finalreport

FEEDLOTS

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Integrated management of nuisance fly populations on cattle feedlots

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Abstract

Nuisance flies are recognised as a problem on Australian feedlots despite improvements in manure management. The impacts of various management tools on populations of flies breeding in feedlots were determined. Feedlot sanitation and two new biological fly control agents, parasitic wasps and fungal biopesticides, were shown to be important components in fly control. Research and development of parasitic wasps and fungal biopesticides in collaboration with commercial companies advanced these tools to commercial production and pre-registration stages respectively. A strategy for feedlot nuisance fly control based on integrated pest management (IPM) principles, including the use of biological agents, has been devised and formulated for industry use. Implementation of the IPM strategy and further exploration of biological agents will provide the industry with effective, sustainable and economic fly control.

Executive Summary

The feedlot industry has applied a significant amount of effort to improved manure management practices over the past decade as a means of reducing odour emissions and fly problems. There is, however, evidence that fly populations remain a serious problem. Insecticide resistance and a desire to minimise the use of chemicals also drive the need to move to a more integrated approach to fly control.

This project embraced many aspects of research and development with the aim of providing improved control of nuisance flies in cattle feedlots, including the development of new tools, their implementation in feedlot fly control and assessments of the efficacy of new and old tools. This was achieved by laboratory work, bioassays under controlled conditions and applications in commercial feedlots. Feedlots in two areas in south-east Queensland, the Brisbane Valley and the Warwick shire, were used for field work. The major nuisance flies breeding in feedlots are the house fly and the stable fly.

We demonstrated that frequent cleaning of fence lines can effectively reduce fly breeding. Compared with a 3-monthly cleaning interval, monthly, fortnightly and weekly cleaning of fence lines reduces the numbers of fly pupae by 55%, 67% and 84% respectively. These substantial reductions in fly breeding can be achieved through removal of manure accumulated under the fence without the need for a simultaneous cleaning of the whole pen where fly breeding is minimal.

The application of the larvicide cyromazine under fence lines provided a measurable reduction in immature and adult flies only when fence lines had been recently cleaned. The cyromazine treatment did not reduce the rates of wasp parasitism. Spraying of an adulticide (cyfluthrin) on feedlot structures had a small and short-lived effect on stable fly populations but no effect on house flies.

One of the most common parasitic wasps on Australian feedlots, *Spalangia endius,* was selected for mass production and subsequent augmentative releases to improve control of fly populations in cattle feedlots. This wasp is one of several species used in the USA for the same purpose. During this project a laboratory colony of *S. endius* wasps was established and subsequently transferred to the commercial partner Bugs for Bugs. The wasps were extensively characterised, rearing techniques assessed and a quality assurance program for mass reared wasps instigated.

In the initial field trial (2005/06) individual fence line segments where wasps were released were compared with similar segments where no releases were made. Wasp emergence from pupae collected along these segments was the same in release and control segments but fewer flies emerged from the segments where wasps were released. There was also a change in the trend of *S. endius* populations in the segments where the wasps were released indicating an increase due to the released wasps.

In several feedlot trials, there was generally an increase in the parasitism rate in the feedlot with wasp releases compared to the control feedlots. The percentage of *S. endius* in the wasp population was generally higher in the feedlots with wasp releases. These trials demonstrated that mass-reared wasps parasitise fly pupae in feedlots and thus contribute to fly control.

As a result of this project, *S. endius* wasps are now available from the Australian company Bugs for Bugs in Mundubbera as a new biological tool for fly control. The augmentative releases of parasitic wasps in feedlots should be commenced before fly populations increase and continued throughout the expected fly season at recommended rates.

Fungal biopesticides are a novel biological tool with potential for use in nuisance fly control. We have demonstrated that entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* selectively infect and kill flies. The efficacy of many *Metarhizium* and *Beauveria* isolates against adult house flies was high (typically 80-100% mortality) and those providing high spore yields in culture were selected for further investigations. A spore production facility was set up at the DPI&F Yeerongpilly laboratory to produce up to 2kg of spores for testing. Collaboration with Becker Underwood, the only commercial producer of fungal biopesticides in Australia, was initiated during the project and they produced the spores used for feedlot trials.

A range of investigations of selected isolates was conducted using bioassays with adult and immature house flies, including spore uptake from food or sprayed surfaces, efficacy of spray and bait formulations, spore levels required to kill flies and combinations of fungal species. These investigations assisted in the selection of fungal isolates and formulations for feedlot trials.

The impact of formulated spores on nuisance flies was determined in feedlot trials. The mortality of and *Metarhizium* isolations from flies netted after spraying were much higher than in flies netted in control feedlots. This confirmed that flies contacting the freshly sprayed formulation were taking up lethal doses of spores. The increased fly mortality and presence of *Metarhizium* infections were still evident, though at lower levels, in flies netted one week after spraying. This showed that fungal applications can remain effective for at least seven days post spraying.

These findings verify the potential for a fungal biopesticide as an effective and safe tool for fly control. For this purpose, a fungal biopesticide will need to be registered as an agricultural product with the Australian Pesticides and Veterinary Medicines Authority (APVMA) for which additional efficacy and safety data are required. It is recommended that commercialisation of a fungal biopesticide fly control using DPI&F spores be investigated by DPI&F, MLA and Becker Underwood.

During the 2007/08 fly season two comparisons between integrated fly control (IPM) and normal fly control programs were conducted in the Brisbane Valley and Warwick shire. The IPM program included frequent cleaning of fence lines, the release of parasitic wasps, spraying of fungal biopesticides and focused use of insecticidal fly baits. Adult and immature fly populations were monitored in all feedlots. The IPM program achieved reductions of 36% and 40% in adult house fly and stable fly populations respectively, compared to the control feedlots. Increases in wasp parasitism rates and fungal infections and mortality were also observed in the IPM feedlots.

An integrated fly management package for nuisance flies in cattle feedlots has been provided, incorporating knowledge of the major fly pests and their natural enemies, elements producing fluctuations in fly populations, the effects of flies on feedlot operations and results from this project. IPM fly control includes components such as feedlot design, manure management, biological control, fly population monitoring and selective use of insecticides. Details of these components are provided in the report and in MLA tips & tools brochures.

Recommendations from the project include the adoption of an IPM strategy for fly control in feedlots, further development of fungal biopesticides to a commercial fly control product and the expansion of parasitic wasps and fungal biopesticides to other industries with similar fly problems.

Contents

1 Background

Nuisance flies are an on-going problem for intensive animal holdings. Uncontrolled fly populations may lead to reduced production from flies 'worrying' the animals as well as complaints from neighbours. Flies are also potential carriers of diseases and at high density annoying to staff. Common means for controlling nuisance flies on intensive animal holdings include insecticidal sprays and/or baits. Repeated use of insecticides can lead to unwanted residues in produce and the environment and to the development of resistance in flies. Integrated pest management (IPM) can provide control of nuisance flies in intensive animal holdings while eliminating or minimising the above problems.

The cattle feedlot industry has applied a significant amount of attention to improved manure management practices over the past decade as a means of reducing odour emissions and fly problems. An investigation of nuisance fly and parasitoid populations on cattle feedlots has been carried out (DPI&F/MLA Project FLOT.306). This investigation provided a comprehensive overview of feedlot fly and parasitoid species, temporal and spatial distribution of these species, fly breeding sites and the impact of flies on cattle behaviour. The study demonstrated that natural populations of control agents, e.g. parasitic wasps, mites and entomopathogenic fungi, limit fly numbers in the feedlot. Low to moderate levels of resistance towards common insecticidal sprays and baits were detected in house flies collected from feedlots.

In the current feedlot nuisance fly project (B.FLT.0326) we demonstrated and quantified the benefits obtained through implementing the recommendations from the previous study, by:

- Quantitatively assessing the reductions which were achieved in fly breeding and fly populations through changes to management practices such as more targeted cleaning and chemical applications.
- Assessing the impact of releasing parasitic wasps in the feedlot on fly breeding and fly populations. These wasps, which parasitise and kill fly pupae, were identified as important, existing natural enemies of feedlot flies in the previous study. Such augmentative releases of parasitic wasps are proving successful in intensive livestock industries in the USA.
- Investigating the use of fungal biopesticides for fly control in cattle feedlots. It had previously been demonstrated that fungi isolated from feedlot flies kill the larvae and adults of house flies.

Targeted feedlot cleaning and biological agents are ideal tools for a fly IPM system, as they will not compromise existing natural ecosystems and enable feedlot operators to minimise their nuisance fly populations with economically and ecologically sustainable methods.

This final report contains:

- A detailed update on project work carried out since the previous milestone report (No 7, July 2007), including the development of fungal biopesticides and production of parasitic wasps
- An assessment of the effect of an integrated fly control program on fly populations in cattle feedlots
- Recommendations for an integrated fly control program for cattle feedlots
- An outline of the success in achieving objectives and the impact on meat and livestock industry
- Conclusions and recommendations arising from the project work

2 Project Objectives

By 30 November 2008:

1. Demonstrate and quantify the effect of the following measures on feedlot fly breeding activities and/or populations:
    a. Targeted chemical application and cleaning measures, with particular focus on cattle pen fence lines and sedimentation ponds
    b. Augmentative releases of cultured parasitic wasps
    c. Fungal biopesticides
    d. Integrated nuisance fly control

2. Develop IPM system recommendations, based on the outcomes of the research, which can be utilised by feedlot operators to manage their nuisance fly populations.

3 Methodology

3.1 Fungal biopesticides

3.1.1 Flies

House flies used in these tests were from the ARI laboratory colony and during testing they were maintained under standard conditions (27°C, 65% RH, and 12:12 L:D). Three to five day old flies in mixed-sex groups were used. Tests were carried out with either groups of 20 flies in small round plastic containers with gauze lids (90 mm diameter x 90 mm high) or 50 flies in wire mesh fly cages with two clear Perspex sides (30 × 30× 45 cm). Water was supplied via a container with either a sponge or cotton wick.

3.1.2 Spray formulation development

A range of aqueous formulations with different carriers and different concentrations of molasses and fungal spores were tested against flies in small containers and in fly cages.

Small fly container experiments
Fungal spores formulated in emulsifiable vegetable oil or glycerol, with different concentrations of molasses (Table 1), were investigated in small fly containers. The flies were exposed to the formulations as a food source and to formulations applied to the surface of the containers as follows:
a) Food source formulations (3 ml) were applied to a 3 × 3 cm piece of sponge which was placed on the bottom of each fly container. Twenty flies were added to each container with 3 replicates per treatment. A control treatment in which flies were given 3 g of sugar was included. Fly mortality was assessed daily from day 4 until day 7. The experiment was carried out twice.
b) Filter papers (90 mm diameter) were dipped into formulations, dried and then placed on the bottom of each fly container. This experiment was conducted as above except that in each treatment flies were also given 3 g of sugar. The experiment was carried out twice and the data were pooled.

Fungal isolations were carried out on dead flies recovered from the treatments. Flies were surface sterilised with 70% ethanol, rinsed in sterile water, blotted on sterile filter paper and plated on water agar amended with 0.05% Chloramphenicol and then incubated at 27°C without light. Flies were examined for fungal growth at 4 and 7 days.

Table 1: Composition of spore formulations for small container experiments

Treatment	Fungal Spores (g)	Glycerol (ml)	Oil (ml)	Molasses (ml)	Total (ml)
Sp +G+0.1M	1	10	-	10	100
Sp +G+0.2M	1	10	-	20	100
Sp+O+0.1M	1	-	5	10	100
Sp+O+0.2M	1	-	5	20	100
G+0.2M	-	10	-	20	100
0.2M	-	-	-	20	100

Sp = Spores; G = Glycerol; M = Molasses; O = Oil; made up to total volume with water

Fly cage experiments

A series of four experiments were carried out with flies in fly cages to further evaluate the effect of different formulations on fly mortality resulting from indirect spore uptake from a treated surface. Test formulations were sprayed onto cardboard (30 × 45 cm) that was dried and then inserted into the bottom of the cages. All fly cages were supplied with 3 g sugar in a small container. Fly mortality was assessed daily from day 3 until day 7 or until some treatment mortalities had reached at least 80%. Controls in each experiment had clean cardboard inserted into the bottom of the cages. Fly cages were kept at 27°C, except in the first experiment where the temperature over the first seven days was about 23°C.

Experiment 1 investigated the difference between sugar or molasses in the formulation. Experiment 2 compared oil and glycerol as well as two different levels of molasses in the formulation. Experiment 3 compared different levels of spores in the formulation, with spore concentrations of approximately 1×10^8 spores/ml; 2×10^8 spores/ml; 3×10^8 spores/ml and 4×10^8 spores/ml. Experiment 4 compared different carbohydrate sources in the formulation. The different formulations are given in Table 2. Experiment one used five replicate cages of flies for each treatment and three replicate cages of flies were used for each treatment in the other experiments.

Table 2: Composition of spore formulations for fly cage experiments

Treatment	Spores (g)	Oil (ml)	Glycerol (ml)	Molasses (ml)	Sugar (g)	Total (ml)
EXPERIMENT 1						
Molasses	1.5	7.5		30		150
Sugar	1.5	7.5			30	150
EXPERIMENT 2						
Oil + Molasses I	1.0	10		30		200
Oil + Molasses II	1.0	10		60		200
Glycerol + Molasses I	1.0		10	30		200
Glycerol + Molasses II	1.0		10	60		200
EXPERIMENT 3						
Spore level I	0.5	10		30		200
Spore level II	1.0	10		30		200
Spore level III	1.5	10		30		200
Spore level IV	2.0	10		30		200
EXPERIMENT 4						
Molasses	1.0	10		30		200
Sugar	1.0	10			30	200
Molasses & Sugar	1.0	10		20	20	200

Made up to total volume with water

Data were prepared for analysis using Abbot's formula to give effective number treated (ENT) and corrected mortality (CM) for each treatment (Abbott 1925). A t-test was performed on the mortality data in the first fly cage experiment. In the other small container and cage experiments the corrected mortality values at day 7 (days 8 and 11 for second cage experiment) were subjected to ANOVA using the replicate cages as the experimental units. As the corrected mortality is a percentage measure, the arc-sine transformation was considered, but was not required given the observed ranges of the data.

3.1.3 Bait formulation development

A number of potential bait formulations were evaluated. Formulations were based on a paste of sugar and molasses mixed with spores. The addition of either glycerol or peanut oil and milk powder was assessed. The amounts of the formulation components are shown in Table 3. The formulations (approx. 5 ml) were applied to plastic lids (3 cm diameter × 1 cm depth) which were placed in the centre of the floor in the test cages. Flies were also supplied with sugar and water. Fly mortality was assessed after 7 days. The experiment was carried out three times. The data from these three experiments were pooled. After the last bait experiment, samples of the bait formulations were streaked across selective agar media as a qualitative test of spore viability.

Table 3: Composition of bait formulations

	Formulation	Fungal Spores 3 g	Glycerol 7 ml	Peanut Oil 7 ml	Raw Sugar 18 g	Molasses 10 ml	Milk powder 9 g
1.	S				✓		
2.	S+M				✓	✓	
3.	S+G+M		✓		✓	✓	
4.	S+G+M+Sp	✓	✓		✓	✓	
5.	S+G+M+Sp+MP	✓	✓		✓	✓	✓
6.	S+G+M+MP		✓		✓	✓	✓
7.	S+O+M			✓	✓	✓	
8.	S+O+M+Sp	✓		✓	✓	✓	
9.	S+O+M+Sp+MP	✓		✓	✓	✓	✓
10.	S+O+M+MP			✓	✓	✓	✓

S = Sugar; M = Molasses; G = Glycerol; Sp = Spores; MP = Milk Powder; O = Oil

3.1.4 Viability of spore formulation on vegetation

The viability of the spore formulation applied to vegetation in full sun was evaluated. Bougainvillea plants at ARI were sprayed with the same formulation used in feedlots (see section 3.3.6). The formulation was applied to the leaves until run off. Vegetation sprayed in the morning was sampled six hours later and then weekly for up to 3 weeks. The leaf samples (branches approximately 20 cm long with 15 – 19 leaves) were cut from treated plants and placed on the floor of cages containing flies that had been carbohydrate (sugar) starved for 6 hours. A small container of sugar was also placed in each cage when the leaves were added. Leaves were left in the cages overnight for approximately 17 hours, removed the next morning and fly mortality was assessed 7 days later. Moist sponges were provided in all cages. Some of the sampled leaves were pressed onto selective agar to assess the spore viability. The experiment was replicated four times.

3.2 Parasitic wasps

3.2.1 Use of frozen and heat killed *M. domestica* pupae as hosts for *S. endius*

Two day-old *M. domestica* pupae were placed at 55^{o}C 64% RH for 30 minutes or -80^{o}C for 10 inutes. For both frozen and heat killed pupae, 2 lots of 100 pupae were exposed to female *S. endius* (10 wasps / 100 pupae) for 24 hours and then held at 27^{o}C/ 70 % RH for 4 weeks before checking for parasitoid emergence. The remaining pupae were then sealed in plastic bags and stored at 4^{o}C. At 2, 4, 6 and 8 weeks, 2 lots of 100 pupae are removed from the bag and exposed to female *S. endius*. With each assay 2 lots of 100 fresh *M. domestica* pupae with *S. endius* are used as the control, plus 1 lot of 100 fresh pupae to check fly emergence. Wasps used were newly emerged female wasps (0-24hrs old) from the ARI colony, selected under CO_2 and held in a container for 24hrs prior to exposure to the pupae, and given honey as a food source. This gave a wasp age of 24-48 hrs at time of testing.

3.2.2 Use of *M. domestica* pupae frozen for 2 years as hosts for *S. endius*

Batches of 100 *M. domestica* pupae at 0-24, 24-48, 48-72 and 72-96 h old were either vacuum-sealed in plastic bags or placed in 20ml vials and subsequently stored at -20^{o}C for 2 years (2 batches for each treatment). After 2 years the pupae were removed from the freezer, thawed for 30 minutes, and the condition of the pupae was observed before exposing to *S. endius*. The pupae were exposed to female *S. endius* (10 wasps / 100 pupae) for 24 hours and then held at 27^{o}C 70% RH for 4 weeks before checking parasitoid emergence. With each assay 2 batches of 100 fresh *M. domestica* pupae, 24-48 hours old, were exposed to 10 female *S. endius* as the control and one batch of 100 fresh pupae was used to check fly emergence. Wasps used were newly emerged female wasps (0-24hrs old) from the ARI colony, selected under CO_2 and held in

a container for 24 hrs prior to exposure to the pupae, and given honey as a food source. This gave a wasp age of 24-48 hours at time of testing.

3.2.3 Effect of cold storage on emergence of *S. endius*

S. endius parasitised *M. domestica* pupae as supplied by Bugs for Bugs for field release trials were used. Batches of 150-300 pupae 11 to 18 days post-parasitism were placed in 20 ml vials and stored at 10^{o}C. At 0, 4, 8 and 12 weeks, 1 batch of pupae was removed from the 10^{o}C incubator and stored at 27^{o}C 70 % RH for a further 3 weeks before checking for parasitoid emergence.

3.2.4 Large scale production of *S. endius*

A report on the production of *S. endius* is provided as Appendix 1.

3.2.5 Quality assessment of mass-reared wasps

Aliquots of about 100 parasitised pupae from each daily batch were placed into plastic tubes with a screened lid immediately after the pupae had been exposed to wasps. The tubes were kept at 27°C for 7-10 days when the emerged flies were removed and counted. All intact pupae were individually placed into gelatine capsules and stored at 31°C to day 28 (since being parasitised) when the numbers of emerged wasps were counted.

Wasps emerged from the quality assessment samples were inspected under a stereomicroscope to confirm their species. Contaminating species detected included: *Pachycrepoideus vindemmiae*, *Spalangia gemina*, *Nasonia vitripennis* and *Muscidifurax raptor.*

A one-off collection of pupae in- and outside the larval rearing facility at the Bugs for Bugs plant in Mundubbera was carried out in November 2007. These pupae were escapees from the larval production system and the outside pupae had been washed out of the container during the previous weekly cleaning process.

3.3 Integrated fly control on feedlots

3.3.1 Feedlots

All experiments were carried out on two feedlots in the Brisbane Valley (coded feedlot A and C) and two feedlots in the Warwick shire (coded feedlot D and F) in south-eastern Queensland. The carrying capacities of feedlots A-F were 3100, 1000, 1000 and 2000 standard cattle units (SCU) respectively. The Brisbane Valley feedlots and feedlot D primarily supply cattle for the domestic market whereas feedlot F produces cattle for domestic and export markets.

3.3.2 Weather data

Weather data for the trial period and long-term (50 years) data were obtained from the Department of Natural Resources and Water SILO web site, based on the coordinates near the feedlots in the two areas.

3.3.3 Adult fly populations

Four Alsynite traps (Alsynite cylinder covered by a clear sticky sheet) were used in each feedlot to monitor adult fly populations. Sticky sheets were changed weekly (except the last collection in April 2008 which was fortnightly) and flies identified and counted. Fly catches are reported as average weekly trap catches when populations are reported across seasons. The comparison between IPM and control feedlot fly populations was based on an ANOVA of transformed fly numbers (logx+1) and back-transformed values are presented.

Adult flies on the sticky sheets were inspected for the presence of Trombidiid and *Macrocheles* mites.

3.3.4 Immature fly populations

Weekly counts of larvae in feedlots were obtained from transects (about 250 mm wide) through manure under fence lines and “squares” (about 250 x 250 mm) in the sedimentation system. The estimates were obtained by digging up and exposing the manure in the designated area with a trowel. For each feedlot 10 estimates each were obtained for the fence lines and the sedimentation system (except feedlot A where 20 fence line estimates were obtained). The comparison between IPM and control feedlot fly populations was based on an ANOVA of transformed larvae estimates (logx+1) and back-transformed values are presented.

Three samples of approximately 100 pupae were collected fortnightly from identified fly breeding areas at all feedlots, to determine species, emergence and parasitism. Two of these samples were collected from fence lines and one sample from the sedimentation system. Pupae were extracted from the samples, individually encapsulated and stored at 27°C for at least 30 days. Emergence of flies or wasps from each pupa was recorded and species of pupae, flies and wasps were determined.

3.3.5 Parasitic wasp releases

Wasp releases were carried out in the two IPM feedlots A and D. Wasp release containers (PVC pipe 100 mm diameter, 250 mm long with two removable end caps, 12 holes diameter 25 mm evenly distributed along the pipe and covered with aluminium fly mesh to prevent access for flies, ants, birds and other predators to parasitised pupae) were attached with tie wire (or plastic tie) to the fence (400-600 mm above ground) and evenly distributed along fence lines across the feedlots. The release containers were charged weekly (except week between Christmas and New Year) with fresh parasitised pupae (to provide about 100 wasps/per animal) mixed with Vermiculite Grade 3 (ratio about 2:1). Prior to release the parasitised pupae were stored at either 10°C or 27°C to achieve wasp emergence in the days following placement in the feedlot.

From feedlot pupae collected as described in 3.3.4, fly emergence and wasp emergence from all pupal species were determined. The species of the emerged wasps were also identified and their abundance is reported as a percentage of collected pupae or total wasps. Emergence data and proportions of wasps were subjected to a generalised linear model analysis (McCullagh and Nelder, 1989) using GenStat (2008).

3.3.6 Fungal biopesticides

The following fungal formulation (40, 60 or 80 L) was sprayed onto targeted areas in feedlots A and D:

Fungal spores (isolate M16) - 0.75 % (wt/vol)
Emulsifiable vegetable oil - 5 % (vol/vol)
Molasses - 5 % (vol/vol)
Sugar 50:50 white:raw - 10 % (wt/vol)

The fungal spores, supplied as a dry powder, were produced by Becker Underwood Australia. In the laboratory the fungal spores were suspended in the vegetable oil and stored in a sealed container for transport to the field. The sugar and molasses were dissolved in approximately 20 L of water and stored in a sealed container for transport to the field. In the field the spores and sugar were mixed together in the spray tank with extra water to make up the final spray volume.

The spray unit consisted of a 100 L tank, a pump driven by a 12 volt battery operating at 25-30 psi (172-207 kPa) pressure and a long flexible hose with an adjustable spray nozzle. The

spray unit was mounted in the back of a utility vehicle. The formulation was sprayed in areas in each feedlot where large numbers of flies were noted to rest in vegetation and on the front of feed bunks. The spraying schedule is shown in Table 4.

