farrell 1993, Naqqash et al 2016). There are still chemical options available, but some importing countries restrict import of meat that exceed specified residue levels of some chemicals. This means that the livestock industry as a whole is examining ways to reduce chemical use while maintaining quality of meat for export.

Mulesing, the removal of excess skin to reduce skin folds that are fly-entry points is effective but has a direct negative impact on animal wellbeing. Mulesing is also encountering growing consumer resistance. This currently affects trade prices for both wool and meat.

The ecology of SBF makes it a promising target for management by sterile insect technique (SIT), whereby sterile males are released to mate with wild females resulting in non-viable eggs and reduced pest populations in the following generation. This technique has been developed for numerous pest insects worldwide and is already used for SBF in Bangladesh. In geographically isolated areas such as islands, eradication of SBF should be readily feasible. In other areas on the mainland, an efficient reduction will contribute significantly to an acceptable

Integrated Pest Management (IPM) for SBF, combining monitoring, modelling and seasonal releases.

Previous attempts to develop SIT against SBF in Australia (Foster 1993, Mahon 2001) have failed for multiple reasons. These included rearing problems, mating performance of sterile males, release conditions and lack of understanding of local SBF ecology. Eradication of SBF on Flinders island was almost successful, but ultimately failed due to funding constraints (Foster 1993).

In this report we propose to develop area-wide SIT for SBF in an island situation (Kangaroo Island), based on a ‘simple’ mass rearing of flies, irradiation and release of pupae, while simultaneously developing improvements through research in production, quality control, release strategies and SBF ecology.

Because the basic techniques and equipment needed for SIT of SBF are available, it is possible to develop and apply this technique in a very short time span in selected areas of up to 5000 km2. Once this has been achieved, optimisation and upscaling of production and release, for the further deployment over larger areas, can further increase efficiency and reduce costs.

This project could be set up very cost effectively using the experience obtained through the design and operation of the SITplus facility (Queensland fruit fly SIT) in Port Augusta. Moreover, we propose here to create a modular rearing facility based on shipping containers, substantially reducing the costs for the buildings and allowing quick modification to increase capacity if needed. Such a container-based facility could also be relocated and repurposed for subsequent eradication or suppression programs.

Total estimated costs for a project to eradicate SBF on KI would be $5.7 million over 5 years. This is considerably more cost and time efficient than earlier projects, including the $41-million-dollar project for eradication of SBF from Tasmania as proposed by Horton et al. 2002.

The development of SIT capacity for SBF will significantly increase our preparedness in case an incursion of other livestock attacking fly species (e.g., Screwworm Flies *Cochliomyia* and *Chrysomya* ). For most of these species, SIT applications have been developed abroad and successful eradication has been achieved, in some cases using imported sterile flies. However, for SIT eradication of an incursion for any one of these species in Australia, the insects for SIT will have to be produced in Australia since importing sterile insects from abroad will not be possible because of strict biosecurity policies that require culturing of important insects for at least one generation under quarantine conditions.

Several other livestock pests in Australia are candidates for SIT, but for some their ecology is less favourable (e.g., buffalo fly *Haematobia exigua*) or they are of lesser importance.

**In Summary**

The meat and livestock industry are encountering increasing difficulties in sustainable, consumer and market acceptable management of SBF. The industry will need to adapt to changing market expectations, and find alternatives for current practices in order to maintain and reinforce its market position. SIT would be a valuable, efficient and non-chemical tool, improving animal wellbeing and contributing positively to the quality reputation of Australian sheep meat and wool.

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# 1. Background

## 1.2 The Australian Sheep Blowfly Problem

Flystrike (myiasis), a sheep disease caused by invasion of living tissue by the larvae of calliphorid flies has a significant economic impact (Hall and Wall 1995). Calliphoridae, particularly the Sheep Blow Fly (SBF) *Lucilia cuprina* (Wiedemann) and the Common Green Bottle fly *L. sericata* (Meigen), pose a persistent threat to the meat and wool industries in many parts of the world (Arundel and Sutherland 1988; French et. al. 1992, 1995; Hall and Wall 1995). SBF has long been a significant problem for Australian farmers (Mackerras 1933, Wardhaugh, Mahon et al 2001). Larval infestations not only reduce wool quality and quantity (Broadmeadow et.al. 1984; Arundel and Sutherland 1988) but - if left untreated - also rapidly deteriorate sheep health causing physical ailment, coma and death. Flystrike occurs when maggots feed on living flesh creating open wounds. Flystrike can affect sheep of all ages and sizes, and while more common in the breech area, can occur on the body, pizzle in rams and wethers, on wounds, or the poll.

When infection from the presence of SBF maggots occurs, a large distended lesion forms on the skin of the animal (Sandeman et al 1987). Release of protease from the rasping mouthparts of the maggot causes the sheep's skin to degrade and the animal can become distressed, ill, and in some instances die. Reinfection can occur throughout the life of the sheep.

Historically, a joint blowfly committee (1933) reported that the first records of strike, probably caused by *Calliphora stygia* (Fabricius) in Australia, came from Tasmania and dated from before the 1870's. The first strike on Mainland Australia was recorded in 1883 from Queensland, which became widespread only in 1903 in the Riverina district (Joint Blowfly Committee 1933). Increase in strike incidence led to scientific advances to identify the species involved. In 1911, the fly responsible for the flystrike was recognised to be a *Lucilia* and later determined as *L. sericata* (Tryon 1911). However, in 1930 the responsible species was correctly identified as *Lucilia cuprina* by G. H. H. Hardy (Mackerras and Fuller 1937) which was reiterated by Watts et. al. (1979) and Barton (1981). It is now believed that Australian sheep blowfly, SBF might have been introduced to the Australian mainland in the late 1800s from South Africa or India (Waterhouse and Paramonov 1950) and to Tasmania in 1940s (Ryan 1954), whereas *L. sericata* was recorded in Tasmania earlier than this time (Waterhouse and Paramonov 1950).

Among the myiasis caused by SBF, breech strike and body strike (back, flanks or withers) are of the greatest concern (Watts et al 1979; Murray 1980; Barton 1982). While breech strike is considered most common in Australia, body strike occurrence is likely to be predominant during warm and wet weather conditions (Wardhaugh and Morton 1990).

Alongside animal welfare issues, the economic impact of flystrike on farmers and industry can be devastating. Cost of control measures can vary, although economic models have indicated that prophylactic treatments for lambs when flystrike is high is one of the most cost-effective strategies available to farmers (Lihou and Wall 2019). Costs for chemical management are estimated at $1.80 per head per year. Mulesing and crutching add another 3$ per head

(Adamson and Wheeler 2020). Even when chemical control options are available, managing blowfly outbreaks on pastoral properties in flystrike years is often impossible because of the logistics to muster and treat sheep when outbreaks occur. Total costs of flystrike (management costs and losses) in Australia are estimated at $365 million per year (Sacket et al 2006)) Understanding seasonal patterns of fly distribution and flystrike risk, employing integrated pest management strategies including SIT would reduce economic costs.

**In Summary**

Sheep blowfly (SBF) is a widespread and costly problem for sheep producers in Australia. Economic costs to industry can exceed $365 million annually due to reduced wool production and costs associated with SBF sheep treatment and control. Research and development of control measures that are not only cost effective but have long term influence over populations in the field are warranted.

# 2. Project objectives

## 2.1 Outputs

- A literature review and assessment of the potential for SIT to contribute to livestock pest management in Australia.
- Benefit/Cost analysis of the impact of the development of SIT for SBF on the sheepmeat industry.
- Research plans for a full proposal to develop and trial SIT for SBF control at key locations in Australia (for example, Kangaroo Island).
- Preliminary plans for a production facility, release options and assessment criteria for the trial SIT locations.
- Industry fact sheet describing the SIT process for sheep producers in order to inform them of the process to garner their future cooperation and collaboration.

## 2.2 Outcomes for R&D Adoption

- This proposal addresses the problem of sheep blowfly and the associated negative impact that the insects and mulesing have on the industry.
- This is a widespread problem for sheep meat producers across Australia. Most producers would have some interest in the possibility of SIT being used to ameliorate the SBF problem.
- This project is a preliminary scoping study on the use of SIT for Blowfly control and as such there are no barriers to the realisation of value from this activity.
- If on completion of the project, the development of SIT for SBF is found to be a viable option to pursue, eventual barriers to adoption may depend on whether the method is used as an on-farm or area-wide approach. If SIT releases are deemed suitable for individual on-farm treatment, there may be commercial enterprises willing to produce sterile flies and sell them to sheep producers in much the same way as beneficial insects are produced and sold. There are parasitoids sold to control pest flies of agriculture such as *Spalangia endius,* a wasp parasite of nuisance flies such as house fly and stable fly (Bugs for Bugs 2015). This may also be possible for SBF, as SBF is thought to have low dispersal which means releases at on-farm sheep camps may be an effective approach. Alternatively, if regional suppression or eradication is the aim, potential barriers to adoption will be the financial support and cooperation of farmers across broad areas, and the increased cost of large-scale sterile production.
- The development of a viable ‘business model’ for SBF SIT production and deployment should be part of the project in order to secure the project ‘legacy’.
- Sterile production for SBF may not need to be as large scale as other SIT programs. This species is known to cease development at low temperatures at the pre-pupal stage, so it may be possible for a relatively small sterile fly production facility to produce and store pre-pupae continuously throughout winter, thereby having the required large numbers ready to distribute in Spring when the natural emergence of wild flies takes place.

- Producers will be engaged and informed of the project via articles and/or a fact sheet as needed. As part of the development of research and release plans, some producers will be identified and approached to determine their interest in co-operation with those plans – for example identifying release sites for sterile release trials and trapping points for baseline population data.
- For this scoping study, there are no specific skill development activities required by the target market or intermediaries. Eventually, for efficient adoption of any developed SIT methodology via on-farm releases, the target market would need to be educated on simple live insect handling requirements and release methods (for example, keeping insects within the desired temperature range and handling with care). Alternatively, area-wide adoption would see a larger role for advisors and extension specialists to educate the target market on the support for area-wide services (for example releases made by dedicated personnel across large growing areas).
- This project is potentially the start of a major investment going forward. The development of effective SIT for a new species should be seen as a long-term process (5-10 years for establishment, with ongoing development over longer periods). While some methodologies are readily transferable from SIT for other species, the information base required to develop an effective, practical SIT methodology for SBF should not be underestimated. This study delineates those needs.

# 3. Methodology

This report has been prepared using information from diverse sources.

- Scientific literature:
  Scientific publications were sourced using the Web of Science database and Library resources of Macquarie University and University of Adelaide (see references)
- Reports:
  Various reports, prepared for MLA, AWI, CSIRO and other organisations were sourced though these organisations (see references)
- Grey Literature:
  In some cases internal documents were sourced directly from related projects or through personal email exchanges with contacts from research facilities or Sterile insect facilities (for example reports from the Bangladesh SBF SIT facility manager)
- Personal Communication:
  Informal discussions with people involved in current and past SIT projects were held when needed. Terril Marais, the manager of SITplus Port Augusta was extremely helpful in providing information on the current facility and guidance for the design of a possible new facility. The staff of the Bangladesh SBF SIT facility have been very open to sharing of methods and discussion of rearing methods.
- Study visits:
  Earlier IAEA study visits to SIT facilities (Guatemala, Mexico, Israel) by P. Crisp, P. Taylor and T. Marais provided valuable information and photos to explore different options for design of SIT facilities.
- Pre-studies and quotes:
  - A number of pre-studies were conducted through specialised companies to agree on technical requirements and to provide a quote for facilities, equipment and releases.
  - P. Crisp and M.van Helden conducted a study flight over Kangaroo Island on February 29th April 2020 to assess the effects of the bushfire on sheep production areas and distribution of sheep on the island.

# 4. Results- Literature Review

Macquarie University and the South Australian Research and Development Institute

# Sheep Blowfly (*Lucilia cuprina*) in Australia
# Literature Review

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## 4.1 Biology of Sheep Blowfly *Lucilia cuprina*

Study into SBF in Australia began in the 1930s and can largely be attributed to the work done by IM and JM Mackerras, medical scientists and entomologists with the Council for Scientific and Industrial Research during the 1930s/40s (Mackerras 1933, Mackerras and Freney 1933, Mackerras 1936, Mackerras et al 1936, Mackerras and Fuller 1937, Mackerras and Mackerras 1944). These studies focussed initially on biology, ecological surveys and biological effects of this introduced fly species on livestock (including factors that made livestock more attractive to blowflies).

The SBF, *Lucilia cuprina* (Wiedmann), is a fly belonging to the family Calliphoridae (Waterhouse and Paramonovo 1950). Calliphorid flies commonly display carrion-feeding habits. Compared to other blowfly species, SBF is a poor competitor in the race for consuming enough food to complete development on carrion. SBF has adapted to become a parasite of live sheep (Mackerras 1933, Anderson et al 1988) and (dead) fish (Islam 2014). SBF is the major pest blowfly species in Australia, being responsible for over 90% of all flystrike (myiasis) in the country (NSW Department of Primary Industries 2016). SBF is rarely found on carcasses of sheep or other species (Anderson et al 1988). Infestations of sheep with other fly species (Chrysosoma / Calliphora) alone is rare, but co-infestation occurs frequently following initial SBF infestation (Anderson et al 1988).

Gravid female SBF are attracted to sheep, mostly by olfactory cues, and stimulated to oviposit into the fleece and/or on open wounds (Ashworth and Wall 1994). The feeding activity of the resulting maggots, both mechanical and chemical, is responsible for the numerous pathological effects of the flystrike and can prove to be fatal in the most severe attacks (Morris 2000; see Chapter 3).

Data on the biology and developmental rate of SBF are readily available because this species is used in forensic sciences. However, data on field ecology, populations and phenology are scarce.

### 4.1.1. Life Stages

SBF has four developmental stages: eggs, larvae (maggots), pupa and adults. Details on developmental parameters are presented in appendix 9.1.1. Since SBF is used in forensics many details on developmental parameters of the larvae and eggs are available. Data on adults are less detailed.

Adult females lay batches of several hundreds of eggs on sheep, especially focusing on wrinkles, dirty moist wool and existing wounds. Aggregation of egg laying females occurs (Murray 1957; Macfarlane et al 1958) resulting in large numbers of maggots attacking a single susceptible sheep. Maggots feed collectively by producing extracorporeal secretion of proteolytic enzymes that liquefy the host tissue resulting in open wounds. After 4-7 days (Wardaugh and Dallwitz 1984, Wardaugh 2001) maggots fall to the ground where the post-feeding maggots dig into the soil (up to 5cm deep) to pupate immediately, or overwinter before pupating. Pupae can hatch immediately after completing their metamorphosis or stay in the soil until the soil warms up in spring, but this varies depending on region, time of year and climatic conditions (Wardaugh 2001).

The life expectancy of adult flies is under three weeks (Vogt and Woodburn 1979). Adult females can develop a batch of eggs every 4-8 days (Wardaugh 2001) and in order to have eggs of sufficient quality must have a protein rich diet. Mating occurs when adults have ingested sufficient protein after which the release of juvenile hormones produces vitellogenin protein (vitellogenesis—egg production in the females). More than 10 generations can occur during a single season.

Predictive models for flystrike have been shown to accurately predict the start of the blowfly season. This provides farmers a tool for choosing control strategies (Horton and Hogan 2010). Inaccuracies of weather projections (Wall et al 1992, 2003, Rose and Wall 2011) and rainfall events can hamper the precision of these predictions.

### 4.1.2 Ecology

As in all insects, the developmental rate of blowflies depends on temperature (Gunn 1942). Optimal temperature for SBF is 24-30 degrees (Kotzé et al 2016) when the whole lifecycle (egg to adult) can be completed within 9-15 days (Dallwitz 1984) allowing for multiple generations to occur in most Australian regions.

Adults are exposed to ambient temperatures. While on living sheep the eggs and larvae are 'warmed' by the sheep's body temperature and large masses of larvae can also generate heat to increase development rate when ambient temperature is low (Kotzé et al 2016). When in the soil, larvae and pupa are exposed to fluctuating soil temperatures. Lower developmental threshold temperature for pupae in the soil is about 11$^{o}$C.

Winter in Southern Australia results in an arrested development (diapause/quiescence) as prepupa (a post-feeding larval stage occurring after abandoning the host sheep; Mackerras 1993; Norris 1959) or pupae (De Cat 2007, De Cat et al 2012). In Southern Australia, adults of SBF are rarely caught by field traps between July and September (Gilmour et al 1946) mainly due to low temperatures. In field studies conducted in the Canberra region, prepupae pupated within a few days after abandoning their host sheep during summer and early autumn, while pupation dropped to almost zero for prepupae entering the ground in late March (Dallwitz and Wardhaugh 1984). Mortality of prepupae and pupae due to predation and diseases (e.g., entomopathogenic fungi, nematodes, ants) likely exceeds 90%, Pitts and Wall 2005, De Cat et al 2012). Numbers surviving through winter to spring are therefore expected to be low.

#### *Population densities*

Reliable estimates of fly densities are scarce. Gilmour et al (1946) and Readshaw (1982) estimate densities through mark-recapture experiments to less than 10 flies per ha (0.9-9.3/ha). Vogt and Morton (1991) using a different mark-recapture interpretation method in NSW estimated 6-40 males/ha. Smith and Wall (1998), find values for *L. sericata* (the closely related green bottle fly) ranging from 0 to 6 per ha.

Other estimations can be based on work by Urech et al (2009) who used specific traps (Luci-traps) at a rate of approximately 1 trap / 100 ha (Range 15-400 ha/trap, 1 trap/100 sheep on 24 different properties) and obtained a 60% reduction by trapping of 25 (Queensland in September) to 100 (WA in December) Lucilia per trap. The removal of low numbers per ha (<1 fly/ha in these cases) already seems to result in a substantial reduction of fly numbers.

The above estimated range of values can be taken as realistic and as meaning that normal initial fly densities in spring will commonly be 5-10 flies per hectare.

In spring, adult emergence was synchronous amongst surviving individuals that had entered the ground from late March to October. Such a synchronised hatching in spring can be induced by rising soil temperatures (> 11$^{o}$C, possibly in conjunction with rainfall events; De Cat et al 2012).

Opportunity for initiating flystrike in moderate climates is therefore low during the winter period and higher in spring in areas where pupae overwinter in the soil.

In warmer and more humid areas of Australia there is little information available on SBF development and flight periods although laboratory studies have examined this under controlled conditions for *L. sericata* (Wall et al 2009). Warman et al (2000) examined circadian rhythms for comparison with other dipteran species that could be used to further model adult SBF seasonal flight patterns. Fly activity and flystrike have been reported all year round (Hayman 1955) with rainfall and fleece rot associated strongly with overall flystrike pressures. More data are needed since this will influence the probability of successful SBF control through SIT (see chapter 4). We are currently collecting additional flight data in different climatic areas of South Australia.

The risk of flystrike will not only depend on the populations of adult flies, temperatures high enough for flying and egg-laying, and distance between emergence site and the flock, but also on the state of the flock (lambs are more susceptible, shorn sheep are less susceptible) and climatic conditions such as rain (wet soiled wool is very attractive) and wind (flies are less able to approach sheep when wind speed is high). According to French and Morgan (1996) and Broughan and Wall (2007), sheep susceptibility depends on wool length, fleece humidity and the accumulation of faeces and urine. High quality fine wool merino sheep are at high risk due to excessive wrinkling of the breech region.

### Blowfly dispersal

Data on dispersal of SBF are completely lacking. For *L. sericata* Smith and Wall (1998) find mean values of 100-180 meters per day. Over a 3 week lifespan that would potentially result in several kilometres of active dispersal. Wind induced or animal-assisted transport can certainly be much higher. Gleeson and Heath (1997) use isozymes to show that gene flow between populations is limited, and conclude that sheep movement rather than fly movement is primarily responsible for gene-flow between sites. The limited daily dispersion range and isozyme results suggests that natural barriers such as water and/or habitat with no sheep (hosts) will be quite efficient at restraining colonisation of sites where eradication has been achieved. More detailed information on dispersal of SBF will be needed to determine the risk of fertile (mated) females migrating into areas where SIT is applied.

Larval dispersal is insignificant and occurs only in post-deeding (pre-pupation) larvae leaving the host. Dispersal is limited to <20 cm and digging into maximum 10-15 cm (Gomes and Von Zuben 2004).

**In Summary**
Lifecycle, developmental parameters and ecology are all factors influencing the seasonal fluctuation in population densities of SBF. Targeted management strategies for SBF could easily be deployed depending on these seasonal influences. The Sterile Insect Technique uses adult flies and releases of adult strains at peak adult field emergence in spring could significantly reduce population numbers of SBF. Predictive models for adult emergence of SBF in the field could prove useful for this purpose.

## 4.2 Current methods of control

From the start of their work on SBF, IM and JM Mackerras explored viable control options. Developments on control strategies have evolved significantly since then, including abandoned products such as DDT, development of new insecticides, biological controls, cultural and genetic control as well as new developments that could expand the use of Sterile Insect Technique into control of SBF.

In this chapter we present existing control strategies, with the exception of Sterile Insect Technique (SIT) which will be presented in Chapter 4.

### 4.2.1 Insecticides and insecticide resistance

#### *Insecticides*

Insecticides have a long association with the control of flystrike for the Australian livestock industry. Much of the prophylaxis against flystrike, has relied on the application of synthetic insecticides (Sandeman et al 2014, Waghorn et al 2013, Baker et al 2014).

Synthetic insecticides (see appendix 2 for full listing and details) currently approved for flystrike are mostly based on cyromazine or ivermectin, with some alternative options using imidacloprid, dicyclanil or spinosad. Older chemistries (organochlorines, organophosphates) are no longer registered or have become inefficient due to resistance in SBF (Sandeman et al 2014).

Insecticides are currently and predominantly applied through dipping of the sheep or using mechanical means of insecticide application through jetting by hand or machine application to the back of adult sheep. Jetting is widely employed by sheep farmers and can provide adequate protection to animals during periods of high flystrike risk, as long as the animals can be mustered for application.

Insecticides can be applied as a preventative treatment during early season when flies are dormant over winter, or before fly activity in spring. In addition to this, insecticides can be applied to wound/fly struck areas of sheep as part of wound dressing, or treatment after mulesing to prevent further fly strike of vulnerable healing areas. Because of the rather long withholding periods (generally two months before shearing) the protection of sheep (with long wool) during this phase is sometimes insufficient.

Preventive dipping or jetting of flocks generates expenditure associated with the chemicals applied, but more importantly, for handling the flock for application. In the case of curative treatments of affected sheep, the costs per head increase. Concerns have also risen about large volumes of ectoparasiticides entering the environment (Beynon 2012).

Choice of chemical, application time, method of application, and dose are all parameters that should be optimised to obtain effective prevention or healing of flystrike in relation to regional risk. However, clear decision rules and information on flystrike risk are often lacking (Lihou and Wall 2019) and treatments need to be planned together with other activities on the farm. This often results in either excessive treatment effort (overuse resulting in expense) or insufficient treatments (resulting in flystrike and expense).

### *Insecticide Resistance*

The currently available insecticides for SBF control (see appendix 3) are from only four chemical modes of action (IRAC Classification https://www.irac-online.org/modes-of-action/))

The most widely used pesticides for SBF are Cyromazine (IRAC Class 17) and dicyclanil (‘unclassified’ but likely Class 17). These are Insect Growth regulators (IGR) specifically disrupting the moulting of diptera and have a low mammalian toxicity. Resistance to cyromazine has been reported since 2012 (Levot 2012) and cyromazine resistant blowflies have cross resistance to dicyclanil (Levot et al 2014).

Ivermectin (IRAC Class 6), Spinosad (IRAC Class 5) and Imidacloprid (IRAC Class 4a) are all neurotoxins with slightly different modes of action. Increased tolerance of field strains of some Lucilia to ivermectin and spinosad (as reported by Levot and Sales 2008) exists but SBF has not developed resistance to these compounds. The use of the neonicotinoid imidacloprid is more recent and no reports on reduced sensitivity or resistance exist.

### *Fate in the environment and risk of off-target effects*

The use of any insecticide can have off-target effects. During and after application of pesticides on sheep pesticides can enter into the environment (Beynon 2012)

- When dipping sheep, the pesticide will drip from the animals after application, potentially causing high local pollution. The leftover from the dipping needs to be disposed of as prescribed.
- Sheep will excrete part of the applied chemicals through urine and faeces.

Little information is available on the amount excreted, and the effect of insecticide-tainted dung and other excretion products on other organisms in Australia. Species like dung beetles and flies feeding on faeces and urine will be exposed to such compounds, potentially reducing their numbers and their important ecological functions, as has been reported in the UK for several compounds including ivermectin (Gilbert 2019, Sands and Wall 2018, Wall and Strong 1987).

The APVMA is currently reviewing neonicotinoids because of a large amount of overseas research reporting significant off-target effects.

### *New Chemistries*

The probability of new chemical options becoming available soon seems low. The existing chemicals have all been on the market for more than 20 years and no new efficient options are expected to be registered any time soon.

A number of recent studies have examined the effect of natural products (essential oils) and combinations of natural products with insecticides for population control of blowfly (Bedini et al 2019, Callander and James 2012, Chaban et al 2018). Altering blowfly behaviour through application of feeding deterrents such as essential oils, could impede initiation of flystrike. In addition, essential oils possess ovicidal and larvicidal effects, and along with their antimicrobial properties and purported wound healing properties, could further hinder the development of flystrike. However, the efficiency of these products seems low.

**In Summary**

Insecticides remain in widespread use throughout Australia for treatment of sheep for ectoparasites. However, concerns with resistance, residues and environmental run off from chemicals is of increasing concern. The number of chemical options is low, and no new chemistry is expected to become available soon. Lack of good risk analysis leads to over-use of these chemicals accelerating the risk of resistance and off-target effects. More environmentally sustainable ways of dealing with SBF would alleviate these concerns. SIT provides an opportunity to develop an overall SBF control strategy that reduces the need for insecticides, reduces the risk of resistance, provides market opportunities (lower withholding periods for meat and wool and opening up of certain markets) and reduces environmental impact.