Table 4: Treatment and sampling schedule in Feedlots A, C, D and F over the 2007-2008 fly season

Week	Fungal Treatment		Netting			
beginning	A	D	A	C	D	F
17/9/2007			✓	✓		
24/9/2007			✓	✓		
1/10/2007	60 l spray (2/10)		pre; post	✓	✓	✓
8/10/2007	60 l spray (9/10)	40 l spray (11/10)	pre; post	✓	✓	✓
15/10/2007	60 l spray (16/10)	40 l spray (18/10)			pre; post	✓
22/10/2007	80 l spray (23/10)	40 l spray (25/10)	pre; post	✓		
29/10/2007	60 l spray (30/10)	40 l spray (1/11)			pre; post	✓
5/11/2007	80 l spray (6/11)	60 l spray (8/11)	pre; post	✓		
12/11/2007		60 l spray (15/11)	✓	✓	pre; post	✓
19/11/2007					✓	✓
3/12/2007	60 l spray (4/12)	40 l Spray (6/12)	pre; post	✓	pre; post	✓
21/01/2008	60 l spray (22/1)		pre; post	✓		
28/01/2008	60 l spray (29/1)	40 l spray (31/1)	pre; post	✓	pre; post	✓
4/02/2008	Baits (5/2)	Baits (7/2)	✓	✓		
11/02/2008	Baits (12/2)	Baits (14/2)	✓	✓	✓	✓
18/02/2008	Baits (19/2)	Baits (21/2)	✓	✓	✓	✓
25/02/2008	Baits (26/2)		✓	✓	✓	✓
3/03/2008	60 l spray (4/3)		pre; post	✓	✓	✓
10/03/2008	Baits (11/3)		✓	✓	✓	✓
17/03/2008	60 l spray (18/3)		pre; post	✓	✓	✓
24/03/2008	Baits (25/3)		✓	✓		

Flies were sampled by netting to asses the effect of the fungal formulation in the treated feedlots. Samples were taken before (pre) and after (post) spraying in the treated feedlots and in the control feedlots for comparison. The netting schedule is given in Table 4. To avoid contamination, different nets were used for control, pre-spray and post-spray flies**.** In each feedlot flies were netted fortnightly or weekly (Table 4) in three defined locations, transferred to three separate cloth covered cages, supplied with water and sugar and transported to the laboratory. Care was taken not to expose the flies to direct sunlight or high heat during transport. In the laboratory 100 flies from each sample were transferred to new cages supplied with water and sugar and kept at 27°C and 65% humidity with light:dark 12:12 h for 7 days when fly mortality was assessed.

Dead flies from each cage were sampled and investigated for evidence of fungal infection. Flies were surface sterilised with 70% ethanol, washed in two changes of sterile water, blotted dry then plated on water agar amended with 0.05% chloramphenicol and incubated at 27°C without light. Flies were examined at 4 days and 7 days for the presence of fungi, specifically *Metarhizium*.

The data for average percent netted fly mortality and percent *Metarhizium* isolated from dead flies were subjected to a generalized linear model analysis (McCullagh and Nelder, 1989) using GenStat (2008), with a binomial distribution and logit link assumed.

Fly baits using a fungal paste were also trialled in Feedlots A and D later in the fly season during 2008. A stiff paste of fungal spores mixed with peanut oil, sugar and molasses was applied to hessian cloth covering a 10 x 20 cm piece of plastic corflute. The baits prepared in the laboratory

were sealed inside aluminium foil for transport to the feedlots where they were suspended inside large plastic containers (as described in fly bait section 3.3.7) to protect them from the weather. The baits were replaced fortnightly. A post-hoc t-test was performed on the data for fly mortality and *Metarhizium* infection during the time the baits were in the feedlots.

3.3.7 Insecticidal fly baits

Insecticidal fly baits were used in the IPM feedlots when trap catches indicated rapid fly population increases (see Table 14 for bait application schedule). Agita 10 or DyFly Plus granules were applied to both sides of Corflute sheets (180 x 100 mm) to which a thin layer of starch based glue had been applied. The granule layered sheets were suspended from a wire hook inside a used 10 or 20 litre plastic container with windows (approx. 150 x150 mm) cut into four sides. This system protected the baits from rain and prevented dead flies from covering the bait. Eight or nine bait stations were used in each feedlot.

4 Results and Discussion

4.1 Fungal biopesticides

Laboratory investigations to improve the spray formulation for application to feedlots during 2007-2008 aimed to make the formulation more attractive to flies, and lower the cost of the formulation.

The formulation sprayed in feedlot A during the 2006-2007 fly season consisted of fungal spores suspended in emulsifiable vegetable oil which was mixed with a sugar solution and diluted before spraying. There was evidence that when this formulation was sprayed onto the vegetation flies picked up lethal doses of spores.

It was proposed that the uptake of fungal spores from the vegetation could be increased by making the formulation more attractive to flies. Molasses has been reported to contain fly attracting components (Quinn *et al.* 2007) and is a cheaper source of carbohydrate than sugar which was used in the 2006-2007 fly formulation. In addition large numbers of flies were observed congregating around spilled molasses in Feedlot A. Different levels of molasses as the sole carbohydrate source and in combination with sugar were tested in the laboratory to develop a suitable formulation for spraying in feedlots.

Glycerol has been reported as an agent for fungal spore suspension and as a cryo-protectant for fungal spores (Burges 1998). Glycerol is also cheaper than the emulsifiable vegetable oil used in the spray formulation. Laboratory investigations compared the fly killing efficacy of formulations with spores suspended in either glycerol or vegetable oil. The most expensive component of a fungal biopesticide is usually the fungal spores, so finding the lowest effective spore dose is critical for developing a commercially acceptable formulation. Laboratory investigations also compared different concentrations of spores in the formulation.

Formulations with glycerol caused a similar fly mortality to those with emulsifiable vegetable oil. However investigations with glycerol were discontinued when there was not time to properly evaluate the effect of glycerol on the viability of fungal spores under field conditions. A new formulation was chosen with a lower spore dose and sugar concentration than that used in the 2006-2007 fly season but with the addition of molasses. The new formulation was sprayed onto vegetation in the treatment feedlots in the Brisbane Valley and Warwick shire weekly for six weeks and then as required in response to high fly numbers. There was clear evidence that lethal doses of the fungal spores were being taken up by flies in both treated feedlots. Later in the fly season a prototype fungal bait, similar in function to insecticidal baits, was trialled in the feedlots. The spray formulation was adapted for the fungal baits. The effect of these baits on flies in the feedlots was difficult to assess on the limited data gathered. An investigation of the

effective residual period of the formulation when sprayed onto vegetation found that the spores were still able to kill flies after two weeks and could remain viable for up to three weeks.

4.1.1 Spray formulation development

A number of laboratory investigations were carried out to test the effect of different formulations on the capacity to kill flies, primarily through indirect uptake of fungal spores. Initial experiments were carried out in small containers and subsequently larger fly cages were used for later experiments.

Small fly container experiments
All formulations containing fungal spores caused high fly mortalities in the small container experiments whether the spores were taken up from the food source (Fig. 1) or contaminated walls (Fig. 2). Overall there was no significant difference (P>0.05) in the fly mortality caused by spores formulated in glycerol or oil. Modifying the concentration of molasses in the formulation gave variable results and there was no conclusive evidence that higher concentrations increased fly mortality.

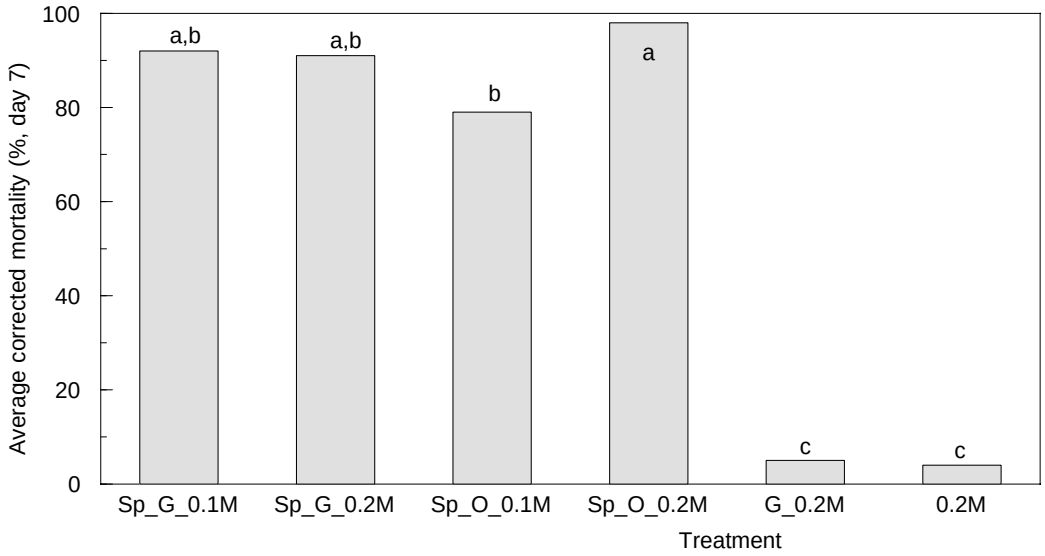

Figure 1: Average percent fly mortality after 7 days exposure to fungal spores in different food source formulations (Sp = Spores; G = Glycerol; M = Molasses; O = Oil; values with different letters are significantly different P<0.05)

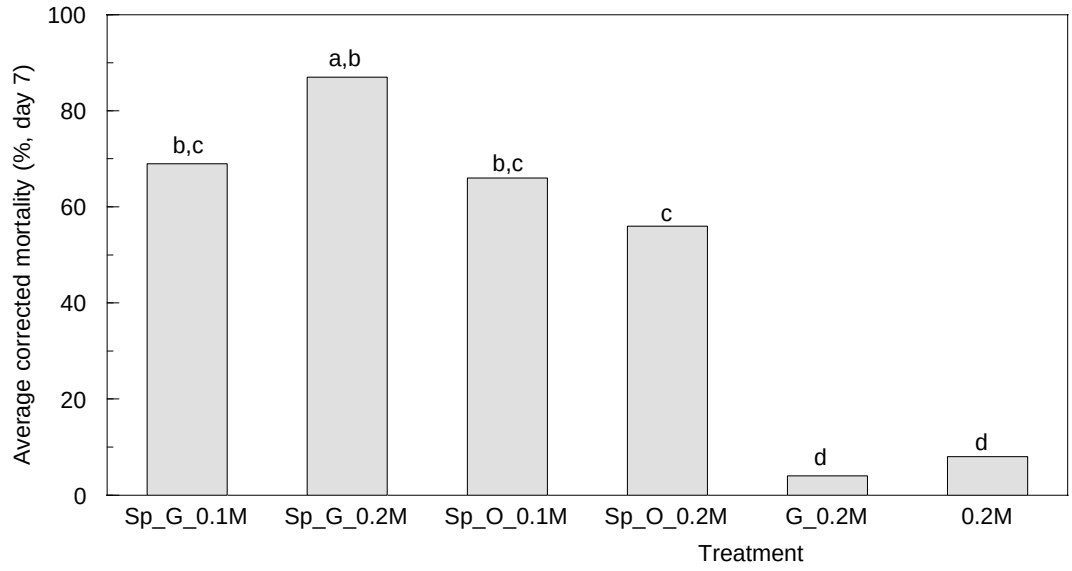

Figure 2: Average percent fly mortality after 7 days exposure to fungal spores in different formulations applied to a surface (Sp = Spores; G = Glycerol; M = Molasses; O = Oil; values with different letters are significantly different P<0.05)

Isolations carried out on dead flies confirmed that most flies in the spore treatments had taken up spores and died from *Metarhizium* infections (Table 5). However there were some anomalies with a low level of infection in flies exposed directly to the formulation with spores in oil with the lower molasses level. In addition the level of *Metarhizium* infection in flies indirectly exposed to only molasses or glycerol and molasses was unexpected.

Table 5: Average percent Metarhizium isolated form dead flies in the small fly container experiments

Treatment	Average % *Metarhizium* isolated	
	Food source	Surface
Sp+G+0.1M	100	80
Sp+G+0.2M	80	100
Sp+O+0.1M	20	100
Sp+O+0.2M	100	100
G+0.2M	0	20
0.2M	0	40
Control	0	0

Sp = Spores; G = Glycerol; M = Molasses; O = Oil

Fly cage experiments

In the first cage experiment with indirect uptake of spores, the formulation with sugar caused a significantly higher (P<0.05) fly mortality (86 ±5 %) after 12 days than the formulation with molasses (68 ±3 %). The cool ambient temperatures during the first 7 days were well below the optimal temperature for fungal growth. Once this was realised heaters were used to increase the temperature. The fungal formulation then had a marked effect on the flies for the five days until the experiment finished.

In the second cage experiment there was no significant difference (P>0.05) between any of the treatments to day 8 (Fig. 3). The spore treatments caused between 60.1 % and 72.3% fly mortality. However by day 11 the glycerol formulation with the higher molasses level caused a significantly lower (P= 0.002) fly mortality than all other treatments.

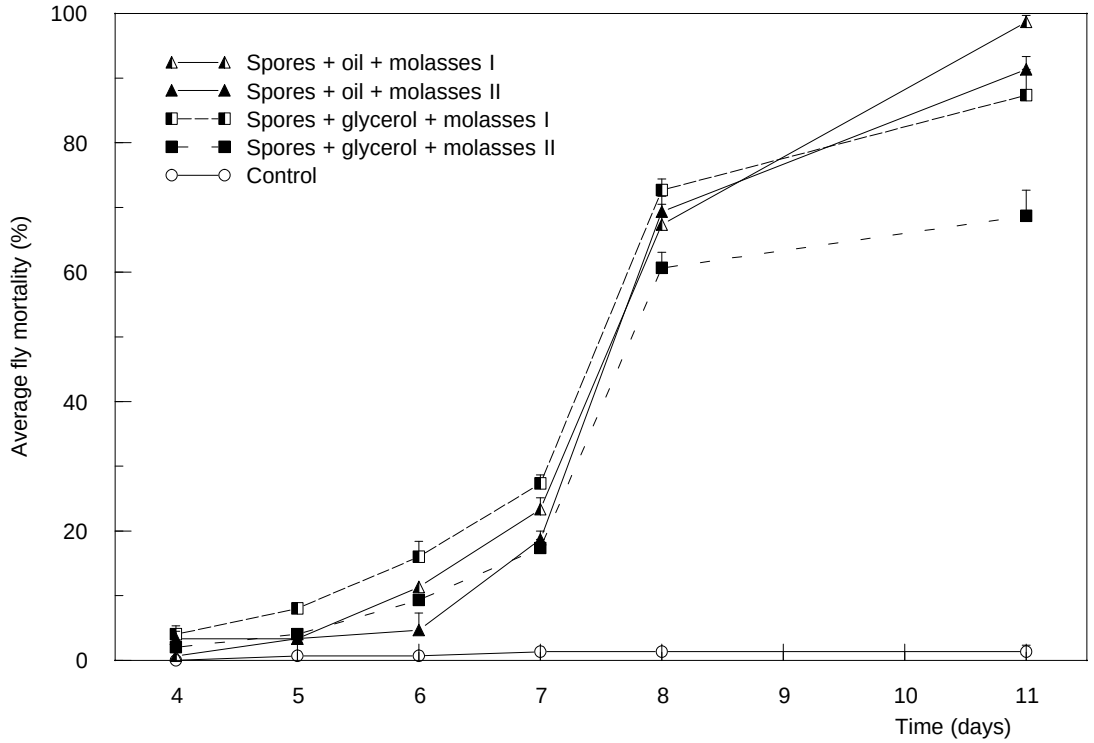

Figure 3: Average (+se) percent mortality in flies indirectly exposed to formulations with spores in either oil or glycerol and one of two levels of molasses

In the third cage experiment (Fig. 4) there was no significant difference (P = 0.112) between the formulations with different levels of spores. However the lowest spore level (~1 × 10^8 spores/ml) appeared to be slower in killing flies prior to day 7.

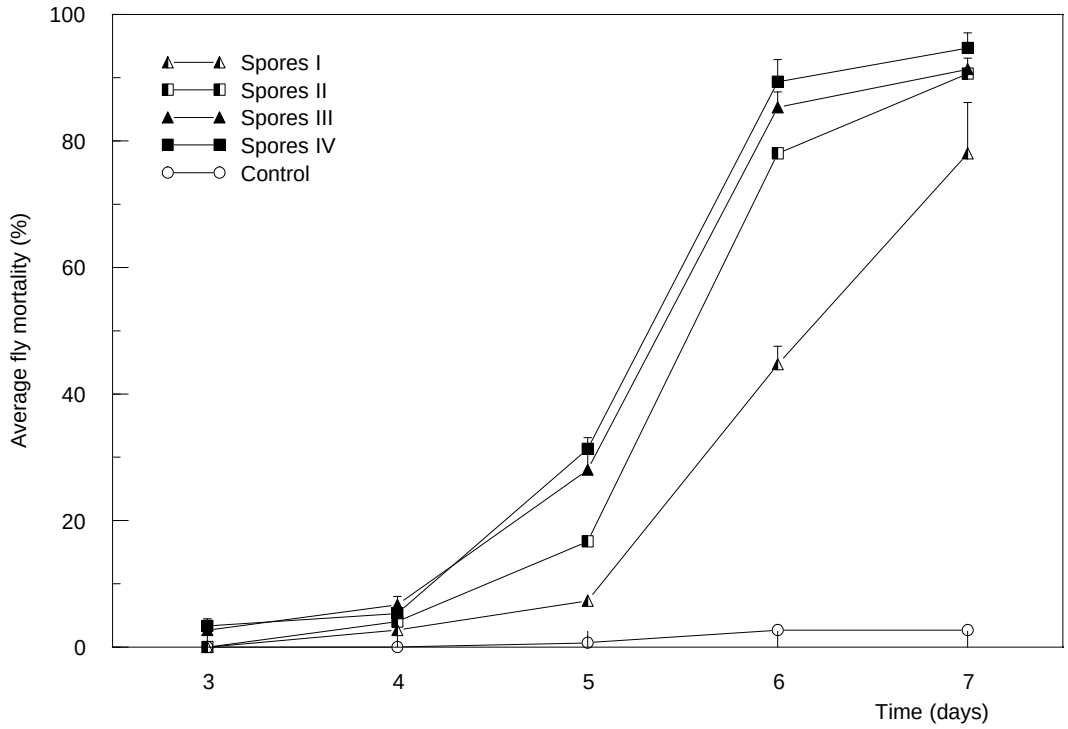

Figure 4: Average (+se) percent mortality in flies indirectly exposed to formulations with different levels of fungal spores

In the last cage experiment (Fig. 5) there was no significant difference (P>0.05) at day 7 between sugar (77±6%) and molasses (81±3%) in the formulation, although the combination of sugar and molasses resulted in a significantly lower fly mortality (42±2%). However by day 11 there was little difference between treatments.

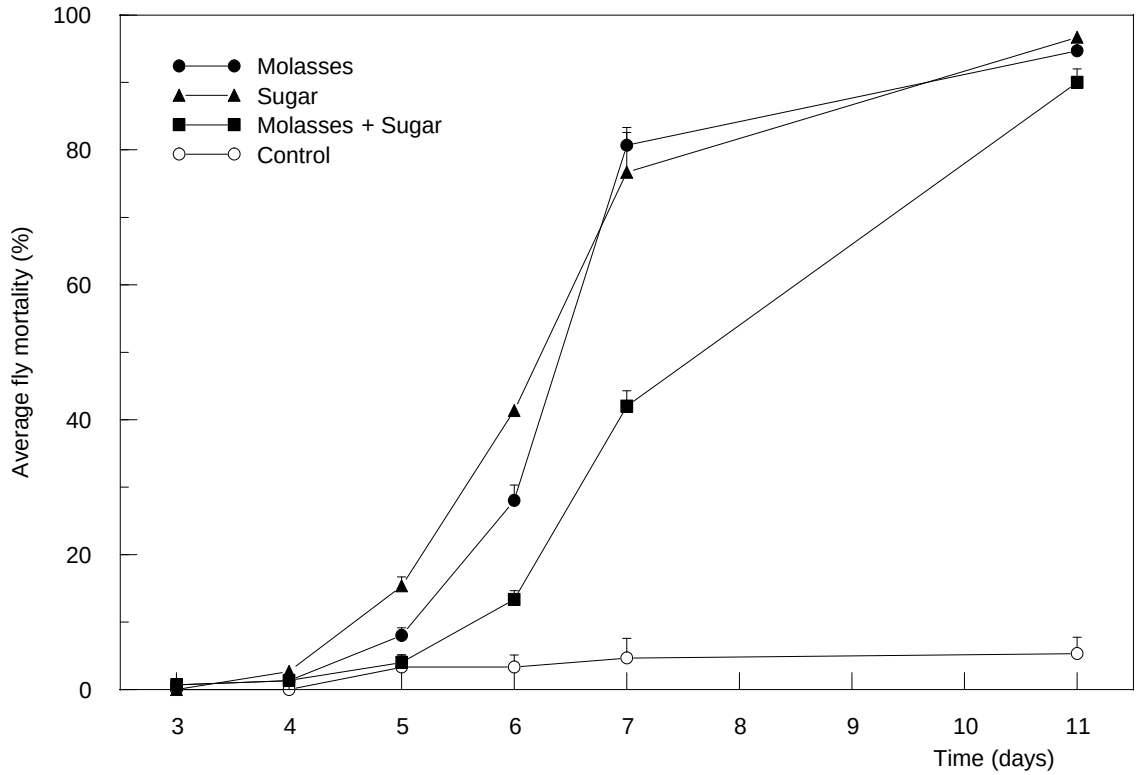

Figure 5: Average (+se) percent mortality in flies indirectly exposed to fungal spores formulated with different carbohydrate sources

Laboratory investigations showed there was little difference in the effect on flies between oil and glycerol in the formulation. However further investigations are needed into the effect of glycerol on spore viability before a decision should be made to replace oil in the formulation with glycerol. The preservative effect of oil on fungal spores under field conditions is well established (Bateman *et al.* 1993); however the effect of glycerol under similar conditions is yet to be evaluated. The investigations also showed that molasses could be added to the formulation, although there did not appear to be any advantage in increasing the level of molasses from 10% to 20%. The laboratory results also suggested that the spore concentration in the formulation could be decreased without compromising the efficacy against flies through indirect uptake. Based on these results the spray formulation used in the field trials differed from the formulation used in the previous season (2006-2007) by the addition of molasses as a fly attractant and cheaper carbohydrate source and decreasing the concentrations of sugar and spores.

4.1.2 Bait formulation development

All bait formulations containing spores caused 100% mortality by 7 days. However some baits without spores also caused high mortalities (Fig. 6). The combination of sugar and molasses with either glycerol or peanut oil had a toxic effect on flies, killing 73(±8)% and 78(±8)% of flies respectively after 7 days. The addition of spores increased this mortality to 100 % for both glycerol and peanut oil. Glycerol was considered as a cheaper alternative carrier to peanut oil that might better preserve spore viability. When baits were tested for spore viability after 7 days exposure to flies, no spores in the glycerol baits germinated compared to a high rate of germination of spores in the oil baits. This effect of glycerol needs further investigation. Because of the short time frame for the bait development it was decided not to use glycerol based on the

viability tests. Milk powder was trialled in the formulation as an attractant to encourage flies to feed. However the addition of milk powder appeared to have a protective effect, with the formulations producing lower fly mortality up to day 5. The milk powder also reduced the mortality effect of sugar and molasses mixed with either peanut oil or glycerol. The high mortality effect of sugar and molasses mixed with either oil or glycerol was not observed in the spray formulation investigations carried out previously. A bait formulation with spores, oil, molasses and sugar was adopted for trialling in feedlots A and D.

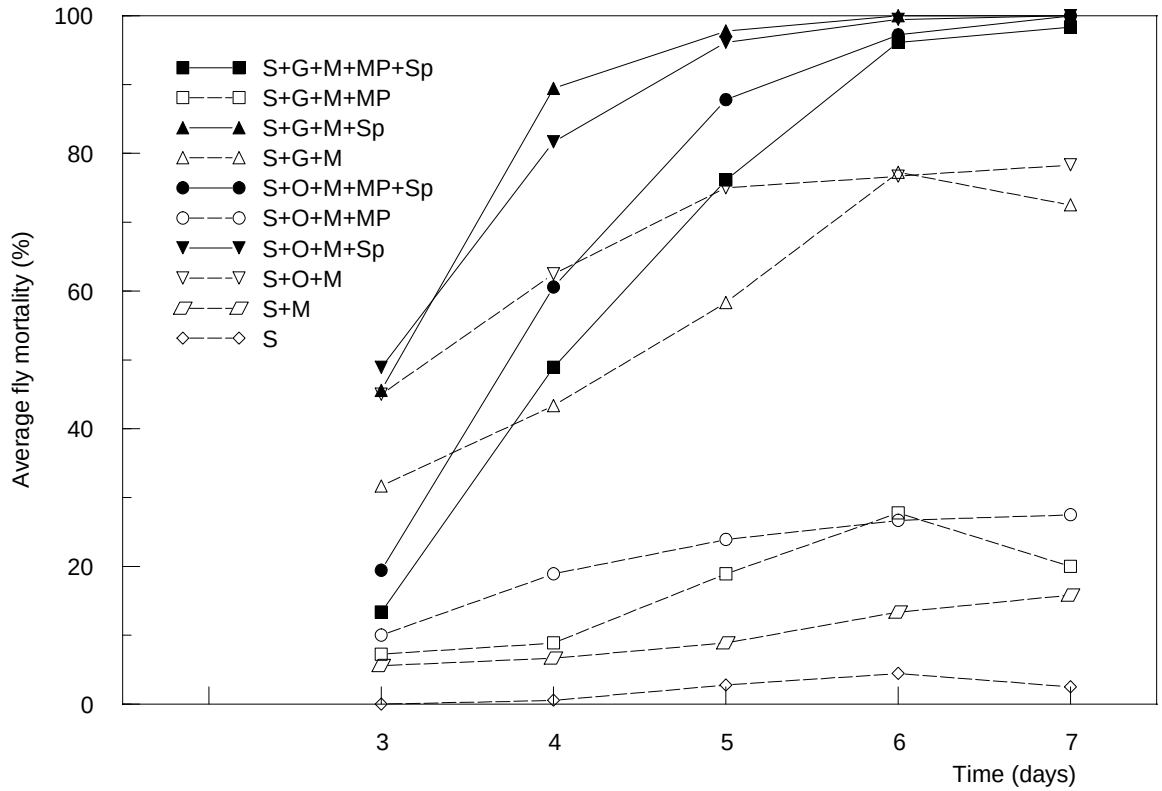

Figure 6: Average percent mortality in flies exposed to different bait formulations in the laboratory (S = Sugar; M = Molasses; G = Glycerol; Sp = Spores; MP = Milk Powder; O = Oil)

4.1.3 Viability of spore formulation on vegetation

The viability of spore formulations which had been sprayed onto vegetation was assessed by fly mortality and spore culture at different time points after application. Spores applied to vegetation caused between 48% and 75% mortality in flies exposed to leaves for 17 hrs overnight when the leaves were sampled on the day of spraying. One week after spraying the mortality in flies exposed to the leaves was between 43% and 52%. In one trial the formulation was still able to kill 15% of flies after 2 weeks on the vegetation (Fig. 7). However the length of time the formulation remained effective against flies varied between trials and may have been affected by rain. It was noted that any effect against flies disappeared after heavy rain. Spores germinating on the selective agar indicated that spores on the leaves could remain viable for 3 weeks. Figure 8 shows a colony growing from a *Metarhizium* spore that had remained viable on vegetation for 3 weeks.

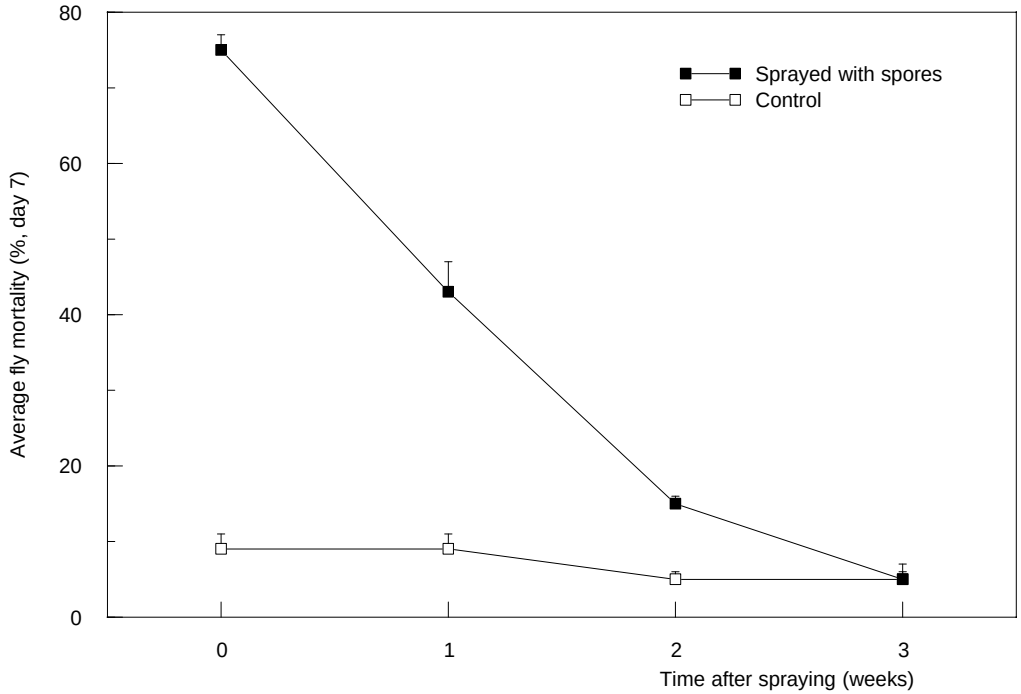

Figure 7: Average (+se) percent fly mortality 7 days post exposure to vegetation sprayed with the fungal formulation and sampled over three weeks

Figure 8: *Metarhizium* colony growing from where a leaf sprayed with a *Metarhizium* formulation 3 weeks previously was pressed into the *Metarhizium*-selective agar

These experiments demonstrated that spores sprayed onto vegetation remained viable and continued killing flies that came into contact with it for at least a week after spraying and in some cases for up to two weeks after spraying. This was a stringent test of the formulation because of the short term exposure of flies to the sprayed leaves once the formulation had dried. The loss of efficacy observed after heavy rain indicates that future development of a fungal formulation for fly control should include a component to make the formulation rain-fast.

4.2 Parasitic wasps

A colony of *Spalangia endius*, the most common parasitic wasp found on feedlots in south-eastern Queensland, was established at the DPI&F Animal Research Institute in 2003 (ARI colony). The ARI colony uses fresh house fly pupae as hosts. The large difference in development times of flies and wasps makes it possible to produce parasitised pupae which will exclusively produce wasps, an important factor for field releases. The ARI colony has been continuously maintained and has provided wasps for a variety of exploratory experiments. Several batches of wasps were transferred to the commercial partner, Bugs for Bugs, for the

establishment of their large-scale culture. Bugs for Bugs subsequently provided wasps for feedlot experiments in quantities which would not have been produced without the availability of commercial facilities. A detailed report on Bugs for Bugs production of the *S. endius* parasitic wasps is provided in Appendix 1. In this section, laboratory experiments with *S. endius* wasps, a brief description of the Bugs for Bugs wasp production and the wasp quality assessment, which was carried out at ARI, are described.

4.2.1 Use of frozen and heat killed *M. domestica* pupae as hosts for *S. endius*

It was previously found that *M. domestica* pupae that are killed either by heating at 55^{o}C or freezing at -80^{o}C were suitable for *S. cameroni* production for up to 2 months when stored at 4^{o}C (Geden and Kaufman 2007). Similar work had not been done on *S. endius.* The results from our study are shown in Table 6.

Table 6: Average *S. endius* emergence and emergence relative to control for frozen and heat killed pupae after different times of storage

Time of storage (weeks)	Average wasp emergence (%) (percent relative to control)		
	Frozen	Heat killed	Control
0	41 (80)	45 (88)	51
2	31 (70)	38 (86)	44
4	33 (56)	17 (29)	59
8	2 (3)	9 (16)	56

Control fly emergence 96-100%

Preliminary testing confirmed that exposing 2-day-old *M. domestica* pupae to 55^{o}C and 64% RH for 30 minutes or -80^{o}C for 10 minutes resulted in 100% mortality (no fly emergence). Pupae subjected to both treatments were successfully parasitised by *S. endius*. Wasp emergence was good from pupae stored for two weeks or less (70-88% of control emergence). Thus, pupae killed by heat (55°C) or freezing (-80°C) can be stored under refrigeration for a few weeks and then used for wasp production. This may be useful to shorten time lags in the production of commercial quantities of *S. endius* wasps.

4.2.2 Use of *M. domestica* pupae frozen for 2 years as hosts for *S. endius*

The suitability of fly pupae stored at -20°C for the production of *S. endius* was investigated. The age of pupae at the time of placement in the freezer may be an important factor, therefore pupae aged from less than one day to four days were tested.

The average emergence and the conditions of pupae after two years storage at -20°C are provided in Table 7.

Table 7: Average *S. endius* emergence and condition of pupae after two year storage at -20°C)

Treatment	Pupal age (h)	Wasp emergence (% relative to control)	Condition of pupae
Vacuum sealed, frozen	0-24	10.5 (32)	50% indented/collapsed
	24-48	17.5 (53)	All indented/collapsed, look dried out
	48-72	8.5 (26)	Good condition
	72-96	9.5 (29)	Good condition
Frozen	0-24	3.0 (9)	25% 'freezer burnt'
	24-48	23.5 (78)	Good condition
	48-72	7.5 (23)	Good condition
	72-96	8.0 (24)	Good condition
Control	24-48	33.0	

The highest wasp emergence from pupae stored in the freezer over a long period was obtained when 24-48 h old pupae were frozen with 53% and 78% emergence relative to the control. Vacuum sealing did not improve the suitability for wasp production, except when the pupae were less than 24 h old. Visual inspection of pupae did not provide a reliable predictor for their suitability for wasp production. Storage of *M. domestica* pupae in the freezer could be another useful means of increasing the flexibility of wasp production, but pupal age has an impact on their suitability.

4.2.3 Effect of cold storage on emergence of *S. endius*

Parasitised fly pupae should be deployed in the feedlot just prior to the wasps emerging from the pupae. The development time of wasps is highly dependent on temperatures during the process, eg at 27°C the majority of wasps emerge from day 21 to 23 after being parasitised. The development process can be prolonged by keeping the pupae at lower temperatures and at 10°C development is virtually stopped. We carried out an experiment to determine whether wasp emergence was reduced by keeping parasitised pupae at 10°C for various periods and whether the pupae's developmental age when placed in cool storage was important.

The average percent emergence, relative to pupae which were not placed in cool storage, was 87%, 76% and 57% for 4, 8 and 12 weeks storage at 10°C respectively. There was a reduction, increasing with the length of storage, in the wasp emergence. However, the reduction was small in the first month and cool storage for such a period is a feasible tool for management of parasitised pupae. There was no trend in changes to wasp emergence from pupae placed in cool conditions at different ages. Therefore pupae aged from 11 to 18 days old can be held at 10°C for a period of one month with only a small loss in wasp emergence.

4.2.4 Large scale production of *S. endius*

Bugs for Bugs is a Queensland company dedicated to the concepts of integrated pest management (IPM). Biological control is a valuable and widely used tool in IPM and Bugs for Bugs have been producing beneficial insects for more than 20 years. They possess extensive knowledge and experience with IPM for horticultural and agricultural crops. Bugs for Bugs agreed to collaborate with the DPI&F based group on the development and production of parasitic wasps for nuisance flies.

Bugs for Bugs role in the project is to mass produce the fly pupal parasitoid *Spalangia endius*. In order to achieve this several prerequisites were to be met. These included the installation of specialised buildings for the purpose of insect rearing, the purchase and/or manufacture of highly specialised production equipment and the development of reliable and efficient production techniques for the required insect species. Although some of the basic techniques used in mass rearing of other organisms could be adopted for *S. endius*, much of the equipment and

techniques for fly and parasitoid production has necessitated an entirely new approach. Some of the equipment is highly specialised and has been manufactured on site.

The *S. endius* rearing system encompasses two separate cultures, the hosts and the parasitoids. The host species used at Bugs for Bugs is the house fly *Musca domestica*. The procedure for rearing house flies was originally based on DPI&F procedures and has since been modified to suit large scale production. Input from Jerry Hogsette (USDA) has been immensely helpful in increasing the reliability of house fly production. The parasitoid culture uses *M. domestica* pupae which are exposed to wasps for 24 to 48 h to be parasitised. The procedures for rearing the parasitoids were originally roughly based on those developed by Phil Morgan *et al.* (1978) and others in the United States, but have been modified extensively in recent times to suit local conditions. Much trial and error has gone into the development of the parasitoid culture.

The major improvements and modifications implemented during 2007/08 included:

- The use of a pre-mixed diet for the house fly culture; this reduced labour requirements and dust issues
- Installation of corrosion-resistant air conditioners and humidity control equipment to withstand the corrosive atmosphere in the larval rearing room
- Installation of a pupae/larvae collection device at the back door of the larval rearing room to reduce escapees during room washout (escapees enable wild parasitic wasps of undesirable species to breed and potentially contaminate the colony).

A report provided by Bugs for Bugs on activities and innovations applied to the *S. endius* production during 2007/08 is provided as Appendix 1.

4.2.5 Quality assessment of mass-reared wasps

Mass production of the house fly parasitoid *S. endius* requires a comprehensive quality assurance program for both host and parasitoid. Contamination by other undesirable parasitoids can often be a recurring problem and a QA program was put in place to monitor the *S. endius* culture.

Aliquots of approximately 100 parasitised pupae from daily production batches were provided to assess fly and wasp emergence and checking for contamination by other parasitic wasp species. The results from the emergence assessments are provided in Table 8. Since the beginning of the mass production in August 2005, Bugs for Bugs has exposed about 90 million fly pupae to wasps and produced over 41 million *S. endius* for trial work. About 16 million wasps were produced during the 2007/08 summer to meet demands for the feedlot trials. The emergence of wasps during the latter half of 2007 and 2008 was about 52%. During the same time, fly emergence was typically around 17% compared with 92% from pupae which had not been exposed to wasps. This indicates that the majority of pupae are utilized by the wasps either for egg laying or feeding. Non-emergence is approximately 31% and can arise from non viable pupae, from pupae which had been used for feeding by wasps or unsuccessful development of wasps in the pupae.

The ratio of pupae to wasps during the 24 to 48 hours exposure is monitored because it is an important factor in optimising parasitoid production. Although a wide range of ratios has been used by other groups, ratios of 5 to 15 fly pupae per wasp are generally used. The higher the ratio, the fewer wasps have to be used in the production cycle and therefore more wasps are available for release. In the Bugs for Bugs production of *S. endius* the pupae to wasp ratio was generally between 5 and 10 (average 2005-08: 7.2; late 2007-2008: 7.7; Table 8). The ratio of pupae to fresh wasps (added on the same day) was usually between 15 and 20.

Table 8: Average pupae to wasp ratios, emergence of flies, *S. endius* wasps or nil emergence from house fly pupae exposed to *S. endius* wasps, and total numbers of wasps produced by Bugs for Bugs 2005 to 2008

Year	Quarter	Ratio*	Emergence (%)			Number of
		Pupae:wasp	Flies	Wasps	Nil	wasps produced
2005	3	18.3	24.9	30.5	38.9	307,975
	4	7.5	9.2	32.6	58.2	1,157,456
2006	1	5.8	15.0	53.2	31.9	1,842,135
	2	4.7	10.8	51.1	38.0	1,548,815
	3	5.4	11.6	44.7	43.7	2,138,638
	4	5.1	4.4	43.1	52.5	3,367,214
2007	1	7.0	5.8	40.0	54.2	4,584,373
	2	9.0	20.8	48.6	30.5	4,196,908
	3	6.7	13.4	57.7	28.9	6,400,976
	4	8.2	17.1	50.7	32.2	9,190,337
2008	1	8.1	19.7	48.5	31.7	6,376,357
Overall		**7.2**	**12.7**	**45.7**	**41.3**	**41,111,183**

* Calculated using 30% daily wasp mortality.

The species of emerged wasps were regularly checked and identified in aliquots of parasitised house fly pupae from Bugs for Bugs and from early emerging wasps in bulk pupae. In August 2005 *Pachycrepoideus vindemmiae* was identified with 10 to 78% of the emerged wasps being this solitary species. The colony was subsequently destroyed, changes made to the rearing facilities, and a new colony started. Since this time only very few *P. vindemmiae* have been detected in the QA samples and in bulk pupae supplied for field releases. While *P. vindemmiae* occasionally infests the colony, improved culture techniques appear to prevent it from establishing.

While *P. vindemmiae* was relatively easy to visually differentiate from *S. endius*, a second pupal contaminant, also found in August 2005, was more difficult to detect. Initially thought to be *S. cameroni*, it was subsequently identified by a world expert on parasitic wasps (Dr Garry Gibson, Agriculture and Agri-Food Canada, Ottawa) as the morphologically and biologically similar *S. gemina*. Due to its similar life cycle to *S. endius* it has persisted in the colony but its numbers appear to be declining (Table 9). *S. gemina* has been identified as having potential for nuisance fly control and should have no detrimental impact on the colony.

Table 9. Percent *S. gemina* of wasps emerging from the *S. endius* colony

Year	Number of wasps examined	% *Spalangia gemina*
2005	1637	5.7
2006	2629	5.0
2007	2082	2.9
2008 #	471	1.7

January and March 2008

In October 2006, along with *P. vindemmiae*, a small number of *Nasonia vitripennis,* a gregarious species, was identified from bulk pupae for the first time. In 2007, along with *P. vindemmiae* and *N. vitripennis*, a small number of *Muscidifurax raptor,* a solitary species, was also identified for the first time.

To try and locate the source of the contamination, on a number of occasions sentinel pupae have been placed around the various facilities used to produce *S. endius*, but no parasitoids were ever recovered. Insight into the potential for contamination was obtained during a visit to Bugs for Bugs in November 2007. It was noticed that cleaning of the larval rearing facility resulted in escaped larvae and pupae being flushed out of the container and accumulating on the ground. Collection of these pupae yielded a number of nuisance fly parasitoids (Table 10).

Table 10: Parasitoids recovered from house fly pupae collected outside and inside the larval rearing facility (November 2007)

Number	Number of parasitic wasps				
of pupae	*M. raptor*	*S. endius*	*S. nigroaenea*	*Dirhinus* sp.	*P. vindemmiae*
4815[A]	1872	24	1	13	169
416[B]	12	0	0	1	0

[A] Collected outside larval rearing facility; [B] collected inside larval rearing facility

Outside the larval rearing facility, 43% of the pupae were parasitised by 4 species of wasp, including those species found to occasionally infest the colony. As might be expected, pupae from inside the container (escapees from the larval collection device) were only 3.1% parasitized. Collection and subsequent destruction of pupae hosed from the rearing facility should reduce the chances of further contamination. Bugs for Bugs installed such a collection device late in 2007.

4.3 Integrated fly control on feedlots

4.3.1 Introduction

An integrated program for fly control was put in place and maintained over spring, summer and autumn in two commercial feedlots, one in the Brisbane Valley and one in the Warwick shire. Adult and immature fly populations, the extent of parasitism and the impact of fungal biopesticides were measured during these trials and compared with values obtained from control feedlots situated in the same areas. The integrated fly control program included monitoring of fly populations, release of parasitic wasps, application of fungal biopesticides and the use of insecticidal bait stations.

4.3.2 Weather

Temperature and rainfall data and the corresponding 50-year averages are shown for the Brisbane Valley and the Warwick shire in Figures 9 and 10 respectively.

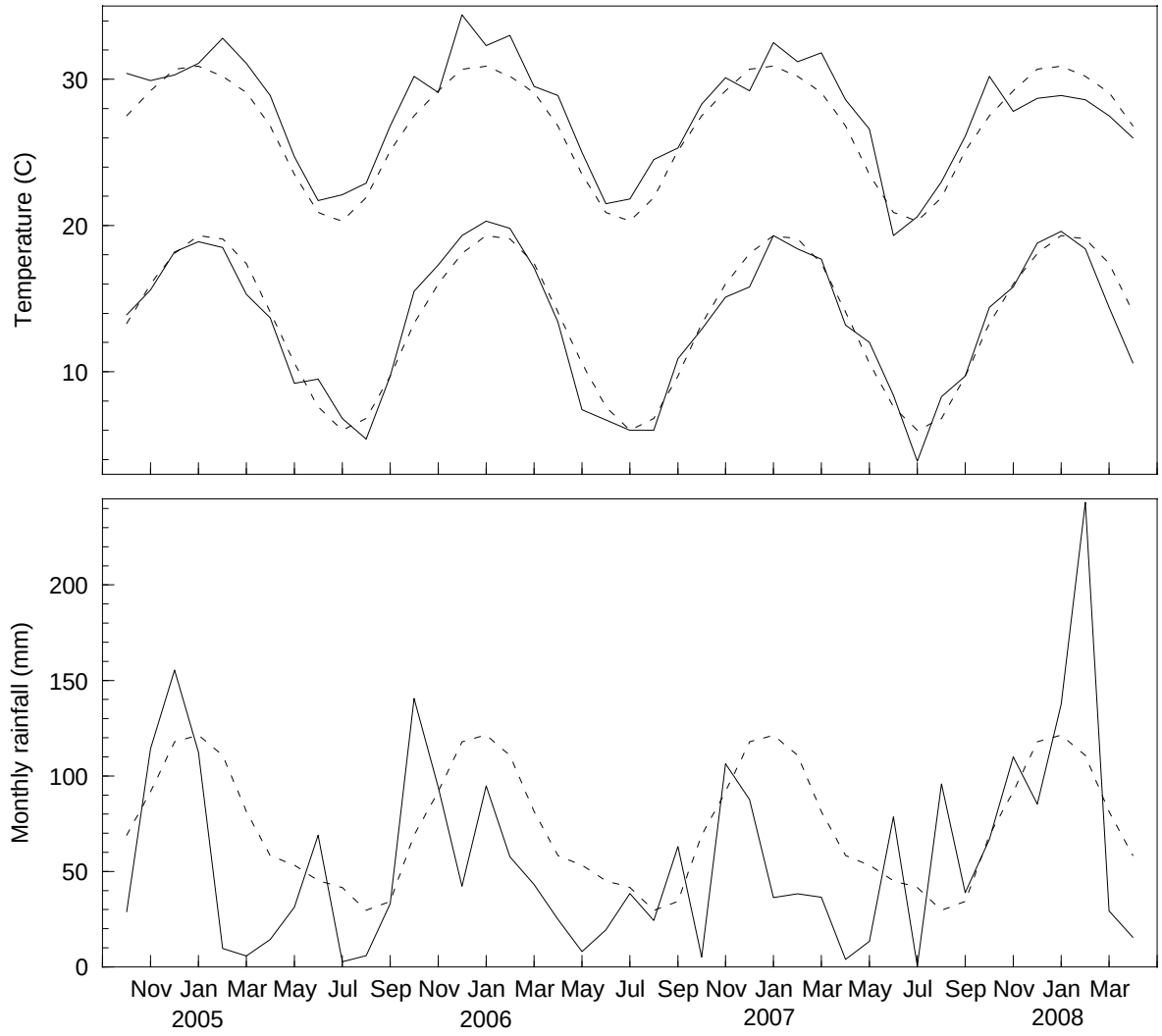

Figure 9: Monthly average maximum and minimum daily temperatures and rainfall from October 2004 to April 2008 (solid lines) and the corresponding 50-year averages (dotted lines) for the Brisbane Valley

The average daily maximum temperatures were below the long term average for most months during the 2007/08 fly season. The minimum temperatures were close to the averages. Both average maximum and minimum temperatures were 1 to 3ºC higher in the Brisbane Valley than in the Warwick area. Rainfall was above the long-term average for both regions during the 2007/08 seasons. Total rainfall for the period June 2006 to May 2007 was 914 mm (long term average 854 mm) and 712 mm (679 mm) for the Brisbane Valley and Warwick shire respectively. Weather conditions during the 2007/08 were favourable for fly populations and higher fly pressure than in previous years was expected.

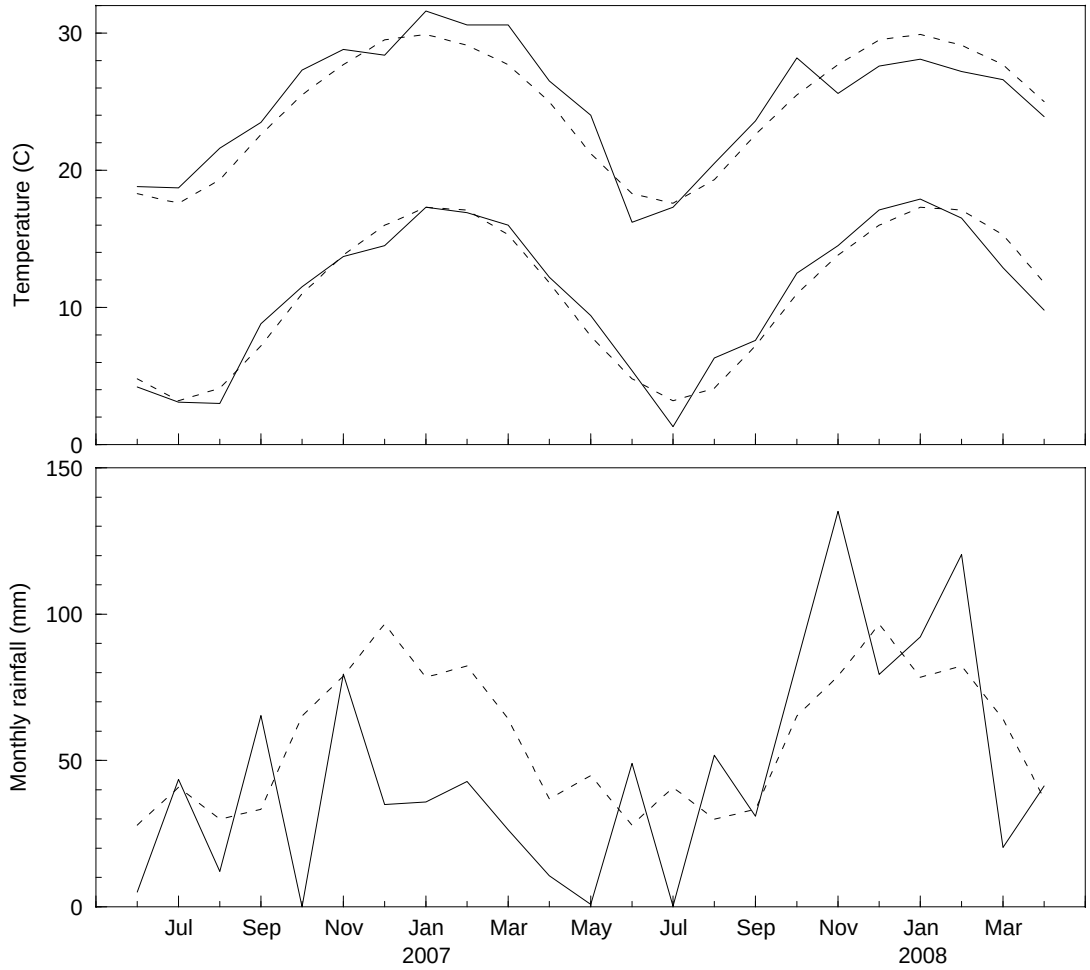

Figure 10: Monthly average maximum and minimum daily temperatures and rainfall from June 2006 to April 2008 (solid lines) and the corresponding 50-year averages (dotted lines) for the Warwick shire

4.3.3 Fly and parasite populations

Monitoring of adult fly populations has been undertaken in the two Brisbane Valley feedlots since October 2004. The catches of adult flies on sticky Alsynite traps on the feedlots A and C are shown in Figure 11. House flies were the most commonly trapped flies and were generally present in relatively high numbers during late spring, summer and autumn. Stable flies were also trapped in both feedlots with the highest numbers during spring and autumn. Bush flies were trapped in appreciable numbers only during October and November. Other flies were trapped in smaller numbers (not shown in Figure 11), including *Physiphora clausa* (November to April). Fly numbers were generally higher during the 2007/08 season than in previous years, as expected from weather data.