### 4.2.2 Mulesing

Mulesing (named after Australian Woolgrower, John Mules, Beveridge 1984, Morely and Johnstone 1983) is the removal of strips of wool-bearing skin from around the breech. This technique limits access of SBF to areas on a sheep vulnerable to flystrike – primarily the breech (behind) area where fleece and the perineum (dags) can remain wet and accumulate urine and faeces- a perfect environment for gravid SBF females to lay eggs (Watts 1979).

Mulesing is performed mainly on lambs and differs from crutching as it removes the skin to provide permanent protection against flystrike (crutching removes only the wool). Wool bearing skin and folds are removed by sheers or with a knife. As the wounds heal the skin stretches around the area and no longer has folds or wool for blowflies to lay eggs. The procedure is often done without any form of anaesthesia or post-operative pain relief, though the industry is trying to develop the use of analgesia and anaesthetics and Queensland is likely to impose this soon as a requirement (Stock Journal June 2020).

Over the last twenty years public opposition to the practice of mulesing has mounted. The first organised campaign within the community was in 2004 where a retail and consumer boycott were organised against the Australian sheep industry (Sneddon and Rollin 2010). However, mulesing continues to be a method of control despite research into alternative practices such as extensive breeding programs and non-surgical alternatives (James 2005, Atkins and McGuirk 1979, Beattie 1962.)

Since a failure to implement a complete ban on mulesing by 2010, there had been ongoing pressure to develop alternatives to mulesing from animal welfare groups and the retail and consumer industry. This may gain traction in the near future with reports that markets in non-mulesed, ceased mulesing, and flocks where pain relief was used when mulesing, have increased to approximately 50% of the Australian fleece production (Woods 2019).

### 4.2.3 Biological control

Biological control against SBF has not shown promising results so far.

4.3.2.1 Parasitic wasps

A larval parasitoid *Alysia manducatory* (Hym.: Braconidae), introduced from Europe and a native pupal parasitoid *Nasonia vitripennis* (Hym.: Pteromalidae) were amongst the first biocontrol agents investigated for biological control of SBF (Tillyard and Seddon 1933; Fuller 1934). Bishop et al (1996) collected and identified larval parasitoids from flystrikes on sheep in New Zealand. The overall parasitism rate of identified parasitoids *Tachinaephagus zealandicus* (Hym.: Encyrtidae) and *Aphaereta aotea* (Hym.: Braconidae) was only 1.1% which is unlikely to be sufficient to contribute in any significant measure to control fly populations (Sandeman et al 2014). The use of parasitoids in combination with pesticide application might result in reduced efficiency of biological control as reported for Nasonia and Imidacloprid (Tappert et al 2017)

4.3.2.2 Micro-organisms

A range of pathogenic microorganisms have been described for SBF and related species, but, as yet, none have been developed into commercial biopesticides for SBF control (Wright et al 2019).

*Bacillus thuringiensis* (BT) strains specific to Diptera have been tested as a possible preventive treatment since the bacteria can survive in the wool on the sheep. Further study is needed to investigate whether these can be developed into efficient control options.

Other potential biopesticides (microsporidia, entomopathogenic fungi) have been studied but have not developed into commercial applications (Leathwick et al 2019). Such products certainly have potential for integration into an integrated pest management package targeting SBF. However, the rate of spread of pathogens and parasites is almost invariably density-dependent, while SBF occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. Therefore, most recent research towards a biological control agent has focussed on inundative approaches or the development of biopesticides (Wright et al 2019, Leathwick et al 2019).

### 4.2.4 Genetic control

In the late 1980s, a “field female killing” strain of SBF was developed and tested successfully on an island (Foster, Maddern et al 1985, Foster, Vogt et al 1988). However, a subsequent larger island trial was not successful. The strain was found to be unstable; males were not competitive for matings with wild fertile males, and there were difficulties in mass rearing. The onset of low wool prices limited funds for further research.

More recent research has led to the development of transgenic female-lethal lines in the United States (Li, Wantuch et al 2014, Scott 2014). While these lines are based on North American SBF genetics, it may be possible, with the appropriate permits, to import a strain in quarantine, cross it with a field strain from Australia. It could then be mass-reared as a male-only strain for sterilization and release to suppress local population through SIT. SBF has a low tendency for dispersal when in favourable habitat conditions such as those that exist in New Zealand (Gleeson and Heath 1997). In such limited dispersal locations localized control measures such as mass trapping or genetic control techniques may have potential for controlling SBF numbers. However, the prospects for operational use of a transgenic fly for SBF SIT would be prohibited by current regulations and also constrained by social licence. While such approaches are likely to become more available and acceptable in future, and may be adopted later, SIT based on unmodified organisms can be established immediately.

Appendix 9 contains more options for improvement.

**In Summary**

SIT programs preferably use male only strains as female insects are not effective as control agents. This need for male only strains for SBF has driven research into transgenic strains of SBF that limit the development of female strains. Earlier research into the feasibility of developing female-killing (FK) systems looked at providing supposed benefits for less cost than SIT (Foster 1985). However, a recent study (Yan et al 2019) showed that new embryonic SBF transgenic sexing strains hold promise for genetic control programs and for SIT.

### 4.2.5 Integrated pest management (IPM)

Management of SBF should not solely depend on the preventive or curative use of insecticides. Additional information on flystrike risk, observation of the herd, and weather should all be used to optimise management and reduce insecticide use to a minimum. Such an approach of IPM is a form of applied ecology that enhances control by combining insecticides with other practices. These include appropriate husbandry, application of knowledge of SBF behaviour and ecology all of which present a more holistic approach to flystrike prevention (Cole and Heath 1999, Wall and Ellse 2011). IPM also incorporates each farm’s characteristic features of topography, microclimates, past experience of flystrike and general farm management into a workable prevention program (Sandeman et al 2014). IPM is a best practice approach using all available information to obtain efficient management with optimal economic, animal welfare, environmental and social outcomes.

In general, the control of SBF strike relies on two main approaches: suppressing the SBF population and reducing the susceptibility of sheep to SBF strike.

A number of methods that can be combined in the control of flystrike include (Scholtz 2010 and others):

- Tail-docking (Gill and Graham 1939, Riches 1942, Graham et al 1947);
- Crutching (McCulloch and Howe 1935, McCulloch 1932 1933, 1937, 1938, Graham et al 1947, Shanahan and Morley 1948, Belschner 1953, Graham 1954, Anson and Beasley 1975);
- Dagging (French et al 1992, Scobie et al 1999);
- Mulesing (Bull 1931, MacKerras 1935, Seddon 1935, Gill and Graham 1938, 1939, 1940, Graham et al 1941, Dun 1964b);
- Strategic shearing just before times of high fly activity makes the sheep less attractive to SBF (Belschner 1953, Mangano 1986, French et al 1996);
- Management of stocking rates and sheep movements to reduce sheep to high SBF areas and/or high flystrike weather conditions can contribute to a ‘clean’ or low-risk flock.
- Having topographical variations on farms allows for sheep, at times of high strike risk, to be grazed on sites with lower temperature and higher wind speed which are less attractive to the blowfly population (Cole and Heath 1999).
- The use of specialist forages that reduce faecal soiling also have a useful role in reducing flystrike (Leathwick, D.M., Heath, A.C.G. 2001).
- Control of internal parasites a such as gastro-intestinal nematodes reduces faecal staining and reduces areas where SBF can lay eggs: Morley et al 1976)
- Breeding for resistance, aiming at reducing skin folds in the breech area (Belschner 1953, Watts et al 1979);
- The selective application of pesticides (Russell 1994).
- Vaccines against Fleece rot (Tellam and Bowles 1997) and blowfly larvae (Colditz et al 2006)

Methods to control blowfly populations include:

- Fly traps (French et al 1992). The use of mass-trapping can reduce flystrike risk. Trials in Australia and New Zealand have shown it is possible to reduce flystrike prevalence (Heath and Leathwick 2001, Urech et al 2009). There is strong evidence in the literature that the use of fly traps in combination with other management systems can keep flystrike at low levels (Scholtz 2010, Urech et al 2009). The luci-traps used for such mass-trapping are however no longer commercially available in Australia (Bugs for Bugs pers. comm. August 2020)
- Sterile Insect Technique (Horton et al 2002, Bell and Sackett 2005) (see chapter 4)

Application of aerial insecticides to control SBF area-wide are not a viable option since the pupae in the soil will not be reached by the pesticide.

### *RISK Monitoring*

These management options should be completed with a good knowledge of the SBF population dynamics and flystrike risk in a given area in relation to weather conditions.

Predicting when flies are active, and anticipating their activity, affords farmers time to plan control strategies, including insecticidal treatments. Small offal-baited monitor traps provide an indication of fly activity (Norris 1965, Dadour and Cook 1992, Cole 1996) and can also be used as a means of assessing the relative abundance of each species in the flystrike fauna as the season progresses. More recently luci-traps with specific lures have been developed.

Computer modelling enables prediction of risk periods (Horton 2014, Percival and Horton 2013) and is available online to compare management strategies (Flyboss system). Modelling allows prediction of when flies will be active and, combined with weather predictions, if climatic conditions will be favourable for flystrike. However, these programs do not predict actual fly population size.

Observing the sheep regularly (twice weekly) for signs of flystrike will enable intervention when needed, but is not feasible in large surface, low stocking rate farming systems.

### *Existing IPM approaches*

An IPM tool (FlyBoss system) has been developed to prevent and control flystrike. It consists of inclusive information on flystrike management, decision making, and lists of products for preventing and treating flystrike (Horton and Hogan 2010). Extensive use of technology has enabled services such as Flyboss (Horton and Hogan 2010) to become readily available to farmers and has also enabled computer simulation models which have an important role to play in flystrike IPM program (Sutherst 2001). MLA and AWI present the FlyBoss system as the premier system for flystrike control information, providing easily accessible content to farmers on flystrike, breeding and selection for flystrike resistance, management, treatments and information on products, comparison management tools and optimise treatment tools. In addition to this “AskBill” a web-based program to enhance sheep wellbeing and productivity (Kahn et al 2017) has been incorporated into the FlyBoss system.

However, the application of IPM is labour intensive, requires farmers to be aware of blowfly ecology and biology, and willing to fully apply their husbandry skills and knowledge of their properties while reducing reliance on insecticides. The more widespread use of computers for communication, modelling and education increases the chances that IPM will become more accepted (Sandeman et al 2014) and the increased use of smartphones will add to these increasingly important farm tools.

The use of modelling shows differences in risk, resulting in differences in control strategies. In areas where SBF (larvae and pupae) have overwintered, early sheep treatment (14 d before modelling indicated first strikes would appear) with long acting insecticide has been found to be economically better for flystrike management in places where the strike risk was high enough to require treatment over the season (Horton 2014).

- The model indicates that in the Gunning area (with a high flystrike risk), reduction in seasonal risk would reduce total costs related to flystrike, reduce the overall use of preventive chemical treatment and reduce the number of sheep struck.
- In a lower risk area (Flinders Island), the value of early treatment would depend on a number of other factors, including flock and fly density and current treatment strategies for flystrike reduction.

**In Summary**
Efforts to combat SBF have resulted in an array of chemical, physical and cultural control methods readily available to farmers. Whilst beneficial, many of these methods have drawbacks and constraints to their use. Chemical treatments are less environmentally sustainable and are at risk of becoming obsolete through resistance or regulations. Physical and cultural methods are laborious and time consuming and in the case of mulesing, the distress to animals and decreased marketing potential of treated flocks have animal welfare and economic drawbacks.

## 4.3 SIT as a promising technique for SBF management

The Sterile Insect Technique (or SIT) is an environmentally sustainable technique for insect control. It involves area wide management of a pest through release of sterile insects of the targeted pest species (Dyck et al 2006). The science behind SIT has shown that mass rearing, irradiation and release of sterile insects leads to a high degree of mating competition leading to a reduced incidence of wild flies. Because the wild fly pest will not have progeny from mating with a released sterile insect, the overall population numbers are greatly reduced. The result is a severely impacted population or eradication.

Although SIT has been trialled for SBF in the past in New Zealand (Scott et al 2004), and field trials in NSW and Flinders Island showed great promise (Foster et al 1985), no ongoing projects exist in Australia.

Analysis of previous attempts identified a number of problems. These include (Vogt et al 1985; Foster et al 1993, Mahon 2001):

- Rearing problems due to capacity of the facilities, and diseases mainly in the egg stage
- The use of a rather complicated transgenic sexing system with inherited partial sterility in males, that needed several successive generations to achieve sufficient control.
- Release of larvae that need to dig into the soil, pupate and emerge, requiring at least 3 weeks before adults appear, resulting in high mortality
- Problems in maintaining a sufficient ‘overflow’ of sterile males
- Lack of capacity of sterile males to compete with wild males
- Releases in (subtropical) areas where populations of SBF are often high.

These problems can be avoided in the first stage of this project (also see proposals of Horton et al 2002) aiming to eradicate flies in island situations. Simultaneously, preparations can begin to further develop SIT for SBF through research and to increase the potential for control with reduced costs for fly production and successful deployment of SIT in other, larger and less isolated, areas.

Recent experience obtained through the production and irradiation facilities for fruit fly in Port Augusta (South Australia) suggest this as a potential starting point for a new programme of SIT for SBF. The success of the SITplus facility and associated logistics allows us confidence that this project will achieve its goals.

**In Summary**
SIT has a great potential for adding to the arsenal of control strategies for SBF. SIT is environmentally sustainable and has been widely successful for other dipteran pest species such as fruit flies, Screwworm fly and Tsetse fly. Here we propose to develop a pilot project using simple, existing technology, while conducting a research program for further optimisation. As an example: Current research into genetic strains of SBF that are compatible with IPM and area wide management through SIT is ongoing and could potentially be incorporated into a larger programme of research and delivery to industry.

### 4.3.1 Principles of SIT

In SIT, pest insects are mass reared, sterilised using radiation and released in large numbers to flood the wild populations within a defined area (Area Wide management).

Mating of fertile wild females with sterile males results in no production of fertile eggs and suppression or local eradication of the target insect population (Gilmore 1989; Knipling 1960). Using SIT for SBF was investigated in the early 1980’s (Foster et al 1985) but a lack of suitable mass rearing facilities halted further work on the programme.

SIT has been used effectively for a number of pest species including fruit flies, screwworm, tsetse fly, moths and mosquitos (see 4.2: success stories). SIT for SBF is used in Bangladesh to control SBF attacking drying fish (Islam et al 2014)).

To evaluate the feasibility of establishing SIT several factors need to be considered (Knipling et al 1955, White et al 2010, Tsitsipis 1977, Lux et al ,2002), including:

- Knowledge on ecology of the species (population density, population dynamics, mobility) in order to:

  - Select the areas with the highest probability of success (low pressure areas, low risk of re-establishment)
  - Determine the best time for release (usually when populations are low)
  - Determine the parameters for release (numbers to release, frequency of release, number of release points)
- Developing an economic rearing method to produce high enough numbers of insects to ‘flood’ the existing population (usually millions of insects are needed for large areas)
- Efficient irradiation of flies resulting in sterile insects with good capability of irradiated sterile male insects to compete with wild fertile males to mate with the virgin females in nature
- Development of cost-effective transport and release strategies (land or aerial releases)
- The ability of sterile matings to outnumber fertile matings.

In the rest of this chapter we will present why SBF is a very promising candidate for SIT in Australia for each of these points.

For expansive assessment of SIT programs, a thorough understanding of population ecology is needed (Barclay 2005, Dyck et al 2005). In relation to SBF, evaluation of any SIT programs should first take into account sterility of released insects is an environmentally safe strategy for SBF suppression in a range of climatic areas and livestock management practices. Secondly, SIT is compatible with all current SBF management strategies. Evaluation after trials should also highlight economic gains from SIT use and gaps for further research on technologies benefiting SIT.

## *SIT in relation to SBF ecology*

### Release numbers

It is important that wild populations of candidate insects for SIT are low enough to be able to produce and release enough sterile flies to outcompete the wild males. Sterile males need to outcompete the local population when females appear and mate. Literature indicates that optimal results will be achieved with release densities being at least 10x the pest population (Gilmour et al 1946, Vogt et al 1983, 1985, Wardaugh et al 1983), but this ratio should be further investigated with more details on field population density and reproductive rate (FAO 2016, 2019) allowing to model the expected result of release rates and frequency.

In ‘cool climate’ situations where SBF overwinters in the soil, SIT releases should initially target the first spring generation since this is the lowest population and it is well synchronized. This means the number of sterile flies needed to outnumber the wild spring population, and the number of successive releases needed to cover this activity period for sufficient control or eradication, will be low. As explained in Chapter 2, SBF populations are expected to commonly be between 5 and 10 flies per ha during the spring emergence in areas where SBF overwinters in the soil. This means that release quantities for sterile SBF would need to be around 50-100 flies per ha per week (5,000-10,000 per Km$^2$).

In warmer and higher rainfall situations the population of SBF is expected to be present most of the year and possibly in higher numbers. In many of the dry central Australian pastoral properties the activity of the flies will be rainfall dependent. These conditions will need to be considered in more detail to optimise release strategies for those areas. Before starting SIT in such areas more data on SBF population size, dynamics and distribution needs to be available. In low rainfall areas, it is expected that releases will be most effective when at the onset of rainfall events, focussing on areas where sheep are concentrated (near waterholes, camps and mustering/shearing stations).

### SBF Dispersal

As explained in chapter 2, SBF are considered to be not a highly mobile insect. Low mobility of the adults is an obvious advantage for SIT since the recolonisation of areas where SIT has been deployed will be limited. This will especially be the case for isolated (island) situations where flies would be unable to fly for more than a few hundred meters over unsuitable habitat (water). Island situations would be the first to target in the first SIT demonstration attempts (see chapter 6) as has been the case in earlier attempts (Foster et al 1978, 1985, Mahon 2001, Horton et al 2002). If eradication is achieved in such situations, the only risk of reintroduction is through movement of livestock.

However, given the low SBF mobility, a relatively small (several kilometres) separation between favourable habitats (sheep farms) would already be enough to enable local suppression/eradication with low risk of unassisted recolonisation (Gleeson and Heath 1997). Given the spatial scale of Australian sheep farming this means that even eradication at a farm scale/pastoral property, aiming at areas where sheep are present but separated from other flocks is feasible. It also means that an approach for area-wide management of SBF in large areas of the mainland based on a moving front of releases (e.g., as for Screw-worm flies in America [Scott et al in 2017] and as proposed for SBF in Tasmania [ Horton et al 2001]) is feasible.

Based on the existing ecological information, SIT will certainly be highly effective to eradicate SBF in isolated areas where sterile insects can be released area-wide. Before deployment of SIT in larger and less isolated areas more data on the ecology of SBF is needed in order to develop optimal strategies. Studies on the efficiency of SIT in initial pilot sites and candidate areas will enable collection of such additional more precise information on SBF ecology and population density, allowing improved SIT releases.

### *SBF rearing*

The rearing of SBF is relatively simple. Ideal rearing conditions are 28°C and high humidity. Flies lay eggs into a diet, usually consisting of offal such as liver. Larvae develop in 4-6 days and ‘jump’ out of the rearing diet into a collection tray filled with substrate (vermiculite) where they burrow to pupate. Adult flies emerge after another 5-6 days. After mating and a protein meal the flies start laying eggs. Females can produce several batches of >300 eggs (Readshaw and van Gerwen 1983) resulting in a very high reproductive rate, allowing them to produce large numbers of offspring. Individual cages or rearing chambers can be highly synchronised. Pupae can be easily separated from the pupation medium for irradiation.

This simple rearing technique, very similar to the one used for Queensland fruit flies, will be sufficient to produce sufficient numbers of flies (up to 100,000 flies per week, enough cover 1000-2000 hectares) for initial small-scale demonstration SIT releases in the first year of the project. Further improvement of rearing conditions, diet, and other operational activities will be investigated to increase production capacity and to reduce costs in preparation for wider potential deployment. Existing Australian mass-rearing facilities (for fruit-flies) are capable of producing 50 million flies weekly. Similar numbers of SBF would allow eradication of SBF on a 4-5000km$^2$ area

### SBF Sterilisation

The sterilization of SBF by irradiation is straightforward. Irradiation of pupae is carried out shortly before release to reduce the risk of damage to other than reproductive tissues and to allow emerging flies to have maximum fitness for competition in the field. The dose of radiation needed for SBF sterility while maintaining sufficient mating competitiveness in the field is about 3-5 Krad (30-50 Gray), which is lower than for many other insects.

One big advantage of SIT for SBF over moat other insects is that it is possible to cold-store pupae over considerable time periods (weeks to months). This would allow accumulation and storage of pupae until needed. However, the optimal storage conditions and quality of the flies emerging from stored pupae will need to be investigated more in detail (see Quality Control section later in document).

Irradiation equipment is costly, and irradiators require a power generator backup system. Irradiators for fly pupae can be either Gamma or X-ray based, and volumes for irradiation are relatively small if pupae are used. The SITplus facility in Port Augusta uses two X-ray irradiators with sufficient capacity to sterilize SBF for this project.

### Storage, Transport and release

SBF will be irradiated in the late pupal stage. This will allow small volumes (of pupae) irradiated, packed and shipped to release sites. No rearing out of the pupae would be needed since pupae (rather than adult flies as for fruit flies) can be released just before adult emergence, further facilitating the transport and release process. Pupae, once on the ground, should hatch quickly, and therefore predation should be low. Female flies need protein and carbohydrates to produce eggs (van Gerwen et al 1987); however, male flies are sexually mature upon emergence and do not require a protein source, only carbohydrates (Williams et al 1977). Studies into the best release method and timing will be needed to optimise large scale release strategies and SIT efficiency.

Since irradiation will be carried out just before hatching of the pupae it is essential to have a quick and efficient transport to the release site and subsequent release in the field. The best method for release will need to be decided on, anything from on-ground release using cars (for small scale releases) to drones or planes. When upscaling the areas under SIT, release from planes will be the preferred method since it allows minimal the time between irradiation and release and allows efficient coverage of large areas. It is essential that infrastructure for transport, storage and release are organised efficiently.

When releasing sterile insects, it is important (1) to target the areas where wild flies are expected to emerge, (2) to target the time when wild flies emerge. Competitive mating by sterile males needs to take place as soon as possible after females emerge from the soil. Modelling of SBF development and hatching dates is available (Horton 2015, Flyboss system). This, combined with knowledge of where SBF larvae are most prevalent in the period before SIT releases will enable targeting of the right areas at the right times.

Because of the limited mobility of SBF the exact positioning of release sites is essential. Ideally sterile pupae should be positioned close to areas where wild females are expected. The actual distribution of the releases should focus on areas where sheep (and SBF) were present in the previous autumn.

### *Quality control*

One of the risks related to mass-rearing insects for SIT is that the rearing conditions will produce flies that are best adapted to the rearing conditions, but not adapted to the field situation. This was one of the main issues with the previous attempts of SIT on Flinders Island, where males from the reared strain were unable to compete with wild males for matings with wild females. Therefore, it seems a better option to use ‘normal’ wild strains for SIT and use irradiation to sterilise them. Though the irradiation dose that is likely to be needed for SBF (30-50 Gray, Huda et al 2007) is low compared to other organisms (60-70 Gray used for Fruit Fly, Bloomfield et al 2017), irradiation can still modify the flies’ competitiveness in the field. Therefore, a quality control methodology will need to be developed to make sure that the rearing is efficient (egg production, egg and larval survival, pupal emergence, diet conversion etc) and the mass-produced sterile flies will be competitive in the field. Standard methodology is available for dipteran species (Mutika et al 2019) and QC protocols that have been developed for various insects at Macquarie University are now routinely used in SIT facilities.

Because of the large differences in climate over the area where SBF is present in Australia (see distribution map figure App 3.1 in Appendix 3) it is possible that flies will need to be ‘conditioned’ differently for the climate conditions they will encounter when they are released. Alternatively, the strains to be used for local SIT releases might be collected on site and/or rearing facilities can be moved to and used in the region where SIT is deployed.

### 4.3.2 Feasibility of using SIT for SBF – Practical Concerns and Considerations

SIT is more efficient than any of the existing control methods

The development of SIT for SBF is expected to be relatively straightforward, based on existing knowledge, experience and facilities. Before developing this into a full proposal it is important to quickly highlight some of the considerations for using SIT over other means of SBF control. These include:

- The expected efficiency of SIT will be superior to any of the other means. In those cases where eradication is achieved, other management methods are no longer necessary.
- Improved animal welfare and with that industry, market and consumer acceptance.
- SIT is not sensitive to resistance development.

- SIT causes no environmental hazard, and will result in a significant reduction in the use of pesticides that can have off-target effects on native populations of vertebrates and invertebrates both in agricultural land and sensitive co-located environments.
- Reduction of the handling sheep for preventive or curative insecticide applications (dipping, jetting, wound dressing), resulting in less costs, fewer WHS risks and less labour costs for farmers.
- Reduced need for costly and slow to develop alternative treatments such as vaccines or breeding costs for sheep resistance to flystrike.
- SIT is compatible with any of the other existing means (see table 4.3.1.), and in the case of reduction of population by SIT all these can still be used as part of SBF IPM where needed.
- Used in an Area Wide Integrated Pest Management Program residual populations of SBF could be eliminated.
- Improved market access through reduced chemical use and alternatives to mulesing.
- Improved consumer image of the industry.

**Table 4.3.1: Comparison table of SIT treatments with current available treatments**

| Treatment | Effects | Drawbacks | SIT Comparison |
|---|---|---|---|
| **Chemical treatments** | Kills insects and treats mulesing wounds. | Environmental concerns with off label use/environment dispersal and disposal of chemistries<br>Widespread resistance to chemistries (already occurring)<br>Misuse of chemicals<br>Export effects due to withholding periods needed for certain markets (fleece and meat) | Species-specific and non-polluting.<br>No resistance.<br>Easily structured for effective use in large scale campaigns.<br>No withholding periods. |
| **Physical** | Mulesing, crutching, shearing | Labour intensive<br>Community backlash to animal husbandry techniques and concern for animal welfare<br>Market considerations (export markets will not take sheep meat from farms using mulesing) | Medium labour intensive for duration of program.<br>Considered more environmentally friendly by general public<br>Markets are generally favourable to this technique |
| **Immunological** | Herd vaccinations | Sheep genome research is still ongoing in an attempt to unlock a vaccine<br>Australian trial likely not for 2-3 years. | Trial easily set up for production and release based off new available facilities around the country |
| **Genetic** | Selective breeding of sheep<br>Fly transformations | Breeding programs Intensive and lengthy process and focused not just on vaccinations<br>Gene editing technology still in infancy for blowfly | Process is rapid and adapted easily within a season. Labour is rearing and releasing.<br>Research into SIT is well established |
| **Biological** | Entomopathogens | Host-pathogen interaction complex and not fully understood as yet. No commercially available strain | Pathways for SIT for SBF understood and researched. Ready for further field trials |

## *Other considerations affecting SBF SIT Bushfire Recovery and Restocking*

The summer of 2019-2020 catastrophic bushfires tore through large tracts of Agricultural land in Queensland, New South Wales, Victoria and South Australia and resulted in the declaration of Bushfire Emergency affecting many red meat and livestock producers. Millions of hectares in regional and rural areas of Australia's vast livestock areas have been affected. People, animals and infrastructure have been severely affected by loss.