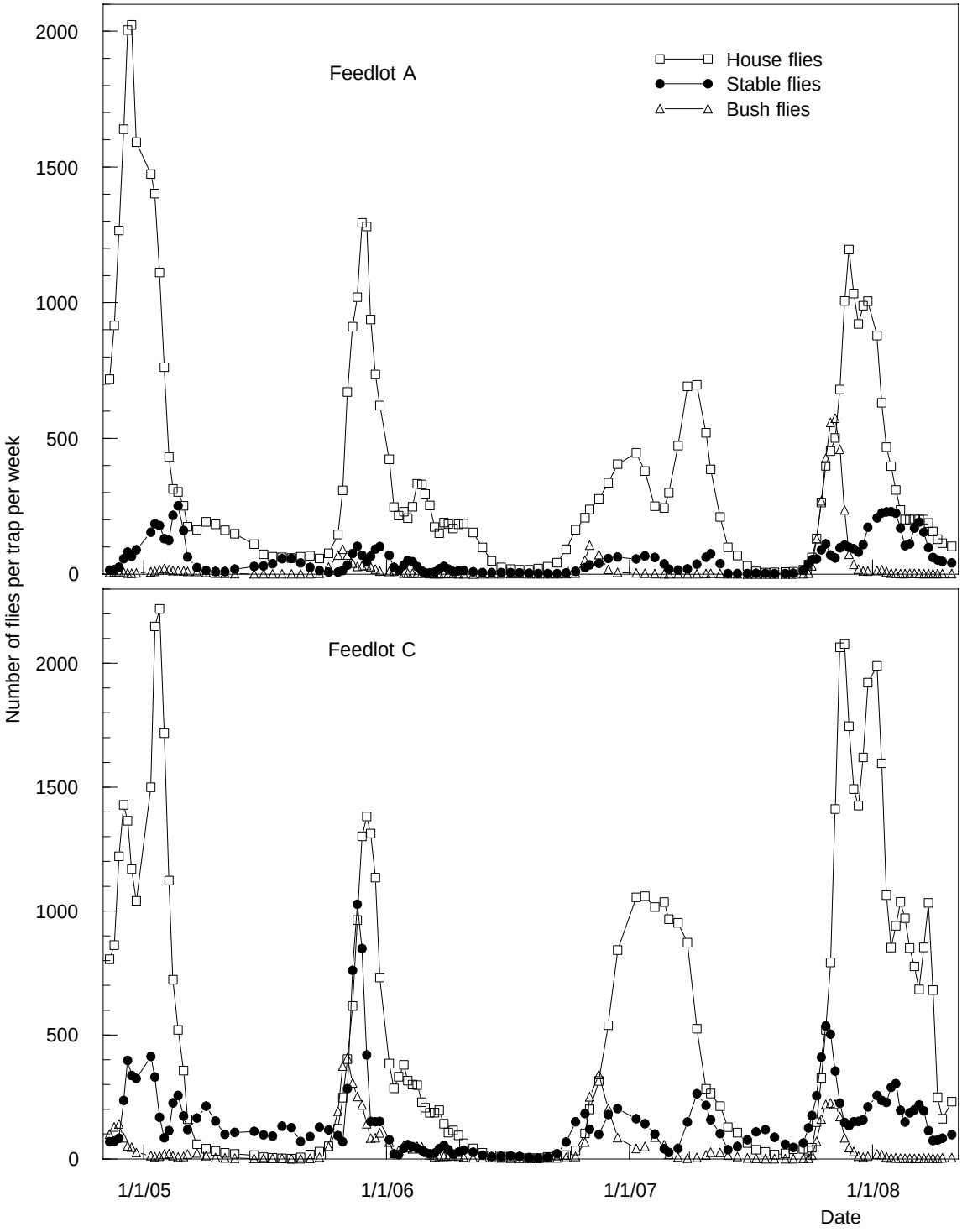

Figure 11: Fly catches on sticky Alsynite traps on Brisbane Valley feedlots A and C (3 point averages of mean weekly trap catches)

Fly catches for the feedlots in the Warwick shire have been recorded since November 2006 (Fig. 12). During the 2007/08 season, bush flies were trapped in highest numbers during October and November (and December in feedlot F). The increased populations of bush flies, which do not breed in feedlots, were due to favourable weather conditions. Stable fly populations were also higher than in the previous season. House flies were trapped over autumn, summer and spring but they were less abundant in the Warwick feedlots compared to the Brisbane Valley feedlots. Stable flies contribute much more to the total fly populations in the Warwick area than in the Brisbane Valley. In summary, 2007/08 fly populations in the Brisbane Valley and the Warwick

shire were at or above average levels and provided a good opportunity to assess integrated fly control programs in feedlots in those areas.

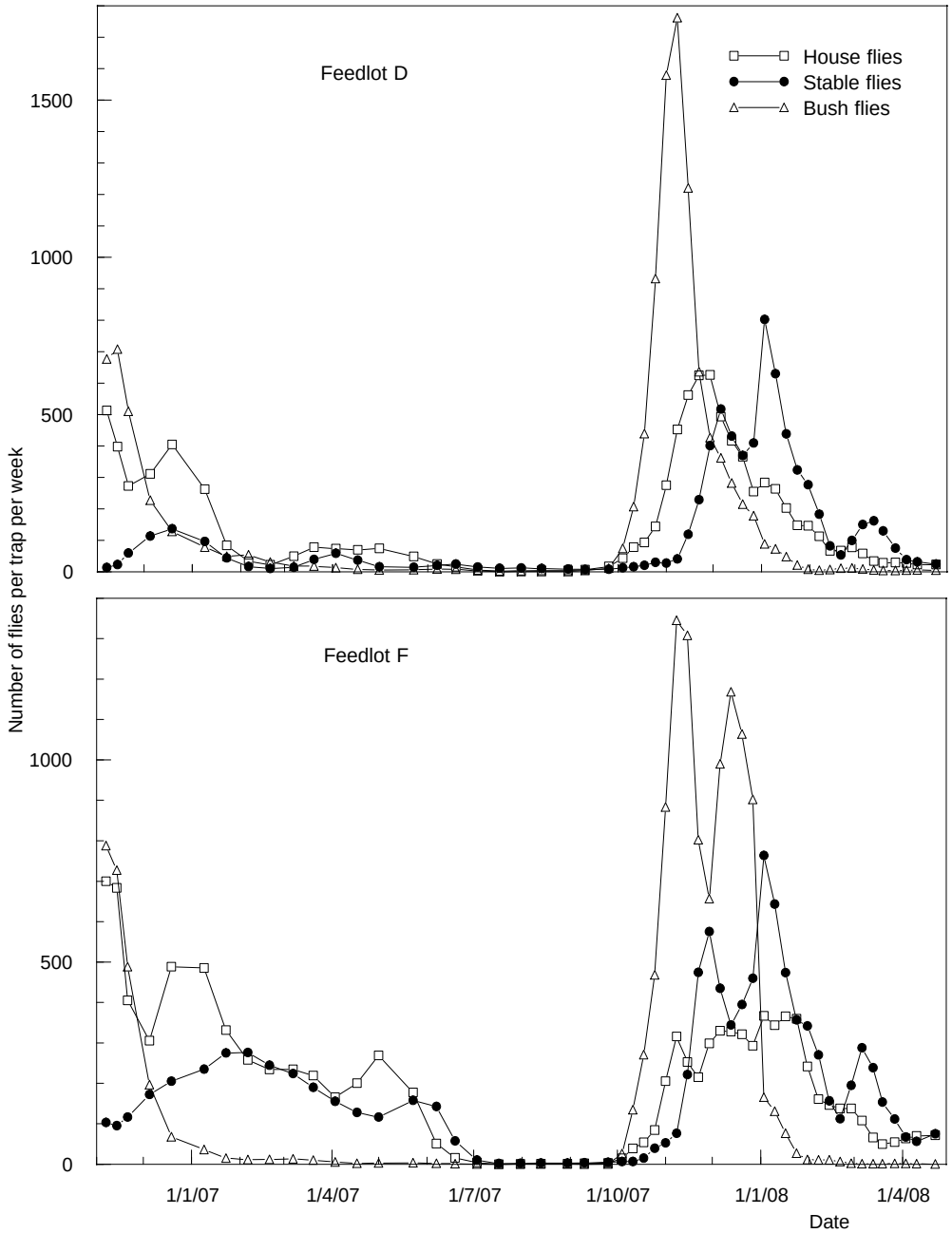

Figure 12: Fly catches on sticky Alsynite traps on Warwick shire feedlots D and F (3 point averages of mean weekly trap catches)

The species of parasitic wasps that emerged from pupae collected on the feedlots are given in Tables 11 and 12.

Table 11: Wasp species from pupae collected on Brisbane Valley feedlots

Feedlot	Wasp species	Species composition (%) #								
		2007				2008				**2007-**
		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	**2008**
A	*S. endius*	7.5	57.1	70.0	96.9	45.8	22.2	59.1	60.2	**54.6**
	S. cameroni	57.7	10.2	3.3	3.1	14.6	33.3	27.3	36.1	**17.1**
	S. nigroaenea	0	3.1	3.3	0	17.4	33.3	4.5	3.6	**6.6**
	M. raptor	32.5^{A}	29.7	23.3	0	21.5	11.1	9.1	0	**21.4**
C	*S. endius*	45.5	55.0	74.6	94.7	55.7	0	70.0	33.3	**56.4**
	S. cameroni	40.9	19.0	3.2	0	6.6	66.7	30.0	33.3	**17.4**
	S. nigroaenea	9.1	2.0	6.3	5.3	6.6	33.3	0	22.2	**6.7**
	M. raptor	0^{B}	24.0	15.9	0	31.1	0	0	11.1	**19.1**

Both feedlots had small numbers of *Trichomalopsis* sp. in September 2007;
Values with different superscript within column and species differ significantly (P<0.05)
Releases of *S. endius* from September 2007

Table 12: Wasp species from pupae collected on Warwick shire feedlots

Feedlot	Wasp species	Species composition (%)								
		2007				2008				**2007-**
		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	**2008**
D	*S. endius*	na	75.7	67.5	87.5	91.7^{A}	33.3^{B}	20.0	86.4	**75.0**
	S. cameroni	na	5.7^{B}	2.6	0	0	0	40.0	6.8	**4.8^{B}**
	S. nigroaenea	na	1.4	23.4^{A}	12.5	8.3^{B}	66.7^{A}	40.0	6.8	**14.4**
	M. raptor	na	17.1	6.5	0	0	0	0	0	**5.9**
F	*S. endius*	na	56.7	86.5	73.3	48.1^{B}	100^{A}	33.3	57.9	**67.4**
	S. cameroni	na	26.7^{A}	0	6.7	0	0	25.0	21.1	**11.7^{A}**
	S. nigroaenea	na	0	0^{B}	13.3	51.9^{A}	0^{B}	41.7	21.1	**12.0**
	M. raptor	na	16.7	13.5	6.7	0	0	0	0	**8.8**

Values with different superscript within column and species differ significantly (P<0.05)
Releases of *S. endius* from September 2007

Parasitoid complexes were similar on both feedlots in the Brisbane Valley with an overall average of 80% *Spalangia*, 20% *Muscidifurax* and small numbers of *Trichomalopsis* emerging from predominately *M. domestica* pupae. *Spalangia* spp. were the most common parasitoids, with *S. endius* the most common species of *Spalangia* (50-60% of all wasps), reaching a peak of >95% on both feedlots in December 2007. The species composition of the parasitoid complex was broadly similar to those found in previous reports, but with percentages of *S. endius* increased.

Parasitoid complexes in the Warwick shire feedlots overall had higher *Spalangia* (93%) and lower *Muscidifurax* (7%) percentage and again *S. endius* was the predominant species of *Spalangia* (65-75%) emerging from predominately *M. domestica* pupae on feedlot D and predominately *S. calcitrans* pupae on feedlot F. No *Trichomalopsis* were found on the Warwick feedlots and *M. raptor* only was found in 2007. The mix of *Spalangia* species was broadly similar to previous reports from Warwick shire with parasitism by this genus higher than in the Brisbane Valley. The small numbers of *Physiphora clausa* pupae collected on both feedlots were largely parasitized by *S. nigroaenea*.

Flies caught on the Alsynite traps were inspected for predatory mites. Trombidiid and *Macrocheles* spp. mites were detected on trapped flies from all feedlots (Table 13). About 2% to 8% of house flies and 1% to 2% of stable flies were infested with Trombidiid mites. *Macrocheles* mites were found on a small proportion of house flies (0.1% to 0.2%) but more frequently on stable flies (typically 1% to 2%). Almost all mites on house flies were red Trombidiid mites,

whereas on stable flies Trombidiid and *Macrocheles* mites were found in about equal numbers. The percent mite infestations were higher from January to March than earlier and later during the observation period.

Table 13: Percentage of trapped flies infested with mites from four feedlots

Feedlot	Average mite infestation (%)			
	House fly		Stable fly	
	Trombidiids	*Macrocheles*	Trombidiids	*Macrocheles*
A	3.5	0.2	1.5	1.2
C	1.9	0.1	0.9	2.9
D	2.4	0.2	1.1	0.7
F	7.8	0.2	2.3	1.4

4.3.4 Integrated fly management programs

The integrated fly management programs which were instigated in Feedlots A and D (IPM feedlots) used a variety of fly management tools. These tools and the timing of their applications are summarised in Table 14.

Table 14: Fly management tools used in feedlots A and D

Tools	Application	
	Feedlot A	Feedlot D
Fence line cleaning	Approx. 3-weekly	Approx. 4-weekly
Sedimentation system cleaning	Approx. 3-monthly	Approx. 2-monthly
Fly population monitoring (adults)	4 Alsynite traps Weekly	4 Alsynite traps Weekly
Fly population monitoring (larvae)	Estimate larvae on fence lines and sedimentation system Fortnightly	Estimate larvae on fence lines and sedimentation system Fortnightly
Parasitic wasp releases	Weekly	Weekly
Fungal biopesticides (spray)	2/10/2007 9/10/2007 16/10/2007 23/10/2007 30/10/2007 6/11/2007 4/12/2007 22/01/2008 29/01/2008 4/03/2008 11/03/2008 18/03/2008	11/10/2007 18/10/2007 25/10/2007 1/11/2007 8/11/2007 15/11/2007 6/12/2007 31/01/2008
Fungal biopesticides (baits) (Bait stations containing hanging strip wrapped with hessian soaked with molasses and spores)	8 bait stations 5/02/2008 12/02/2008 19/02/2008 26/02/2008 25/03/2008	9 bait stations 7/02/2008 14/02/2008 21/02/2008
Insecticidal baits (Bait stations containing hanging strip coated with DyFly Plus or Agita 10)	8 bait stations 4/12/2007 11/12/2007 18/12/2007 2/01/2008 22/01/2008	9 bait stations 22/11/2007 29/11/2007 6/12/2007 13/12/2007 20/12/2007 3/01/2008 17/01/2008
Insecticidal treatments (larvae)	No application	No application
Insecticidal treatments (adults)	No application	No application

Some of the tools were used continuously at predetermined intervals while others were only applied when the monitoring systems indicated that fly populations were increasing beyond a desirable level. The desirable frequency of fence line cleaning was fortnightly, however in practice it happened at about 3 to 4 weekly intervals. The cleaning of the sedimentation system was carried out less frequently and only occurred once or twice during the fly season. Both these tasks were carried out by feedlot operators who made a sustained attempt at meeting our requested schedule. There were a variety of reasons for the discrepancy between desirable and actual frequencies: dry weather periods during which fly breeding was minimal; wet periods where fence lines and sedimentation systems could not be cleaned because the ground was too soft; shortage of staff.

The fly monitoring, animal observations, parasitic wasp releases and spray and bait applications were conducted as indicated in the table by project staff and carried out regularly with very few exceptions (Christmas - New Year break, feedlot inaccessible due to wet weather).

Some of the management tools were also used in the control feedlots. The population monitoring for adults and immature flies was carried out in the control feedlot to the same extent and at the same time as in the IPM feedlots. Fence lines and sedimentation systems were also cleaned in the control feedlots but somewhat less frequently than in the IPM feedlots. With regards to fly population control tools, only insecticidal baits were occasionally used with no other insecticidal treatments applied.

4.3.5 Fly population monitoring (adults)

The monthly geometric mean trap catches on Alsynite sticky traps are provided in Figures 13 and 14 for the Brisbane Valley and Warwick areas respectively.

In the Brisbane Valley, the house fly populations were low in both feedlots in October. The house fly population was higher in the control feedlot than the IPM feedlot in all subsequent months with this difference being significant ($P<0.05$) except for December 2007. The use of an IPM system reduced the house fly populations over the whole season by 55%.

The stable fly population was higher in the control feedlot than the IPM feedlot in October. It remained higher through most months (significant only in October and November) but in autumn the populations were at similar levels in both feedlots. Overall the stable fly population was 51% lower in the IPM feedlot. On the other hand, the IPM feedlot had a 1.35 times higher bush fly population than the control feedlot. Bush flies do not breed in the feedlot and IPM programs had no impact on their populations.

In the Warwick feedlots (Fig. 14) house fly populations were significantly higher in the IPM than the control feedlot during the first two months (October, November). However, that trend was reversed and from January onwards populations were lower in the IPM feedlot (significant February to April). Stable fly populations were similar in both feedlots from October to December but consistently lower in the IPM feedlot from January onwards. Overall both populations were lower in the IPM than the control feedlot (10% and 27% for house and stable flies respectively). House fly numbers were consistently lower in the Warwick feedlots than the Brisbane Valley feedlots. The Warwick feedlots were inundated with bush flies from October till November (December for Feedlot F; see Fig. 12). These high numbers of bush flies may have led to some distortion of trapping values for these months. Traps were often saturated by bush flies and this could have resulted in underestimating the house and stable fly populations.

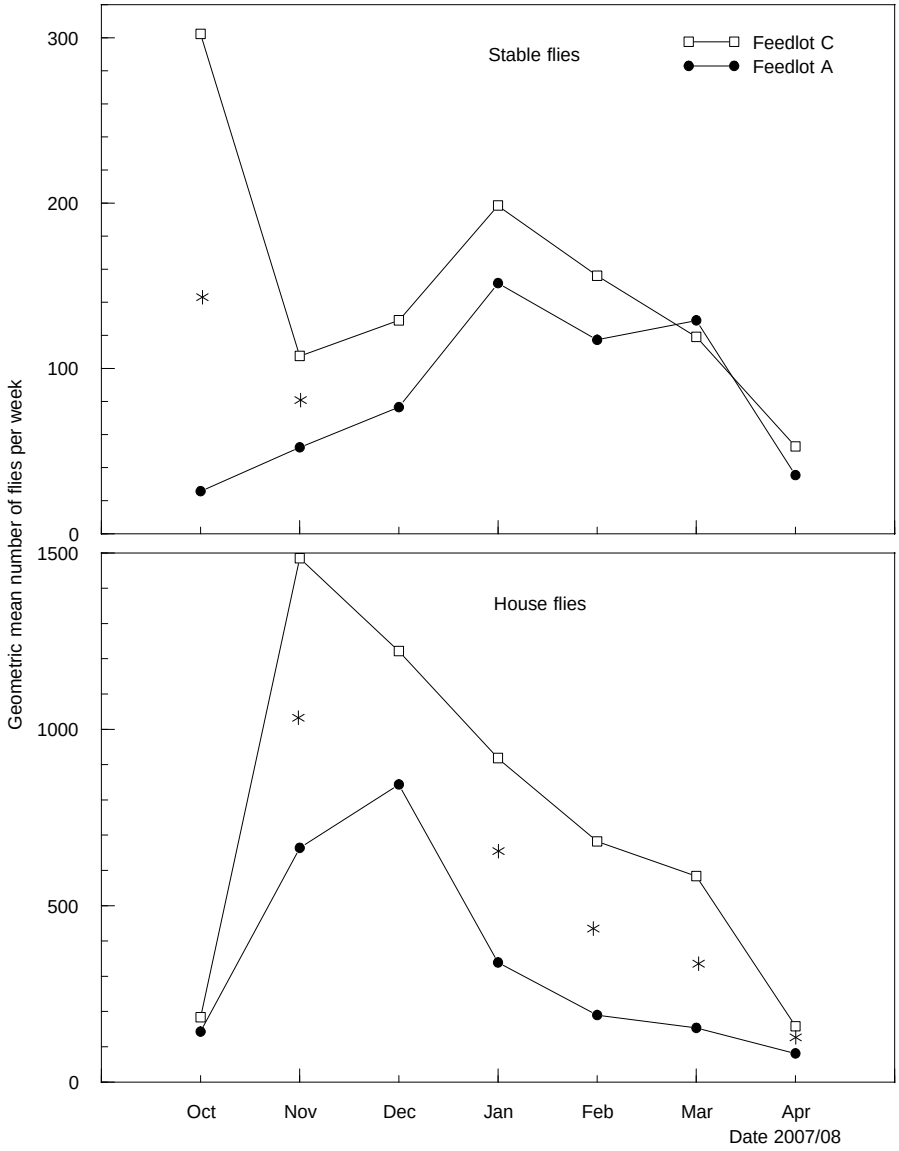

Figure 13: Monthly geometric mean fly catches on Alsynite traps on Brisbane Valley feedlots A (IPM program) and C (control) (* values differ significantly P<0.05)

The results from the analysis of the combined data from both areas are shown in Fig. 15. The house fly populations were identical in the control and IPM feedlots during October and November, but from December to April were consistently (and significantly from January to April) lower in the IPM feedlots. The reduction in house fly population achieved by the IPM program across both areas and season was 36%. Stable fly populations in the IPM feedlots were below those of the control feedlots for every month, significantly so in four out of seven months. The overall reduction in the stable fly population was 40%. The overall treatment effect (control versus IPM) was not significant in the combined analysis but the time by treatment interaction was, indicating that the treatment effect was not consistent across time. It is expected that the biological tools (parasitic wasps, biopesticides) take some time to have an impact on adult fly populations. The data support this assumption with most of the population suppression and significant differences observed later during the fly season.

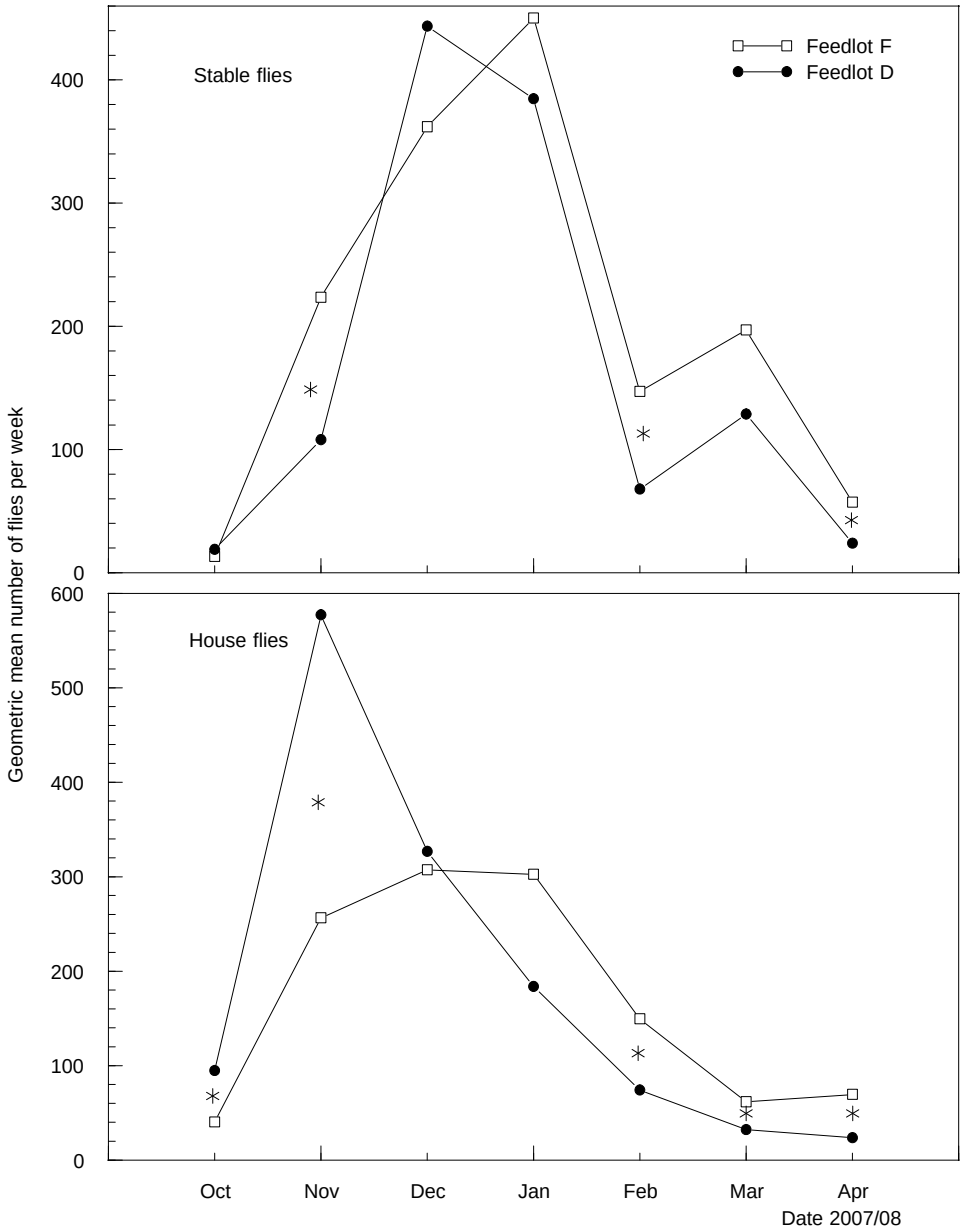

Figure 14: Monthly geometric mean fly catches on Alsynite traps on Warwick feedlots D (IPM program) and F (control) (* values differ significantly P<0.05)

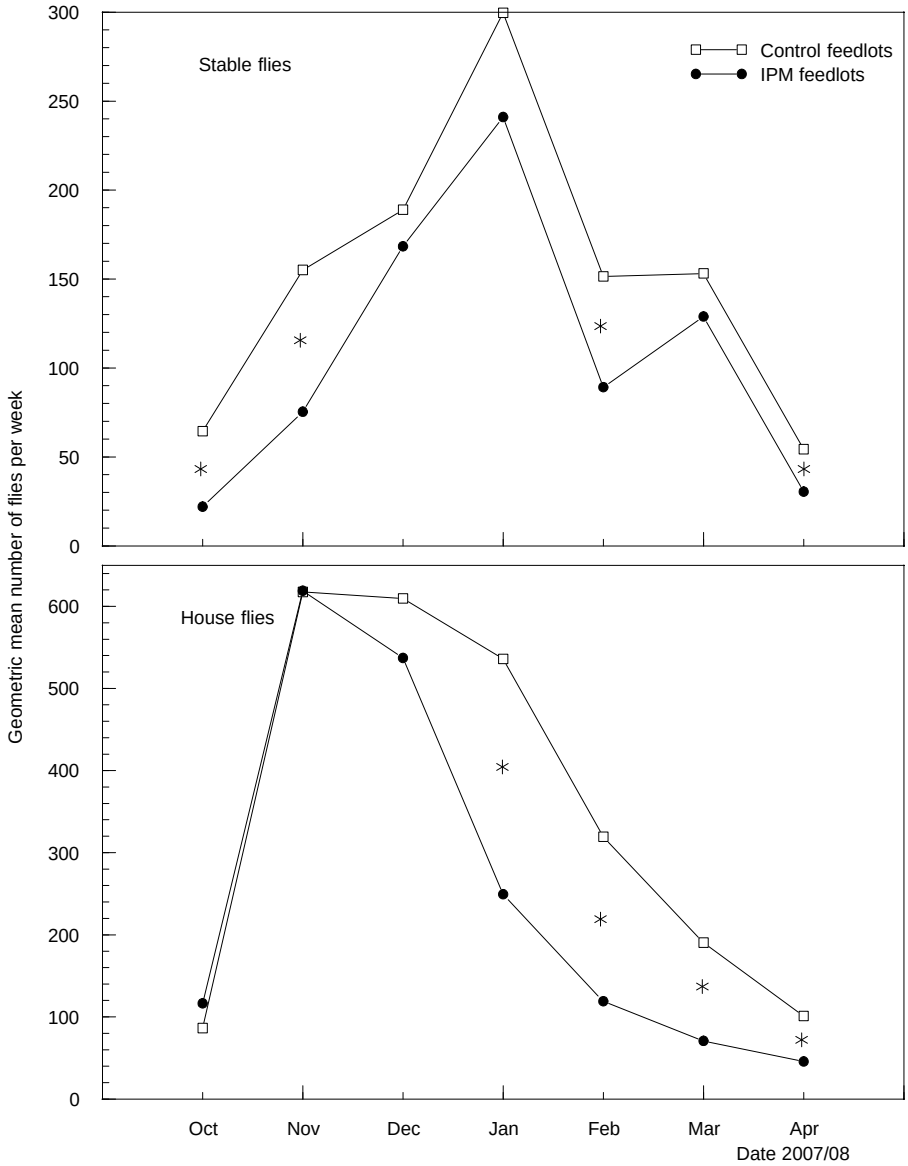

Figure 15: Monthly geometric mean fly catches on Alsynite traps on IPM and control feedlots (* values differ significantly P<0.05)

4.3.6 Fly population monitoring (immature)

The monthly geometric mean numbers of larvae found in the Brisbane Valley and Warwick feedlots are provided in Figures 16 and 17 respectively. In the Brisbane Valley significantly more larvae were present in the control feedlot (32.7) than the IPM feedlot (7.7) throughout the season. However, in the Warwick feedlots the reverse was found with more larvae in the IPM (9.6) than the control feedlot (2.6). There were more larvae in the Brisbane Valley than the Warwick feedlots. An analysis of combined data from both areas indicated no impact of the IPM on immature fly populations.

In the Brisbane Valley feedlots, most of the larvae (94% and 96% in feedlots A and C respectively) were found in manure under the fence lines and the rest in the sedimentation system. In the Warwick shire, 82% and 49% of the larvae were found under fence lines in feedlots D and F respectively and the reminder in the sedimentation system. Feedlot F had a particularly large proportion of larvae in the sedimentation system. Feedlot F had an increased pen slope compared to others and this may have reduced the suitability for fly breeding. The high pen slope may have increased the amount of manure moving from the feedlot pen into the sedimentation system during runoff events. This may explain the greater immature fly population

in the sedimentation system. Feedlot designers generally consider a pen gradient of 3% to be optimal in terms of promoting good drainage characteristics without excessive movement of manure off the pen surface during runoff events. It is generally preferable to routinely harvest manure from the feedlot pens in a relatively dry state rather than to remove large amounts of semi-solid (sloppy) manure from feedlot drains or sedimentation systems. Large amounts of manure that settles in drains and sedimentation systems can take significant periods of time to dry out sufficiently for mechanical removal. During this time, the wet manure deposits can be significant odour emission sources and an ideal breeding ground for nuisance flies.

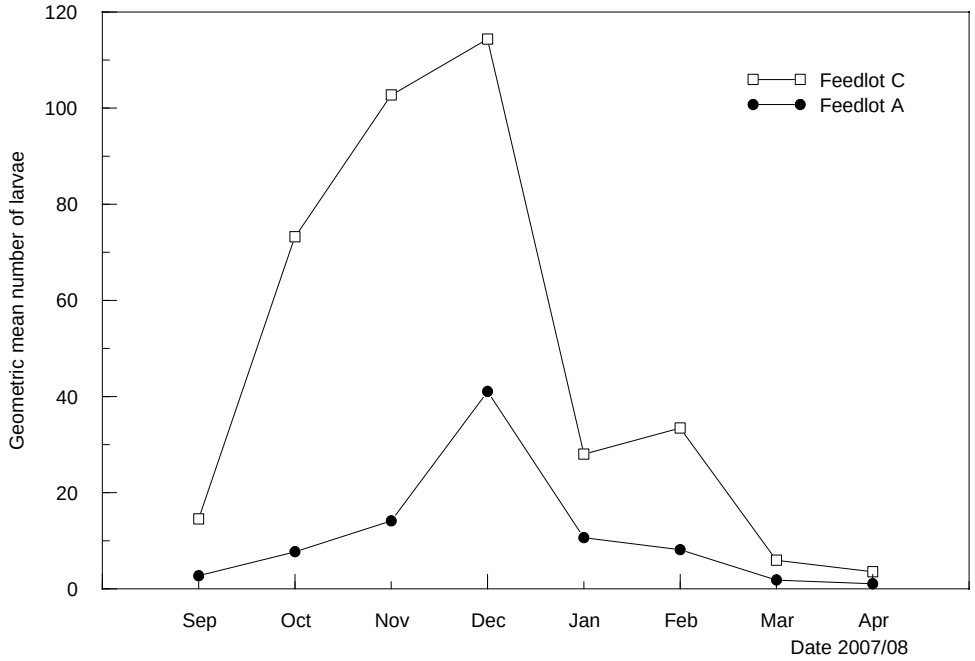

Figure 16: Monthly average number of larvae in Brisbane Valley feedlots A and C (values differ significantly (P<0.05) for each month)

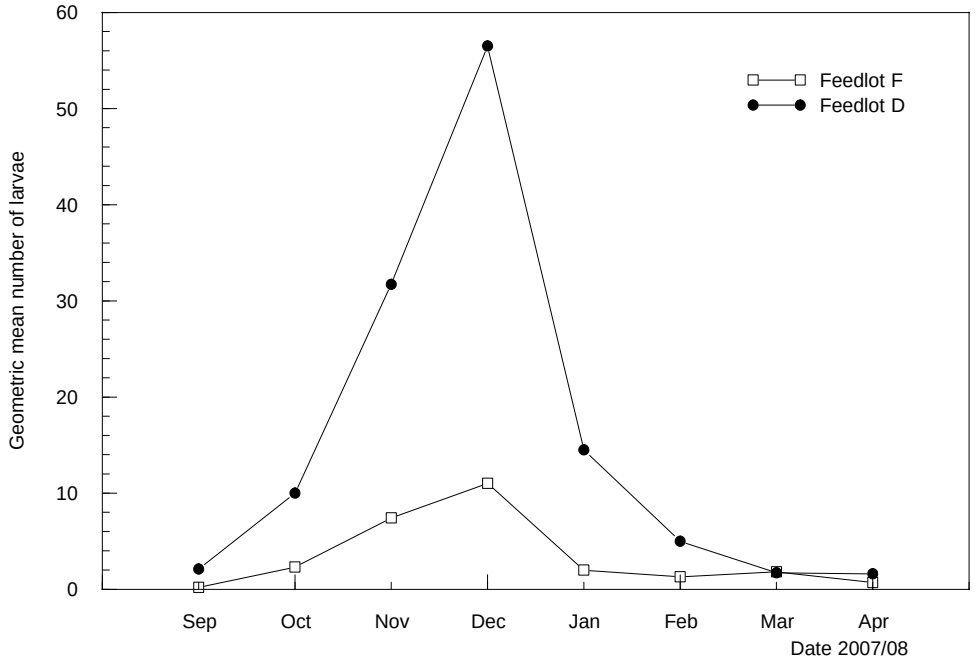

Figure 17: Monthly average number of larvae in Warwick feedlots D and F (values differ significantly (P<0.05) for each month except March and April 2008)

The species compositions of the breeding populations were determined from pupae collected under the fence lines and the sedimentation systems. A summary of the monthly and overall percentages is provided in Table 15. In the Brisbane Valley feedlots A and C the species compositions were fairly similar. House flies were the dominant species (total averages 92% and 91%) and stable flies made up almost the remainder (7% and 9%). All other fly species, including *Physiphora* constituted less than 1% of the sample.

Table 15: Species composition of pupae collected on feedlots

Feed lot	Fly species	Species composition (%) 2007					2008			**2007-2008**
		Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	
A	House fly	93	90	85	95	96	92	95	99	**92**
	Stable fly	7	9	14	5	4	8	4	2	**7**
	Physiphora	0	0	1	0	0	0	0	0	**0.1**
C	House fly	82	88	96	100	97	100	94	80	**91**
	Stable fly	18	12	2	0	3	0	5	18	**9**
	Physiphora	0	0.3	2	0	0	0	1	3	**1**
D	House fly	-	95	97	83	45	69	22	69	**86**
	Stable fly	-	5	3	17	46	3	22	20	**11**
	Physiphora	-	0	0	1	9	27	56	11	**3**
F	House fly	-	37	55	50	14	30	17	13	**39**
	Stable fly	-	61	43	39	35	70	59	79	**51**
	Physiphora	-	1	1	11	50	0	24	7	**9**

The species distributions in the Warwick feedlots D and F were different. In feedlot D, house flies were the major species (86%), followed by stable flies (11%) and *Physiphora* (3%). While this was similar to the Brisbane Valley feedlots, feedlot F had stable flies as the major species (51%), followed by house flies (39%) and *Physiphora* (9%). This difference between feedlots D and F was also reflected in the adult fly trap catches (Fig. 14) and it confirms that there were differences in fly breeding and populations between these two feedlots.

4.3.7 Parasitic wasp releases

As part of the IPM program, *S. endius* wasps produced by Bugs for Bugs were first released on the 19 September 2007 in feedlot A in the Brisbane Valley and a feedlot D in the Warwick shire. The numbers of pupae and wasps released on feedlots A and D from 2007 to 2008 are shown in Tables 16 and 17.

Table 16: Number of parasitised pupae, average *S. endius* emergence and number of wasps released in the Brisbane Valley feedlot trial

Year	Month	Number of parasitised pupae	Emergence[#]	Number of *S. endius*
2007	Sep	1,039,345	61.3	639,873
	Oct	2,833,209	54.2	1,524,712
	Nov	2,639,755	44.9	1,139,873
	Dec	1,717,997	55.5	962,434
2008	Jan	3,718,450	50.7	1,864,842
	Feb	2,362,639	48.1	1,115,797
	Mar	2,687,961	49.7	1,280,370
	Apr	1,858,330	53.3	938,769
Total		**18,857,685**	**51.9**	**9,466,671**

[#] based on laboratory quality assurance data

Table 17: Number of parasitised pupae, average *S. endius* emergence and number of wasps released in the Warwick feedlot trial

Year	Month	Number of parasitised pupae	Emergence[#]	Number of *S. endius*
2007	Sep	168,411	51.6	86,900
	Oct	714,856	49.6	343,565
	Nov	1,144,963	55.6	641,621
	Dec	597,567	52.2	319,127
2008	Jan	1,216,821	42.4	605,554
	Feb	952,534	53.8	419,566
	Mar	833,530	58.5	493,948
	Apr	807,026	49.6	410,396
Total		**6,435,708**	**51.4**	**3,320,677**

[#] based on laboratory quality assurance data

The available parasitised pupae were divided amongst the two feedlots (according to cattle numbers) and placed into the feedlots weekly from September 2007 to April 2008 (with the exception of 25/12 and 22/4 in feedlot A and 27/12 and 17/4 in D). The numbers of wasps, on a per head (SCU capacity) basis, released at the two feedlots are shown in Figures 18 and 19. No generally accepted standards are available for release rates of the various species of wasps for use in different production systems. For previous parasitoid trials in North America a wide range of wasps per animal ratios (20 to 500) have been used (as well as releases per fly breeding area rather than animal numbers). We aimed at releasing a minimum of 100 wasps per head per week and this target was generally achieved. For the Brisbane Valley feedlot A an average of 99 *S. endius* wasps were released per head per week while for the Warwick shire feedlot D this was 110.

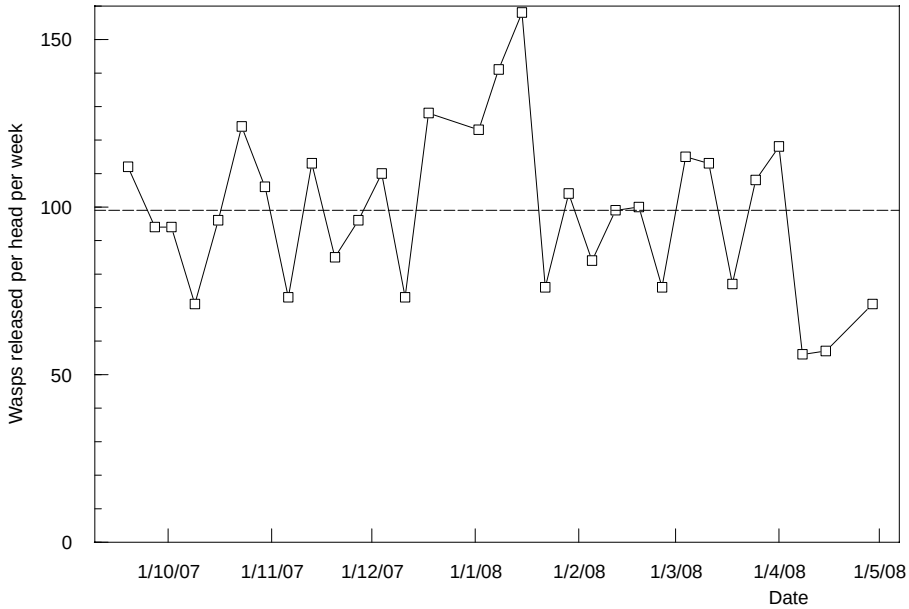

Figure 18: The numbers of wasps per head of cattle released weekly in feedlot A

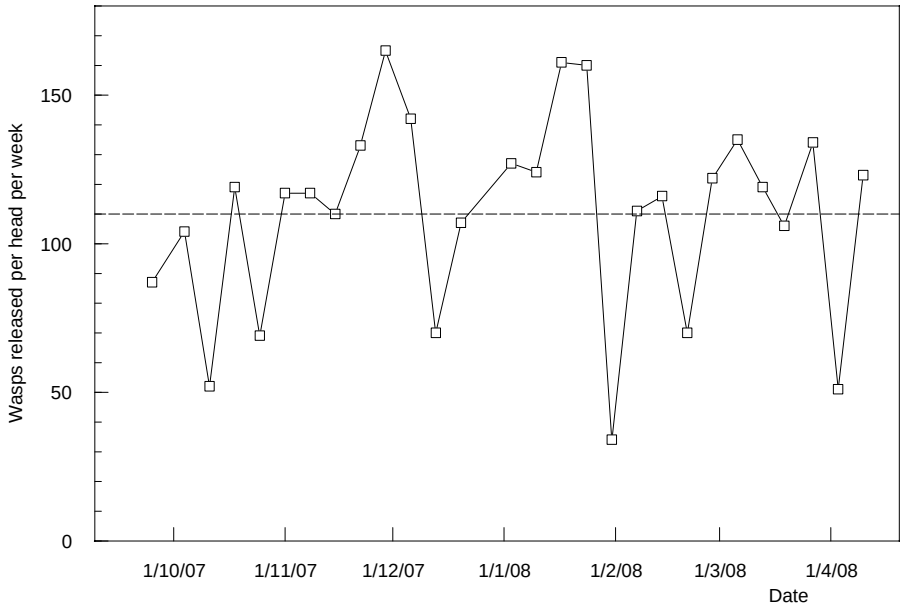

Figure 19: The number of wasps per head of cattle released weekly in feedlot D

The regular collection of pupae from all feedlots and the subsequent determination of their parasitism rates and identification of parasitoids and pupae were used to assess the impact of releasing wasps into cattle feedlots. The mean monthly fly and wasp emergence and non-emergence from feedlot pupae in the Brisbane Valley and Warwick area are given in Tables 18 and 19 respectively. In both areas there was a control feedlot with no releases (feedlots C and F) and one feedlot with *S. endius* releases (A and D).

Table 18: Percent emergence of flies and wasps and non-emergence from pupae collected on Brisbane Valley feedlots A (releases of *S. endius* wasps) and C (no releases)

Year	Month	Fly emergence (%)		Wasp emergence (%)		Non-emergence (%)	
		A	C	A	C	A	C
2007	Sep	40.4	52.4	14.0	4.3	46.0	43.3
	Oct	38.4^{A}	61.2^{B}	22.3^{A}	8.4^{B}	39.2	30.3
	Nov	30.4	16.8	7.0	10.8	62.6	72.2
2008	Dec	28.5^{A}	6.7^{B}	11.4	6.3	60.1^{A}	87.0^{B}
	Jan	15.4	13.6	21.3^{A}	11.5^{B}	63.1	74.9
	Feb	19.5	5.4	4.5	2.7	76.0	91.9
	Mar	22.4	20.2	25.9	10.6	51.8	69.2
	Apr	27.0	37.7	32.1^{A}	3.8^{B}	40.9	58.5
2007/08		**30.5**	**35.5**	**18.0^{A}**	**8.1^{B}**	**51.5**	**56.3**

Values with superscripts differ significantly (P<0.05) within rows and variable

Table 19: Percent emergence of flies and wasps and non-emergence from pupae collected on Warwick shire feedlots D (releases of *S. endius* wasps) and F (no releases)

Year	Month	Fly emergence (%)		Wasp emergence (%)		Non-emergence (%)	
		D	F	D	F	D	F
2007	Sep#	47.4	0.1	0.0	0.0	52.6	99.9
	Oct	48.3	57.9	7.3	7.2	44.3	34.8
	Nov	33.9	50.1	12.3	9.9	53.6	40.0
2008	Dec	29.8	29.2	10.3	11.0	59.9	59.9
	Jan	26.9	42.2	6.0	18.4	67.2	39.5
	Feb	51.1	5.4	10.2	10.8	38.6	83.8
	Mar	48.2	52.2	18.5	26.1	33.3	21.7
	Apr	39.0	34.2	31.2	16.7	29.8	49.1
2007/08		**39.0**	**45.9**	**10.8**	**10.6**	**50.1**	**43.6**

Values with superscripts differ significantly (P<0.05) within rows and variable
Small number of pupae

In the Brisbane Valley the overall parasitism rate in all pupae in 2007/2008 was significantly higher in feedlot A (18.0%) than in the control feedlot C (8.1%). Overall fly emergence was higher in the control feedlot than the treated feedlot but this difference was not significant (Table 18). A comparison of parasitism over the duration of the trial indicated increased parasitism in the treated feedlot A except for November 2007. Percent parasitism showed increased divergence from the control feedlot in March and April 2008 (Fig. 20) in the face of falling numbers of *S. endius* released into the feedlot in April 2008. Overall parasitism in house and stable fly pupae (Table 20) is following similar trends with parasitism higher in *Stomoxys calcitrans* than *Musca domestica*. No parasitoids were recovered from small numbers of *Physiphora clausa* pupae collected in the Brisbane Valley feedlots.

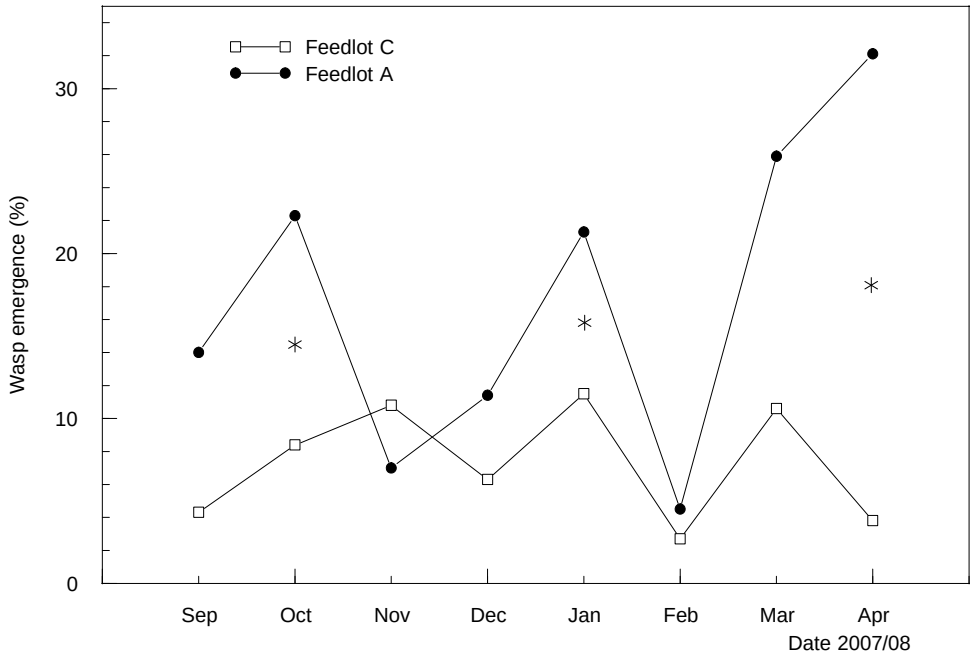

Figure 20: Wasp emergence from all pupae collected in feedlots A and C from September 2007 to April 2008 (* values differ significantly P<0.05)

Table 20: Percent parasitism of three species of pupae collected in feedlots A, C, D and F from September 2007 to April 2008

Host species of pupae	Overall percent parasitism of 3 species of pupae			
	Feedlot A	Feedlot C	Feedlot D	Feedlot F
M. domestica	17.4[A]	7.6[B]	10.1[A]	2.2[B]
S. calcitrans	24.7	12.8	15.5	16.4
P. clausa	[#]	[#]	19.1	18.6

Values with superscripts differ significantly (P<0.05) within A and C and D and F
[#] Very few pupae collected largely from C and none were parasitised.

In the Warwick trials, overall wasp emergence was similar in the two feedlots (Table 19). Non-emergence was 15% higher and consequently fly emergence 15% lower in the IPM feedlot (D). The higher non-emergence could have been due to the feeding activity of the released wasps. Both feedlots (Fig. 21) had initially low parasitism rates (7%) in October 2007 but by April 2008 the treated feedlot (D) had almost twice the rate of the control feedlot (F). *P. clausa,* normally found at levels lower in the manure profile than the two major feedlot species, showed overall increased parasitism.