State and commonwealth government along with Meat and Livestock Australia, Peak Industry Councils, State Farming Organisations and livestock producers on the ground are now focusing on helping those in need of immediate assistance and working towards the challenges to be faced by industry during the recovery process.

At this time, welfare of suffering animals is a priority for MLA and farmers. Current assessments for livestock loss in sheep are around 13% of the national flock severely impacted, and a further 17% partially affected.

Kangaroo Island, one of the trial sites being considered for SBF SIT trials has been severely affected with almost half the landmass of the island affected by bushfire. An estimated 52,000 sheep have been killed or severely wounded during the December 2019/January 2020 fire, around one-tenth of the island's total flock count. The South Australian government, through the Department of Primary Industries and Regions, are still working with producers on Kangaroo Island to complete estimates of stock losses.

Diversity studies in invertebrate abundance post bushfire show that the reestablishment of invertebrate animals plays a primary role in product nutrient cycling and uptake, population and community level interactions and energy storage and transfer. Contributions of invertebrates provide substantial benefits to the overall recovery to affected ecosystems. This includes a number of native species of blowflies. Currently no data exists on the establishment of SBF after bushfire.

Many of the farms affected by fire losses are already in the process of restocking and an opportunity to remove/suppress SBF from farms will enhance the economic regrowth of regional areas.

**In Summary**

Considering the devastation to sheep numbers in certain regions of Australia, farmers are looking to restock and increase sheep numbers. SIT could benefit farmers at this time through the suppression and management of SBF. An opportunity exists for further research into establishment and dispersal of SBF during the fire regeneration period as well as establishing preventative SIT protocols to rapidly increasing stock numbers.

## *Recent SIT success stories in Livestock*

SIT has been developed successfully for a number of livestock pests, mostly flies. Here we provide an overview of some of these projects (see appendix 4 for a more complete listing) since these provide valuable information on successful project design and set-up, allowing us to shape a proposal in several phases (chapter 6) that would have the highest probability of success.

The International Atomic Energy Agency lists approximately twenty countries having taken part in active/pilot programs using the sterile insect technique especially for issues surrounding livestock (see Table 4.3.1)

**Table 4.3.2: Insect pests for which SIT was used or is being developed in Livestock**

| Insect | Previous sites | Current sites |
|---|---|---|
| **New World Screwworm** | Curaçao, USA, Mexico, Puerto Rico, US Virgin Islands | Guatemala, Belize, Libya |
| **Old World Screwworm** | Papua New Guinea* | No SIT currently |
| **Tsetse flies (Four species)** | United Republic of Tanzania*, Nigeria, Zanzibar, Burkina Faso* | |
| **Sheep blowfly** | Australia* | Bangladesh (fish) |
| **Mosquitoes** | El Salvador* | |
| **Stable fly** | St Croix, USA* | |
| **Cattle fever tick** | St Croix, USA* | St Croix. USA |

* SIT Pilot trials

The success of screwworm eradication programs in the United States during the 1950s and 1960s (Baumhover et al 1955; Knipling 1979) encouraged wider use of the SIT to control other insects, including at least 13 species of tephritid fruit flies (Steiner 1969; Klassen et al 1994). By far and away the most widely used of SIT currently is for dipteran insects with large scale successful eradication throughout the world. Some examples of successful past SIT programs include the eradication of Queensland fruit fly (*Bactrocera tryoni*) from Western Australia (Fisher 1994), melonfly (*Zeugodacus cucurbitae*) from the Okinawa islands (Kakinohana 1997) and tsetse fly (*Glossina spp*) from Zanzibar (Vreysen et al 2000). SIT has also been used for eradication or suppression of the Mediterranean fruit fly (*Ceratitis capitata* - medfly) in various parts of the world (Gilmore 1989; Hendrichs 1994), pink bollworm *Pectinophora gossypiella* in Californian cottonfields, and codling moth (*Cydia pomonella*) in Canadian pome fruit orchards. More details are provided in appendix 3

## *Tsetse Fly*

Tsetse flies (Diptera: Glossinidae) are active in livestock populations throughout west and central Africa and are vectors for sleeping sickness – human African trypanosomiasis (HAT) and African animal trypanosomiasis (Nagana). Trypanosoma spp are the primary pathogens responsible for the disease and affect cattle and other ruminants. The pathogen has also been known to cause high mortality in domestic pigs (Abd-Alla 2013). If left untreated, the condition is fatal and, in some livestock, can devastate up to 50% of stock numbers in severe outbreaks (Holmes 2013). Affected livestock can produce little milk (in the case of cattle) and prevent the availability of manure to assist remediate soils.

SIT programs for tsetse flies have been run in various parts of Africa, including Tanzania, Nigeria, and Zanzibar. 'Area-Wide Integrated Pest Management in the Niayes Region of Senegal' for tsetse fly has recently been at the forefront of long-term efforts in an eradication and suppression program for tsetse fly populations. In the recent campaign, tsetse fly was declared eradicated by the government of Senegal (2018) and subsequent reduction in effects on livestock have enabled increased incomes for cattle farmers through improved milk and meat production and cattle reproduction rates.

Irradiation was carried out in IAEA approved facilities in Slovakia and irradiated male pupae were sent to Senegal for release on a weekly basis. Some of the flies were produced in neighbouring Burkina Faso. The mass rearing centre in Bobo Dioulasso (Burkino Faso) was provided with fresh blood from abattoirs to facilitate improved rearing of sterile males to help strengthen the program.

One of the most notable outcomes of the eradication program was improvement in the methods of delivering SIT to the region. This is a particular concern to livestock areas that stretch over large grazing areas.

As SIT is often considered not to be a stand-alone method for tsetse fly species control, eradication has often not been the focus of such programs. Instead, SIT has been used in combination with other methods within an area wide integrated pest management strategy.

## *Screwworm fly*

### USA - Florida

Re-emergence of the primary screwworm *Cochliomyia hominivorax* (Coquerel) is of great concern to USA livestock industries. Screwworm fly was eliminated from the continental USA in 1959 using SIT which continues to be part of the arsenal against incursions. The screwworm fly SIT program is an ongoing effort in Central and South America, but re-emergence of the pest occurred in 2016 in Florida (Skoda et al 2016) resulting in reestablishment of the SIT program. USDA officials released almost three million transgenic (FL11/FL12 transgenes) male-only screwworm flies weekly over a five-month period from October 2016 to March 2017. Successful eradication of the fly from the outbreak was declared soon after. Such transgenic male only lines have been considered to be economically viable means of reducing eradication costs in modern day programs and provide more efficient population suppression (Concha et al 2016).

The molecular characterisation of screwworm detected in the Florida 2016 outbreak determined that the flies were from one geographic source (Dupais et al 2018. This enabled specific focus on risk abatement strategies against incursions from this region to prevent further introductions.

## Central and South America

Through cooperation with United States government, in 1991 Mexico was declared free of New World Screwworm Fly (NWSF). This was an important result for animal and public health in the region as well as economically significant impact to agriculture. Programs for control have been ongoing in Mexico since 1966 with one of the biggest focuses of the campaigns involving research into preventing further spread into northern Mexico and the USA. A geographical—critical line—was established and a sterile insect barrier set up to prevent further incursions (Vargas-Terán 2005). Mated NWSF females have a greater dispersal capacity than males (Gutierrez et al 2019).

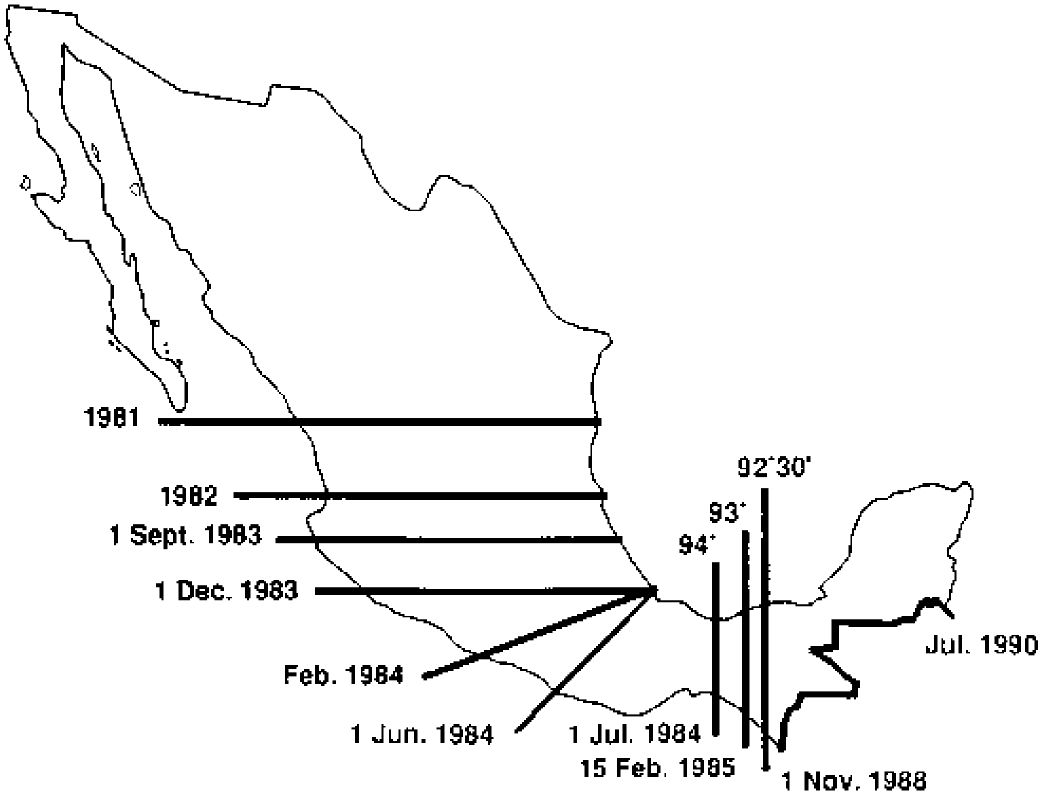

**Fig 4.3.1: 'Critical lines' dividing Mexico's screwworm-free northern zones from southern control zones (from M. Vargas-Terán 2005)**

Through the eradication program, microclimates were detected as a barrier to successful eradication as flies could overwinter in these areas. A range of control measures assisted in ensuring a successful SIT program.

Other areas of Central and South America have had success with eradication using SIT – Puerto Rico eradicated NWSF in 1975, Curacao in 1959 and again in 1975 (following re-incursion). Eradication is still in process in Guatemala and Belize, although populations are already greatly suppresssed (Graham 1985, M. Vargas-Terán 2005).

Eradication and suppression of New World Screwworm fly in Central and South America is still considered one of the world's most successful SIT programs and a model for incursions on other continents.

### Africa – Libya

The New World Screwworm fly was first introduced to Libya in 1988, likely through importation of livestock from South America. The presence of the fly posed a serious risk to all livestock industries in North Africa (Vargas-Terán 1994, 2005). Control of the incursion by chemical means alone was considered not sufficient enough to eradicate the pest so SIT was used for eradication and suppression.

Sterile insects were imported from Mexico and ‘boxed fly transports’ were flown through several transits to Tripoli. In a two-year program, over thirteen hundred million sterile screwworm pupae were sent to Libya. The control and suppression program instigated by the Libyan government covered over six thousand square kilometres. It involved large scale control at ground level and included animal inspection by veterinarians and training programs for farmers (Lindquist and Abusowa 2012). As a result of the program, eradication was declared in 1991.

Continued surveillance ensured that NWSF had not re-established and continued support from the FAO and other UN organisations has ensured that the insect did not spread to neighbouring North African countries and increase overall economic costs of control.

**In Summary**

Success stories of dipteran pest control using SIT are well documented. SIT for such pests has been in place and ongoing for many decades in the USA, Mexico, other central and South American countries, as well as in several African countries. Screwworm fly in particular, a devastating pest of livestock and wildlife, is one of the most successful entomological SIT stories of all time. The success of this noxious dipteran pest demonstrated that SIT has great potential for other dipteran pests of livestock.

Based on the available information, SIT seems a very promising technique for SBF management in moderate climate conditions where SBF overwinters in the ground. Current existing and available techniques and facilities for rearing, irradiation etc allow to establish small scale demonstration projects in the first project phase. During this initial phase additional ecological research on SBF biology, populations, dispersal, and distribution in the field can be carried to complete existing information. Improvement in sterile fly production capacity is warranted and can be gained through further trials on SBF.

## 4.4 Collaborations with other countries

Progress in this project will be achieved through collaboration and exchange with other facilities such as the SBF SIT in Bangladesh and NWSW SIT in Panama. Nearly all SIT projects and facilities are linked to the Joint FAO/IAEA Programme on Nuclear Techniques in Food and Agriculture and have regular meetings, information exchange and collaborations.

### 4.4.1 Bangladesh SBF SIT

SIT is used for control of SBF in coastal fishing areas of Bangladesh and shows promising potential for reducing blowfly issues (Islam 2014). International collaboration on the method would be beneficial to both the Australian Sheep industries and Bangladesh fishing industries.

Sun drying of fish is a common practice in Bangladesh, particularly in the remote coastal depressions and offshore islands where chilling and freezing facilities are unavailable (Islam, Islam et al 2014). Fish and fishery products are damaged or reduced in quality due to larval infestation of a number of fly species during the process of sun drying. SBF eggs are often laid around the gills and mouth of the fish, and control has been achieved through salting, smoking and insecticide dipping. There is a limited demand for salted and smoked products and there is a move away from the practice of insecticide dipping or spraying of the heads of the fish, which while effective, has health implications for the producers and consumers (Walker and Greeley 1991)

A study of the fly species infesting sun-dried fish in Sonadia, an offshore island on the Bay of Bengal, found that SBF is the species dominating infestations. A SIT release program was conducted over two years (2002 – 2004) on the island of Sonadia (Shahjahan, Saifullah et al 2005). In the first season (Sept 02 – Nov 02), 500,000 sterile flies were released and a 60% reduction in egg hatch was observed. In the second season (Nov 03 – Feb 04), 15 million flies were released in batches of 2.5 million over six months. Post-release sampling in Nov 03 – Mar 04 indicated no occurrence of blowfly.

Subsequent work has involved the development of an artificial larval diet (Shefa, Hossain et al 2013) and a new mass rearing facility has been constructed at Cox’s Bazaar, nearer to the release site to improve pupal pre-release survival (M. Yasmin, pers. comm.). A new irradiation source from Hungary will be installed soon to facilitate the production of sterile pupae for the 2018/19 fly season.

Rearing conditions, release methodology and other operational practises certainly differ between the Bangladesh SIT program and the needs of an Australian program but exchange of information with the local staff has already contributed to this review and optimisation of the current small-scale rearing.

## 4.5 Other candidate livestock species for SIT

There are a number of other species that could be considered suitable candidates for SIT in Australia. SIT for SBF could be used as a model to combat potential incursions of New World Screwworm Fly (NWSF) and Old-World Screwworm Fly (OWSF) as well as to control pest species that are already established in Australia, such as Buffalo Fly.

Chapter 4 documented examples where SIT has been successful in other countries for suppressing or eliminating pest fly species. Particularly Screwworm fly (both Old World and New World). Given Australia's unique geography, flora and fauna, any preparedness program for these pests would benefit from SIT research conducted on SBF.

Australia currently runs a Screw Worm Fly Surveillance & Preparedness Program through Animal Health Australia. The Program, in consultation with stakeholders from industry and government departments aims to detect Screwworm Fly before it establishes. The program runs surveillance, mainly through parts of Northern Australia, provides screwworm fly maggot collection kits, manuals for diagnosis, and advice on current chemicals registered that can combat Screwworm Fly. Currently, no preparedness plans have been set for eradication using SIT. However, given the success of overseas programs using SIT, a new approach to preparedness for these pests could easily be adapted from existing technologies and from outcomes and learnings from SBF SIT research programs

### 4.5.1 New World Screwworm Fly Genus: Cochliomyia

*C. macellaria, C. hominivorax, C. aldrichi, Wohlfartia magnifica and C. minima.*

*Cochliomyia hominivorax*, at larval development, are obligate parasites of mammals causing wound myiasis, is one of the most economically damaging pests of livestock in the Americas (Lindquist et al 1992).  Female *C. hominivorax* lay up to 200 eggs at a time to open wounds, enough to kill small animals with a single oviposition event, and multiple infestations can kill cattle (Robinson 2009). Eggs hatch after 11-24 hours and larvae feed on living tissue for 4-8 days before dropping to the soil to pupate (Robinson 2009). There are reports of *C. hominivorax* adults travelling up to 290km to find new hosts and use the wind to aid dispersal (Spradbury 1994). All *Cochliomyia* spp. Have similar biology and would present a similar threat to the Australian cattle industry. The screwworm fly that could most readily establish if introduced to more temperate areas is *Wohlfartia magnifica* and while generally of lower economic importance in its home range in Europe and across to China there have been incursions into new areas in Asia and Northern Africa (Robinson 2009). The biggest biological difference between *W. magnifica* and other screwworm species is that it is larviparous and deposits 1st instar larvae into open wounds and body orifices of host animals.

*Cochliomyia hominivorax* has been the target of an international eradication program based around area-wide releases of sterile insects. The highly successful control and widespread eradication of *C. hominivorax* from most in central and North America was estimated in 2005 to benefit producers in the US US$796m, Mexico $US292m and Central America $US77.9m annually (Vergas-Teran et al 2005). A 1988 incursion of *C. hominivorax* into Libya was initially managed by insecticide treatment but in 1990 3.5 - 40m sterile flies per week from the Mexican-American program were released over a 25,000 km2 area around Tripoli. The program eradicated *C. hominivorax* from the region with the last detection in May 1991, avoiding damage and human health across the entire African continent (Lindquist 1992).

The production of *C. hominivorax* in America has now moved to Pacora in Southern Panama as all areas north of that are now considered free of the pest.

If an incursion of this species occured in Australia, current regulations would likely prevent the import and release of sterile flies or pupae for biosecurity reasons. Therefore, to allow for this technology to be deployed as part of an area wide incursion response program Australia needs to have systems in place that could quickly be moved to the incursion area and be functional at short notice. A SBF program could be used to develop this type of transportable facility that could be deployed if needed. While the introduction of *Cochlimonyia* spp. into Australia is less likely that *Chrysoma* spp., which are present in a number of neighbouring countries, *C. hominivorax* was detected in a tourist's neck wound in 1992.

### 4.5.2 Old World Screwworm Fly Genus: Chrysomya

*Chrysomya rufifacies and Chrysomya megacephala*

Old World Screwworm fly, Chrysomya sp., causees primary and secondary myasis of domestic and wild animals and some species such as *C. bezziana, C. megacephla* and *C. rufifacies* have the potential to cause economic losses exceeding $300m (1989 estimate) annually to the Australian livestock industry (McKelvie et al 1993; Spradbury et al 1989). *C. rufifacies* is distributed throughout the US, Japan, New Guinea and India and has been found as far north as Ontario suggesting that it could establish and spread quite widely if introduced to Australia. *Chrysomya megacephala* also is widely distributed in South Africa and Asia, including New Guinea, which are not dissimilar to Australian cattle production regions. *Chrysomya bezziana* is also present in South Africa and some of Australia's near neighbours such as Papua New Guinea. Modelling of dispersal of *C. bezziana* after a potential incursion through Darwin predicts rapid dispersal and establishment across much of northern Australia within 3 years of arrival, whereas dispersal would be marginally more limited if the incursion was through Brisbane (Welch et al 2014).

*Chrysoma* spp. prefer to lay eggs at the edge of wound sites or body orifices of living hosts in the hours preceding dusk (Norris and Murray 1964) to reduce mortality caused by the heat and radiation levels of daylight hours (Spradbury 1979).

It is estimated that the eradication of *C. bezziana* from South Africa through area wide control including the application of SIT would benefit their economy by $US3.6 billion annually (Vargas-Teran et al 2005). Trials carried out in Papua New Guinea by Spradbury et al (1989) demonstrated that SIT would be a viable management and eradication tool for *C. bezziana*.

The distribution of the OWSF in Papua New Guinea presents a persistent threat to Australia. Infestation levels over a ten-year period in Papua New Guinea (1973-1985) is thought to be a foundation for concern for the Australian livestock industry, especially in areas of Northern Australia. The Torres Strait is considered a high-risk entry point for OWSF although the only incident of introduction was through a case of human mysias in 1992 (Searson et al 1992). A Screw-worm Fly Surveillance and Preparedness Program exists at various entry points in Australia with fly trapping areas and active livestock monitoring taking place across northern Western Australia and Queensland and the Northern Territory.

### 4.5.3 Buffalo Fly *Haematobia exigua*

The Buffalo fly *Haematobia exigua* was introduced to the Northern Territory in 1825 (Seddon 1967). It is widespread in the tropical regions of Australia from Robe Pt in Western Australia to Kempsey in NSW. Buffalo fly has permanently established within 500km of the coast but can disperse as far inland as Birdsville and as far south as Taree in NSW in favourable seasons. In 1992 it was considered the main cause of cattle health problems in Northern Australia in both beef and dairy production (Bock et al 1995). In 2006 the cost of H. exigua to the Australian beef cattle industry was $75m annually and higher in wet years (Sackett et al 2006). Climex modelling by Sutherst and Maywald predict that by 2030 their range may extend as far south as SA’s southern production regions further increasing the costs to Industry through increased management costs and reduced yields. Buffalo flies cause irritation to the cattle by creating lesions and feeding on the animal’s blood resulting in reduces weight gains, lower milk yields, transmit nematodes (*Stephanophilaria* sp.) and they create oviposition sites suitable for screw worm fly species if these would be introduced into Australia (James 2011). The lesions caused by *H. exigua* and *Stephanophilaia* sp. also devalue hides (Sackett et al 2006). Estimates of the losses in production vary partly due to the infestation level and tolerance of the breed assessed but increases of up to 13kg in 5 months were observed between animals treated with insecticidal ear tags and untreated, other published results record increased weight gain of between 4 and 15% after treatment of over untreated animals have been reported (Bean et al 1987; Spradbury and Tozer 1996).

#### *Buffalo fly Biology*

*Haematobia exigua* are obligate permanent parasites of cattle causing open suppurating lesions from which they feed up to 40 times a day (Anon 2011). Adult flies live 10-20 days on the host animal with females leaving the host for short periods to lay eggs in fresh dung. Eggs usually hatch within 24 hours of laying and larvae develop in the dung pat. Larval development is temperature dependant, but normally pupation occurs 4-5 days after eclosion from the egg, similarly pupation duration is also temperature dependant and varies from 5-7 days resulting in an optimal period of egg to egg laying adult of 14 days but this can extend up to 40 days (Macqueen and Doube 1988, Anon 2011). Emergence ratio is normally 1:1 female:male with development of juvenile females generally one day quicker than for males. Wing hardening takes approximately one hour followed by host search and infestation of hosts usually overnight (1600-0800h) (Macqueen and Doube 1988).

Adult emergence generally occurs in the afternoon (1200-2000h). Adults can fly 5-8 kilometres to find a suitable host (cattle). Adults can only live short periods away from the host (1-3 days, Anon 2011) although this period is humidity and gender dependant, females netted away from cattle survived 8 hours (low humidity, no water availability) to 20 hours (high humidity) and 2 and 11 hours respectively for males (Macqueen and Doube 1988). While flies move upwind and across wind to find hosts approximately 80% of flies located on hosts in release and recapture experiments were downwind from their release point (Macqueen and Doube 1988).

*Haematobia exigua* competes for the dung resource with several other dipteran and coleopteran insects but responds earlier than other species during late winter, allowing it to establish damaging populations before other species emergence. *Haematobia exigua* is able to exploit declining quality autumn dung better the dung beetle and house flies giving it further advantage over them competition for breeding resources (Macqueen et al 1986).

### Management

There are a currently a range of chemical and non-chemical options for the control of *Haematobia exigua*, however, widespread resistance to synthetic pyrethroids has been reported with the first reports in 1980 finding >1000X reduced sensitivity, with some evidence of cross resistance (Schnitzerling et al 1982; Farnsworth et al 1997). Resistance to organophosphates is anecdotally increasing (Anon 2011). With the development of resistance to insecticides and the physiological advantages that *H. exigua* has over its rivals for reproduction resources the cattle industry has been relying heavily on insecticide impregnated ear-tags and the development of new chemistries for management of the ectoparasite. It's likely that the availability of new chemistry options in the future will slow and, with the fragmentation of the industry, resistance management strategies are unlikely to be widely adopted (Holdsworth 2005).

The use of tunnel traps has shown a good effect, reducing pest pressure on the animals.

### SIT for Buffalo fly

The use of SIT for buffalo fly is less likely to be successful than for other species. The main issues hampering SIT for Buffalo fly are the fact that the population size of this species is generally high (tens to hundreds of flies per head of cattle) and the high mobility of the flies (5-8 km). Therefore, releases of very high numbers of sterile flies over very large areas will be needed to have a good effect.

More detailed work on buffalo fly field ecology is needed to determine if and when populations will be low enough for SIT. Eradication of buffalo fly through simple SIT using irradiated flies seems unlikely/uneconomic. Buffalo fly management will rely on a combination of management options which are minimal but likely sufficient for limited outbreaks.

However, as control becomes more challenging and *H. exigua* spreads south, the development of a SIT based on "field female killing" strains or other genetic modification could become an economically viable option, rearing methodologies, diets and facility design developed for SBF could be adapted. Development of a program suitable for *H. exigua* will also provide a basis for a SIT based eradication program if an incursion of Horn fly *H. irritans* were to occur.