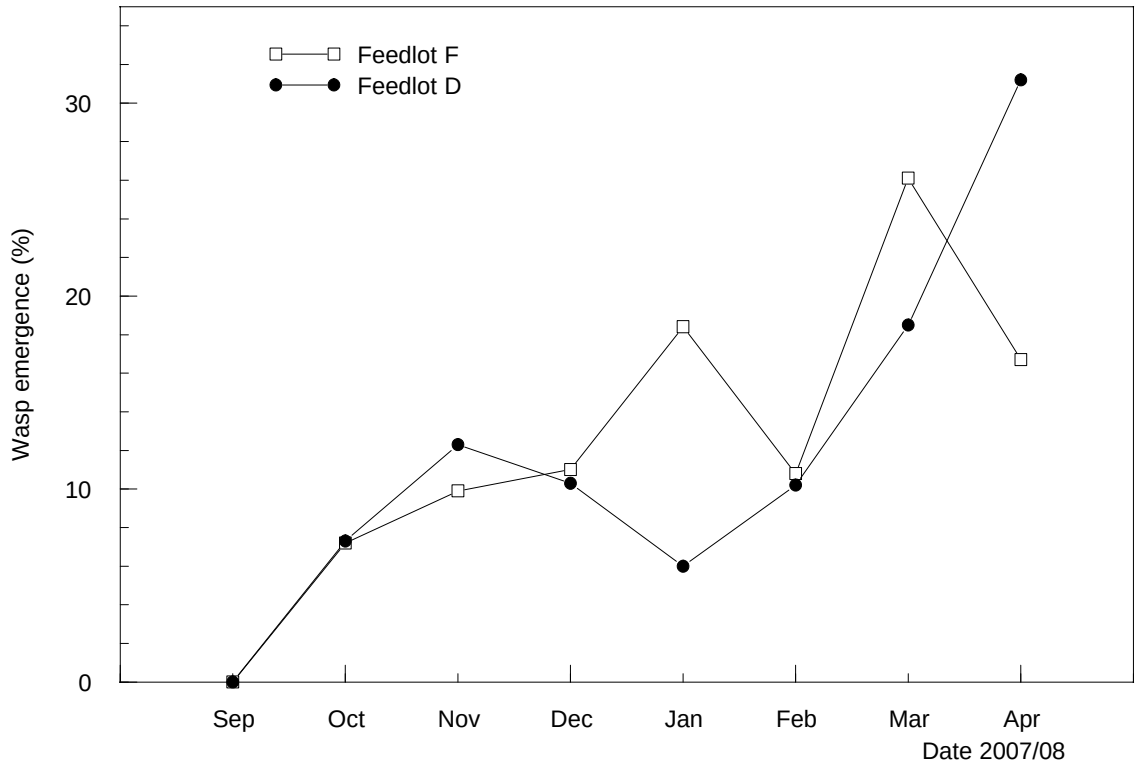

Figure 21: Wasp emergence from all pupae collected in feedlots D and F from September 2007 to April 2008

An analysis of combined data from both trial areas indicated that parasitism was significantly higher in the IPM feedlots (14.7%) than the control feedlots (9.0%). The release of one parasitic species did increase the parasitism rate compared to the controls.

The impact of *S. endius* releases was also assessed by comparing the emergence of *S. endius* (Fig. 22). In the Brisbane Valley significantly more *S. endius* emerged from pupae collected on the IPM feedlot (9.2%) than the control feedlot (4.8%). Apart from November 2007, parasitism by *S. endius* was increased in the IPM feedlot pupae and by April 2008 there was 19% and 1.3% parasitism by *S. endius* in treated and control feedlot pupae respectively (Fig. 22). In the Warwick trial, overall parasitism by *S. endius* was also higher in the treated than control feedlot (8.1% and 6.9% respectively; difference not significant). From September to December 2007

both feedlots showed similar levels of parasitism by *S. endius*. The levels of parasitism by *S. endius* in the treated feedlot subsequently declined below that of the control feedlot until March 2008. In April 2008 parasitism by *S. endius* in the IPM feedlot was more than twice that of the control feedlot (Fig. 22). Across both trials and season, *S. endius* emergence was significantly higher in IPM than control feedlots (8.7% and 5.6% respectively).

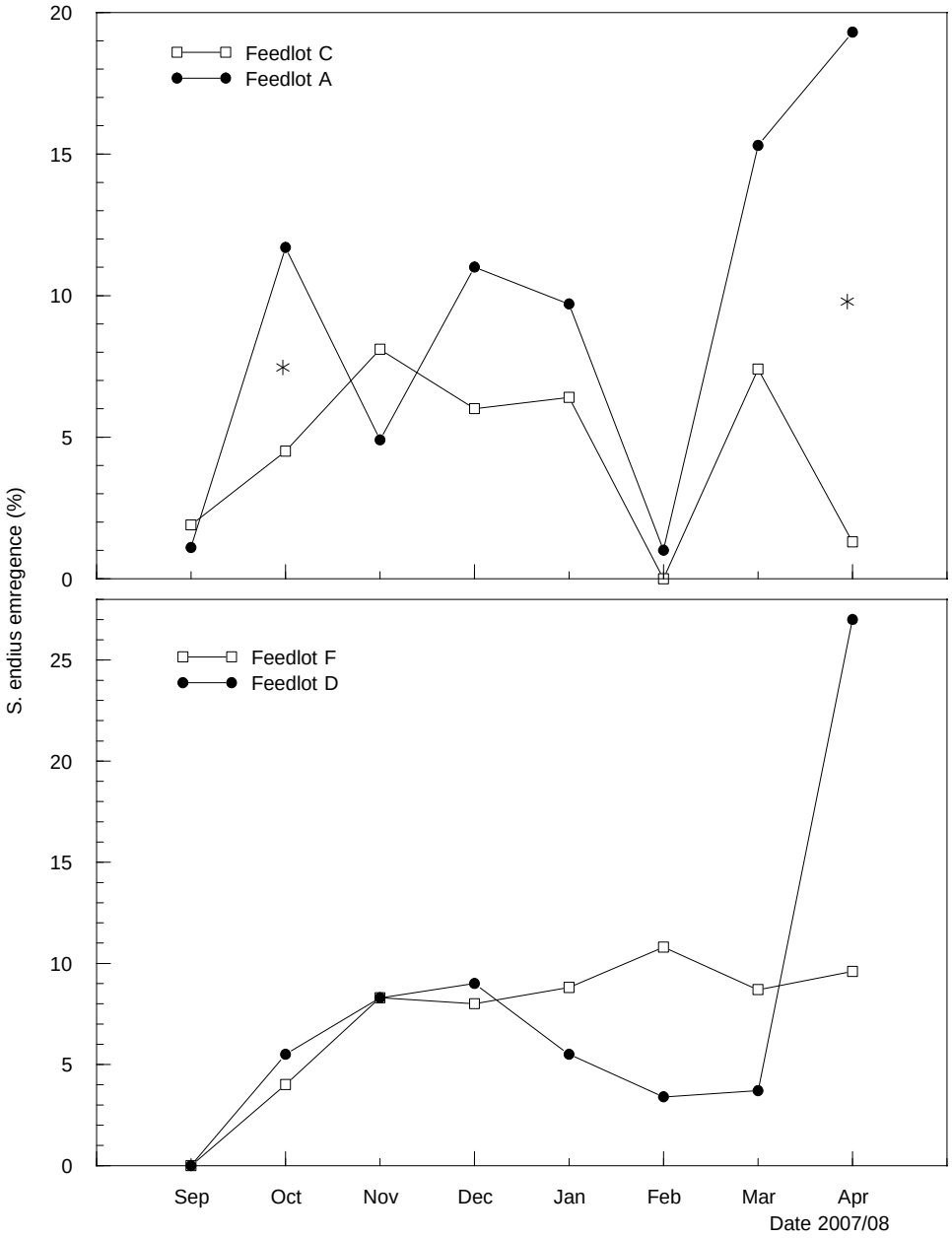

Figure 22: Percent *S. endius* emergence from pupae collected on feedlots A and C (top) and D and F (lower) from September 2007 to April 2008 (* values differ significantly P<0.05)

The other species of wasps emerging from the feedlot pupae were also examined and overall composition of the parasitoid complex is given in Table 21. *S. endius* was the predominant species in all feedlots, accounting for 55-75% of all wasps. In both Brisbane Valley feedlots, the proportion of *S. endius* has increased from the previous observations (MS 3 & 7), however the release of wasps on the IPM feedlot (A) has not further increased the proportion of *S. endius*. Possibly due to previous releases of *S. endius* on the Warwick feedlots, proportions of *S. endius* were similar to previous observations (MS 7) however with the treated feedlot showing an increase (11%) in *S. endius* over the control (Table 21). Parasitism by *Muscidifurax raptor* was

higher in the Brisbane Valley than Warwick feedlots (in line with previous findings, see MS 7). Small numbers of *Trichomalopsis*, a gregarious species, were present in the Brisbane Valley feedlots only.

Table 21: Overall composition of wasp species from all pupae collected on feedlots in the Brisbane Valley (A, C) and Warwick area (D, F)

Species	Wasp species (% of total wasps)[#] on feedlot			
	A	C	D	F
S. endius	54.6	56.4	75.0	67.4
S. cameroni	17.1	17.4	4.7^{A}	11.7^{B}
S. nigroaenea	6.6	6.7	14.4	12.0
M. raptor	21.4	19.1	5.9	8.8
Trichomalopsis sp.	0.3	0.3	0.0	0.0

[#] Average over 2007/08; Values with superscripts differ significantly (P<0.05) within A and C and D and F

The impact of *S. endius* was also examined for *M. domestica* and *S. calcitrans* separately (Tables 22 & 23). As might be expected, in the Brisbane Valley feedlots where house flies accounted for >90% of the population, *S. endius* was the dominant species and mirrored the levels seen in Table 12. *S. endius* was also the dominant species parasitising stable flies in both feedlots. *M. raptor* appears to prefer house fly over stable fly pupae.

Table 22: Percent wasp species of all wasps parasitising house and stable fly pupae in the Brisbane Valley feedlots

Wasp species	Species composition (%) Host pupae[#]			
	M. domestica		*S. calcitrans*	
	Feedlot A	Feedlot C	Feedlot A	Feedlot C
S. endius	54.8	53.0	58.3	66.0
S. cameroni	16.0	18.8	26.5	22.0
S. nigroaenea	6.7	5.4	7.6	4.3
M. raptor	22.1	22.8	7.6	5.5

[#] very few pupae of *Physiphora clausa* collected; no sig differences

Table 23: Percent wasp species of all wasps parasitising house and stable fly pupae in the Warwick feedlots

Wasp species	Species composition (%) Host pupae					
	M. domestica		*S. calcitrans*		*P. clausa*	
	D	F	D	F	D	F
S. endius	76.6	71.2	84.7	73.3	62.9	32.5
S. cameroni	4.0	2.4	0^{B}	13.5^{A}	11.4	4.1
S. nigroaenea	11.8	6.4	17.7	6.4	25.8	68.1
M. raptor	7.6^{B}	26.6^{A}	0^{B}	6.8^{A}	0	0

Values with different superscripts within row and variable differ significantly (P<0.05)

In the Warwick feedlots, the pupae collected were predominately house fly (86%) in feedlot D and stable fly in feedlot F (51%). The Otitid *P. clausa* was also present on feedlots D (3%) and F (9%). *S. endius* was the dominant species on both feedlots (Table 23) with increased levels of *S. endius* in all three species of pupae in the treated feedlot (D). *M. raptor* again appeared to prefer house fly pupae, while *S. nigroaenea* was a dominant species parasitising *P. clausa* in the control feedlot.

Pupae were collected from two main areas of the feedlots. Previous studies had indicated that accumulated manure under the fence lines and along the edges of the sedimentation ponds contained most of the fly breeding population. Pupae were generally collected separately from

these two principal sites; however on occasions when numbers of pupae were low they were combined. The overall percent parasitism and percent parasitism by *S. endius*, obtained from all pupae in the two areas is shown in Table 24. In the Brisbane Valley most of the wasps in the sedimentation pond were *S. endius* in both feedlots A and C but there was increased *S .endius* in the sedimentation pond compared to the fence lines in A but not C. Generally there was increased *S. endius* and overall parasitism in feedlot A compared to C. As shown previously, there was an increase in *S. endius* and all wasp species, particularly in March and April 2008 in A.

Table 24: Percent parasitism by *S. endius* and all species of wasps from all pupae collected from two areas of the Brisbane Valley and Warwick feedlots

Feedlot	Fence line		Sedimentation pond		Total average	
	S. endius	All wasps	*S. endius*	All wasps	*S. endius*	All wasps
A	7.2	14.6	14.5	15.9	9.5	18.3
C	6.1	10.3	5.4	6.2	4.9	8.1
D	6.8	9.3	6.9	9.3	7.8	10.6
F	7.9	10.6	4.8	11.1	7.1	11.1

In the control Warwick feedlot (F) there was increased *S. endius* under the fence line (7.9%) compared with the sedimentation pond (4.8%) while for the treated feedlot (D) the areas were similar. This may simply reflect the suitability of both areas in D compared with F or an increased activity due to released *S. endius*.

Summary

Weekly releases of *S. endius* produced by Bugs for Bugs, commenced on control and treated Brisbane Valley and Warwick feedlots in September 2007 and continued until April 2008. The target release rate of 100 wasps per head was generally achieved over the trial period. Assessment of the impact of these releases was achieved by the regular collection of pupae from all feedlots and the subsequent determination of parasitism rates and identification of parasitoids and pupae.

In the Brisbane Valley feedlots, the overall parasitism rate from all pupae was significantly higher in the treated feedlot A (18.0%) than in the control feedlot C (8.1%). Emergence of *S. endius* was also significantly higher in the IPM feedlot compared to the control feedlot (9.2% and 4.8% respectively, a 92% increase). Fly emergence was consequently lower in the treated feedlot. Parasitoid complexes were similar on both feedlots with an overall average of 80% *Spalangia*, 20% *Muscidifurax* and small numbers of *Trichomalopsis* emerging from predominately *M. domestica* pupae. *S. endius* was the most common species of *Spalangia* (55% of all wasps). In the Brisbane Valley, overall there was ample evidence of increased parasitism in both house and stable fly pupae due to released *S. endius*.

In the Warwick shire, the overall parasitism rate from all pupae was similar in the IPM and control feedlots, however fly emergence was 15% lower in the IPM feedlot. Both feedlots had initially low parasitism rates but by April 2008 the IPM feedlot had twice the parasitism rate of the control feedlot. Parasitism by *S. endius* was also increased in the IPM (8.1%) compared to the control feedlot (7.1%). Parasitoid complexes had higher *Spalangia* percentage (93%) and lower *Muscidifurax* (7%) than the Brisbane Valley feedlots and again *S. endius* was the predominant species of *Spalangia* (67-75%) emerging from predominantly *M. domestica* pupae on the IPM and *S. calcitrans* on the control feedlot. Overall the percent *S. endius* was significantly increased on the treated feedlot (75%) compared with the control feedlot (65%).

Analysis of the combined trials demonstrated that the percent of parasitism by *S. endius* and all wasps was significantly increased by the release of mass produced *S. endius*. It can be concluded that such releases are a useful tool for controlling nuisance flies on feedlots.

4.3.8 Fungal biopesticides

The new fungal formulation, modified from that used in field trials during the previous fly season (2006-2007), was sprayed onto vegetation and the front of feed bunks in Feedlots A and D. Spraying commenced in both feedlots during early October when fly numbers started to become noticeable. After an initial 6 weeks of spraying weekly, Feedlot A was sprayed another five times while Feedlot D was sprayed another two times in response to high fly numbers.

In both treated feedlots there was a significant increase ($P<0.001$) in the day 7 mortality of flies netted after spraying (post spray) (Fig. 23 & 24) compared to the flies netted either before spraying (pre spray) or in the control feedlots. Correspondingly, *Metarhizium* was isolated from a significantly higher percentage ($P<0.001$) of flies netted after spraying than from either the control flies or flies netted before spraying (Fig. 25 & 26) in both the Brisbane Valley and Warwick shire.

In Brisbane Valley 79(±1.6)% of flies died within 7 days when netted after spraying compared to 21(±1.1)% in the control feedlot (Fig. 23). In the Warwick shire 74(±2.4)% of flies died within 7 days when netted after spraying compared to 23(±2.3)% in the control feedlot (Fig. 24). Although the 7 day mortality of flies netted pre spraying was slightly higher than the corresponding control mortalities in the Brisbane Valley (24±1.6%) and the Warwick shire (28±2.2%) the difference was not statistically significant ($P>0.05$). However when the data from two seasons of spraying in the Brisbane Valley feedlots are combined (Table 25) the difference between the mortalities in flies netted pre spray and those netted in the control feedlot are statistically significant ($P<0.05$). Thus overall there is evidence that flies are dying from both the direct uptake of spores when hit by the fungal spray and the indirect uptake of spores from the sprayed vegetation. The decrease in the effectiveness of indirect spore uptake during the 2007-2008 season may have been due to the difference in the formulation or weather patterns. The 2007-2008 season was wetter with more storms. These storms, in the first days after spraying, may have washed much of the formulation and spores from the vegetation. This conclusion would be supported by the results obtained from the investigations into the viability of the spores sprayed onto vegetation carried out at ARI.

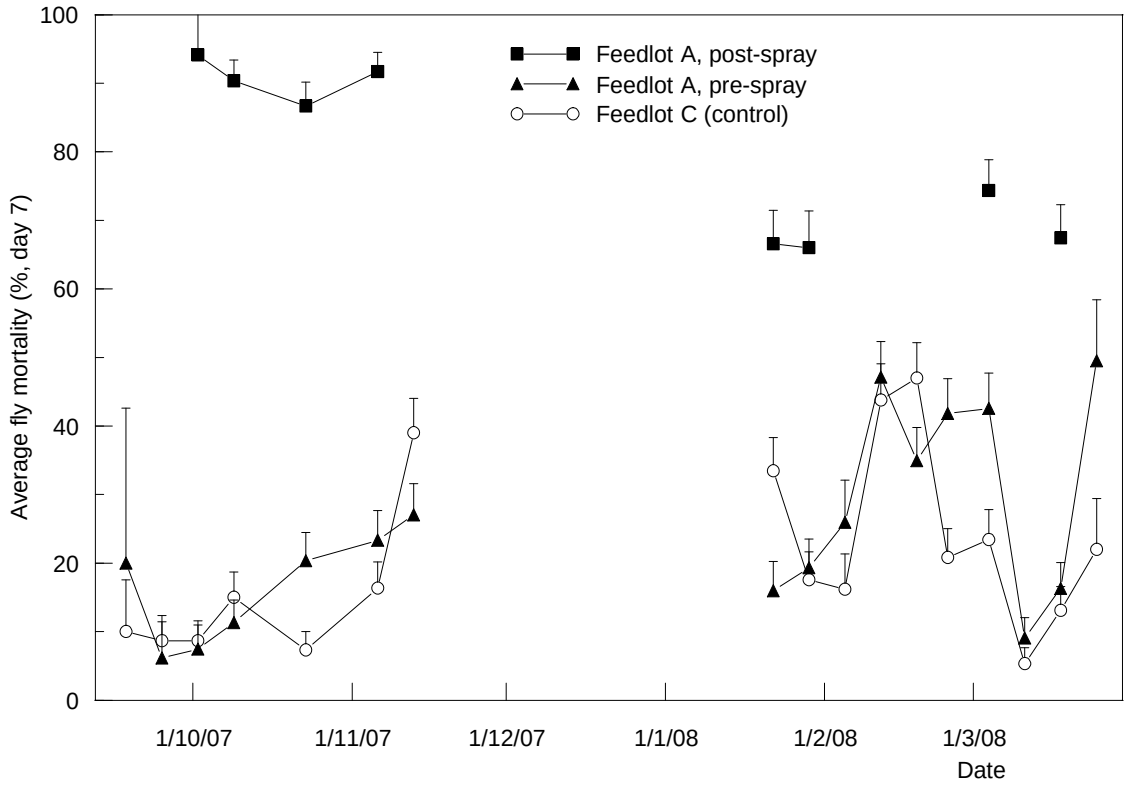

Figure 23: Average (+se) percent corrected mortality in flies netted in the Brisbane Valley from Feedlot C (control) and Feedlot A (pre-spray and post-spray) after 7 days laboratory incubation

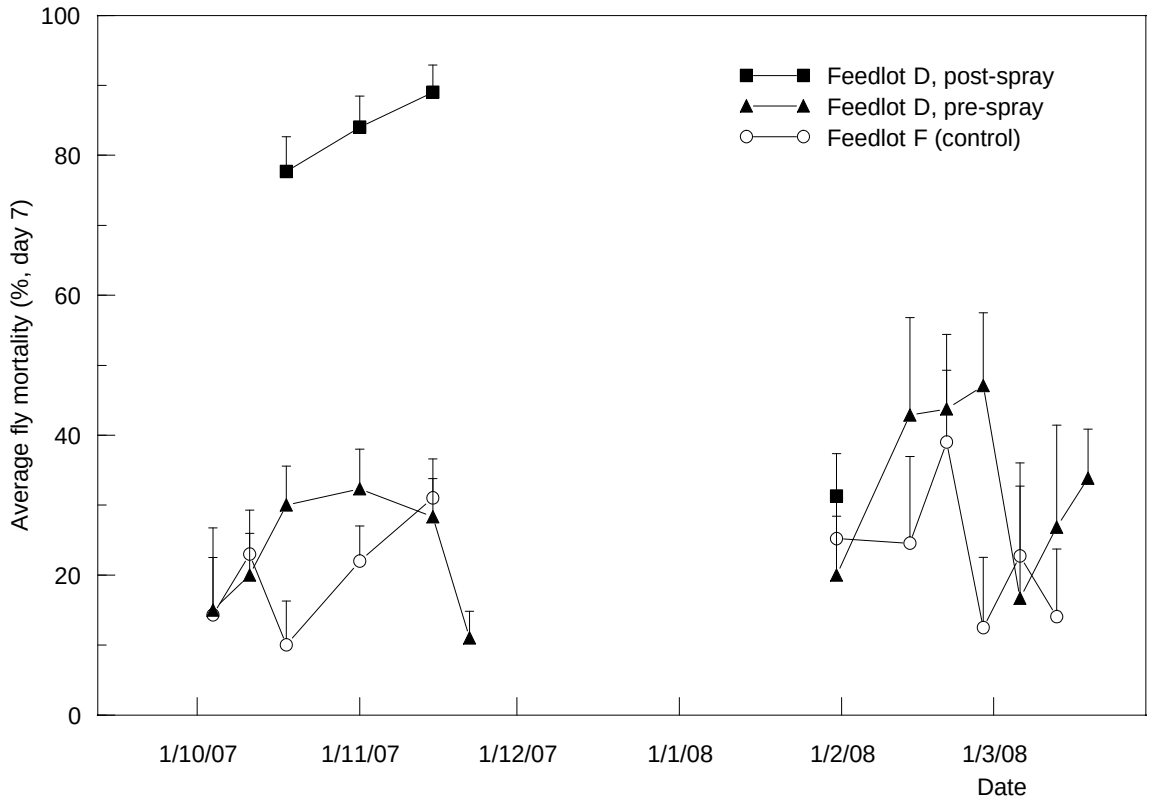

Figure 24: Average percent corrected mortality in flies netted in Warwick from feedlot F (control) and feedlot D (pre-spray and post-spray) after 7 days laboratory incubation

Table 25: Average percent mortality in netted flies and percent flies infected with *Metarhizium* in Brisbane Valley feedlots during 2006-2007 and 2007-2008

Feedlot Sample	Av % Mortality in netted flies (7d)	% dead flies with *Metarhizium*
A - post	76.9 (±1.0) [a]	77.0 (±1.7) [a]
A - pre	27.9 (±0.7) [b]	19.8 (±1.2) [b]
C	20.7 (±0.6) [c]	5.8 ((±1.7) [c]

Value with different superscripts are significantly different (P<0.05)

Significantly higher levels (P<0.05) of *Metarhizium* were isolated from the flies netted after spraying in the Brisbane Valley (72±2.1%) and the Warwick shire (80±3.4%) compared to those netted in the respective control feedlots (9±1.7% and 5±1.7%) (Fig. 25 and 26). The level of *Metarhizium* isolated from flies netted pre spraying was also significantly higher (P<0.05) for the treated feedlots in Brisbane Valley (26±2.5 %) and the Warwick shire (13±3.4%) than the respective control feedlots. This indicated that *Metarhizium* was being taken up by flies through direct application when sprayed and indirectly through the subsequent uptake of spores applied to the vegetation.

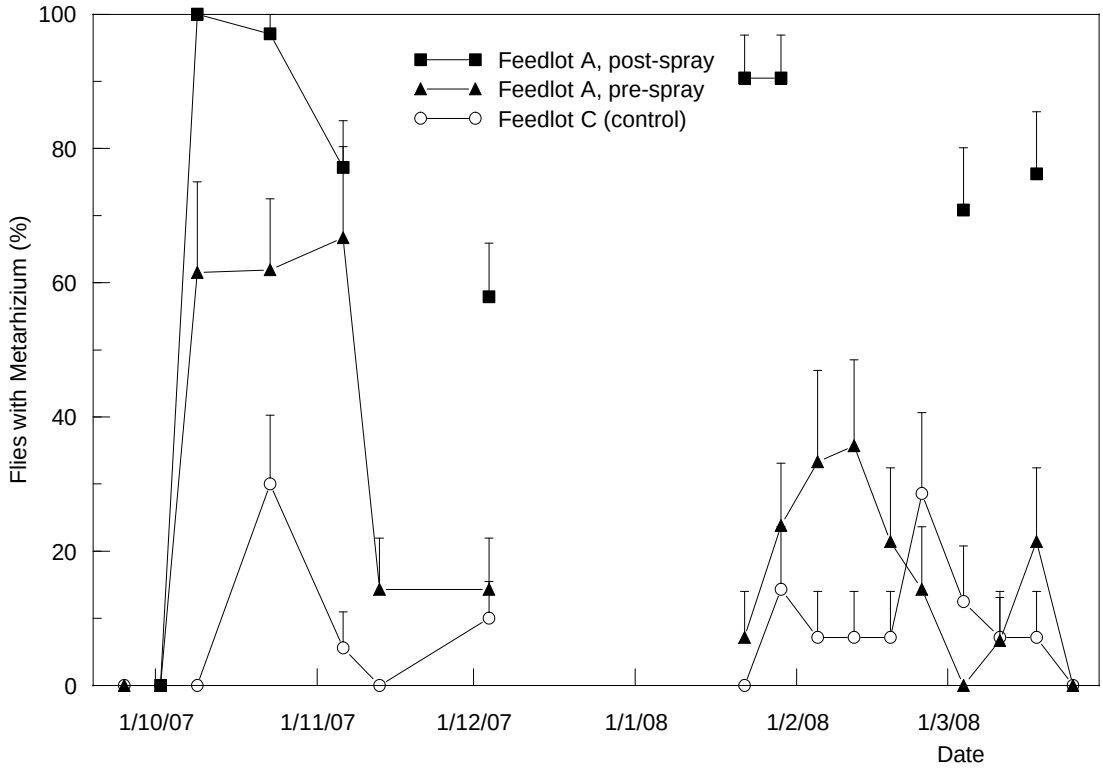

Figure 25: Percentage of flies netted in Feedlot C (control) and Feedlot A (pre-spray and post-spray) infected with *Metarhizium*

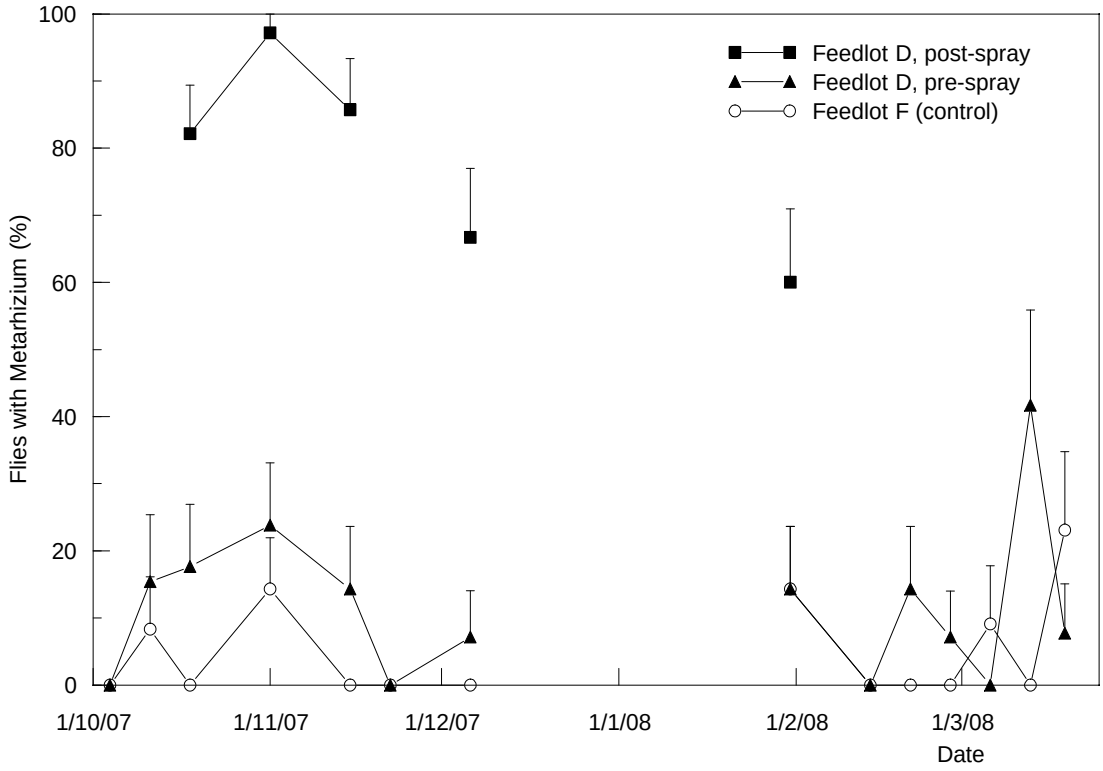

Figure 26: Percentage of flies netted in Feedlot F (control) and Feedlot D (pre-spray and post-spray) infected with *Metarhizium*

An assessment of the efficacy of baits through analysis of netted fly mortalities and subsequent *Metarhizium* isolation gave positive results. The mortality in flies netted in the Brisbane Valley in Feedlot A (36.6 ± 2.2%) was significantly higher (P = 0.02) than in Feedlot C (26.2 ±1.7%). Although the mortality of flies netted in Feedlot D was also higher (35.8 ± 7.3%) than those from Feedlot F (24.7 ± 6.9%), this difference was not statistically significant (P=0.384). The level of *Metarhizium* infection in flies in Feedlot A (22.3 ± 4.4%) was not significantly higher (P = 0.167) than the level of *Metarhizium* infection recorded for Feedlot C (12.5 ± 3.8%). However in the Warwick shire *Metarhizium* infection was only found in flies from the treated feedlot (33.2± 1.3%). The analysis was based on data from only a small number of samples, five in the Brisbane Valley and three in the Warwick shire. This limited data set restricted the power of the t-test and ability to statistically prove any difference in fly mortality and *Metarhizium* infection due to the fungal baits.

During the 2007-2008 fly season it was demonstrated that the new fungal formulation sprayed in the two IPM feedlots A and D was taken up by the fly population and killed flies. Evidence from two seasons of spray trials showed that the direct up take of fungal spores consistently killed large numbers of flies. In addition the combined data from two seasons of trials in the Brisbane Valley showed flies are also dying from the uptake of spores deposited on the vegetation. The prototype fungal baits used later in the 2007-2008 fly season appeared to increase the mortality of netted flies with a corresponding increase in *Metarhizium* infection, however due to limited sampling the statistical evidence was not completely unambiguous.

5 Success in Achieving Objectives

The project objectives were to demonstrate and quantify the effect of a) focused chemical application and cleaning measures, b) augmentative releases of cultured parasitic wasps, c) fungal biopesticides and d) integrated nuisance fly control on feedlot fly breeding activities and/or populations and to develop integrated fly management programs, which can be utilised by feedlot

operators. All objectives were addressed in this project and the results from the research provided the basis for the development of two new biological tools for fly control in Australia, the design of an IPM fly program, information in feedlot industry tips & tools and recommendations to the industry on fly control.

We have quantified the impact of fence line cleaning at different intervals on fly breeding. This demonstrated that short fence line cleaning intervals, fortnightly or weekly, can reduce fly breeding by up to 84%. Such frequent cleaning does not need to be extended to areas with low fly breeding potential (e.g. cattle pens) but can be restricted to the relatively small major breeding areas.

The release of mass-reared parasitic wasps to augment natural populations in feedlots increased parasitism of fly pupae, thus assisting in fly control. Production of wasps by the commercial company Bugs for Bugs was scaled up to meet demands for feedlot trials and these wasps are now commercially available. Quality control checks for the wasp production were put into place and conducted during this project. Recommendations for the applications of the wasps have been provided.

The development of a fungal biopesticide as a novel tool for fly control progressed. Fungal spores were formulated for spray and bait applications in feedlots and these formulations were assessed in laboratory and feedlot experiments. It was demonstrated that lethal doses of fungal spores were taken up by flies contacting the freshly sprayed formulations. It was also shown that the lethal effect of the formulations can continue in the days post spraying as spores are taken up by flies resting on sprayed surfaces. Recommendations have been made to advance these formulations to commercial products.

The principal objective was to develop guidelines for integrated fly management strategies in cattle feedlots. Such guidelines were derived from the results obtained in this project, specialist knowledge in the research team and publications from other experts in this field. The guidelines are presented as elements of an integrated pest management (IPM) for nuisance flies on cattle feedlots. The guidelines are presented in this report (in Conclusions and Recommendations) and, somewhat abridged, in extension material for industry use (MLA tips & tools). A second tips & tools brochure, containing information on nuisance flies (life cycle, identification, natural enemies) which is relevant to the feedlot industry, is also available.

All project objectives were completely met. Experimental work in commercial feedlots, laboratory investigations and subsequent processing, compiling and interpretation of project data provided the information to fill knowledge gaps and answer critical questions. The information on the impact of control measures on nuisance fly populations and their natural enemies in Australia was greatly increased. Guidelines for integrated fly control in cattle feedlots were developed on the basis of new data and new tools. By adoption of these guidelines, the Australian feedlot industry is now in a better position to manage nuisance fly problems.

6 Impact on Meat and Livestock Industry – now & in five years time

The feedlot industry has applied a significant amount of effort to improved manure management practices over the past decade as a means of reducing odour emissions and fly problems. There is, however, evidence that fly populations remain a serious problem at many feedlot sites despite the improved manure management practices. Insecticide resistance and a desire to minimise the use of chemicals also drive the need to move to a more integrated approach to fly control.

This project has delivered results on the efficacy of existing and new control tools on nuisance fly populations in cattle feedlots. We have provided guidelines for integrated fly control in cattle feedlots and relevant information on life cycle and identification of nuisance flies.

Adoption of the integrated fly control strategy by the feedlot industry will lead to improved control of nuisance flies on feedlots. This strategy integrates cultural (mechanical/physical), biological and chemical methods to optimise fly control. The second publication on nuisance flies and their natural enemies increases the feedlot operators' knowledge and understanding of nuisance flies and their habitat. It enables them to make more informed decisions and thus to improve their fly control program. The project has thus delivered relevant information and guidelines for immediate implementation of integrated fly control on cattle feedlots.

Improved feedlot fly control will deliver a suite of benefits to industry. Lower fly populations in the feedlot will lead to increased production gains resulting from a reduction in biting and annoyance of cattle by flies. It also assists in avoiding animal welfare issues, resulting from undesirably high fly populations on intensively farmed cattle. Working conditions for feedlot employees will improve and transmission of diseases decrease with lower fly numbers. The risk of complaints from neighbouring landholders is also reduced by effective fly control. This results in better community relations and generally enhances the image of the industry.

The integrated fly management strategy minimises the use of insecticides while taking full advantage of cultural and biological control methods. Lower insecticide usage minimises the risk of residues in beef products and the environment, thus enhancing the clean-and-green image of the Australian feedlot industry and the beef industry, as a whole. Furthermore, the detrimental impact of insecticides on beneficial insects is reduced, workplace health and safety standards in the feedlot are improved and the costs for purchase and application of insecticides are lowered.

The project work also demonstrated the efficacy of new biological tools for controlling nuisance flies on Australian cattle feedlots. Parasitic wasps and entomopathogenic fungi, two biological control agents which are naturally present in the feedlot ecosystem, either play an important role, or have the potential to do so, in reducing fly populations. We have demonstrated that augmentation of populations of both these natural agents can increase fly control. Parasitic wasps are now commercially available in Australia for biological fly control and they could become a common industry tool as they are in the USA. Fungal biopesticides, which were also shown to be effective in fly control, have to be registered with the APVMA prior to their use for fly control. The availability of both agents would enable industry to further improve fly control and reduce the use of insecticides.

The reduction of fly breeding sites through improved feedlot sanitation was also shown to be a critical component of an IPM strategy. It was recommended that frequent cleaning of major fly breeding areas be carried out. Such cleaning will also reduce odour emission.

The project has delivered to industry two new biological tools, quantitative information on fly control and guidelines for integrated fly management. The implementation of the findings and recommendations of this project will enable the feedlot industry to make major advances in the management of nuisance flies, leading to a wide range of ensuing benefits for industry and the wider community.

7 Conclusions and Recommendations

7.1 Major project results

This project embraced many aspects of research and development with the aim of providing improved control of nuisance flies in cattle feedlots, including the development of new tools, their

implementation in feedlot fly control and assessments of the efficacy of new and old tools. This was achieved by laboratory work, bioassays under controlled conditions and applications in commercial feedlots. Feedlots in two geographical and climatic distinct areas in south-east Queensland, the Brisbane Valley and the Warwick shire, were used for field work. A brief summary of the major results is provided in this section.

7.1.1 General fly control tools

We demonstrated that frequent cleaning of fence lines can effectively reduce fly breeding. Compared with a 3-monthly cleaning interval, monthly, fortnightly and weekly cleaning of fence lines reduced the numbers of fly pupae by 55%, 67% and 84% respectively. These substantial reductions in fly breeding were achieved through removal of manure accumulated under the fence without the need for a simultaneous cleaning of the whole pen where fly breeding is minimal.

Two trials using the larvicide cyromazine under fence lines were carried out, one on recently cleaned fence lines and one on lines with manure accumulation. Although deformed non-viable pupae were found in the manure in both trials, a reduction in immature and adult flies was observed only when fence lines had been recently cleaned. Larvicidal treatments are more effective when used in conjunction with good sanitation. The cyromazine treatment did not reduce the rates of wasp parasitism.

Spraying of an adulticide (cyfluthrin) on feedlot structures had a small and short-lived effect on stable fly populations but no effect on house flies.

7.1.2 Parasitic wasps

One of the most common parasitic wasps on Australian feedlots, *Spalangia endius,* was selected for mass production and augmentative releases to improve control of fly populations in cattle feedlots. This wasp is one of several species used in the USA for the same purpose. A laboratory colony was started from wasps collected on a Queensland feedlot. During this project wasps produced in the colony have been extensively characterised and rearing techniques assessed, including:

- Suitability of fly host species, which included house fly, stable fly, blowflies and buffalo fly.
- Longevity at different temperatures and food regimes
- Optimal age of fly pupae for parasitism
- Optimal fly pupae to wasp ratio for mass production
- Method of presentation of fly pupae to wasps for parasitising
- Suitability of frozen or heat killed pupae as hosts
- Cool storage of parasitised fly pupae to program wasp emergence

Wasps from the laboratory colony were transferred to the commercial partner (Bugs for Bugs, Mundubbera) which has set up a large scale culture using house flies as the host. Bugs for Bugs has supplied *S. endius* wasps since December 2005 for field evaluation. A quality control scheme to assess wasp emergence and contamination of the colony with unwanted wasps was carried out by DPI&F. The capacity of mass produced wasps to parasitise fly pupae was equal to wasps from the laboratory colony.

Wasp release containers for field work were devised and constructed. The purpose of the containers was to optimise the release of wasps from parasitised pupae placed in the field. The containers protect the pupae from the sun and predators such as birds and ants. They were constructed from sturdy PVC pipes containing multiple meshed windows and were attached to fence posts. They were filled with a mixture of parasitised pupae and vermiculite.

In the initial field trial (2005/06) individual fence line segments where wasps were released were compared with similar segments where no releases were made. Wasp emergence from pupae collected along these segments was the same in release and control segments but fewer flies emerged from the segments where wasps were released. There was also a change in the trend of *S. endius* populations in the segments where the wasps were released indicating an increase due to the released wasps.

In two trials with wasp releases over the entire feedlot (2006/07), there was an increase in the parasitism rate in the feedlot with releases when compared to the control feedlot at one of the localities but not at the other. However, in the second trial there was an increase over time in the parasitism rate, suggesting a positive impact of the released wasps. The percentage of *S. endius* in the wasp population was much higher in the feedlots with wasp releases. With the exception of stable flies in one trial, reductions in the adult fly populations from wasp releases could not be demonstrated by our monitoring methods. The number of wasps released in these trials was below the desirable release rate for most of the trials.

7.1.3 Fungal biopesticides

Fungal biopesticides are novel biological tools with potential for use in nuisance fly control. It has been demonstrated by several groups that entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* can selectively kill insect pests. There are currently four registered *Metarhizium* based products used to control locusts, grasshoppers, pasture grubs and cane grubs in Australia. However, no work on the application of fungi to control nuisance flies had been reported at the start of this work.

A range of *Metarhizium* and *Beauveria* isolates, including some isolated from flies collected on feedlots, were screened for growth at various temperatures up to 35°C to select isolates capable of growing at the elevated temperatures expected in the feedlot environment. *Metarhizium* isolates were also characterised by DNA analysis.

The efficacy of twenty-three *Metarhizium* and eight *Beauveria* isolates against adult house flies was determined. Fly mortality was high (typically 80-100%) for many isolates and those selected for further investigations also produced high spore yields in culture.

A spore production facility was set up at the DPI&F Yeerongpilly laboratory to produce up to 2 kg of spores for testing.

A range of investigations with selected isolates was conducted using bioassays with adult house flies, including:

- Spores mixed with sugar (no avoidance)
- Optimal spore levels and length of exposure for high fly mortality
- Combination of *Metarhizium* and *Beauveria* (no benefit)
- Direct (spray flies) versus indirect (spores on surfaces) uptake of spores
- Comparisons of efficacy of formulated spore isolates

The efficacy of fungal spores against immature flies (larvae) was also investigated. Although larval mortality was observed in some assays, the results were inconsistent. The best performing spore isolates were not the same as for adult flies. It appeared that spores did not effectively adhere to 3rd instar larvae (the stage before pupation).

In an initial feedlot trial in 2006-2007, using several fungal bait stations containing a mixture of sugar and *Metarhizium* no differences in fly mortality or *Metarhizium* infection in netted flies were observed. It was possible that too few flies were attracted to these baits stations for any fungal spore uptake to be detected through random fly netting.

To maximise their uptake by flies in the feedlot, the fungal spores needed to be formulated and applied to areas where flies congregate. In general, this involved suspending the spores in an emulsifiable vegetable oil to extend spore viability and adding a food source to make it attractive to flies. Spores can be taken up through direct contact with the spray formulation or ingestion of the contaminated food deposited on surfaces.

Insectary trials demonstrated high mortalities from *Metarhizium* infections in caged fly populations exposed to boards sprayed with formulated spores.

Two feedlot trials were conducted with formulated spores sprayed onto the front of feedlot bunks and vegetation. In both trials the mortality of and *Metarhizium* isolations from flies netted after spraying were much higher than in flies netted in control feedlots. This demonstrated that flies contacting the freshly sprayed formulations were taking up lethal doses of spores. The increased fly mortality and presence of *Metarhizium* infection were still evident, though in lower levels, in flies netted one week after spraying. This shows that the fungal formulation can remain effective on sprayed surfaces in the days post spraying.

7.1.4 Integrated fly control

During the 2007/08 fly season two comparisons between integrated fly control (IPM) and normal fly control programs were conducted in two areas, the Brisbane Valley and Warwick shire. The IPM program included frequent cleaning of fence lines, the release of parasitic wasps, spraying of fungal biopesticides and focused use of insecticidal fly baits. Adult and immature fly populations were monitored in all feedlots using traps and larval number estimates respectively.

Across the two comparisons, the IPM program achieved reductions of 36% and 40% in adult house fly and stable fly populations respectively, compared to the control feedlots. The overall treatment effect (control versus IPM) was not significant in the combined analysis but the time by treatment interaction was, indicating that the treatment effect was not consistent across time. It is expected that the biological tools (parasitic wasps, biopesticides) take some time to have an impact on adult fly populations. The data support this assumption with most of the population suppression and significant differences observed later during the fly season. The fly suppression was more pronounced in the Brisbane Valley (55% and 51% for house and stable flies respectively) than in the Warwick shire (10% and 27%).

There was only a small reduction (5%) in immature fly populations in the IPM feedlots compared to the control feedlots. A significant reduction observed in the Brisbane Valley (75%) was negated by higher larval populations in the IPM feedlot in the Warwick shire. This finding was probably due to the steeper pen slope in the control feedlot which assisted in rapid drying of manure during this high rainfall season, thus reducing the suitability for fly breeding.

Fly pupae were parasitised at a significantly higher rate in the IPM feedlots (14.7%) than in control feedlots (9.0%) and fly emergence was lower for IPM (34%) than control (37%) feedlots. The percentage of parasitism by *S. endius* was also significantly higher in IPM (8.7%) than control (5.6%) feedlots indicating that the increase in parasitism was due to the releases of *S. endius* in the IPM feedlots.

The fungal biopesticide used in the IPM feedlots infected and killed adult flies. Flies collected in IPM feedlots showed an increased mortality and were infected with *Metarhizium* spores at a higher rate than flies from control feedlots. It was demonstrated that both fly mortality and infection rates were still elevated one week after application of the fungal biopesticide.

7.2 Implications for feedlot fly control

7.2.1 General fly control tools

The implications of the results, presented in the previous section, for fly control in feedlots are discussed in this section. Firstly, and most importantly, good sanitation is critical for efficient management of flies. Particularly, feedlot areas with a high potential for breeding flies should be cleaned frequently. Such areas include fence lines, sedimentation systems, drains and hospital areas. We have demonstrated that reductions of up to 84% in fly breeding can be achieved by frequent cleaning of fence lines without the need for concurrent pen cleaning. Targeted and frequent cleaning of fly breeding hot spots will provide a good basis for efficient fly control.

Monitoring of fly populations is an important part of fly control in feedlots. It provides objective information on fly population trends and forms the basis for decisions on preventative or curative fly control measures. Fly populations can be assessed in many ways. The most reliable tools are sticky traps or sheets which are deployed at strategic locations over set time intervals. Fly traps with liquid bait can also be used as an indicator of fly populations but fly numbers are harder to determine because flies tend to drown and decompose in the bait. The baits do not provide constant attractancy over time and this adds another variable. Other, less reliable tools for measuring fly populations include repetitive counting of flies on pre-defined surfaces, sticky ribbons and spot cards. In the latter, the spots left by flies on a white surface are counted. An estimate of fly populations can also be obtained by checking fly breeding hot spots for larvae and pupae by turning over an area and counting (estimating) their respective numbers. The advantage of obtaining estimates of immature flies is that rapid fly population increases can be detected about a week earlier than by monitoring adult flies.

The major nuisance flies in Australian feedlots are house flies, stable flies, bush flies and blowflies. Bush flies do not breed in feedlots. To make relevant decisions for fly control, the species of trapped flies should be known. The most reliable method is to use entomological keys which involve a stepwise evaluation of morphological criteria to identify fly species. A simplified pictorial key for common nuisance flies was created by our project team. This is available from MLA (tips&tools FL14: Feedlot flies – identifying the problem and some solutions, http://www.mla.com.au/NR/rdonlyres/90BBDCF6-7B49-4C78-8A24-4A0C3A6DDC09/0/TipsToolsFeedlotfliesidentifyingtheproblemsandsomesolutionsJuly2006.pdf). Less reliable tools for species identification may be the use of specific fly behaviour. For instance, the propensity of bush flies to seek moisture by landing near eyes and nose and of stable flies to land and bite animals on their lower legs.

We have also confirmed that structured observations of animals can give an indication of nuisance fly populations. Counts of movements such as head tosses, ear flicks and tail swishes are indicators for house fly and bush fly numbers. The number of leg stomps correlate well with the number of stable flies. These counts depend on the sensitivity of the animals, which can vary between breeds and seasons. Therefore, animal observations provide a relative measure of fly activity.

Fly population monitoring should be an ongoing activity, as one-off monitoring is not of much value. The same measure should be used at regular intervals to obtain information on population trends. An easy way to visualise fly population fluctuations and trends is to graph the fly numbers against time. Such graphs will provide valuable information for making decisions on fly control.

The experiments with insecticides also provided useful information for feedlot fly control. The application of the larvicide cyromazine to fence lines was reasonably successful in controlling fly breeding over about two weeks when it was applied to recently cleaned fence lines. A cyromazine application to fence lines with several weeks of manure accumulation achieved little control. This further demonstrates that good sanitation is an important adjunct to other fly

management practices. Spraying of the adulticide cyfluthrin to feedlot structures, where there was no contact with feed or animals, achieved a short-lived reduction in stable flies but had no effect on house flies, the major target of the treatment.

Insecticides should only be used in feedlots when all other fly control measures cannot keep the fly populations below an acceptable level. If insecticides are required, larvicides are the better choice. They target larvae which represent a higher percentage of the total fly population than the adults and precede the adult flies in time. Larvicides are easier to apply in feedlots than adulticides as the major breeding areas (e.g. manure under fence lines) can be targeted. Larvicides will also have a smaller impact than adulticides on natural fly control agents, such as wasps and mites, which play an important role in suppressing fly populations.

7.2.2 Parasitic wasps

Several species of parasitic wasps are commercially produced and used in fly control in intensive livestock facilities in the USA. During this project, the naturally occurring parasitic wasps in Australian feedlots were surveyed and identified. One species of these wasps, *S. endius,* was selected on the basis of its abundance in Australian feedlots, its capacity to parasitise fly pupae and its common use in commercial wasp production. An *S. endius* colony was established and later transferred to a commercial company producing biological control agents. The company built separate rearing facilities for house flies (host) and wasps and can now produce large quantities of *S. endius* wasps. This wasp can now be purchased from the company for releases into feedlots (see http://www.bugsforbugs.com.au/product/33 and Appendix 1). The availability of this new biological tool for nuisance fly control is a major achievement of this project. The longstanding and ongoing use of parasitic wasps for fly control in the USA suggests that this is an effective tool in the control of flies.

We have established that the rate of parasitism of fly pupae can be increased by releasing *S. endius* in cattle feedlots. The wasp releases were an integral part of the IPM feedlot management where significant reductions in fly populations were achieved. There was also an increase in *S. endius* parasitism, indicating that this increase was due to the wasp releases.

These results were achieved with releases of about 100 wasps per animal per week. Recommendations for wasp releases vary widely with 50 to 200 wasps per animal per week as the more common range. Although these wasps breed in feedlot fly pupae, it is generally considered necessary to have repetitive and ongoing releases of wasps to achieve fly control. It is therefore recommended that releases of 100 wasps per animal per fortnight should be considered as the starting point for wasp releases. The emergence of wasps from parasitised pupae is variable; the emergence of *S. endius* from parasitised pupae produced by Bugs for Bugs is about 70%. As a consequence, about 140 parasitised pupae are required for a release of 100 wasps. The parasitised pupae are sent to users by overnight express mail and they should be placed into the feedlot soon after arrival, as the wasp emergence is timed to occur shortly thereafter.