# 5. Discussion

## 5.1 Conclusions from Literature Review

The ecology of SBF makes it a very good target for SIT. Low population densities and limited dispersal, existing rearing methods, irradiation and release methods, knowledge and models on development and spring emergence, are all available through current projects making it highly likely that a project based on 'standard' area-wide SIT in island situations using irradiated flies will be successful.

In the past SIT has been attempted for SBF during the late seventies early eighties but these attempts were hindered by the lack of facilities for mass rearing, irradiating and releasing sterile insects (Foster et al 1978, 1985). In the case of Flinders island, the self-sexing strain that was used was a 'field female killing' strain that unfortunately was unstable in the field and in the rearing process (Scott 2014), males were unable to compete successfully with wild males and therefore the project was not extended to other islands or mainland Australia.

Based on existing knowledge of SBF, experience from historic studies and attempts; the experience acquired in the use of SIT for several fruit fly species (Q-fly, medfly) and SBF in Australia, and for management of numerous fly species elsewhere in the world (including SBF in Bangladesh); the availability of existing facilities and equipment for rearing, irradiation and release, SIT is a very promising technique for a cost effective eradication (in 'island' situations) or suppression of SBF (in high pressure areas of the mainland) with significant advantages. The technique certainly can be improved, but the current knowledge level is sufficient to start using SIT very rapidly as a field control method in small and medium size demonstration sites (see chapter 6).

Improvements in the SIT process are certainly possible (see appendix 5). Research should accompany early SIT attempts in pilot areas, to obtain increased understanding of SBF field ecology and improve production and release capacity. This is expected to increase the potential area under management and to reduce the costs considerably. This will then enable development of a plan for the use of SIT against SBF over larger areas, including mainland Australia, similar to the current NWSW work in America (Vargas-Terán 2005)

**In Summary**

Early research investigating SIT for SBF during the late seventies early eighties was hindered by lack of facilities for mass rearing, irradiating and releasing sterile insects. Since then, further research into the insect's biology, ecology and genetic makeup has allowed reconsideration of the advantages of SIT for further SBF control programs. When compared with current methods of control, SIT provides an environmentally sustainable method that would easily complement existing IPM strategies for SBF management and could be a regular tool used for eradication and suppression.

# 6. Conclusions/recommendations

## 6.1 Proposal for the development of SIT for Australia

Australian meat and wool producers struggle with the cost-effective and acceptable management of SBF and flystrike. Here we propose to develop SIT for SBF as a sustainable alternative, compatible with existing methods and likely to be very efficient.

### 6.1.1 General

- Current research and development on SBF provide a sufficient basis to commence implementation of SIT for SBF.
- The research partners proposing this project (listed hereafter) have substantial experience with the development and implementation of SIT for other insects and in animal husbandry:
    - Macquarie University, Professor Phil Taylor and colleagues
    - PIRSA-SARDI Adelaide, Dr Peter Crisp and colleagues
    - NSW-DPI, Dr Polychronis Rempoulakis and colleagues
- Current existing facilities and methodologies can be adapted and made available to develop a cost-effective project and reduce initial investments.
- A new rearing facility based on shipping containers would be very cost-effective and allows use of the facility elsewhere if needed after the project. A modular setup could also be equipped with standard ‘off the shelf’ climate control options (split system air conditioning, thermo-hygrostat, humidifiers, that would reduce costs and would make repairs easier.
- Partnerships between industry and research should be strengthened and support from industry should be backed up by relevant and involved research to improve existing technology and cost effectiveness.
- Should SIT reach an area wide management approach for SBF - commercial interests may come into play and investigation into potential partners should be done through relevant industry bodies.

### 6.1.2 Specific

- Pilot studies over small demonstration areas should be initiated to investigate optimisation of mass rearing and release of irradiated SBF, aiming at eradication.
- A research effort should be associated to these pilot studies to further develop SIT efficiency and cost-effectiveness before deployment over a larger area
- In the larger candidate areas, research into SBF ecology should be initiated from the start of the project to establish the optimal SIT strategy (such as eradication following a release front).

- Communication:
    - Representatives from the livestock industry are considered the key stakeholders in any future research proposals.
    - Meetings between collaborators and industry should be held regularly to discuss the range of research and formulate research projects through a steering committee.
    - Efficient communication such as an information sheet / newsletter should be created and regularly updated throughout the programme's lifetime to inform farmers and industry on what is happening with SIT.
- Final project outcomes would be:
    - Eradication of SBF in pilot areas and progressing eradication in first large-scale deployment area.
    - Optimised capacity (including rearing facility) for expansion of SIT-SBF control to new and larger areas.
    - A mid-term review of the project after two years, before the large area SIT deployment starts.
    - Follow up action plan on further research and development strategy, twelve months before project end.

## 6.2 Detailed project proposal

Based on the literature preview we propose the possibility of launching the development of SIT for area-wide SBF management since this is technically and economically feasible and will improve economic performance and market opportunities.

We propose such a project over 5 years, in two major phases with a go-no-go decision between these phases (phase 1 = Demonstration and optimisation, phase 2 = Medium scale SIT deployment), depending on results and demand from the industry. If successful, the project would result in the creation of a cost-effective and sustainable methodology. After these 5 years the technique can then be deployed further, for example over two more phases aiming at eradication over a larger island area using a moving release front (phase 3), and progressive regulation/eradication on the Australian mainland using a similar approach.

We expect that improvement of SIT during phase 1 and 2 will result in sustainable SBF eradication and/or suppression, reducing the need for other less efficient and less acceptable management options and resulting in a significant cost reduction for the industry.

### 6.2.1 PHASE 1: Demonstration and Optimisation

The first phase (2020-2023) includes the following elements:

1. **Deployment of SIT in paired pilot studies:**
    - **Proof of concept (Spring 2020)**
        - Sheep pen trials using sheep and releases of different sterile male / wild female ratios in the presence of sheep made ‘attractive’ for egg laying by wet conditions.
        - In these trials known numbers of non-irradiated (fertile) and sterile males and non-irradiated females would be released in a situation favourable for the development of flystrike (sheep with moist wool in caged pens).
        - Mating success, egg-laying and hatching and flystrike symptoms will be monitored to compare success of control.
        - Research through NSW DPI and existing facilities.
    - **Paired pilot studies (2020-2022)**
        - Selection of 2 or 3 sets of paired pilot areas (with/without SIT) in different climatic contexts to demonstrate the feasibility and success of SIT. Possible sites:
            - Islands with sheep grazing near Port Lincoln (Mediterranean climate)
            - Experimental Sheep farms in NSW (DPINSW/ CSIRO) (subtropical)
            - Farms / farmers interested in low rainfall areas
        - In these sites SIT would be deployed with the aim to eradicate (islands) or suppress the existing populations on one of the paired sites while using the other site as an untreated control.

- Test-releases of pupae should be carried out at these trial sites to obtain data on spread and survival of dropped pupae.
- The size of these pilot sites should be small enough to cover with SIT releases based on existing rearing facilities (SARDI/NSW DPI). Current rearing can be upscaled without major investment to produce 50-200,000 sterile flies per week, allowing SIT to be deployed on two sites of 500-2000 hectares maximum from September 2020 onwards.

2. **Field studies on SBF ecology (2020-2025)**
   Field studies are needed to increase knowledge on SBF ecology in order to optimize release timing, quantity and conditions (suitable for a PhD subject) for pilot and future areas, more detailed data on actual SBF population dynamics and ecology is needed.

- The flight periods and density of the target population, dispersal and survival.
  The density of flies is an important parameter for optimising SIT. Exact knowledge on fly densities is lacking. It seems highly likely that densities are very different between regions and seasons. Therefore, multiple observations should be carried out in the initial test areas before and during SIT.
  - Trapping of SBF (adults and larvae) to obtain reliable data on population dynamics, spatial distribution in relation to landscape and sheep movement, dispersion, diapause etc)
    - Weekly trap monitoring data (adult trap and soil-emergence traps) should be collected
    - Mark recapture experiments (using sterilized and unsterilized flies) from marked cohorts.
    - These same experiments can be used to measure the dispersal range of the insect when using mark recapture experiments from a point source and short-term trapping incidents.
    - All these results can also be used to quantify survivorship of released insects.
- Observation of sheep to quantify flystrike and egg and fly numbers (maggots) in the areas under study
- These field observations will allow us to know when to release sterile flies, where to release them, what quantity to release per ha, at which frequency and over which length of time etc. As explained before there is still a lack of good data on SBF ecology and these issues will need to be addressed to improve understanding and the success of SIT.
- Observations on the success of SBF SIT in the pilot areas
  - During the deployment of SIT observations on the fate of released flies, and the wild populations need to be collected to be able to monitor SIT success.
  - Sterilise flies will be marked so they can be easily distinguished from wild flies. In the field, trapping and checking insects will

        allow us to track the populations of marked and unmarked flies and successful and sterile matings.
    - Data on the longevity and mating success of sterile males can then be used to fine-tune release rates and frequency.
- Studies to be conducted in the pilot study areas (years 1-3) and larger areas targeted later in phase 2 (year 3).
- This part of the project is suitable for a PhD subject (Macquarie University/ SARDI).

3. **Laboratory studies**
    - **Quality control protocols (2020-2023)**
    - Quality control of reared flies, egg laying capacity, larval and pupal survival. Adult longevity and weight parameters, flight capacity, mating capacity and competitiveness etc.
        - Rearing conditions can modify the population through 'domestication', resulting in reduced performance for field releases. Only a rigorous QC protocol will be able to detect such subtle and gradual changes.
        - Initial choice of irradiation doses and developmental stage will be based on information from previous work and the methods used in the Bangladesh facility. QC will allow us to further fine-tune the sterilisation in order to optimise male sterilisation without affecting competitiveness
        - This laboratory QC of flies will be completed by extensive surveillance of populations in pilot and future SIT areas.
        - We should be prepared for the possibility that future deployment in different climatic conditions might require different production conditions.
        - This part of the project is suitable for a PhD subject (Macquarie University)
    - Optimisation of rearing conditions (diet, egg production), irradiation, transport and release conditions (2020-2022, Ongoing research Macquarie and SARDI).
      To upscale the rearing to 10-40 million flies per week (as needed for the second phase of the project) it is crucial to optimise rearing conditions.
        - This means that all phases of the rearing need to be designed to increase (optimise) productivity and reduce costs.
        - At this stage it should be possible to produce flies for a price similar to that for Queensland fruit flies of around $1000-1500 per 1 million flies (T. Marais pers. comm) in full production mode (see CBA for more details).

The following aspects will have the highest priority for improvement in order to achieve upscaling for the second project phase:

- **Diet**
  The diet currently used for SBF rearing is a mix including offal and

commercial (wet) pet-food. This diet is prone to bacterial and fungal contamination, variation in quality due to changes in food constituents etc. Multiple diets have been described for SBF, ranging from offal-based to semi-synthetic diets based on yeast. For other flies we have developed improved diets, yielding more flies and with a higher longevity, based on rigorous testing of dietary needs of flies. In the SBF SIT project we propose to develop a new, improved diet, based on SBF nutritional needs, while reducing costs of diet ingredients and preparation. The development of a non-offal based, fully synthetic, diet would also improve conditions for workers in the rearing, and assist in reducing odour emissions

- **Irradiation**
  The irradiation of insects before release needs to be optimised. The dose of irradiation should be sufficient to obtain sterility, but not too high to maintain fitness. Huda et al (1983) carried out a comprehensive assessment of sterility and competitiveness following gamma irradiation. Complete sterility in males was achieved when pupae were irradiated 1 day before emergence by 5 krad (50Gy) in air and a dose of 3 krad (30 Gy) eliminated egg production in females. Similar results have been reported for *L. sericata* (Meigen) by Donnelly (1960) and *C. hominivorax* (Coquerel) by Crystal (1979). Mortality of irradiated males and females was not significantly affected by any irradiation regime when assessed 5 weeks after treatment. They also showed that irradiated males and females did not differ in their preference for, or acceptability to, untreated partners, so assortative mating did not occur, while in other species assortative mating has been found to occur (Ohinata et al 1971; Schroeder et al 1973).
  The effect of the sterilising irradiation dose on fly fitness is not currently known. In the development of quality control tests (see above) this aspect needs to be considered as a priority. The same test can be used to determine at what irradiation levels or irradiation developmental stages, male mating competitiveness is negatively influenced.
- **Egg production and collection**
  The production of eggs of SBF is currently done by providing the flies with a protein meal (which is the same as the larval rearing medium), followed by a two-three day egg-maturation period. After that time dishes/trays with larval diet are presented as egg-laying substrates. This results in a variable and irregular egg production, possibly due to competitive interaction of the flies during egg-laying. Resulting egg numbers can be too low (diet not fully exploited by the larvae) or too high (resulting in small larvae). Though sufficient for the first phase of the project, this technique will be unsatisfactory for future scaling up of production.
  Egg production should be organised differently. Eggs should be separated from the substrate after egg laying, to be able to transfer them in known amounts to the rearing medium. This would also allow quantification of the number of eggs and optimisation of the egg/diet ratio for rearing, improved efficiency and reduced waste.

- **Storage and diapause (2020-2022)**
  Research should be directed towards storage of larvae or pupae for seasonal releases.
    - SBF does have a dormancy in the soil over winter in cooler climates. During this soil hibernation mortality is high (through predation and diseases), but emergence of the small spring population is synchronized
    - This spring emergence should be the primary target for SIT because this is the smallest seasonal population.
    - If conditions can be established for artificial hibernation in storage (low temperature, regulated humidity), but without the high mortality, it will be possible to produce flies over a longer period (in winter) and store them for mass release when required in spring. This would reduce the peak production capacity needed for SIT and reactivity to conduct releases when emergence of flies occurs (temperature and rain dependant)
    - It is important that flies emerge quickly upon release of the pupae to avoid any mortality due to predation or pathogens. Therefore, optimal conditioning of pupae before release should be investigated. The actual weather conditions during release campaigns are essential to get a quick emergence.
    - This part of the project is suitable for a PhD subject (Collaboration Macquarie University/ SARDI)

- **Other research questions 2021--2023**
  Several other questions related to competitiveness need to be investigated.
    - Are wild females able to distinguish and ‘refuse’ mass reared males.
    - Is there chemical attraction (pheromones) to either sex? If so, this may create an opportunity for sterile flies to be “treated” with chemical attractants to increase efficiency.
    - Is there re-mating? Ideally females would mate only once (as in the case of Screw-worm flies).
    - Are males limited in their mating capacity?
    - If SBF females are capable of multiple matings or in the case of limited mating capacity of the males, it is even more important to maintain a high overpopulation of sterile males.

### 4. Design of a mass rearing facility (2020-2022).

The current rearing facilities are insufficient to produce enough flies for a large-scale project in phase 2 (see hereafter), when demand will increase to 10-40 million flies per week. Therefore, a prototype of a bigger mass-rearing facility will be needed for phase 2 of the project, and this facility needs to be finalised in year 2 of phase 1 in order to start large scale production needed for the phase 2 SIT releases (Kangaroo Island or similar).

**Existing Permanent facilities**

In 2016, Primary Industries South Australia opened the Sterile Insect Technology (SIT) facility in Port Augusta to develop fruit fly management and response methods. The facility is a state-of-the-art rearing and irradiation facility for fruit fly. The SITplus factory is involved in significant research to improve the production and quality aspects of SIT. Through this facility and its associated programs several successful pilot programs have been launched including sex selection from fruit fly species for use in SITplus, post factory pilot studies into fly production, implementation of RapidAIM realtime monitoring for the presence and location of fruit fly and raising the standards for fruit fly production to international levels.

Investment into the facility has allowed swift response to fruit fly outbreaks especially along South Australian border regions and in prime growing areas such as the economically important Riverland Region of the state.

This facility could easily be used as a basis for pilot studies into early stage rearing and production trials for SBF control. Its proximity to potential field sites on Kangaroo Island would allow for easy transport of irradiated flies and a convenient facility for investigations into mass rearing. This includes developing facility operating procedures for SBF, refining breeding and sterilising methods, optimising SBF health through diet and environment, assessing SBF fitness and staff training.

Outcome: Preliminary plans for a production facility should be established based on release options and assessment criteria for the trial SIT locations.

**Mobile rearing facilities**

- **Design of rearing facility (2020-2021)**
  The design of the new SBF rearing facility will be greatly assisted by the experience with the SITplus facility in Port Augusta. The new facility will be designed to combine optimal technical performance with minimal infrastructure and operational costs. A provisional design is presented in chapter section 6.3.
- This facility will be designed to be mobile and modular (container modules, ATCO modules) so it can be moved and extended in the future if SIT will be deployed elsewhere. The existing SIT facilities in Port Augusta (Queensland fruit fly) and Perth (Mediterranean fruit fly), the container based rearing facility in Sydney (all designed and used by project members), and information about the existing SBF SIT facility in Bangladesh will allow us to design a cost effective and efficient facility.
- Technical specifications (size, climate control needs, rooms for egg laying, larval rearing, storage, (irradiation), washing etc.) will be designed based on what will be needed in the second phase of the project (see below). Because of the modular structure the facility could then easily be expanded when needed and/or moved to a new location.
- Since SBF are present all over Australia the facility will not have to be modelled as a quarantine facility, simplifying the design. If eradication is

    aimed at and the rearing facility is in the targeted area it should be designed to avoid any escapees. This would add to the costs
  - As with larger mass rearing facilities a mobile facility needs to fulfill certain criteria in order to be effective (Cáceres Barrios et al 2012). These include:
    - Site selection.
    - Size restrictions and flow design for optimal processing of large numbers of insects.
    - Research requirements.
    - Storage of equipment and consumables.
    - Utilities and back-ups for controlled temperature (generator, possibility of solar cells for power etc).
    - Staffing levels and accommodation (if needed).
    - Equipment automation and energy efficiency.
    - Backup power generator.
    - Waste treatment (water, leftover diet).

We propose to construct this facility using insulated shipping containers with rooms for egg laying, diet preparation, diet inoculation, larval rearing, storage of raw materials, storage of pupae, cleaning equipment, cold storage, (irradiation) and research and staff accommodation. Ideally in the barest of facilities (no irradiators included), a rearing facility will have

- One “Site Office” (changing rooms, toilet facility).
- Several (4) insulated containers for pupae production (28°C).
- An area for fly and egg production (28°C).
- Storage room for diet etc.
- Washing room (industrial washer).
- Diet preparation and egg inoculation room.
- Cool-room for storage and transport of pre-pupae.
- Cold room for sanitizing leftover diet and maggots.
- Fit Out materials include:
  - sinks and benches
  - shelving for cages and larval trays
  - adult egg-laying cages
  - Racks and towers for larval development
  - Industrial washer for trays and towers
  - Basic lab for QC activities
  - Water heater for sink

- **Construction of a rearing facility (2021-2022).**
  - For simplicity and cost reduction we propose to base the prototype rearing facility in Port Augusta, it can then be moved to other locations as needed later in the project.
  - The location of Port Augusta is optimal for several reasons
    - We will profit from local experience and knowledge on mass rearing (Queensland Fruit fly SITplus facility).
    - The irradiators from the existing SITplus facility can be used for sterilisation of the flies. This will avoid additional costs for

    irradiators (two irradiators plus automatic backup power supply = 1.5 million dollars) at this phase of the project). If larval rearing is carried out locally for future SIT areas, this would require a sterilisation facility. This could be added to a modular facility to allow deployment in different areas over time.
    - Existing capacity for transport and release can be used (planes and pilots available from Port-Lincoln, deployed for fruit-fly releases in SA.)
  - See 6.3. for initial design parameters of such a facility.

### 6.2.2 PHASE 2: Large scale deployment

The second phase (2023-2025) of the project would develop a large-scale SIT project on an area of 2500-5000 Km$^2$. A detailed case study, based on deployment of SIT on Kangaroo Island (4.500 Km$^2$) is presented in appendix 6.

- Such a large area would require a large number of sterile flies to be released (releasing at 100 flies per hectare would require 45 million flies weekly. This requires scaling up the production of sterile SBF. The existing SITplus facility in Port Augusta has a similar maximum production (50 million Q-flies/week)
- Based on existing data and rearing efficacy (Qfly, SBF in Bangladesh, NWSW in Panama) it should be possible to produce 400-500,000 pupae per M2 per week. Therefore, rearing area surface would be about 100M2 (= 4 shipping containers)
  - Improvement of current rearing techniques should be achievable based on what was achieved for Qfly.
  - This will also improve the quality of the flies (as was done for Qfly) so we can reduce the number to be released per ha or the release frequency.
  - If we can cold-store pupae before release this would greatly reduce the required production capacity and allow a more regular production over a longer period rather than peak production over a short spring period.
- The initial design of the facility should be based on a facility capable of producing 30 to 50 million pupae per week. Not only will this number of pupae be needed, but this will also impact on the design of egg-laying areas, washing areas and other facility components.
- Because of the low mobility of SBF we can attempt eradication in a large area by using the ‘moving release front’ approach. This is another reason why Kangaroo island would be a suitable site for the second phase. Starting from the west point of the island, releases can be done according to the available pupae over a north-south band of the island that can be moved eastwards between years or even during the season according to success ratio.
- A more thorough optimisation will be possible using the results of the initial studies before larger scale SIT-releases commence. This should also take into account parameters of the actual releases (numbers and volume of flies to transport in plane, equipment for GPS guided release, release paths, flight paths of the plane. The current aerial releases are done through Wright aviation who have experience and knowledge to develop this further.
- Suppression or eradication of SBF in a large area such as KI is expected to take 3-4 years of releases.

## 6.3. Design of the rearing facility

Based on current estimates and performance we think a design such as presented in figure 6.3.1 below would be appropriate. This is based on the following assumptions:

1. Eggs are produced in the 'fly and egg rearing room' by offering the flies in cages an egg laying substrate for a number of hours after which the eggs are collected.
2. The egg production of the day is put into suspension in (aerated) water and is used that same day to inoculate diets
3. Diets are presented in trays (approximately 700 x 500 mm and 25mm deep. Each tray can receive approximately 7-8 kg of diet allowing an estimated 60.000 eggs to develop.
4. During egg to pupae a loss of 15-20% is expected, leaving 50.000 per tray
5. Towers can stack up to 25 trays, so produce 1,250,000 pupae each
6. All towers inoculated on a single day will be kept in one rearing room. A rearing room can potentially contain 25 towers, leaving room for manipulation, climate control and for the staff to move around.
7. Larval development at 28°C takes about 5-6 days till the larvae 'jump' and are collected in vermiculite at the bottom of the tower.
8. During this larval development, climate control will need to be adapted according to the amount of evaporation and ammonia production of the rearing, particularly important in the last larval stage.
9. After 7 days the towers are dismantled and cleaned, ready to be re-used the next day.
10. Inoculation of towers occurs on Monday, Wednesday and Friday, filling 3 climate rooms (with each an independent climate control).
11. The fourth room (and the towers it contains) is available for necessary cleaning and decontamination on the intermediate days and can be filled with towers again the next day.
12. Cages for egg laying are prepared, cleaned and maintained on Tuesday and Thursday/Flies can lay three batches of eggs over a two-week timespan. Each fly can lay 3 batches of 300 eggs, so approximately 150.000 adult flies (males and females) should be available for egg laying each week. This would require a single container room with appropriate cages.
13. When towers have produced pupae, they will be transported out the other end of the container room into the area where they can be disassembled and pupae can be conditioned (packed) for either short storage and irradiation (when used immediately) or longer storage (cold room).
14. Towers then go to the washing facility where the trays are immediately washed and ready to be used again the next day.
15. This allows a circular 'circuit' where each phase of the production (diet preparation, inoculation, larval rearing (and different developmental stages), pupal conditioning, and washing occurs in separate rooms. This reduces the risk of contamination and increases efficiency.

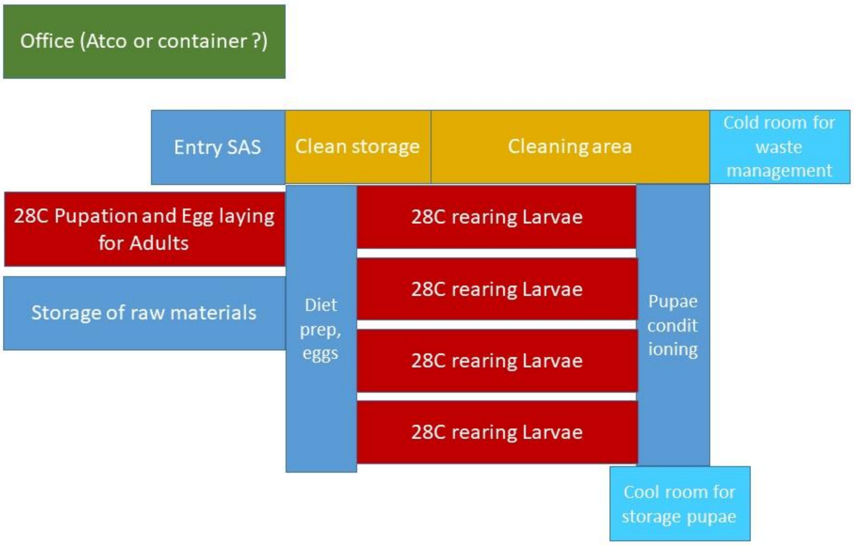

**Figure 6.3.1 Initial proposal for design of rearing facility based on shipping containers.**

The design of the facility will need to be finalized early in the project to allow timely construction. The modular design will allow for adaptations when needed.

## 6.4 Differences of this project with earlier attempts

Previous attempts to use SIT for SBF have either not been successful or have not resulted in a continued use of SIT for SBF. We are not the first to propose SIT for SBF, and Horton et al (2002) proposed the use of SIT for SBF for Tasmania as recently as 2002. In this section we will list the main differences between this proposal and previous attempts to explain why the present proposal has a high chance of success and with limited costs compared to earlier attempts. As a general guideline for this project proposal we have adopted a KIS (Keep It Simple) approach.

1. **Fly strain**

   The proposed project will use a 'wild' type strain of SBF, with no genetic modifications. This means that the flies are much more likely to be competitive in the field. Previous attempts using self-limiting strains suffered from reduced fitness and competitiveness of males.

2. **Rearing**
   a. Efficiency of the recently created SITplus facility is one of the highest ever achieved for the mass rearing of flies. Upscaling of the SBF rearing will be using the same techniques (towers, separation of pupae, egg laying devices).
   b. Proximity of the new facility to the existing SITplus will ensure efficient knowledge transfer.
   c. While we will be using a basic (dog food) diet in the for SBF rearing, we will also develop research into diet optimisation as we have done for fruit flies. This has resulted in a strong increase in both rearing production and longevity of sterile males in the field.
   d. Storage of pupae. None of the earlier SIT attempts have used the storage of diapausing pupae. We expect that this will allow us to easily build up the numbers available for SIT releases just before spring generations occur.
3. **Irradiation**
   a. The main reason for installing the SBF in Port Augusta is the fact that we can profit from existing radiators reducing costs by about 1.2 million.
   b. The X-ray irradiators of the SITplus facility are modern, high quality and well-maintained machinery
4. **Design of the facility**

   Design of this new facility is largely profiting from lessons from the existing SIT facility.

   a. Container based design (quoted at 500,000 dollars) is a very substantial cost reduction. For comparison:
      i. Horton et al proposed a 23-million-dollar facility
      ii. The existing SIT-plus facility did cost 4.5 million.
   b. The existing rearing technology already allows for a very compact design (10 40-foot containers is approximately 300M$^2$ (700M$^3$)

   c. With existing techniques this facility should be able to produce 50-75 million flies per week. With expected improved performance of the rearing (as achieved for Q-fly) we expect to be able to produce > 100 million by the end of the project.
   d. The modular design will allow it to adapt if needed, to further increase capacity.
   e. Standard equipment will be used where possible (e.g., climate control) to avoid high installation and maintenance costs
   f. Separation of larval rearing units into 4 rooms will simplify workflow, avoid contamination and simplify climate control
   g. Flow of material (towers, diet, cleaning, insects) has been optimised
   h. Climate control has been adapted (simplified) to have a cheaper but equally efficient design

5. **Quality control**
   a. Quality control of mass-reared insects is essential to monitor and avoid any genetic drift due to rearing conditions that might reduce competitiveness in the field.
   b. QC for other flies has been developed by our team and will be adapted for SBF.
   c. We will especially focus on longevity and competitiveness of irradiated males.
6. **Releases**
   a. We will release sterile insects in the pupal stage, just before adult emergence. This will reduce mortality after release compared to earlier attempts using larvae.
   b. Releasing pupae close to emergence of adults will result in almost immediate increase in sterile males, this reactivity is essential to maintain overflow of males at all times.
   c. Releasing pupae rather than adults will reduce the need for rearing out the pupae before release (adding extra time and costs, more space needed and more complex release units.
7. **Choice of demonstration sites**
   a. The demonstration sites of both phase 1 and phase 2 of this project will be in cool climate areas where SBF ecology is better understood than in warmer climates.
   b. This will allow to target the spring population with inundated SIT releases. Overflow of sterile males will be achieved from the start of season
   c. KI had an intermediate density of sheep (approximately 1 sheep/ha) , spread relatively evenly over the entire agricultural area of the island, and has an intermediate rainfall pattern (450-900 mm).
   d. Research into the ecology of SBF in both higher and lower sheep density situations and in different climates is needed to optimise success rates
8. **Research**
   a. This project will be combined with a limited volume of applied research projects in order to prepare for SIT applications after the eradication of SBF on KI.

# 7. Key messages

## 7.1 Sheep Blowfly management

- Sheep Blowfly management is a major constraint in wool and meat sheep production
- None of the existing methods is able alone to achieve complete control of SBF
- Existing chemical control methods are encountering resistance and residue issues, new chemistries are unlikely to become available
- Existing physical methods (including mulesing) are costly, have limited efficiency and cause animal welfare issues impacting product value.
- Development of new approaches through other methods (Sheep breeding, Vaccines) are likely to take time to develop and be operational
- Having SIT for SBF as a complementary tool would be a substantial improvement, allowing eradication in certain areas, and contributing to long term sustainable management in others.

## 7.2 Eradication of SBF in island situations is achievable through classic SIT

- PIRSA-SARDI, Macquarie University and the Port Augusta SIT-plus facility have the experience, know-how and capacity to develop classic area-wide SIT for SBF in a cost-effective way.
  - Rearing can be done by a cheap and versatile container based rearing facility
  - Sterilising and releases can be organized efficiently using existing infrastructure and logistics.
- Classic SIT for SBF can be applied on a ‘high density’ island situations in cool climate areas of up to 5000 Km$^2$ as soon as the SBF rearing has been scaled up
  - eradication of SBF on Kangaroo Island is a realistic pilot project that would be achievable in 4-5 years (with 3-4 years of releases on KI)

## 7.3 Research should be directed towards improving efficiency allowing deployment of SIT on larger areas and the mainland

- Applied Research is expected to substantially increase the cost-efficiency of sterile fly production (e.g., diet, rearing conditions, storage of diapausing pupae)
- Ecological research can further improve efficiency with knowledge of fly activity and distribution, allowing improved efficiency in low density/low rainfall areas
- Fundamental research should be directed towards the development of self-limiting strains (such as Field Female Killing strains) that are genetically stable and competitive
- The combination of research activities is directed towards increasing the efficiency of SIT for use in other areas after the KI demonstration project
  - Control program strategies on the mainland could be either local reduction of SBF or eradication using a moving front approach.
  - The business model for such actions needs to be developed during the initial 5-year project phase.

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# 9. Appendices (additional information)

## 9.1 Appendix 1: Australian Sheep Blowfly (additional information)

### 9.1.1 Life Stages

I. Eggs

SBF eggs are ca. 1.09 mm in length and 0.25 mm in width, elongated in shape and creamy-white in colour (Sukontason et al 2007). The egg chorionic ultrastructure is an important taxonomic character for the identification of insect species (Erzinclioglu 1989; Sukontason et al 2004). Scanning Electron Microscopy (SEM) revealed that the eggshell structure of SBF bears a dorsal plastron, an unadorned micropyle and a hexagonal pattern of chorionic sculpture (Martins Mendonça et al 2008). Egg size appears to be constant and irrespective of the size of the ovipositing female (Williams and Richardson 1983). This strategy ensures that the individuals of the next generation are provided with the same amount of energy to initiate larval development and reduces early larval competition. Female SBF prefer to oviposit in cavities of the fleece, preferably in moist areas, and lay egg-masses close to skin, where temperatures range from 34 to 42.5 °C (Murray 1957; Macfarlane et al 1958). The best conditions for egg hatching are found to be 35 °C and high relative humidity (Vogt and Woodburn 1980). At a constant temperature of 25 °C, the duration of the egg stage is ca. 24 h (Smart and Gilmour 1974).

II. Larvae

SBF first instar larvae hatch from eggs and two moults occur before pupation during their development (Barrit and Birt 1971). Larval body length is 1.88-2.75 mm for first instar larvae (L1), 3.71-8.62 mm for second instar larvae (L2) and 7.21-11.69 for third instar larvae (L3) (Day and Wallman 2006). Similar to other dipterans, the larvae (maggots) are pyriform and transparent and larval size is influenced by the substrate on which larvae develop. Since egg hatching, larvae form discrete feeding clusters and feed on tissue and tissue fluids of the host sheep (Williams and Richardson 1983). When mature, L3 larvae abandon their host and drop on the ground where they pupate. It has been estimated that 90 % of the larvae abandon the host between 3 and 7 days post-strike initiation, the point at which larvae begin feeding on the host, (Smith et al 1981). Although larval developmental time can vary, the number of days from strike initiation to the last drop of mature larvae from the host is usually consistent regardless of ambient temperature, presumably due to sheep body temperature having the greatest impact on this parameter (Kotzé et al 2015). Most of the larvae will pupate within 2 m from the drop spot (Vogt and Woodburn 1982). Before pupating, larvae bury into the soil, up to 4 cm in summer and 1 cm during autumn and winter (Smith 1985).

III. Pupae

Pupation of SBF takes place within the hardened skin of L3 larvae, the puparium. Size of puparia varies from 5.99 to 6.90 mm in length, puparia have a barrel shape and a white-yellow colour that turns light brown and then dark brown within a few hours from pupation (Day and

Wallman 2006). Pupation starts when mature L3 larvae retract their mouthparts and become immobile, and continues with a series of complex biochemical and physiological processes. During development, SBF pupae display changes in morphology and respiratory structures that reflect variations in water loss and oxygen uptake from the environment (Gilby and Rumbo 1980). Under natural conditions, the duration of pupal development is comprised between 10 and 220 days, depending on the season (Dallwitz and Wardhaugh 1984). Pupae seem to be insensitive to light and it is hypothesised that adult emergence is influenced by temperature (Smith 1985). While adult emergence was observed to start preferably in the morning, some strains have also been demonstrated to begin emergence independently from the time of the day (Smith 1987). Under laboratory conditions with a constant temperature of 23 °C, pupal development of SBF lasted 8.75 days (Barros-Cordeiro et al 2016).

IV. Adults

SBF adults are metallic green in colour, with bronze reflections, and a medium size of ca. 9 mm (NSW Department of Primary Industries 2016). Adult size depends on pupal weight, which in turn is correlated with the adequacy of larval food (Webber 1955). In females, adult size, measured as headwidth, is correlated with the number of ovarioles and hence fecundity (Vogt et al 1985a). Morphological characters adopted for identification purposes are described in detail by Holloway (1991 Adult SBF display a circadian rhythm showing an increased flight activity during the day while being mostly inactive at night (Smith 1983). Under laboratory conditions with 30±1.5 °C and 65±5 % RH, the average longevity of SBF was 20.56 days for females and 12.76 days for males (Zied et al 2003).

### 9.1.2 Developmental parameters

I. Day Degrees

Insects are poikilothermal animals, therefore their physiological processes are greatly influenced by temperature (Gunn 1942). It is possible to infer the chronological age of an insect by considering a particular physiological change and the ambient temperature of the environment in which the insect develops (Detinova 1962). Degree days models assume that insects follow a predictable developmental pattern based on heat accumulation and consider the rate of development between lower and upper temperature limits (Wagner et al 1984). Age of SBF adult females can be determined by observing changes in their reproductive system. Vogt et al (1974) divided female development in 16 ovarian stages and determined a correlation between actual age and stage of ovarian development at four constant temperatures: 20, 25, 30 and 35 °C. A subsequent study by Woodburn et al (1978), adopted both ambient temperature and solar radiation in field cages to determine the age of female SBF and established 8.2 °C as a lower threshold temperature for development. It has been estimated that females require a minimum of 57-degree days above 8 °C to mature their first complement of eggs (Vogt et al 1985b). A thermal accumulation model for the development of SBF was calibrated by Kotzé et al (2015) by using chicken liver for larval rearing and adopting six constant rearing temperatures, from 18 to 33 °C. In the same study, the lower developmental threshold was determined to be 12 °C. Understanding the relationship between development and temperature is useful to develop models that predict the seasonal abundance of SBF in the field (Régnière 1996; Briere et al 1999). Such predictions are powerful tools for an effective prophylaxis against flystrike (McKenzie and Anderson 1990).

### II. Winter Development arrest

In studies conducted in Southern Australia, adult SBF are rarely caught by field traps between July and September (Gilmour et al 1946) mainly due to lowered temperatures. Opportunity for initiating flystrike is therefore low during this period. In the same region, SBF is reported to overwinter as prepupa, a post-feeding larval stage occurring upon abandoning the host sheep (Mackerras 1993; Norris 1959). In field studies conducted in the Canberra region, prepupae pupated within a few days upon abandoning their host sheep during summer and early autumn, while pupation dropped to almost zero for prepupae entering the ground in late March (Dallwitz and Wardhaugh 1984). It was also observed that, in spring, adult emergence was synchronous amongst individuals that had entered the ground from late March to October. In the same study, larvae kept at 16 °C pupated on average in 8 days between the spring and autumn equinox and 15 days between the autumn and spring equinox. Conversely, 96 % of pupation occurred by 4 days when prepupae were held at 25 °C, all year round. Dallwitz and Wardhaugh (1984) concluded that the two main factors affecting the overwintering of SBF are the temperature experienced by post-feeding larvae and the photoperiod experienced by their parental generation. A field study conducted by De Cat et al (2007) in Victoria showed that SBF emergence followed a biphasic pattern: prepupae put in the ground during April developed immediately and emerged in late autumn or, alternatively, they arrested their development until the following spring. Overwintering prepupae resumed development in September, when soil temperatures increase 1.5 °C over a period of 4 days and when soil temperatures remained above 11 °C for a minimum of 7 consecutive days. The mechanism for overwintering of SBF requires further studies to better understand the conditions that induce its facultative developmental arrest during the unfavourable season. Information on seasonal abundance of SBF is essential to develop control strategies against flystrike, so that graziers can implement preventative measures to limit blowfly populations early in the season (Anderson et al 1984; 1990). Predictive models for adult emergence of SBF in the field can prove useful for this purpose, although they require continuous testing and refinement (McLeod 2001).

## 9.2 Appendix 2: Insecticides and insecticide resistance (additional)

### 9.2.1 Organophosphates

Organophosphates inhibit acetylcholinesterase, an enzyme that degrades the neurotransmitter acetylcholine, found in the central and peripheral nervous system of most animals including humans.

In 2003, a review of diazinon was carried out by the APVMA to ensure that formulation of products curbed breakdown products leaking into soils and to address concerns about operator handling exposure. As a result, recommendations for the cancellation of certain products were made (APVMA Review Series 2003). It was not long after this review that restrictions on the use of OPs as dipping and jetting treatments were imposed (Sandeman et al 2014) and subsequent registration of diazinon and other OP products reflect this.

A large number of organophosphate (OP) insecticides were introduced to the livestock industry in the 1950s and replaced the increasingly resistant organochlorines in the 1960s. They include diazinon, fenthion-ethyl, coumaphos, chlorfenvinphos, carbophenothion and malathion (Tellam and Bowles 1996). Organophosphates are fast acting insecticides and can be readily absorbed by the skin of sheep providing protection as well as killing SBF larvae.

Diazinon, chlorfenvinphos and propetamphos are currently the only OPs registered with the Australian Pesticides and Veterinary Medicines Authority for use in the control and treatment of SBF as it pertains to flystrike. Registered veterinary use of organophosphates on sheep is currently limited to the treatment of affected fly struck sheep and as a post shearing spray for lice or as part of wound dressing – not as an ongoing preventive treatment (limited to direct sprays on affected areas or as a wound preventative dressing for struck sheep and not registered for use on newly pregnant or lactating ewes.)

Since the development of organophosphate resistance within populations of SBF (Shanahan 1966) many OPs are no longer efficacious. Despite issues with resistance (Tellam et al 1997) and human handling exposure (Stephens et al 1995), organophosphates are still used by many farmers within a wider program of integrated pest management for flystrike and general SBF control.

### Table 9.2.1: Current registered organophosphate chemistries used for Flystrike (APVMA PubChris data)

| **Active** | **Method used** | **Alternative uses** | **Registered** |
|---|---|---|---|
| **Chlorfenvinphos** | Wound and mules dressings formulated with antiseptics, and surfactants | Cattle spray, Cattle tick control, Buffalo fly control | All states |
| **Propetamphos** | Wound and mules dressings formulated with antiseptics, and surfactants |  | All states |
| **Diazinon** | Wound and mules dressings formulated with antiseptics, and surfactants | Off shears sheep lice control<br>Cattle tick | All states |

### 9.2.2 Insect Growth Regulators

Insect growth regulators (IGRs) such as benzoylphenyl ureas and triazine and pyrimidine derivatives - diflubenzuron, triflumuron, cyromazine and dicyclanil - act to inhibit the normal development of the juvenile stages of insects.

IGRs have the advantage over organophosphates in being a targeted insecticide affecting the juvenile hormones needed for development from larval to adult stage. Application of IGRs to sheep can halt the progress of SBF from maggot to adult stage limiting further development and expansion of their populations. IGRs have nil or low mammalian toxicity effects (Sandeman et al 2014). Residues of IGRs also have limited persistence in the environment (Siddall 1976), which is an advantage for wool growers sending to overseas destinations with strict Maximum Residue Limits (MRLs) for insecticides.

I. Cyromazine

This IGR is currently used widely on sheep with less than six weeks wool and has efficacy on all larval stages of SBF but not on adult flies (Hart et al 1979). Conflicting evidence exists on the effect of cyromazine on egg oviposition and subsequent hatching, with some studies showing no effect (Kotze 1992, Levot and Shipp 1984) and other studies showing the reverse (Friedal and McDonell 1985, Yen 1998). However, all studies demonstrated larval development was reduced to a similar degree to that reported in the original research carried out by Hart et al (1979, 1982). Cyromazine is the only chemical treatment recommended for sheep dipping.

II. Diflubenzuron

Used both as a ‘spray on’ treatment against flystrike and as a wound dressing and protection after mulesing, diflubenzuron has routinely been used as part of the suite of chemistries available to sheep farmers since the early 1980’s (Anstead et al 2017). It acts to weaken the exoskeleton of developing SBF making it difficult for the insect to moult and for eggs to hatch (Grosscurt 1978). However, its use has since come under scrutiny due to high levels of resistance and OP cross-resistance (Kotze et al 1997, Sales et al 2001). Although diflubenzuron has low mammalian toxicity, it does have significant toxicity to marine invertebrates so adequate disposal safeguards are necessary (Christiansen 1978, Christiansen and Costlow 1982).

III. Dicyclanil

Another recent addition to the suite of IGRs used against SBF for flystrike, dicyclanil, has a similar mode of action to that of cyromazine. Bowen et al (1999) showed when larvae were exposed in vitro, dicyclanil was ten times more effective against SBF larvae when compared to cyromazine. The research also showed dicyclanil treatments could prevent flystrike on sheep for up to twenty weeks and beyond; longer than other IGR’s.

**Table 9.2.3: Current registered Insect Growth Regulator chemistries used for Flystrike (APVMA PubChris data)**

| **Active** | **Method used** | **Alternative uses** | **Registered** |
|---|---|---|---|
| Cyromazine | Spray on treatments, jetting, dipping and wound and mules dressings formulated with antiseptics, and surfactants | General fly larvicide | All states |
| Diflubenzuron | Spraying and jetting treatments | Cattle and sheep lice (not SBF) | All states |
| Dicyclanil | Spraying and jetting treatments | | All states |

### 9.2.3 Other Chemistries for treatment of flystrike

A number of other chemical treatments are available for the treatment of flystrike in sheep.

Synthetic pyrethroid alpha-cypermethrin has medium mammalian toxicity and its mode of action is to block the closure of the voltage-gated sodium channels which results in overexcitation of the fly’s nervous system causing paralysis. It works on all stages of insects.

However, pyrethroids in general are known to work less well against larvae relative to adults but are strong oviposition suppressants (Sales et al 1996). When pyrethroids are combined with IGRs, the outcome has been effective flystrike treatments against both larval and adult flies.

The macrocyclic lactone active ivermectin is an effective treatment against flystrike (Anstead et al 2017) and used often in jetting treatments. It is also an effective active ingredient in many flystrike wound dressings currently registered with the APVMA. It can be used as an alternative to IGRs where resistance has occurred (Levot and Sales 2008, Levot 2013). Ivermectin's mode of action is to disrupt glutamate-gated chloride ion channels in insect muscles resulting in paralysis. When used in jetting, ivermectin can be applied on long wool coats (up until 6 weeks prior to shearing). It has a low toxicity to mammals making it a more palatable option for sheep and handlers.

Spinosad, is a novel mode-of-action insecticide derived from a family of natural products obtained by fermentation of the bacterium Saccharopolyspora spinosa. It has been used widely as agricultural insecticide in broad acre and horticulture since it's registration as a pesticide in 1997. Applied to sheep through jetting or as part of wound dressings it has been effective insecticide used in a rotation with other treatments. The chemistry acts to overexcite the insect's nervous system causing paralysis. Spinosad has remained an effective treatment since its recent introduction (Anstead et al 2017).

The neonicotinoid active imidacloprid, acts to overstimulate SBF nerves by disrupting post-synaptic nicotinic acetylcholine receptors, and is currently used as a spray on treatment for fly struck sheep. It's fast acting and efficacious at very low concentrations. However, in recent years, neonicotinoids such as imidacloprid have been banned from use due to concerns of off-target effects on wild and cultivated bees (Bortolotti et al 2003, Krupke et al 2003, Wood and Goulson 2017).

### *Novel chemistries and non-systemic treatments*

Products containing magnesium fluorosilicate, amitraz (non-systemic insecticide), piperonyl butoxide, rotenone, and sulphur are all additives that enhance the activity of other chemical controls. Often used in high-circulating pumps use of these products has been shown to lead to desiccation of other sheep ectoparasites such as the sheep lice *Psorobia ovis* (Cotter 2013).

A number of recent studies have examined the effect of natural products (essential oils) and combinations of natural products with insecticides for population controls against several blowfly species aligned with animal myiasis. Bedini et al (2019) showed that the essential oils of medicinal plant species *Clinopodium nubigenum* and *Lavandula angustifolia* deterred *L. sericata* from egg laying. In addition, both extracts were found to be toxic to eggs and adults. 1% Tea tree oil (from *Melaleuca alternifolia*) applied to eggs of SBF showed 100% mortality (Callander and James 2012) and close to 100% kill of larvae in feeding assays. Dipping was less effective, but a significant repellent effect was observed, which is an important attribute for effective flystrike dressings.

A South American plant species essential oil extract - *Piper gaudichaudianum* was also effective against SBF in laboratory conditions and demonstrated considerable biological activity against larvae (Chaban et al 2018)

Essential oils (EOs) of vetiver (*Chrysopogon zizanioides*), cinnamon (*Cinnamomum zeylanicum)*, and lavender (*Lavandula angustifolia*) and their blends on *Lucilia sericata* –killed flies and vetiver oil deterred flies from oviposition and reduced longevity (Kahater et al 2018). EOs with insecticidal, repellent, and oviposition-deterrence against *L. sericata* that could be used for suppression of other blow fly species populations.

Altering blowfly behaviour through application of feeding deterrents such as essential oils, could significantly impede initiation of flystrike. In addition, essential oils possess ovicidal and larvicidal effects, and along with their antimicrobial properties and purported wound healing properties, could further hinder the development of flystrike.

### Insecticide Resistance and SBF

SBF resistance to a variety of insecticides has been widely documented (Sandeman et al 2014). Organophosphate resistance was detected within the first few years of use (Shanahan and Hart 1966, Shanahan and Roxburgh 1974, McKenzie and Fegent 1988) but did not inhibit the continued use of a number of these insecticides (Levot 1995, 1999). Until the discovery of alternatives for flystrike, the livestock industry was limited in its choice of chemistries in which to treat sheep. This allowed for increasing resistance over that time to render the OP diazinon 98% ineffective and the OP malathion with severely reduced effectiveness (McKenzie et al 1982, McKenzie and Batterham 1998). In one New Zealand study diazinon resistant populations of SBF were shown to be weakly resistant to other organophosphates (Wilson 1999). The implication for this is that if Australian SBF populations have the same level of cross-OP resistance, the protections offered by further use of these chemistries is limited.

With the introduction of IGRs in the late 1970s populations of SBF could once again be effectively controlled. Sheep producers could achieve at least 14 weeks of flystrike protection for sheep after treatment (Sandeman et al 2014). However, by the end of the nineteen eighties, resistance to widely used diflubenzuron products had risen (Levot and Sales 2002). Low level resistance to another widely used IGR, cyromazine, has also begun to appear in SBF populations (Levot 2012). Although the newest IGR, dicyclanil, is still very effective, its mode of action is very similar to that of cyromazine and cyromazine resistant individuals have been shown to establish on dicyclanil treated sheep (Levot et al 2014). Potential consequences for the livestock industry could be significant if further resistance or cross-resistance develops.

Early field monitoring for synthetic pyrethroid resistance in SBF populations reported no indication of specific resistance despite the chemical’s (deltamethrin) frequent use against sheep lice (Sales et al 1989). However, laboratory resistance was subsequently extended to other pyrethroids, including cypermethrin, cyhalothrin and cycloprothrin, and since then several instances of resistances have been noted in SBF populations which render some formulations ineffective (Levot 1995). Synthetic pyrethroid resistance for treatment of sheep ectoparasites has been partially mitigated with the addition of the synergist piperonyl butoxide.

There have been no recorded instances of SBF resistance to either ivermectin or spinospad. Resistance to other insecticides has been limited with an effective rotation strategy, with the consequence that the livestock industry's reliance on chemistries remains high (Levot 2013).

### 9.2.4 Biological Control

The most applied biopesticide against SBF is *Bacillus thuringiensis*, of which there are limited strains of Bt effective against *L cuprina*. HD 12E and JC 292-18 strains were shown to be the most efficacious against larvae and pupae (Gough et al 2001). Bt can be commonly isolated from sheep fleece (Lyness et al 1994, Heath et al 2004). It has long been considered that a *B. thuringiensis* strain, or another bacterial species engineered to produce *B. thuringiensis* toxins, that is able to colonise the fleece and exert on-going larvicidal activity is most likely to be successful in strike control (Sandeman et al 2014). Recently the knowledge of the range of toxins and coding genes known to confer activity has been expanded (Heath et al 2004, Gough et al 2005, Kongsuwan et al 2005). Recent studies (Chilcott et al 1998) showed that full-length and trypsin-digested Cyt1Aa proteins were toxic to at least three species of sheep blowfly.

A microsporidian, *Octosporea muscaedomestica*, was imported from the United States (US) for potential release as an inoculative biocontrol for SBF (Cooper et al 1983). Following oral infection, the pathogen infects midgut epithelial tissue of both adult flies and larvae, causing mortality to both stages and reducing fecundity in infected females. Infection is spread mainly at feeding sites through spores in faeces passed by infected adult flies. SBF was found to be highly susceptible (Cooper et al 1983, Smallridge et al 1995). However, it took a number of days for infection to develop and it is likely that infected flies would be able to successfully oviposit before infection could impair fecundity. Even in populations of the black blowfly (*Phormia regina*) which is highly susceptible to *O. muscaedomestica* and has life habits seemingly more amenable to transmission than SBF, average annual infection rates are generally only of the order of 4.0–4.5% (Kramer 1968). Maintaining sufficient transmission to significantly reduce fly numbers is likely to be a difficulty. Although attractive baits could be used to disseminate spores (Cooper and Pinnock 1983), with a probable low impact on strike incidence this is unlikely to be economically feasible

In Australia 24 isolates of *Metarhizium anisopliae* and eight of *Beauveria bassiana* isolated from soils and infected insects in Queensland were selected on the basis of characters suggesting suitability for mass production and then screened against adult and larval (L1 and L3) SBF. Adult flies exposed to conidia in their food died faster than those challenged topically with three strains giving 100% mortality within 5 days. Twelve isolates added to the pupation medium killed 100% of third instar larvae and three isolates killed 100% of first instar larvae. These isolates are yet to be tested on sheep.

Wright et al (2004) considered *M. anisopliae* for use as an inoculative biocontrol and concluded that with appropriate attractant systems sufficient levels of infection could be introduced into *L. sericata* populations to induce the 20%-30% daily mortality required to effect field control. However, under Australian conditions use of Metarhizium as a biopesticide for application to sheep, targeting first instar larvae, may have greater potential for practical control than inoculative approaches.

In New Zealand the fungus, *Tolypocladium cylindrosporum*, isolated from blowfly cadavers showed promise in the laboratory as an entomopathogen (Wright et al 2009).

Biopesticides appear to have ample potential for useful integration into an integrated pest management package targeting blowflies. A range of pathogens including the fungi *M. anisoplae, O. muscaedomesticae, T. cylindrosporum, E. muscae* and *C. coronatus* along with the bacteria *B. thuringiensis, B. laterosporus, S. marcescens* and *S. liquefaciens* has been shown to cause significant mortality of *Lucilia* species. Each of these, used alone or in combination, has the potential to be a useful adjunct to blowfly control if they can be delivered in an appropriate format (Leathwick et al 2019). The advantage of using pathogen-based biopesticides is that resistance to them may be slower to develop (Ruiu 2015). However, there are some factors which present difficulties for any classical biocontrol agent to persist and impact on SBF populations, or more particularly to reduce strike incidence.  For example, the narrow host range of many pathogens and the lag time results in low toxicity to the user and fewer issues of non-target effects. Also, the rate of spread of pathogens and parasites is almost invariably density-dependent, while SBF occurs at low population density at most times and flystrike is episodic with fly populations building rapidly when conditions become suitable. Therefore, most recent research towards a biological control agent has focussed on inundative approaches or the development of biopesticides (Leathwick et al 2019).

Entomopathogenic nematodes (ENs), are commercially available and have been shown to readily infect and kill SBF larvae. They are mobile and can actively seek out hosts and kill them through the release of mutualist bacteria. *Heterorhabditis* spp. infect first instar SBF larvae by entrance through the mouth and direct penetration through the cuticle (Bedding and Molyneux 1982). As the majority of the strains of ENs are most active at relatively low temperatures, it has been suggested that ENs could be used to increase mortality in the overwintering soil stages of SBF (Bedding 1983). Since, most ENs require a humid microenvironment to achieve high levels of infection may exclude this approach in many Australian situations.

Despite widespread interest in biopesticides to reduce chemical pesticide use and to assist access to organic or low pesticide markets, no biopesticide formulation has yet been commercialised. This is likely to be due to a combination of factors including costs associated with registration, the current availability of benign and very effective chemical pesticides, comparatively short protection periods with most biopesticides and the limited price premiums currently available for wool and meat produced in organic and low pesticide production systems (Sandeman et al 2014).

## 9.3 Appendix 3: Drivers for SIT approaches and management of SBF

### 9.3.1 Fly population studies

Field population estimation of SBF has been long sought. In the past, several studies attempted to estimate the population size of SBF, which is driven by climatic factors and interspecific competitions. Most of these studies focussed on determining the species of flies' present, the spatial distribution of field populations, and the species breeding in carrion (Vogt and Woodburn 1982; Vogt et. al. 1983; Williams 1987). The Australian sheep blowfly is attracted to carrion but extreme interspecific competition with the native species impedes its breeding success on the carrion (Waterhouse 1947). Carrion-baited traps, however, delivered an estimation of seasonal changes in fly abundance as a simple and effective means for sampling of field populations of SBF (Tillyard and Seddon 1933; Vogt and Haven- stein 1974, Vogt et. al 1983). Nonetheless, the value of trap catches as indicators of population trends was questioned as the trap catch was affected by the differences in weather conditions (Whitten et al 1977; Kitching 1981). Alternatively, mark-recapture techniques were employed (Gilmour et al 1946; Foster et al 1975; Whitten et al 1977), but without much advantage (Kitching 1981). As a solution, it is suggested that the differences in weather conditions to be taken into account for the calibration of trap catches (Williams 1940). Following Williams (1940), Vogt et al. (1983) formulated a model relating catch rates of SBF to various environmental variables.

Vogt and Woodburn (1982) studied mobility and spatial distribution of the post-feeding SBF larvae leaving the hosts. A preliminary assay was run to determine the depth that larvae can burrow. The larvae were released during daylight hours onto two different soil types- moist, bare, undisturbed soil and moist, finely powdered soil. Most of the larvae were found to burrow soil as soon as they were released and were found to dwell in the top 25 mm of soil. None of the larvae penetrated beyond 50 mm and there was very little movement away from the release site. The movement pattern recorded during daylight in the study was not indicative of the natural dispersal because Smith et. al. (1981) had reported that the most post-feeding larvae leave the host animal at night. So, Vogt and Woodburn (1982) undertook further experiments in which post-feeding larvae could disperse naturally from their host. Larvae were reared on live sheep and on carrion excluding other Calliphoridae species to avoid interspecific competition (Waterhouse 1974) such that the SBF larvae can complete their development (Fuller 1934). Regardless of the strains, laboratory bred, or field collected, the pattern of larval distribution suggested that post-feeding larvae are likely to enter the soil and pupate within 2 m of where they leave the host (carcass or live sheep). Larvae recovery declined rapidly with increasing distance from the host.

Vogt et al (1983) sampled field populations of SBF in New South Wales for periods of 3 h on numerous occasions between 1975 and 1982 using West Australian blowfly traps (Vogt and Havenstein 1974). They reported that the ambient temperature, wind speed, relative humidity and solar radiation explained 77·4% of the within-day deviance of the catches. However, temperature alone accounted for 74·9% of this deviance, indicating that the other variables, although significant, did not greatly affect trap catches. Vogt et al (1983) also found that the logarithm of flight activity rates increased linearly up to 26°C, remained constant up to 38°C and then declined at higher temperatures when assessed under constant temperatures in the laboratory. This relationship between catch rate and temperature was very similar to that described by Kitching (1977, 1981) for the effects of temperature on the flight activity of SBF. Wind effects though were significant; wind speed above 2.5 m/s caused a linear decline in logarithm catch rates, but within-day changes were not high enough to noticeably lower the expected mean catch. There was no evidence that intrinsic behavioural changes with time of day affected catch rates. Vogt et al (1983) were of the view that the standardised trap catches which are relative measures of population size differ from absolute measures by a constant scaling factor and the factor can be determined from mark-recapture experiments. They also opined that the temperature and wind speed are the main factors to consider when standardising trap catches.

The 'standardized' trap catches (Vogt et. al. 1983), the catches adjusted to standard set of weather variables to provide relative measures of the size of field populations, had an assumption that both sexes responded similarly to environmental variables. However, the trap catches had twice or more as much female to male (>2:1) ratio. Since the sex ratio at emergence is approximately 1:1, the female skewed catches echoed differences in the behaviour and/or mortality rates of the two sexes (Vogt et. al. 1985). In order to address this issue, Vogt et. al. (1985) conducted another experiment which examined catches of males and females of SBF in relation to the various environmental variables. However, they found that the accuracy obtained by using separate catch rate models is very small compared with day-to-day variation in catch rates. Therefore, they reached to a conclusion that the model of Vogt et al (1983) is enough for standardizing daily trap catches of both sexes of flies.

Vogt et al (1985) conducted field trials with translocation/eye colour strains of SBF aimed at developing production and release procedures of SBF males for flooding field population of the conspecifics, assessing their mating ability and developing methods to measure the response in field populations to increased levels of genetic death. Larvae were released weekly by plane flying at 220km/h at 100-300 m above ground and Western Australia traps were used to monitor the population. They found that the males produced had low mating competitiveness compared to the field-reared males and were less than the required to sustain overflooding ratio which was aggravated by low field survival rates of the released larvae. High pupal mortality has been linked to high soil temperatures (Dallwitz 1984; Dallwitz and Wardhaugh 1984) which explains the summer declines in field populations of SBF (Vogt et. al. 1985).

A two-year study of sheep and fly populations in the Southern Tablelands of New South Wales was conducted by Wardhaugh et al (1989) to correlate strike incidence and fly density and to determine the best method to assess sheep susceptibility to fly attack. They reported a high occurrence of hoof strike followed by crutch and pizzle strike and that the probability of strike increased with the increase in the soiled area. In addition, the study found weather variables as dependent factors influencing female SBF density on strike incidence, but the combined effects of weather and density were negligible. However, since the traps were spread over a wider area than that used by sheep, trapping data may not have been particularly relevant to the sheep strike data.

Wardhaugh and Morton (1990) assessed methods to explain variations in strike incidence brought by fly abundance, flock management and weather conditions in a three-year study carried out in Southern tablelands of New South Wales. They installed 48 West Australian traps in 4 trapping grids, at 2km intervals which were set weekly from October – April/May and opened for either 5 – 7 hours or 24 h. They found that the body strike was more predictable than crutch strike, and correlated with monthly rainfall, cloud cover and the rate of pasture growth. Frequent light rain was contributing more to the occurrence of flystrike than the occasional heavy rains. Both dry conditions and low fly densities, regardless of the weather conditions, favoured crutch strike to replace body strike. The analysis of total strike suggested that rainfall determined overall levels of strike, whereas pasture conditions and cloud cover regulated type of strike. Previous studies could not separate density and fly activity effects, but this study (Wardhaugh and Morton 1990) provided evidence that a reduction in fly population is likely to lead to a reduction in strike incidence.

A comprehensive study was run for 3 years at Fowlers Gap in New South Wales to record numbers and species of blowflies breeding in carcasses and on live merino sheep (Anderson et. al. 1988). SBF was found to be the dominant species in more than 87% of strikes and the native species, *Calliphora nociva* Hardy and *C. Augur* (Fabr.) and *C. stygia* (Fabr.), were found to be associated with SBF and were present in 7% of strikes. Small mob (c. 70) sheep were regularly inspected and it was found that the covert strike was higher than incidence of overt strike, supporting the view that a grazier’s estimate of flystrike is generally an underestimate. On carcasses, *C. rufifaces* was produced in very large numbers. No SBF were produced from either small or large carcasses at any time, however, larvae were recovered from live sheep most of the year. The study suggested SBF as an obligate parasite of live sheep in the arid zone.

### 9.3.2 Field recovery from releases

Quite a few releases and recovery studies that involved sheep blowfly were conducted in the early 1940s (Newman and Clarke 1941; Gilmour et. al 1946). Readshaw (1982) re-examined the data from a mark-recapture experiment related to sheep blowfly conducted by Gilmour et al (1946) to estimate population density using a proposed modification of the Lincoln index (Fletcher et al 1981) that normalizes lost marked flies from the sampling area during trapping. The marked flies were released in 1941-42 summer, near Canberra, at the centre of a large grid of uniformly spaced traps (27 traps) within a 9.6 km diameter circle and an additional 13 traps outside the circle. Analysis from the proposed model provided maximum and minimum estimates of population size according to the survival characteristics of the marked flies. The analysis revealed a ratio of about 3.3 to 1 between the average flies per trap per day and flies per ha.

An experiment was conducted to calibrate trap catches of field flies by comparing the recovery rates of laboratory and field strains of SBF (Vogt and Morton 1991). Field caught females, and females of two laboratory strains (Gen 14 and Gen 29), were marked through emergence by different fluorescent dusts. Emerged adults, held at 10°C to slow down maturity, collected over 3 days, were provided with sucrose and water prior to release following the methods described by Vogt et al (1985). Adults emerging on the first day were mostly male. Percent recovered from releases was generally between 1 – 4.5%. It was advised for seasonal comparisons of wild fly densities, either within or between sites, to have traps installed at fixed locations and to exclude catches for the inconsistent traps.

Wardhaugh et al (1983) compared aerially released larvae (undyed) and ground released (dyed) pupae in southern ranges of ACT (Gudgenby) and in southern Queensland (Cunnamulla). After being corrected for environmental conditions, ground released pupae yielded twice the catch rate in ACT and four times catch rate in southern Queensland. Ground temperatures measured in summer indicate that soil temp at a depth of 5cm are likely to exceed 40°C when air temperatures are above 32°C. Therefore, larval releases in summer are inadvisable in the areas where summer temperatures are high.

### 9.3.3 Distribution

SBF is currently found in many parts of Australia and it is primarily responsible for the myiasis of sheep in Australia (Tellam and Bowles 1997). The early records of blowfly strike during late 1800s to early 1900s in sheep in Tasmania, New South Wales, Victoria, South Australia, Western Australia and Queensland states of Australia was believed to be caused by attack of native necrophagous species or from attack by SBF in a relatively unsusceptible sheep population (Tillyard and Seddon 1933; Norris 1990). A major outbreak of the strike occurred in 1903 in Riverina of New South Wales which then spread throughout the state and into Victoria (Tillyard and Seddon 1933). The strike made its way to Queensland at Barcaldine and Gindie in 1909, and in the Springsure district between 1910 and 1913. Sheep myiasis in the extreme South-west of Queensland also indicated SBF invasion (Waterhouse and Paramonov 1950). In eastern Australia, SBF was the primary blowfly inflicting the majority of flystrikes on sheep (Watts et. al. 1976).

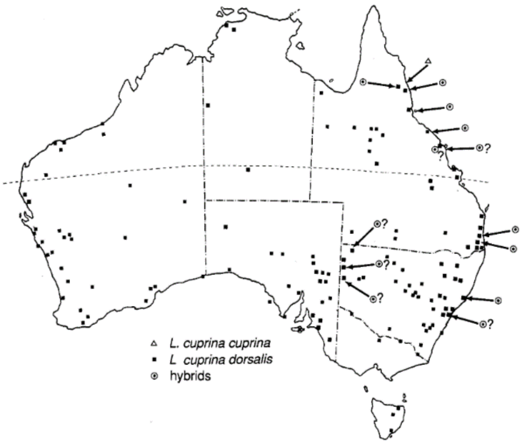


Fig. 1. Distribution in Australia of capture localities of the specimens of *L. cuprina cuprina*, *Lucilia cuprina dorsalis* and their hybrids that were studied.

**Fig 9.3.1: Distribution of SBF (Norris 1990)**

## 9.4 Appendix 4: Short summary listing related insects and efficient programs for other insects

### 9.4.1 Cattle Fever tick

The microscopic parasites *Babesia bigemina*, *B. bovis*, and the rickettsia, *Anaplasma marginale*, can cause tick fever, a potentially fatal disease in cattle. These parasites are spread by cattle ticks *Rhipicephalus* (formerly *Boophilus*) *annulatus* and *R. microplus* (Smith and Kilbourne 1893).

These tick-borne diseases destroy an animal's red blood cells and cause ‘tick fever’ (anemia, fever, organ failure and potential death of susceptible animals) and complete their lifecycle in a single host (Murillo 2019) meaning that if livestock are the only available host, insecticide treatment is an effective suppressant.

Fever tick has been an ongoing issue for Cattle Farmers throughout the world’s livestock regions. Economic losses in outbreaks have occurred throughout cattle regions in Texas and down into Mexico with all breeds of cattle susceptible.

In 1989 the US Department of Agriculture released almost six million female and sterile hybrids (two species) male cattle tick larvae on St Croix Island (US Virgin Islands). Although the female larvae are capable of breeding, they pass sterility on to male offspring with a resulting population drop in overall numbers.

### 9.4.2 Stable fly

Stable fly *Stomoxys calcitrans* is an irritant pest of livestock throughout the world. In Australia it is a declared pest and management falls under the Biosecurity Management Act 2007.

It is smaller than a housefly with a prominent proboscis. The insect irritates humans and livestock and, in the latter, often draws blood after initiating a painful bite. Cattle and horses are predominantly affected. Implications for livestock include weight loss, reduced milk production, allergic reactions and in hot weather cattle have been known to congregate in groups in order to ward off the flies causing additional problems such as heat stroke.

Field tests in St Croix (US Virgin Islands) looked at the feasibility of wide spread releases (Patterson et al 1981). One-hundred thousand flies a day (five days a week) for eighteen months from 1976-77. Reduction in population numbers by 99.9% was achieved with wild flies remaining only in a few isolated pockets of the island.

## 9.5 Appendix 5: Longer Term improvements

### 9.5.1 Chemosterilants

The use of chemical sterilants, either replacing irradiation or adding to release efficiency could be considered. This could increase efficiency since males and females carrying chemosterilants could transfer this to male and female wild individuals. See details of work by Beattie and co-workers (Beattie et al 1972, 1979; Beattie 1975). The use of chemosterilants will have to be assessed thoroughly for environmental fate and risk of off-target effects.

There are obvious advantages in using the insect itself to distribute destruction, as may be done by releasing vigorous, sterile, attractive, but genocidal females. The genocidal agent may be either a chemosterilant or an insecticide, and its transfer to the natural population is expected as a result of attempts by wild males to copulate with the 'booby-trapped' females (Smart and Gilmour 1974).

From the classical and recent experiments there is strong evidence that chemosterilants affect testes and spermatogenesis. The practical application of strong mutagens, and the possibilities of using chemicals to produce azoospermia or inactivation of sperm for male sterilization have been studied in animals. These chemicals degenerate spermatogenic cells and affect growing spermatocytes undergoing the division (Sengupta 2013; Dutta et al 2013) so, the entire testes become smaller (Chandra et al 2013). Earlier experiments on the inductions of dominant lethal mutations also reported that the effects of chemosterilants on spermatogenesis are often followed by lowered vitality of sperm, which may lead to its immotility or even death (LaChance and Crystal 1965; LaChance1967). Ascher et al (1968) studied the degree of motility of sperm in spermatheca of Musca domestica (common housefly) females sterilized with Brestan and Tinicide. They found that the degree of fertility is in direct relation to the degree of motility of sperm.

It has been shown that the aziridinyl chemosterilants N,N '-hexamethylenebis (I -aziridinecarboxamide) (ENT 50172; HMAC) and N,N '-bisaziridinyl-N″-cyclohexylphosphine sulphide (ENT 62488 = A 13-62488, p,p '-bis( 1 -aziridinyl)-N-cyclohexylphosphinothioic amide) are effective sterilants of male and female SBF (Beattie et al 1972; Beattie 1975; Beattie et al1979).

The idea of booby-trapping with chemosterilants, originating from a suggestion by Knipling, were first published by Smith (1963) and have since been examined experimentally in other studies. Booby-trapping with insecticide as a control measure against the Australian sheep blowfly has been discussed by Whitten and Norris (1967), who have also pointed out some theoretical advantages of using a chemosterilant in this operation. Smart and Gilmour 1974 described a series of experiments exploring the possibility of population control in SBF by the introduction of a chemosterilant via booby-trapped females. The ideal sterilant for booby-trapping would need to be capable of sterilizing both sexes in topical application at relatively low doses, but must not be lethal to the female at the high doses, so they would transfer it after mating. Also, it should not reduce the sexual attractiveness of the female nor interfere with the sexual vigour of the male. In the series of experiments, the qualities in the sterilant selected were tested, as well as some laboratory-scale attempts to simulate the introduction of booby-trapped females into a population of flies (Smart and Gilmour 1974; Beattie et al 1974).

Females of the SBF, topically treated with the chemosterilant N,N'-hexamethylenebis (1-aziridinecarboxamide) at a dose of 10 µg per fly. Females tolerated 800 µg per fly. A female 'booby-trapped' with 400 µg of sterilant could transfer a sterilizing dose to a male during copulation. Booby-trapped females were sexually attractive and sterile males were as sexually vigorous as fertile males. The introduction of either booby-trapped females or sterilized males into cage populations caused a greater drop in fertility than expected, but the effect was probably due more to unspecific contact between flies than to mating contacts (Smart and Gilmour 1974).

Treated females with N, N'-tetramethylenebis-(l-aziridinecarboxamide) were released into large field cages containing untreated flies to determine if sterilizing doses of chemosterilant were transferred to untreated flies. Full sterility was induced in cages carrying equal numbers of untreated males, untreated females and females treated with 300 or 600 µg of chemosterilant. In other cages, one female treated with 600 µg of chemosterilant for every five untreated flies of each sex had a similar effect, but lower levels of sterility were obtained with higher ratios of untreated to treated flies. Transfer of chemosterilant appeared to occur through contact during mating, attempted mattings, and between flies of both sexes at the oviposition site (Beattie et al 1974).

Beattie and Mcdonal (1980) showed that doses up to 600 µg of N, N '-hexamethylenebis (I-aziridinecarboxamide) (ENT 501 72; HMAC) had no effect on the longevity of females up to 10 days post-treatment. However, doses of 300 and 600 µg caused significant reductions in longevity at 14 and 21 days post-treatment but did not cause loo% mortality. A dose of 32 µg of N.N '-bisaziridinyl-N ″-cyclohexylphosphine sulphide (ENT 624x8) killed all treated females within 24 hours and a 16 µg dose significantly affected mortality within one day. HMAC had no effect on the competitiveness of males at doses less than 64 µg, but 128 µg induced a highly significant decrease in mating capacity. The sterilant did not affect the receptiveness of females, even when they were treated with 600 µg.

There is some evidence that combined SIT and chemosterilant (Lufenuron) bait stations improved control in a bait-station area for mediterranean fruit fly (Navarro-Llopis, Vacas et al 2011).

A significant reduction in the Medfly population was detected in plots where SIT combined with chemosterilant (Lufenuron) bait station. Likewise, corresponding reduction in the percentage of injured fruit was observed. These data indicate the compatibility of these techniques and suggest the possibility of using chemosterilant bait station system with SIT to reduce *C. capitata* populations in locations with high population densities, where SIT alone is not sufficiently effective to suppress fruit fly populations to below damaging levels (Navarro-Llopis, Vacas et al 2011).

### 9.5.2 Release numbers

Adult SBF will emerge from the sites where larvae have entered the ground in the previous year and mate early before searching for a protein meal (usually sheep dung) needed for egg maturation, move to hosts (sheep) and egg-laying. Therefore, release areas can potentially be adapted based on this knowledge of where sheep were concentrated in previous years (e.g., camps, drinking troughs), in order to increase release efficiency. For the initial small-scale experiments (islands) it is not necessary to have such detailed information. Studies on spatial distribution of the sheep over the year, and with them SFB overwintering sites need to be done before the start of larger scale releases. In the initial phase of a project the first release areas will be small so the numbers to be released will be small.

### 9.5.3 Diapausing individuals

One promising future approach is to exploit the capacity of dormancy in the pre-pupae or pupae produced so that these can be cold-stored during several months and pupae production can be almost year-round for targeted mass-releases in spring. Mackerras (1993) Norris (1959) and De Cat (2007) documented the hibernation of pre-pupae and pupae in the soil when winter temperatures are low. In the soil these larvae and pupae suffer from a high mortality resulting in low spring populations (Pitts and Wall 2005). They also reported a much higher survival rate when pupae were placed in sawdust in the field compared with larvae and pupae in deeper compacted soil.

Research should be directed to the development of induction of the diapause and storage conditions allowing to produce and store prepupae and/or pupae as a way to generate a large stock of individuals for future seasonal releases. This should be studied in conjunction with irradiation and release conditions to make sure releases can be done at the right moment with sterile flies being competitive.

### 9.5.4 Sex-sorting before release

Mass-rearing facilities initially produce equal numbers of the two sexes, females and males are both sterile at the release but females do not add to the efficiency of SIT. In some species it is possible to separate and discard females before releasing (Proverb 1974). The development of male-only strains represents a potentially important step toward improving SIT (Hendrichs et al 1995). Various female-killing and sex-sorting genetic systems have been developed, known generically as genetic sexing (GS) strains. Genetic sexing systems have been constructed in a number of dipteran species, either for use with the SIT or with other genetic methods of population control (Whitten 1969, 1979; Curtis et al 1976; Whitten et al 1977; Foster et al 1978; Kaiser et al 1978; Baker et al 1979, 1980; Rossler 1979; Robinson and Van Heemert 1982; Saul 1985; Kerremans and Franz 1995).

So far, most GS strains in factory production have used radiation-induced translocations to the Y chromosome as dominant selectable markers, complementing an X-linked or autosomal recessive trait such as:

- Pupal colour mutations with males emerging from wild-type pupae and females emerging from mutant pupae (Whitten, 1969; Rossler, 1979, 1980; Robinson and van Heemert, 1 982). Such a system of pupal sorting would still need all larvae to be reared to pupal stage but females would not be released, so released males would not mate with already sterile females, increasing efficiency.
- Temperature-sensitive lethal GS strains, which female zygotes can be killed following a high temperature treatment developed (Kerremans and Franz 1 995). In this case the selection of males could be done in the egg or early larval stage, reducing the costs of rearing and increasing the rearing capacity
- Blind GS strains by using a gene causing slow development during the larval and pupal stages. These genes are the eye color mutation sw-z and sw-y in medfly, in which the traits of slow development and mutant eye phenotype behave as pleiotropic effects of a single gene. The delaying effect of the gene can be used to separate males at the larva stage (Cladera,1 995; Pizarro et al 1997).
- Field-female killing (FFK) strains, which use recessive eye-color mutations as the selective marker. Females are homozygous for the mutations and unable to survive to reproductive maturity in the field, because they are functionally blind. Genetic death in field populations is caused by semi-sterility of the translocation and by homozygosis of the mutations in females and non-translocation males of field origin (Foster et al 1988; Whitten et al 1977, 1979; Foster et al 1985).

Based on fundamental research on female-lethal genetic systems in D. *melanogaster*, Lucilia germ-line transformation and sex determination genes in Lucilia, the transgenic sexing strains of SBF were successfully developed that carry a tetracycline -repressible female lethal genetic system (Scott 2014).

**In Summary**
Sterile Insects used in SIT cannot self-replicate and cannot establish in the environment. SIT is species specific and can easily break the reproductive cycle of pests. SIT has been used effectively for the control of many species of flies and in Bangladesh has shown to be effective for the control of SBF in postharvest fisheries. SIT fits easily within an overall SBF control strategy. It is environmentally sustainable and can be an effective means of pest control. Widening the availability of SIT for SBF throughout Australia would reduce flystrike and decrease economic costs of control and enhance marketability of sheep for meat and wool.

### 9.5.5 Entomovectoring of entomopathogens

If sterile flies could be infested with entomopathogens (such as insect killing bacteria, fungi or nematodes) just before release these ‘booby trapped’ flies could transfer these pathogens to the wild population. This would mean that sterile females would actually contribute to the control of the wild (male) population. This approach has been tested for control of

Mediteranean fruitfly in Brazil to deliver *B. bassiana* conidia for the control of wild populations of *C. capitata (*Paranhos et al 2019).

### 9.5.6 Genome editing and CRISPR-Cas

New research methods to produce sterile flies, or to produce flies that are able to induce sterility in wild populations are being developed. These techniques, if accepted for field releases, could be a valuable addition to SIT (avoiding the need for sterilization) or replace SIT approaches all together. However, these techniques are not yet approved for field releases and are not expected to be approved in the short term.

One possible, more acceptable option could be the production of a sexing strain for rearing in which only the females carry a modified gene construct, allowing their elimination from before release.

## 9.6 Appendix 6: CASE STUDY: Bushfire Recovery and Restocking

The summer of 2019-2020 catastrophic bushfires tore through large tracts of Agricultural land in Queensland, New South Wales, Victoria and South Australia and resulted in the declaration of Bushfire Emergency affecting many red meat and livestock producers. Millions of hectares in regional and rural areas of Australia’s vast livestock areas have been affected. People, animals and infrastructure have been severely affected by loss.

State and commonwealth government along with Meat and Livestock Australia, Peak Industry Councils, State Farming Organisations and livestock producers on the ground are now focusing on currently helping those who need immediate assistance and working towards the challenges to be faced by industry during the recovery process.

At this time, welfare of suffering animals is a priority for MLA and farmers. Current assessments for livestock loss in sheep is around 13% of the national flock have been severely impacted with a further 17% partially affected.

Kangaroo Island, one of the trial sites being considered for SBF SIT trials has been severely affected with almost half the landmass of the island affected by bushfire. Up to 52,000 sheep have been estimated killed or severely wounded during the fire season, reportedly up to one-tenth of the island's total flock count. The South Australian government through the Department of Primary Industries and Regions are currently working with producers on Kangaroo Island to complete estimates of stock losses.

Following the fires, farmers who are busy with fencing and repairs on farm infrastructure are likely to have less time and budget for flystrike treatments and surveillance. Sheep will be kept in confinement in smaller areas awaiting fencing operations. All of these indirect effects could increase flystrike and flystrike impact during the bushfire recovery period.

Diversity studies in invertebrate abundance post bushfire show that the re-establishment of invertebrate animals plays a primary role in product nutrient cycling and uptake, population and community level interactions and energy storage and transfer. Contributions of invertebrates provide substantial benefits to the overall recovery to affected ecosystems. This includes a number of native species of blowflies.

Currently no data exists on the establishment of SBF after bushfire.

**In Summary**

Considering the devastation to sheep numbers in certain regions of Australia, farmers are looking to restock and increase sheep numbers. One benefit SIT could provide to farmers at this time would be the suppression and management of SBF. An opportunity exists for further research into establishment and dispersal of SBF during the fire regeneration period as well as establishing preventative SIT protocols to the rapidly increasing stock numbers.

### 9.6.1 Locations for study

#### *Kangaroo Island*

In this report we propose to develop SBF eradication on Kangaroo island as a first large-scale demonstration. Kangaroo has over half a million head of sheep. 22.5% of the employed population is involved in agriculture with 45% of business in the agricultural sector followed by construction (12.1%). The next highest cohort is 12.4% for the food and accommodation sector (Australian Bureau of Statistics 2017.)

For release purposes, Feral goats and deer have been eradicated so little risk exists of SBF going to other species. Other species such as feral cats and feral pigs are currently seen as problems on the Island and there is a project to try to eradicate cats. (Natural Resources, Kangaroo Island 2018). Little knowledge exists on SBF populations outside of the agricultural area, but SBF has been reported to survive on small wildlife carcasses such as possums in Tasmania.

**Geographic info**

Total area 4400km$^2$ or 440000 Ha

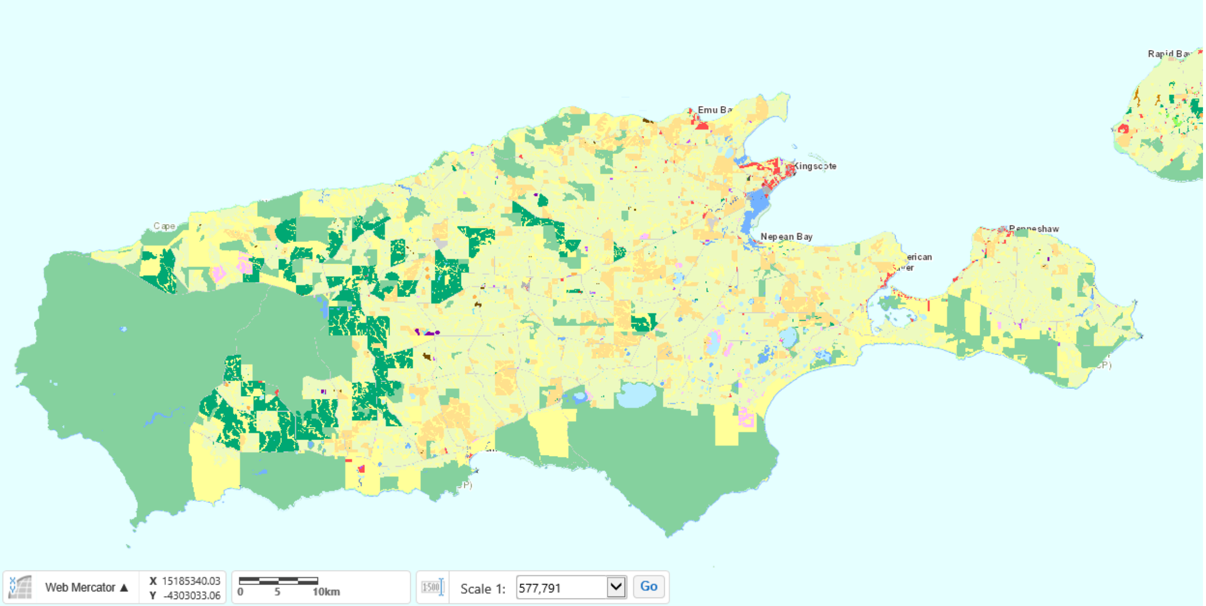
ABS stats put Area of Agricultural commodities at 167,523 Ha

ABS total number of sheep and lambs 2016/17 was 598,889.

167523/440000, ie 38% of the island is in agricultural use. However, this is interspersed with residual native cover and softwood plantations such that the area that would need to be treated is within an area of around 300,000 Ha**,** or 68% of the island. Recent catastrophic fires in the region may affect the end total for this.

By comparison, the area of Adelaide is 71,321 Ha, or about a third of the area that would need to be treated on KI.

**Fig 9.6.1: Land Use map Kangaroo Island (NatureMaps:)**



Land use code 331, Orange, is cropping, cereals

Land use code 320, Pale greenish- yellow, is grazing modified pastures

Land use code 133, Bright yellow, residual native cover

Land use code 117, Bright mid green, nature conservation

Land use code 312, Deep green, softwood plantation forestry

**Fig 9.6.2: Total treatment area, including forestry areas, would be in the order of 300,000Ha**

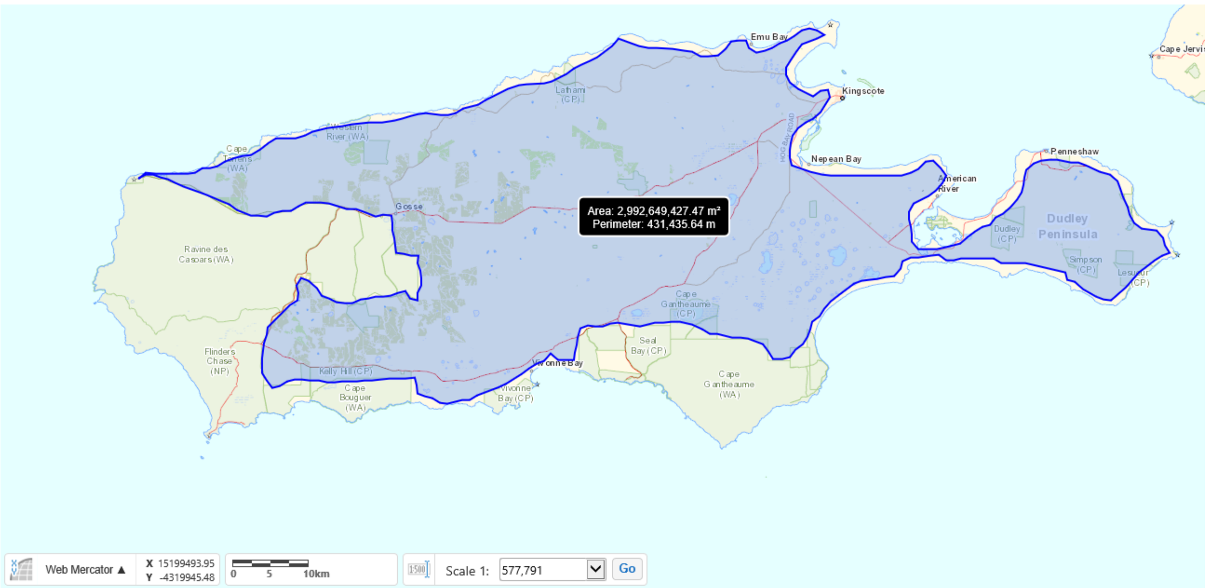
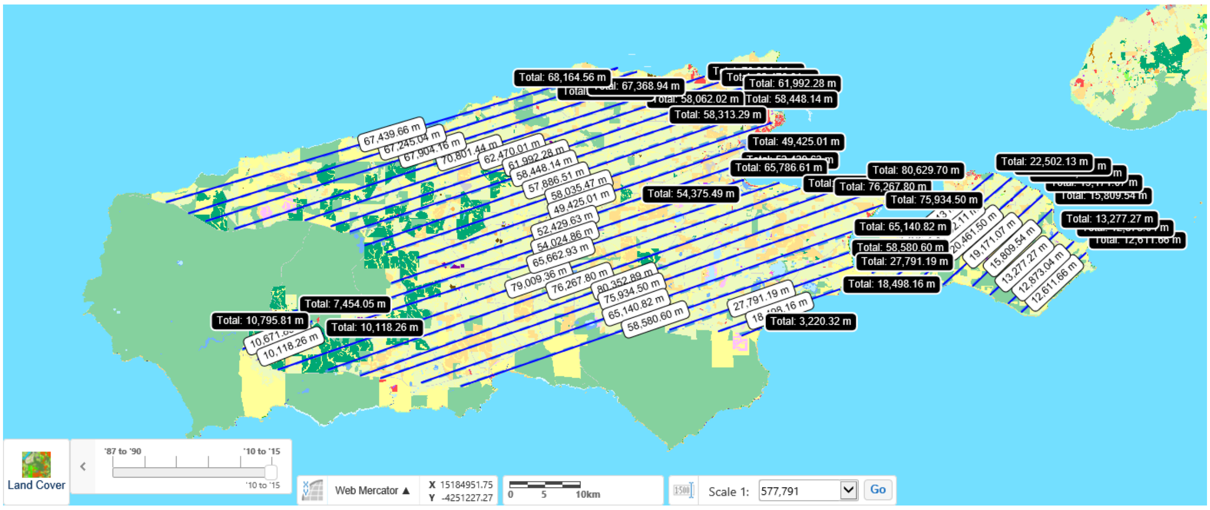
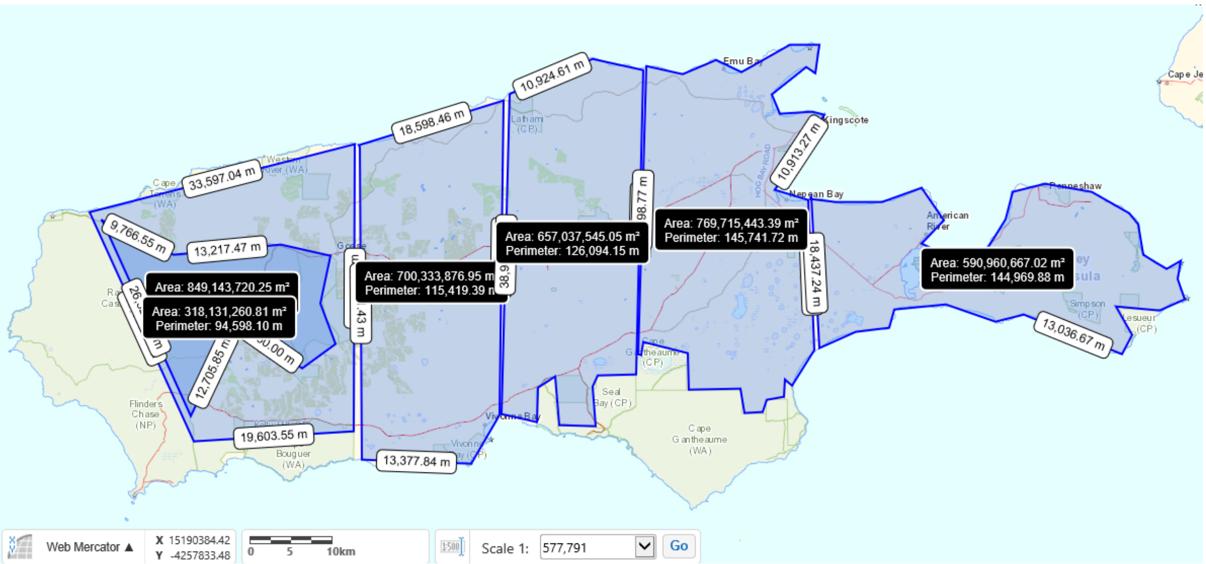


**Fig 9.6.3 :Possible flight lines for whole of area treatment:**



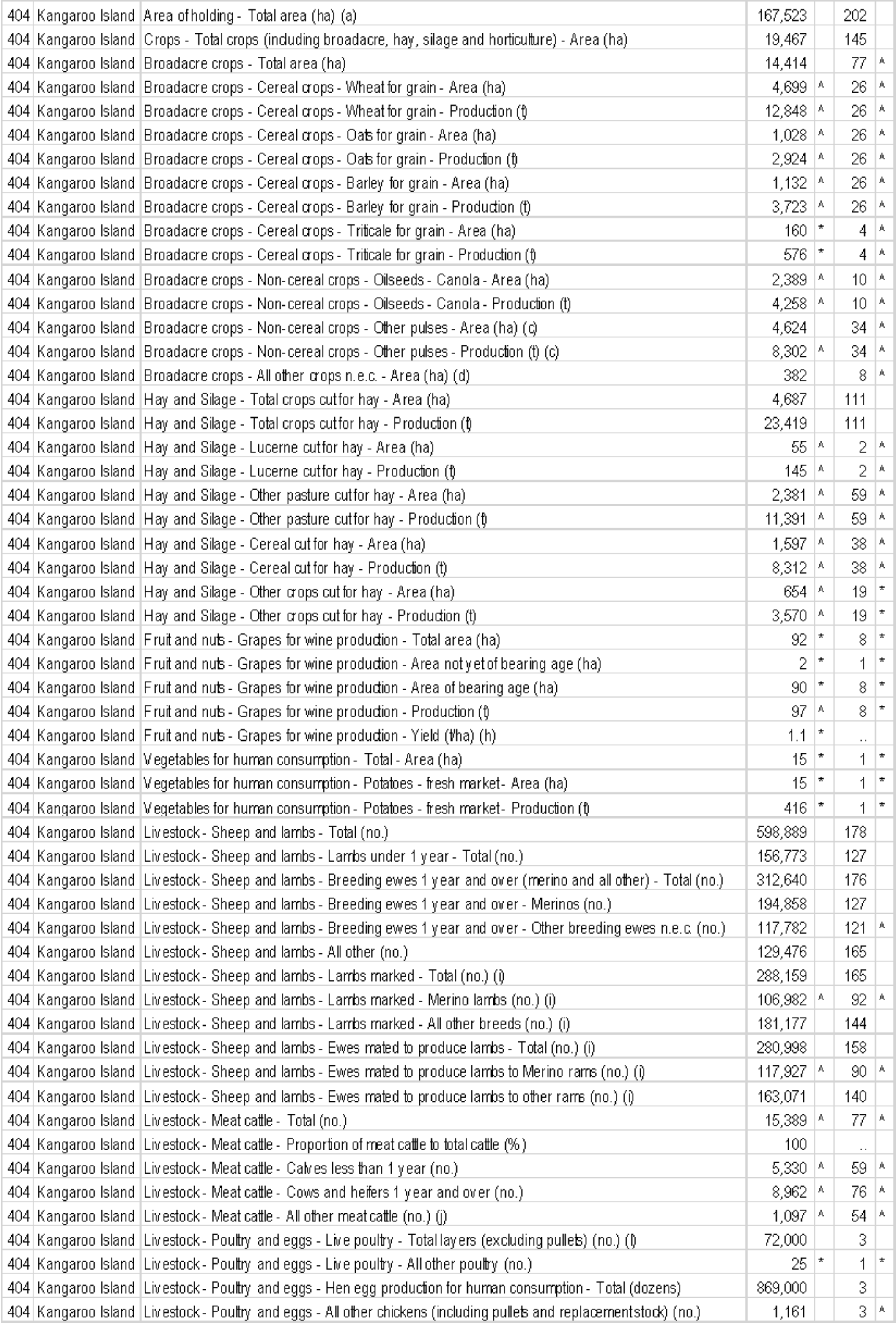
These 32 flight lines are approximately 2km apart (may need to offset release by 1km each week to ensure an even spread of flies), and between 3 and 80 km long. They amount to a total of 1,386 km of flight transect. It may be possible to reduce the fly numbers needed by turning off the release machine while flying over the softwood plantations.

## Fig 9.6.4: Alternatively, treatment of the island in smaller bands may be more feasible



The western-most band of 850 sq km includes 318 sq km of the conservation park that may not need treatment, or need treatment at a lower release rate. The other bands range from about 600 – 770 sq km.

## Table 9.6.1:ABS Agricultural commodities 2016/17



| | | | | | | |
|---|---|---|---|---|---|---|
| 404 | Kangaroo Island | Area of holding - Total area (ha) (a) | 167,523 | | 202 | |
| 404 | Kangaroo Island | Crops - Total crops (including broadacre, hay, silage and horticulture) - Area (ha) | 19,467 | | 145 | |
| 404 | Kangaroo Island | Broadacre crops - Total area (ha) | 14,414 | | 77 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Wheat for grain - Area (ha) | 4,699 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Wheat for grain - Production (t) | 12,848 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Oats for grain - Area (ha) | 1,028 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Oats for grain - Production (t) | 2,924 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Barley for grain - Area (ha) | 1,132 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Barley for grain - Production (t) | 3,723 | ^ | 26 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Triticale for grain - Area (ha) | 160 | * | 4 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Cereal crops - Triticale for grain - Production (t) | 576 | * | 4 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Non-cereal crops - Oilseeds - Canola - Area (ha) | 2,389 | ^ | 10 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Non-cereal crops - Oilseeds - Canola - Production (t) | 4,258 | ^ | 10 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Non-cereal crops - Other pulses - Area (ha) (c) | 4,624 | | 34 | ^ |
| 404 | Kangaroo Island | Broadacre crops - Non-cereal crops - Other pulses - Production (t) (c) | 8,302 | ^ | 34 | ^ |
| 404 | Kangaroo Island | Broadacre crops - All other crops n.e.c. - Area (ha) (d) | 382 | | 8 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Total crops cut for hay - Area (ha) | 4,687 | | 111 | |
| 404 | Kangaroo Island | Hay and Silage - Total crops cut for hay - Production (t) | 23,419 | | 111 | |
| 404 | Kangaroo Island | Hay and Silage - Lucerne cut for hay - Area (ha) | 55 | ^ | 2 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Lucerne cut for hay - Production (t) | 145 | ^ | 2 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Other pasture cut for hay - Area (ha) | 2,381 | ^ | 59 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Other pasture cut for hay - Production (t) | 11,391 | ^ | 59 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Cereal cut for hay - Area (ha) | 1,597 | ^ | 38 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Cereal cut for hay - Production (t) | 8,312 | ^ | 38 | ^ |
| 404 | Kangaroo Island | Hay and Silage - Other crops cut for hay - Area (ha) | 654 | ^ | 19 | * |
| 404 | Kangaroo Island | Hay and Silage - Other crops cut for hay - Production (t) | 3,570 | ^ | 19 | * |
| 404 | Kangaroo Island | Fruit and nuts - Grapes for wine production - Total area (ha) | 92 | * | 8 | * |
| 404 | Kangaroo Island | Fruit and nuts - Grapes for wine production - Area not yet of bearing age (ha) | 2 | * | 1 | * |
| 404 | Kangaroo Island | Fruit and nuts - Grapes for wine production - Area of bearing age (ha) | 90 | * | 8 | * |
| 404 | Kangaroo Island | Fruit and nuts - Grapes for wine production - Production (t) | 97 | ^ | 8 | * |
| 404 | Kangaroo Island | Fruit and nuts - Grapes for wine production - Yield (t/ha) (h) | 1.1 | * | .. | |
| 404 | Kangaroo Island | Vegetables for human consumption - Total - Area (ha) | 15 | * | 1 | * |
| 404 | Kangaroo Island | Vegetables for human consumption - Potatoes - fresh market - Area (ha) | 15 | * | 1 | * |
| 404 | Kangaroo Island | Vegetables for human consumption - Potatoes - fresh market - Production (t) | 416 | * | 1 | * |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Total (no.) | 598,889 | | 178 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Lambs under 1 year - Total (no.) | 156,773 | | 127 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Breeding ewes 1 year and over (merino and all other) - Total (no.) | 312,640 | | 176 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Breeding ewes 1 year and over - Merinos (no.) | 194,858 | | 127 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Breeding ewes 1 year and over - Other breeding ewes n.e.c. (no.) | 117,782 | | 121 | ^ |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - All other (no.) | 129,476 | | 165 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Lambs marked - Total (no.) (i) | 288,159 | | 165 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Lambs marked - Merino lambs (no.) (i) | 106,982 | ^ | 92 | ^ |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Lambs marked - All other breeds (no.) (i) | 181,177 | | 144 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Ewes mated to produce lambs - Total (no.) (i) | 280,998 | | 158 | |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Ewes mated to produce lambs to Merino rams (no.) (i) | 117,927 | ^ | 90 | ^ |
| 404 | Kangaroo Island | Livestock - Sheep and lambs - Ewes mated to produce lambs to other rams (no.) (i) | 163,071 | | 140 | |
| 404 | Kangaroo Island | Livestock - Meat cattle - Total (no.) | 15,389 | ^ | 77 | ^ |
| 404 | Kangaroo Island | Livestock - Meat cattle - Proportion of meat cattle to total cattle (%) | 100 | | .. | |
| 404 | Kangaroo Island | Livestock - Meat cattle - Calves less than 1 year (no.) | 5,330 | ^ | 59 | ^ |
| 404 | Kangaroo Island | Livestock - Meat cattle - Cows and heifers 1 year and over (no.) | 8,962 | ^ | 76 | ^ |
| 404 | Kangaroo Island | Livestock - Meat cattle - All other meat cattle (no.) (j) | 1,097 | ^ | 54 | ^ |
| 404 | Kangaroo Island | Livestock - Poultry and eggs - Live poultry - Total layers (excluding pullets) (no.) (l) | 72,000 | | 3 | |
| 404 | Kangaroo Island | Livestock - Poultry and eggs - Live poultry - All other poultry (no.) | 25 | * | 1 | * |
| 404 | Kangaroo Island | Livestock - Poultry and eggs - Hen egg production for human consumption - Total (dozens) | 869,000 | | 3 | |
| 404 | Kangaroo Island | Livestock - Poultry and eggs - All other chickens (including pullets and replacement stock) (no.) | 1,161 | | 3 | ^ |

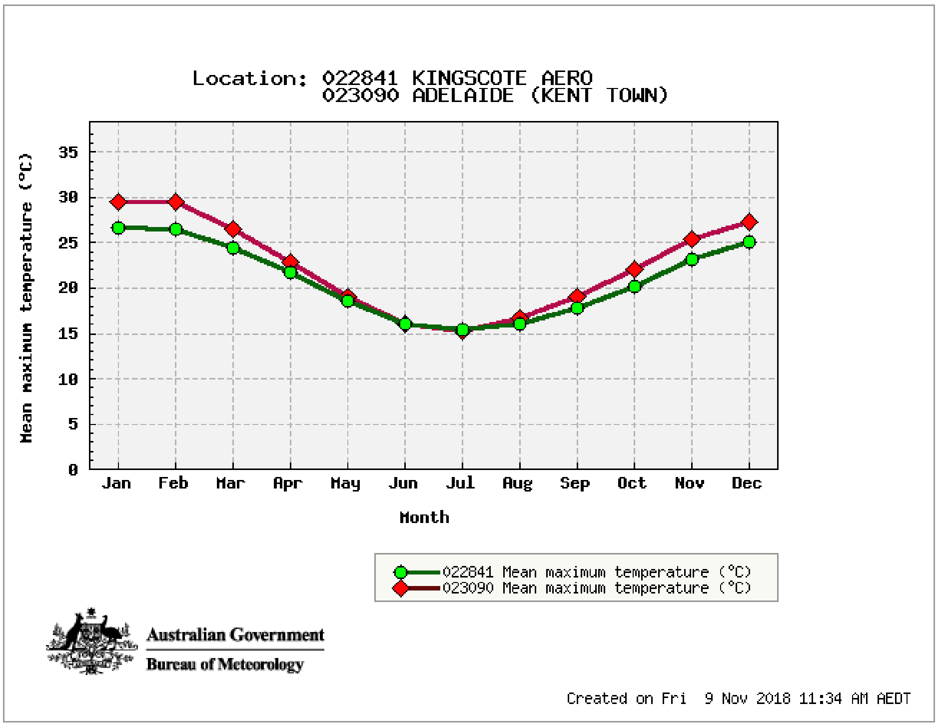
^ estimate has a relative standard error of 10% to less than 25% and should be used with caution

* estimate has a relative standard error between 25% and 50% and should be used with caution

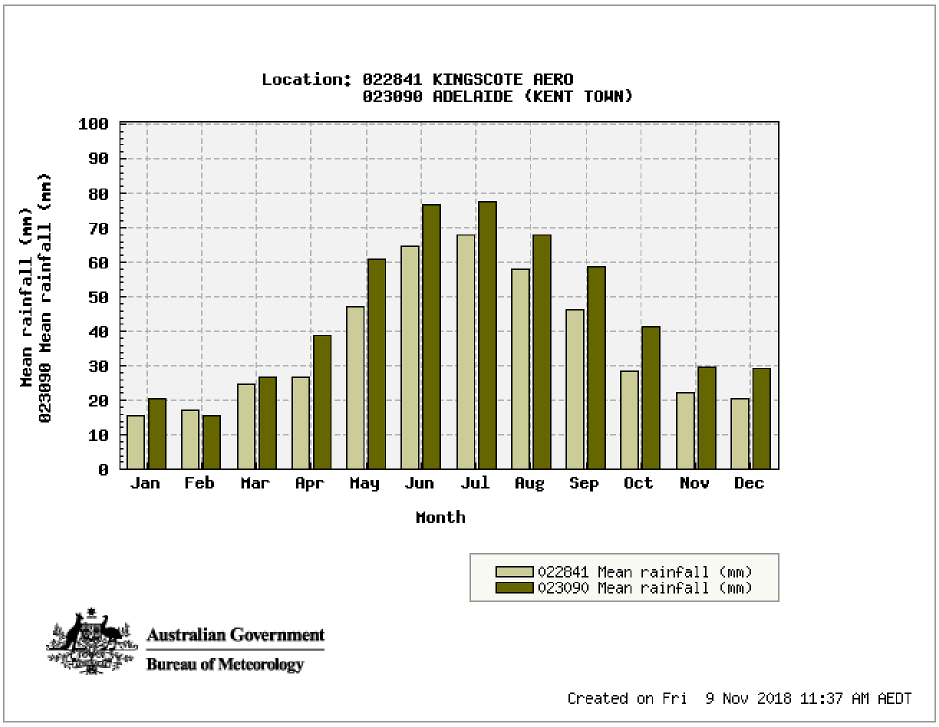
These data suggest that only about 107 Ha of KI used for fruit and veg horticulture. (Latest data as of January 2020)

## General weather conditions

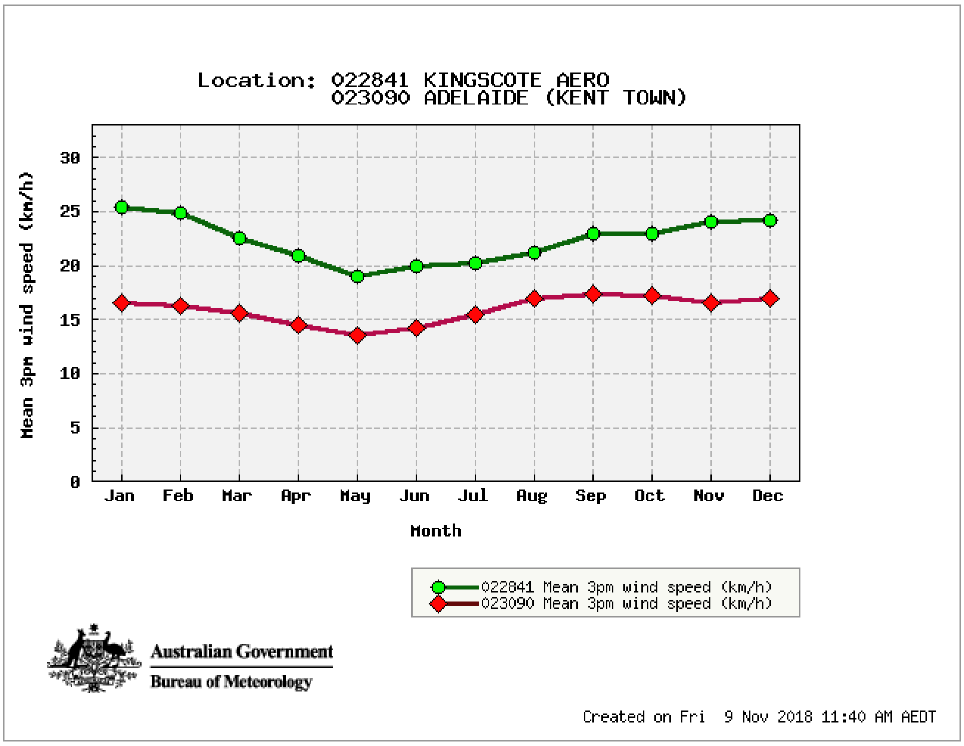
### Fig 9.6.5: Temperatures compared with Adelaide - KI is cooler in summer



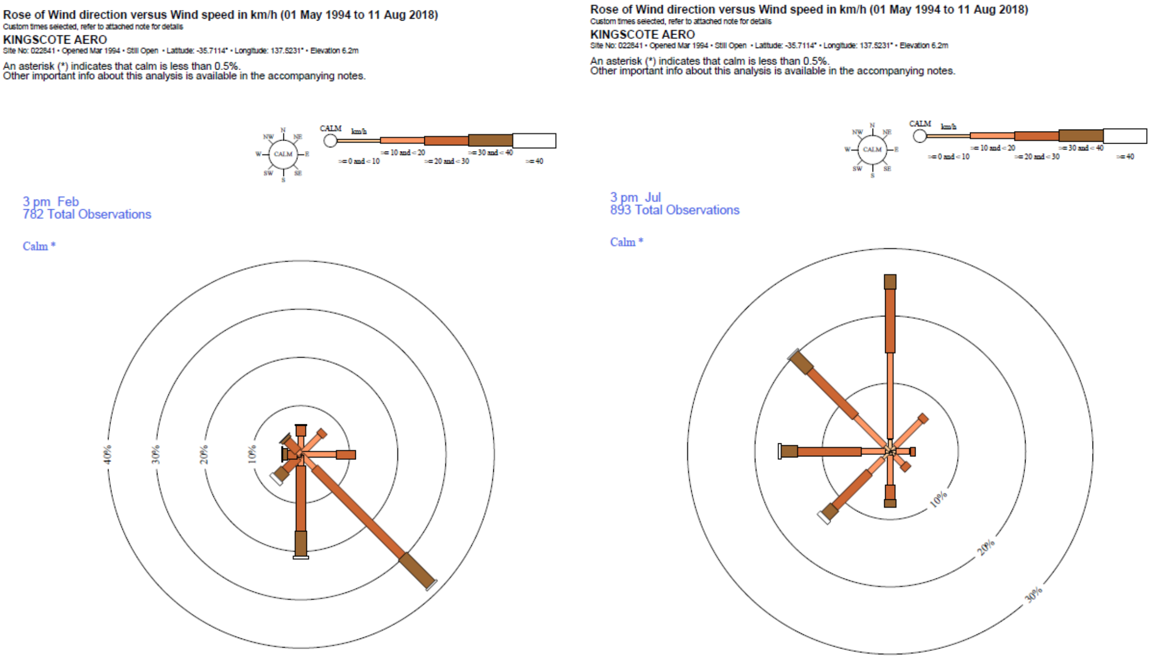
### Fig 9.6.6: Rainfall is slightly lower in each month except Feb.



## Fig 9.6.7: KI has consistently higher wind readings.



## Fig 9.6.8: Directions change from usually strong SE in summer to less strong N-NW in winter.



Rose of Wind direction versus Wind speed in km/h (01 May 1994 to 11 Aug 2018)
Custom times selected, refer to attached note for details
KINGSCOTE AERO
Site No: 022841 • Opened Mar 1994 • Still Open • Latitude: -35.7114° • Longitude: 137.5231° • Elevation 6.2m

An asterisk (*) indicates that calm is less than 0.5%.
Other important info about this analysis is available in the accompanying notes.

3 pm Feb
782 Total Observations

Calm *

Rose of Wind direction versus Wind speed in km/h (01 May 1994 to 11 Aug 2018)
Custom times selected, refer to attached note for details
KINGSCOTE AERO
Site No: 022841 • Opened Mar 1994 • Still Open • Latitude: -35.7114° • Longitude: 137.5231° • Elevation 6.2m

An asterisk (*) indicates that calm is less than 0.5%.
Other important info about this analysis is available in the accompanying notes.

3 pm Jul
893 Total Observations

Calm *

### *Flinders Island (SA) eradication effort*

Flinders Island is an island of 3,642 Ha (9000 acres) in the Great Australian Bight, 30km off the coast of Eyre Peninsula SA. In 1986, Dr Rod Mahon was delivering 10 million flies per week (200kg) to the island.

Flinders Island SA carried 6000 sheep at the time of the eradication effort. Insecticides were not used on the Island. Flystruck sheep observed at shearing in Aug 1985. Release of 1.35million mutant male flies per week from Aug 1985 to May 1986 (34,000/sq km). Trapping indicated a peak of 345 females/ha in October 1985 declined to less than 1 female per ha in May 1986 (Wardhaugh et al, 1990).

### 9.6.2 Tasmanian proposal (2002) for reference:

### *Flinders Island (Tas)*

The largest island in the Furneaux Group. 136,700 Ha (1367 Sq km), 54 km from Cape Portland, Tasmania. Close to Cape Barren Island and Clarke Island.

#### Eradication strategies

1. Pre-treatment of sheep with persistent insecticide

Sheep are the main source of fly breeding in all parts of Australia, including Tasmania. In order to minimize the size of the fly population to be subjected to SIRM, it is intended that all (or as many as practical) sheep be treated with a persistent insecticide such as dicyclanil. Two treatments, one in mid-summer (January) and the second the following spring, could be expected to reduce populations of SBF to a level close to the limit of detection by normal fly trapping. Such a strategy would ensure that a high release:wild ratio would prevail at the outset of SIRM and would allow for savings in rearing costs as well as the size of the SIRM facility.

2. Release numbers and swath widths

The number of flies (bi-sex) needed for controlling fly populations will be based on data from previous studies of fly abundance on Flinders Island (Mahon 2001), supplemented by trapping data accumulated in the season preceding SIRM. Although the pre-treatment of sheep is likely to lead to a substantial reduction in fly population, the laboratory-reared sterile males will be less competitive than field- reared flies, so that estimates of release numbers ($5 \times 10^6$ per week on Flinders Island, and $200 \times 10^6$ per week for Tasmania) should be regarded as approximate.

Flies will be released from the air along parallel lanes spaced 4 km apart (swath width). Releases will occur bi-weekly, with the second lane offset by 2 km.

**Table 9.6.1: Tasmanian eradication proposal**

| | # flies/week | Area to treat (sqkm) | flies/sqkm/week |
|---|---|---|---|
| **Flinders Island(TAS)** | 5,000,000 | 1,367 | 3658 |
| **Tasmania** | 200,000,000 | 68,401 | 2924 |
| **Our proposal:** | | | |
| **Kangaroo Island** | 45,000,000 | 4,500 | 10,000 |

If we carried out the treatment in the area in the bands illustrated above, we would need 3000 x 850 = 2.5 million flies per week for the westernmost band (less if the conservation park were treated at a lower rate) to start with and gradually as the treatment continued eastwards across the island, down to 3000 x 591 = 1.7 million flies/week (bi-sex). However, cannot find any data or information on trapping on Flinders Island for background info on fly population to compare any data we collect.

### 9.6.3 Other Islands to consider

#### *Best options*

**Flinders Island** SA, Area - 36 sq km (3642 Ha); 28 km offshore of the west coast of Eyre Peninsula, SA. Single owner, Peter Woolford, stay@flindersgetaway.com 0428 261 132 PO Box 169 Pt Lincoln, SA 5606.

**Taylor Island**, area from Nature Maps, 2.56 sq km (256 Ha) Looks to be one large holding with a few patches of remnant vegetation, but mostly cleared. Shearing shed within the main vegetation patch. Also holds a navigation tower with flashing light.

- "Ray Watherston has been grazing approximately 600 sheep on this island for many years."http://www.tacoma.org.au/media/documents/news/newsletter/TPS_December_newsletter_date_correction_2.pdf
- "An average of 20 bales of wool from 700 merino sheep are produced on the island annually." - 2017 https://www.portlincolntimes.com.au/story/5077753/tacoma-loads-island-wool-clip/
- LinkdIn has Ray Watherston of Port Lincoln, manager of Logicoil/Liberty Pty Ltd… Port Lincoln Business directory has Logicoil at 46 Pine Freezer Road, Pt Lincoln, 08 8682 6544.

#### *Possible options*

**Boston Island**, 9.6 sq km (960 Ha) in Boston Bay, Spencer Gulf. Re-zoned in 2009 to allow residential development and tourism. Owned by previous mayor of Port Lincoln, Peter Davis. Numerous semi-rural blocks, and a section of suburban size residential blocks too.

**Spilsby Island**, 4 sq km, approx. 3km x 4km, 22 km south east of Tumby Bay. Privately owned, and continues to be grazed by sheep according to Wiki, but website says there are 40 land owners. Naturemaps cadastral shows most of the blocks are large residential blocks at one end of the island, so may also still have sheep. contact@spilsbyisland.com.au Michael Whillas 0427 511 389

**Louth Island**, 1.35 sq km (135 Ha) in Louth Bay, 17.5km NNE of Port Lincoln, and 3km from the coastline, easily accessible by boat. Privately owned, or seems to be up for sale or leasehold http://www.rdawep.org.au/louthisland/

### *Unsuitable*

Neptune Islands SA, two island groups, North and South about 2.4 and 2.0 sq km, about 28km south of the mainland at Cape Catastrophe. Bush rats the only mammal there. Has a BOM station.

**Wedge Island**, 10 sq km, 200 Km west of Adelaide SA. Sheep farmed from 1858 but then abandoned and bettongs, wombats and rock wallabies released there in the 70s and 80s. Conservation and marine wildlife reserve.

**Thistle Island**, 40km from Pt Lincoln. Geoff Freer, geoffreer@hotmail.com, 0427 940030 Cathy contacted Geoff and he confirmed no sheep there now, just bilby's and wallabies.

### *Proposed Sheep Blowfly preliminary trapping trials.*

Pilot trapping program to determine background fly levels and ideally any habitat related density variation.

At four **Locations**

- KI - Kangaroo Island - Richard Glatz – follow up re: fires?
- AH - Adelaide Hills – (Nancy Cunningham)
- EP - Eyre Peninsula – Lindsay Matthews
- FI - Flinders Island – Peter Woolhouse

set 1 Lucitrap with DDVP block in each of three **Environments**:

- SC - sheep camp
- WS - water source,
- GA – grazing area
- NV - native vegetation (if available)

Clear traps weekly. Pour contents into vial labelled with Date, Location and Environment. Place vials in zip lock bag in freezer until they can be transported to SARDI for checking.

## 9.7 Appendix 7: Project Fact Sheet

(see separate document)

## 9.8 Appendix 8: Media Reports on Mulesing since July 2019

| Year | Date | Source/Summary | Title |
|---|---|---|---|
| **2019** | Sep-01 | https://www.countrynews.com.au/livestock/2019/09/01/781490/retailsers-cut-using-wool-from-mulesed-sheep<br>Leading Australian fashion retailers Country Road Group and David Jones have recently made the commitment to phase out mulesed sheep wool from their lines in an attempt to advocate better animal protection in the fashion industry. | Retailers cut using wool from mulesed sheep |
| **2019** | Mar-06 | https://www.sheepcentral.com/german-documentary-on-australian-merino-wool-highlights-mulesing-issues/<br>A NEED for greater transparency about Australian Merino wool production, marketing and sheep welfare trends – especially mulesing and pain relief — has been highlighted in a recent German television and newspaper investigation. | German documentary on Australian Merino wool highlights animal welfare issues |
| **2019** | May-13 | https://www.sheepcentral.com/german-petition-seeks-mandatory-sheep-pain-relief-and-mulesing-ban/<br>A GERMAN filmmaker has launched a petition calling for mandatory use of pain relief for mulesing and other operations, and government intervention to phase out the flystrike prevention practice in Australia | Petition seeks mandatory sheep pain relief for mulesing |
| **2019** | Jul-19 | https://www.weeklytimesnow.com.au/agribusiness/sheep/mulesing-pain-relief-for-sheep-set-to-be-mandated/news-story/a6e83ca4ef0425a71cde8fd765070d05<br>VICTORIA is on track to become the first state to introduce mandatory pain relief for mulesing | Mulesing pain relief for sheep set to be mandated |
| **2019** | 11-Mar | https://www.abc.net.au/news/rural/2019-03-11/liquid-nitrogen-mulesing-alternative/10878280<br>A long-touted alternative to surgical mulesing will be available commercially this season after more than 10 years of development. | Is liquid nitrogen the answer to consumer and animal-welfare objections to sheep mulesing? |
| **2019** | 15-Jul | https://www.abc.net.au/news/rural/2019-07-16/mandatory-mulesing-pain-relief-in-victoria/11312596<br>Victoria is set to become the first state in the country to introduce mandatory pain relief for mulesing | Mandatory pain relief for mulesing in Victoria looks set to |

| | | | |
|---|---|---|---|
| | | | become a reality |
| **2019** | 30-Aug | https://www.abc.net.au/news/rural/2019-08-31/europe-retail-market-drives-demand-for-non-mulesed-wool/11434626 Despite prices in the overall wool market declining, non-mulesed wool is fetching premium prices because of the demand in Europe by retail brands looking for ethically produced wool. | Non-mulesed wool commands premium price during market slump |
| **2019** | 4-Sep | https://www.ragtrader.com.au/news/country-road-takes-stand-on-sheep-wool, Animal protection charity Four Paws has commended Country Road Group's and David Jones' commitments to phase out mulesed sheep wool. | Country Road takes stand on sheep wool |
| **2019** | 18-Nov | https://www.abc.net.au/news/rural/2019-11-16/wool-industry-battles-go-to-the-board-room/11686774 Accusations of dirty tricks, letters from the Agriculture Minister and questions about animal welfare — this is wool industry politics in the lead-up to an election which will help shape the sector's future | Wool industry leadership elections on the way amid claims of dirty tricks |
| **2019** | 22-Nov | https://www.abc.net.au/news/rural/2019-11-22/awi-agm-2019-result-merriman-dumped/11725144 The wool industry has shaken up Australian Wool Innovation, with woolgrowers electing two new candidates to the board and dumping former chairman Wal Merriman | AWI board elections: Industry dumps Merriman, votes in new blood |
| **2019** | 25-Nov | https://www.livekindly.co/mulesed-wool-dropped-target-kmart/ Target Australia and Kmart are dropping mulesed wool. The American retailers have both said they plan to phase out wool from mulesed sheep over the next few years due to animal welfare concerns. Target Australia has said that it will have phased out all mulesed wool by July 2023, while Kmart aims to reach the same target by July 2024. | Mulesed Wool Dropped by Target And Kmart |
| **2019** | 26-Nov | https://www.nationalgeographic.com.au/australia/no-silence-of-the-brands-on-animal-cruelty.aspx Fashion retailers are jumping onboard new animal welfare policies for their fashion lines as brands move to strengthen animal welfare standards or ditch cruel product lines. | No Silence of The Brands On Animal Cruelty |

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|---|---|---|---|
| **2019** | Dec-20 | https://www.miragenews.com/farmers-in-victoria-welcome-new-animal-welfare-regulations/<br>Animal welfare regulations imposing on the spot fines for mulesing sheep without pain relief have been introduced into Victoria | Farmers in Victoria welcome new animal welfare regulations |
| **2019** | 9-Dec | https://www.farmweekly.com.au/story/6526949/lobby-groups-maintain-mulesing-pressure/<br>SHEEP welfare lobby groups supporting retailer moves to ban products made with wool from mulesed Merinos are maintaining pressure on the wool industry to adopt a breeding alternative to any form of mulesing | Lobby groups maintain mulesing pressure |
| **2020** | 18-Jan | https://www.abc.net.au/news/2020-01-18/wool-producers-under-pressure-to-stop-mulesing-fashion-industry/11871706<br>A growing push to stop Australian sheep producers cutting skin off their animals' bottoms, a practice known as "mulesing", is exposing the tension between the farm and the fashion industry | No sheep bottoms were harmed in the making of this fuzzy jumper, but some producers aren't happy |
| **2020** | 22-Jan | https://www.sheepcentral.com/proposed-wool-declaration-changes-generate-mixed-reaction/<br>MORE opposition and some support for proposed changes to mulesing status definitions on Australia's National Wool Declaration has emerged as an industry consultation period continues | Proposed wool declaration changes generate mixed reaction |
| **2020** | Jan-29 | https://www.greenbiz.com/article/how-companies-can-source-wool-more-sustainably<br>In conjunction with the release of the Textile Exchange 2019 Material Change Index, GreenBiz has partnered with Textile Exchange to publish actionable insights for apparel and textile companies looking to source raw materials more sustainably. The entire series may be found here. | How companies can source wool more sustainably |
| **2020** | 19-Feb | https://www.sheepcentral.com/mulesing-alternative-category-proposed-for-wool-declaration/<br>Industry and market opposition to categorising mulesing alternatives as 'non-mulesed' on the National Wool Declaration has let to further consultation on the issue. | Mulesing alternative category proposed for wool declaration |

| | | | |
|---|---|---|---|
| **2020** | 30-Mar | https://www.sheepcentral.com/anger-and-relief-as-awex-keeps-non-mulesed-definition-on-nwd/<br>A DECISION to maintain the current interpretation of non-mulesed wool on Australia's National Wool Declaration has been welcomed by the nation's peak grower body, but condemned by some breeders and animal welfare interests. | Anger and relief as AWEX keeps non-mulesed definition on NWD |
| **2020** | 20-Apr | https://www.sheepcentral.com/awi-leader-garnseys-view-on-non-mulesed-wool-demand-rejected/<br>AUSTRALIAN wool exporters and a leading global sheep welfare standard have rejected statements that non-mulesed wool demand was driven by a few European fashion houses and not garment customers. | AWI leader Garnsey's view on non-mulesed wool demand rejected |
| **2020** | 21-Apr | https://www.sheepcentral.com/australian-wools-lost-transparency-opportunity-in-recent-nwd-review/<br>AUSTRALIA'S wool industry has lost an opportunity to promote unequivocal transparency by allowing wool from freeze-branded sheep to be described as 'non-mulesed', according to Italian processors Reda and Vitale Barberis Canonico. | Australian wool's 'lost transparency opportunity' |
| **2020** | 27-Apr | https://www.sheepcentral.com/nsw-wool-grower-urges-awi-to-promote-non-mulesed-production/<br>In this opinion piece, Mr Gordon puts his case for Australian Wool Innovation to encourage Merino breeders to move to plain-bodied sheep to remove the need for mulesing and increase production of non-mulesed wool. | NSW wool grower urges AWI to promote non-mulesed production |
| **2020** | 17-Jun | https://www.countrynews.com.au/livestock/2020/06/18/1239111/mandatory-pain-relief-rules-for-sheep-mulesing-start-on-july-1<br>The VFF is reminding all sheep farmers that mandatory pain relief regulations for sheep mulesing comes into effect from July 1. | Mandatory pain relief rules for sheep mulesing start on July 1 |
| **2020** | 19-Jun | https://www.sheepcentral.com/mulesing-management-webinar-to-discuss-new-victorian-rules/<br>VICTORIAN sheep producers preparing for the introduction of new mulesing pain relief regulations next month can attend a free webinar on management practices next week. | Mulesing management webinar to discuss new Victorian rules |

| | | | |
|---|---|---|---|
| **2020** | 30-Jun | https://www.abc.net.au/news/rural/2020-06-30/cryogenic-sheep-breech-freezing-as-alternative-to-mulesing/12404082<br>One of Australia's largest brokers and exporters of wool says the industry is "shooting itself in the foot" by not allowing farmers that use cryogenic breech freezing to report it on the National Wool Declaration (NWD). | National Wool Declaration needs to indicate cryogenic alternative to mulesing, broker says |
| **2020** | 1-Jul | https://www.countrynews.com.au/livestock/2020/07/01/1316170/pain-relief-is-now-law-when-mulesing-sheep<br>Victorian producers are now required by law to administer a registered pain-relieving product when mulesing sheep. | Pain relief is now law when mulesing sheep |
| **2020** | 16-Jul | https://www.ecotextile.com/2020071626373/labels-legislation-news/mulesing-legislation-hangs-in-the-balance.html<br>NEW SOUTH WALES – Woolgrowers have been urged to have their say on proposed amendments to the POCTA (Prevention of Cruelty to Animals) Act in New South Wales, as the state’s Legislative Council looks to fast-track new laws that could see pain relief become a mandatory requirement of farmers later this year, with the view to banning mulesing outright by 2023. | Mulesing legislation hangs in the balance |
| **2020** | 30-Jul | https://www.abc.net.au/news/2020-07-30/mulesing-ban-attempt-by-nsw-animal-justice-party/12503440<br>The practice of mulesing sheep could be banned by January 1 next year if the Animal Justice Party gets its desired outcome from a New South Wales Upper House inquiry next month. | Mulesing ban attempt by NSW Animal Justice Party reignites debate and pressure on wool industry |
| **2020** | 4-Aug | https://www.ecotextile.com/2020080426477/materials-production-news/move-from-mulesing-proves-profitable-report-says.html<br>CANBERRA – A new report makes a compelling case for Australia’s wool growing industry to ditch mulesing in favour of breeding flystrike-resistant sheep, having surveyed 97 native producers who’ve already made the switch and found it to yield greater profits, whilst increasing lamb growth rates. | Move from mulesing proves profitable, report says |

| | | | |
|---|---|---|---|
| **2020** | 12-Aug | https://www.sheepcentral.com/nsw-wool-producers-reject-proposed-mulesing-ban-in-2022/<br>EW South Wale's wool industry was not ready or willing for mulesing to be banned within 18 months, a NSW parliamentary inquiry heard yesterday. | NSW wool producers reject proposed mulesing ban in 2022 |