The parasitised pupae should be distributed across the whole feedlot near major fly breeding areas, such as fence lines, sedimentation systems and possibly feed mills. They can be placed in release stations which protect them from direct sunlight and predators such as birds and ants. We constructed release stations from PVC pipe (Fig. 27; diameter 100 mm, about 250 to 400 mm long) with multiple openings (10-20, round, diameter 25-35 mm) covered with fly mesh (this prevents the pupae from falling out but allows wasps to leave the container). Alternatively, pupae can be spread on the ground or into shallow holes but they should be covered with hardened manure to prevent direct sun exposure. It is advisable to place the pupae in the feedlot early morning or late afternoon when temperatures are not too high. Flooding of the pupae by run off or rain water also needs to be prevented.

Figure 27: Release container for parasitic wasps

Like all biological control agents, wasps will not provide an immediate reduction in fly numbers. Wasp releases should be started before fly populations build up to undesirable levels. It is recommended that wasp releases are commenced at the start of the fly season and continued until fly numbers decline due to seasonal conditions.

Care should be exercised if insecticides and wasp releases are concurrently used. Most adulticides and some larvicides will also kill parasitic wasps. The larvicide cyromazine is probably the best choice in this case, as it is reasonably selective for flies.

The costs of parasites depend on the size of the order and details are provided on Bugs for Bugs website (http://www.bugsforbugs.com.au/product/33). The costs of one release of parasitic wasps into a feedlot with 1000 and 5000 head at the recommended rate (100 wasps per head) are provided in Table 26. Releases should be repeated at fortnightly intervals during the fly season as discussed in preceding paragraphs.

Table 26: Costs of parasitic wasp releases at recommended rates (100 wasps per head)

Head	No. of wasps	No. of pupae[A]	No. of packs[B]	Cost ($)[C]	Cost/head ($)
1000	100,000	142,857	57	470.25	0.47
5000	500,000	714,285	286	1653.08	0.33

[A] 70% wasp emergence; [B] 2500 pupae; [C] Bugs for Bugs price list (November 2008); plus post charges $20-30 or $80-90 for 1000 and 5000 head respectively

Wasps are commercially produced through the natural host house flies. Considering the length of the fly and wasp life cycles, there is a minimum time requirement of one month to produce the first generation of wasps. As growth rate per generation is limited it takes longer to build up the wasp production. These constraints can cause problems in a supply and demand situation as there will be inevitable delays. Considering the need for repeated and regular releases in feedlots (as described in previous paragraph), it is recommended that wasp supply contracts between the purchaser and producer are considered. Such contracts specifying the period, frequency and quantity of supply would guarantee the purchaser delivery of wasps as specified and to the producer ongoing sales which allow for a continuous production. The producer, Bugs for Bugs, has stated that additional discounts would be available for such contract deliveries (not included in Table 26).

The parasitic wasps could also be used for fly control in other industries and facilities. Similar nuisance fly problems occurring in other livestock industries such as dairy and poultry, the horse industries, abattoirs and council refuse stations should be investigated. An expansion of fly control with parasitic wasps to these situations would increase the viability of commercial production in Australia.

7.2.3 Fungal biopesticides

Fungal biopesticides are novel biological tools with potential use in nuisance fly control. It has been demonstrated by several groups that entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* can selectively kill insect pests. There are currently four registered Metarhizium based products used to control locusts, grasshoppers, pasture grubs and cane grubs in Australia. The work carried out during this project on the isolation, characterisation and formulation of selected *Metarhizium* isolates and the assessment of their efficacy in infecting and killing flies is a world first. This work has provided the basis for the development of fungal biopesticides as a biological fly control tool. The implications of these findings and how to proceed from here are outlined in this section.

The findings that fungal biopesticides based on *Metarhizium* spores infect and kill flies and can be effectively formulated into sprays or baits to infect and kill flies in feedlots, provide the opportunity to develop an effective and safe tool for fly control. Fungal spore formulations have the advantage that they can be sold and applied in a manner similar to existing chemical products.

Fungal biopesticides need to be registered as agricultural products with the Australian Pesticides and Veterinary Medicines Authority (APVMA) before they can be used for pest control. Efficacy and safety data are required for the registration. There are already products containing *Metarhizium* spores registered for other pests in Australia. Considering the existing registrations and the demonstrated low toxicity of *Metarhizium* spores to vertebrates, a safety clearance should be readily obtainable. Some formulation and efficacy data have been produced during this project, but more work in these areas is required.

Collaboration with Becker Underwood, the only company producing biopesticides in Australia, was initiated during the project. Becker Underwood produced the spores used in feedlot trials in this project and expressed an in-principle interest in producing a commercial product based on fungal isolates from the DPI&F collection. They commissioned a brief report on the commercial feasibility of a product for nuisance fly control in cattle feedlots which concluded that the product would produce marginal returns at best (see Appendix 2). One major impediment was the size of the market but this could possibly be overcome by expanding into other markets with similar fly problems as described in the previous section.

Future development of *Metarhizium* spores into a commercial product will depend on an agreement between DPI&F, MLA and Becker Underwood on how to proceed from here. As mentioned above, additional work on formulation and product efficacy will have to be conducted. The inclusion of other industries with similar fly problems should be considered for future work.

7.3 Integrated pest management (IPM) for nuisance flies on cattle feedlots

7.3.1 Introduction

Fly populations on feedlots are complex systems influenced by many factors such as temperature and rainfall, availability of breeding sites and food resources and abundance and efficacy of natural enemies. One of the common problems with “normal” fly control is that it is reactive to rapid and often massive increases in fly numbers. At this point, it is often too late to achieve effective control and the only hope of success is with insecticides. However, it is much smarter to plan and start fly control when fly numbers are low and prevent the rapid fly population build up. More benign control tools, such as biological agents, can be used for this purpose. To facilitate the implementation of such strategies in feedlots, the following integrated pest management (IPM) package for nuisance flies in cattle feedlots has been provided, utilising published information and results from this project. There is a need of action for feedlot managers to plan and implement such an IPM strategy.

There is a range of general information which is required to design and implement an IPM system for flies:

- Knowledge of the identity and biology of the major fly pests
- Understanding elements producing fluctuations in fly populations
- Effects of flies on production, including economical impact, nuisance value, animal welfare considerations and disease transmission
- Knowledge of biological and chemical control

Integrated pest management (IPM) systems embrace the integration of cultural (mechanical/ physical), biological and chemical control methods to reduce pest populations. IPM strategies need to be tailored for particular situations, incorporating all available approaches and lessening insecticide use (New 2000).

7.3.2 Major nuisance flies in cattle feedlots

The major nuisance flies in Australian feedlots are house flies, stable flies, bush flies and blowflies. These flies vary in their biology and behaviour resulting in seasonal and locality differences in their respective populations. These differences must be considered when devising control strategies.

The first step is to identify the enemy. The most reliable method is to use entomological keys which involve a stepwise evaluation of morphological criteria to identify fly species. A simplified pictorial key for common nuisance flies was created by our research team and is available from MLA (tips&tools FL14: Feedlot flies – identifying the problem and some solutions, http://www.mla.com.au/NR/rdonlyres/90BBDCF6-7B49-4C78-8A24-4A0C3A6DDC09/0/TipsToolsFeedlotfliesidentifyingtheproblemsandsomesolutionsJuly2006.pdf). Less reliable tools for species identification may be the use of specific fly behaviour. For instance, the propensity of bush flies to seek moisture by landing near eyes and nose and of stable flies to land and bite animals on their lower legs.

All flies have similar life cycles which progress from eggs to larvae, then pupae and finally adult flies (see Fig. 28 for house flies). Only a small percentage of the overall fly population is in the adult stage when they become readily noticeable. Due to the short developmental period (egg to adult can be as short as seven days) and the large number of eggs produced per female, adult fly populations can build up rapidly.

House flies and stable flies breed in non-compacted feedlot manure. Areas where fly breeding can be a problem include under the fence lines, in sedimentation systems, drains and the hospital areas. Bush flies breed in undisturbed animal dung and for this reason rarely breed in feedlots. However, bush fly adults can fly to feedlots from outside breeding sources. Blowflies breed in animal carcasses and normal management practices of completely covering these with manure or soil should eliminate blowfly breeding. It has been estimated that 1 kg of manure can produce up to 10,000 house flies.

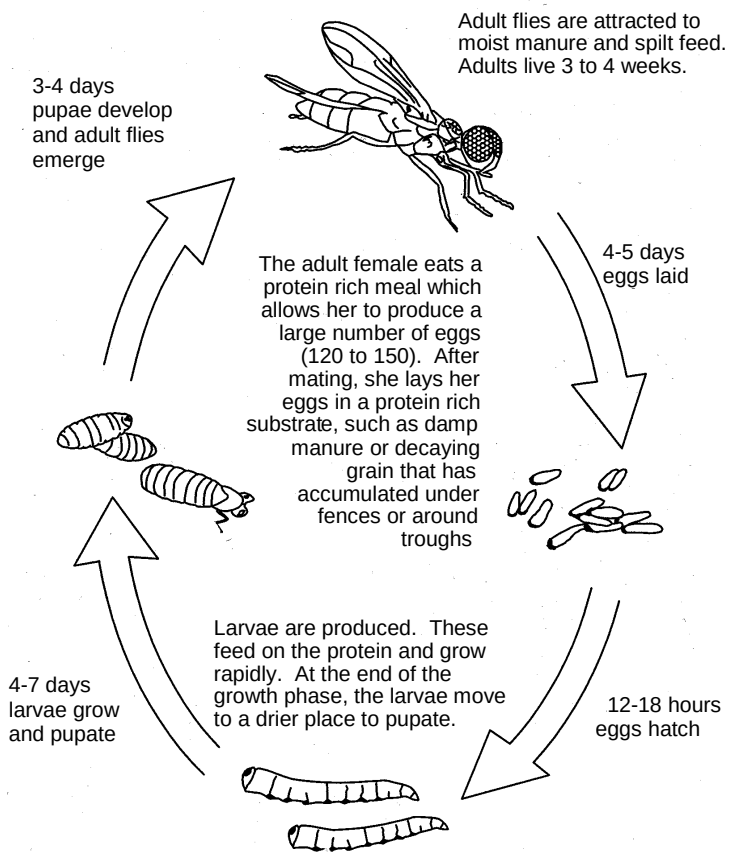

Figure 28: Life cycle of the house fly (*Musca domestica*)

Fluctuations in fly populations are caused by the longevity and fecundity of adult flies, development time and survivability of immature flies, the predation and parasitism by natural enemies (biotic factors), climatic conditions, feedlot design and management practices (abiotic factors). Seasonal changes in the fly populations are, within certain limits, consistent and predictable. Temperature has a major impact on the duration of the life cycle and on the activity of adult flies. Rainfall also plays an important role, as a relatively high moisture content in manure (40-70%) is required for successful fly breeding. Optimising abiotic factors such as pen slopes to keep the manure as dry as possible, targeted and frequent removal of manure under the fence lines to minimise fly breeding sites can greatly assist in reducing fly populations.

Flies can reduce productivity of feedlots by directly reducing weight gains, increasing production costs or limiting marketing opportunities. Fly population thresholds at which action is to be initiated need to be established. Biting flies, e.g. stable flies, have a low economic population threshold (5 flies per animal) above which substantial production losses can occur. House flies, bush flies and blowflies are less likely to cause such direct losses, but in high numbers elicit defensive actions in animals which can lead to lower productivity. House flies can become a nuisance to feedlot staff or neighbours, thus necessitating the setting of an acceptable population threshold on the feedlot. Some blowflies can cause myiasis on animals and all flies can transmit viral, bacterial or fungal diseases either from animal to animal or from animal to human. To minimise disease transmission, management and maintenance of fly populations at or below transmission thresholds is required.

7.3.3 Elements of IPM for nuisance flies

The major components of a fly IPM program are shown in the flowchart (Fig. 29). These components include feedlot design, manure management, biological control and focused use of

insecticides. The need for and efficacy of the IPM components is determined through fly population monitoring.

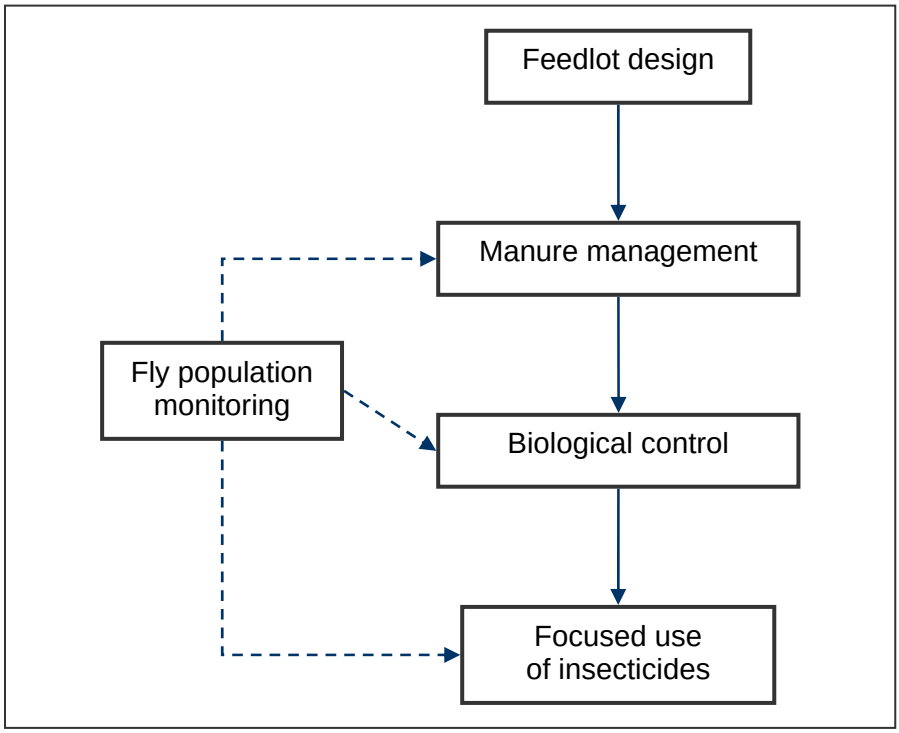

Figure 29: Flowchart showing elements of an integrated pest management system for nuisance flies

7.3.3.1 Feedlot design

Design of many feedlot sections can be optimised to facilitate fly control. The majority of the design features will make cleaning and removal of potential breeding sites easier or more effective. A useful guide to feedlot design is available from the DPIF website (Skerman 2003).

- Employ appropriate pen foundation construction methods and materials to produce a uniform, durable pen surface capable of withstanding the loadings from cattle and cleaning machinery without breaking down to form pot holes and depressions.
- Pen slope should preferably be in the range from 2.5 to 4% to promote rapid drainage and hence drying of the manure pad after rainfall, while limiting manure transport from the pen area. Pen cross-slope should be less than the pen down-slope to avoid pen to pen drainage.
- Feed and water troughs should be designed for ease of cleaning, preferably with enclosed, vertical sides to eliminate any build-up of spilt feed or manure underneath. They should be equipped with durable aprons (generally concrete) sloping away from the trough to promote good drainage while avoiding pen surface degradation (pothole formation).
- Water troughs should be designed for ease of waste water disposal and cleaning, shallow and narrow to minimise waste water volume generated by cleaning. Waste water should be discharged away from the pen, preferably in a durable surface drain or via underground sewer pipe, to prevent the formation of wet patches.
- Fence panels should be relatively widely spaced (up to 3.2 m) to improve the efficiency of under-fence cleaning. The bottom fence cable or wire should be approximately 400 mm above the constructed pen surface to allow easier under-fence cleaning.

- Drains should be designed for flow velocities high enough to minimise deposition of manure.
- Drains should be designed for ease of cleaning, generally with either V or trapezoidal cross-sections and flat batters. A durable base should be provided to enable access by cleaning machinery as soon as possible after rain.
- Sedimentation basins should be designed for ease of cleaning with a durable base to enable access by cleaning machinery as soon as possible after rain.
- Sedimentation systems and holding ponds should be designed to enable mowing and/or spraying of vegetation around the perimeter.
- Manure stockpile and composting areas and carcass composting areas should be established on durable, well-drained earth pads.

7.3.3.2 Manure management

Reduction of fly breeding sites is a critical element of an IPM program. This is largely achieved through adequate feedlot sanitation using cultural methods. House flies and stable flies breed in moist manure, spills of feed and silage and mixtures of vegetation and feedlot run-off, e.g. drains. Compacted or dry manure is not suitable for fly breeding. Bush flies breed in undisturbed dung pads (generally not present in a feedlot) and blowflies breed in carcases. It appears that flies breed largely in a few and relatively small areas in the feedlot. Feedlot sanitation for fly control should be targeting these areas.

- Manure accumulated under fence lines in cattle pens is one of the major fly breeding areas in the feedlot. Frequent removal of this uncompacted manure will reduce the substrate available for fly breeding. To completely stop fly breeding, this would have to be done every 7 days during summer (less frequently in cooler seasons). We have demonstrated that over three months fly breeding under fence lines can be reduced by 84%, 67% and 55% by weekly, fortnightly and monthly fence line cleaning respectively (compared to no cleaning over 3 months). Cleaning involved scraping the fence lines which spreads the manure so that it dried out rapidly and was rendered unsuitable for fly development.
- A similar strategy should be used for the drains and the sedimentation system. The presence of wet manure deposits should be minimised. Thus, regular cleaning of these areas, particularly after major rainfall events, should occur as soon as the manure becomes workable.
- Manure in the stock piles must be managed to minimise its suitability for fly breeding. Composting of the manure in windrows prevents fly breeding because of high temperatures generated in this process. Align and shape manure stockpiles and composting windrows to avoid ponding of rainfall – runoff.
- The hospital area can also be a good fly breeding site, due to a low stocking density, the presence of hay and infrequent cleaning. More frequent cleanouts and a reduction of hay spillage on the manure should achieve a reduction in fly breeding in the hospital area.
- Adequate stocking density in pens is required to compact the manure and render it unsuitable for fly breeding.
- Feed spillages should be avoided where possible and cleaned up regularly. Feed spills were commonly found near feed bunks, in the feed processing area, in the hospital pens and horse stables. Feed spills should be removed promptly and added to composting manure. Feed residues should not be left in bunks for extended periods.
- Moist silage provides a suitable substrate for fly breeding. Spills, particularly along the silage pits, should be avoided and the silage pits should be covered and the edges sealed to reduce fly breeding in this area.

- Cattle carcases should be composted rather than buried. The carcases have to be completely covered with manure to prevent blowflies accessing them to breed. With appropriate manure cover, the temperatures in the pile will kill fly larvae and other organisms.
- General feedlot maintenance will also contribute to fly control through a reduction in breeding sites and resting places for adult flies.
    - Check regularly for water leaks from troughs, as they increase the moisture content of manure pads and thus facilitate fly breeding.
    - Weeds should be controlled and grass and other vegetation kept short, particularly around pens, drains and sedimentation ponds. This makes it more difficult for flies to find resting places and reduces the vegetation–manure interface, a preferred breeding substrate for stable flies.
    - A thorough feedlot clean-up before the start of the fly season, e.g. early spring, will slow down the increase in fly populations

7.3.3.3 Biological control

Biological control agents play an important role in lowering feedlot fly populations. We have demonstrated that naturally occurring control agents in Australian feedlots include parasitic wasps, entomopathogenic fungi and predatory mites. Particularly, parasitic wasps achieved 21-35% control of nuisance flies in three monitored Australian feedlots. Additional natural predators of immature flies which are present in the feedlot include beetles, birds and ants.

It is important that the presence and activity of these biological agents be encouraged or enhanced through appropriate management. Of most importance would be a judicious use of insecticides as most will harm wasps and mites (see below for some exceptions). Releases of biological control agents into the feedlot could enhance the natural baseline control and assist in further reducing fly populations. Unlike some of the chemical fly treatments, biological agents are a soft and slower acting tool for fly control and their use has to be carefully planned and implemented well ahead of the occurrence of major fly waves.

- Parasitic wasps kill fly pupae by stinging them. They lay their eggs inside fly pupae and the resulting wasp larvae feed on the immature flies before they can develop into adults. The wasp larvae subsequently develop inside the fly pupal cases until they emerge as adult wasps after several weeks. One or several wasps can develop inside one fly pupa. Adult wasps also sting and kill fly pupae and feed on pupal contents, but no eggs are laid (dudding); or eggs are laid but wasps die before reaching the adult stage. Parasitic wasps are small (1 to 3 mm), black, flying insects and are normally not seen. These wasps affect only flies, and are harmless to people, pets and other animals.
- Entomopathogenic fungi also limit fly populations. Fungi are unique among the microbial insect pathogens in that they primarily infect their hosts through the external cuticle. Fungal spores have evolved to be picked up and adhere to insect cuticles when contacted. Thus fungal spores applied to the environment of the target pest can be taken up through direct impact with the insect or indirectly through the activities of the insect such as feeding or even resting. Fungi offer additional advantages with robust spores that can be dried and stored either as a powder or formulated in oil until required.
- Predaceous mites feed on house and stable fly eggs and larvae but their impact on fly breeding or populations has not been quantified.
- The primary management strategy is to preserve existing feedlot populations of biological control agents. Insecticide applications should be avoided if possible or used judiciously when necessary. Most insecticidal treatments aimed at adult flies will also kill parasitic wasps and mites. Fly populations will recover quicker from an insecticidal treatment than

parasitic wasps due to their shorter life cycle, resulting in reduced biological control during this lag period.

- Drier conditions in fly breeding substrates favour parasitic wasps and mites and impede fly breeding, hence the aim should be to facilitate the drying of all substrates suitable for fly breeding.
- An additional strategy is to augment the natural populations of parasitic wasps to increase the control achieved by these biological agents. We have demonstrated that such releases increase parasitism of fly pupae. The parasitic wasp, *S. endius,* is commercially available in Australia from Bugs for Bugs. Recommendations for its application in feedlots have been given in a previous section.
- The use of a fungal biopesticide would provide another biological tool for fly control. We have demonstrated that fungal biopesticides infect and kill flies in feedlots. Fungal biopesticides need to be registered with AVPMA before they can be used for fly control.

7.3.3.4 Insecticides

Insecticides can be used to assist in the control of nuisance fly populations on cattle feedlots but they should not be the principal strategy. They should only be used if adequately implemented cultural and biological methods fail to keep fly populations under an acceptable threshold. If insecticides have to be used, the following guidelines should be considered to avoid unnecessary, ineffective or detrimental applications.

- Insecticides should only be handled and used according to label instructions.
- Insecticides should only be applied if a fly monitoring program indicates that a predetermined population threshold has been exceeded. This strategy prevents needless treatments and lowers costs. Insecticides should not be used on a scheduled calendar basis.
- Larvicides and fly baits should be used in preference to adulticides. The use of larvicides will not deliver instant relief but will provide better control over time. We have demonstrated that the impact of adulticides is minimal and short-lived.
- The use of cyromazine is recommended over other currently available larvicides because it does not detrimentally affect beneficial insects. It should be applied to recently cleaned areas to maximise its reduction of fly breeding.
- Fly baits are only effective against house flies as they contain a house fly attractant. They can be applied either in bait stations, scattered or painted on surfaces. Combinations of larvicides with fly baits have been shown to be a successful strategy in delaying development of resistance.
- If an adulticide has to be used, residual insecticides are preferred over knockdown insecticides. Knockdown insecticides are short lived and fly populations are likely to recover quickly after an application. Residual insecticides should be sprayed or painted on major resting sites of adult flies. However, the repeated use of residual insecticides creates a high potential for selection for resistance against them, particularly if a single product is used. Consult product label for information on resistance management strategies.
- Applications of insecticides should be targeted to hot spots rather than broadcast across the entire feedlot. To control breeding, larvicides should only be applied to major breeding sites, e.g. under pen fence lines, drains, sedimentation pond, hospital area. For control of adult flies, treatments should be restricted to resting places, e.g. exterior of feed bunks, pen fences, underside of shade cloth, trees and other vegetation. Insecticides should never be applied to feed or areas which come in direct contact with feed.

- The chemical groups, e.g. carbamates, organophosphates, pyrethroids/pyrethrins, neonicotinoids, spinosyns and cyromazine (moulting disruptor), should be rotated to prevent build up of resistance in the flies (see Table 27). Repetitive exposure of flies to the same insecticide will result in the development of resistance, thus rendering the chemical ineffective.

Table 27: List of active constituents and chemical subgroups which can be used for nuisance fly control in feedlots

Group [#]	Chemical subgroups	Active constituents	Fly control	Use
1A	Carbamates	Bendiocarb	Adult flies/larvae	Residual/Bait
		Methomyl + Z-9-tricosene	Adult flies	Bait
1B	Organophosphates	Diazinon	Adults/larvae	Residual surface
		Dichlorvos	Adult flies	Residual surface
		Fenthion	Adult flies	Residual surface
		Maldison	Adult flies	Residual surface
		Pirimiphos-methyl	Adult flies	Residual surface
		Trichlorfon	Adults/larvae	Residual surface
3A	Pyrethroids/ pyrethrins	beta-Cyfluthrin	Adult flies	Residual surface
		Cyfluthrin	Adult flies	Residual surface
		Permethrin 25:75	Adult flies	Residual surface
		Pyrethrins + piperonyl butoxide	Adult flies	Knock down
4A	Neonicotinoids	Imidacloprid+ Z-9-tricosene	Adult flies	Bait
		Thiamethoxam+ Z-9-tricosene	Adult flies	Residual surface/Bait
5A	Spinosyns	Spinosad	Adult flies	Residual surface
18A	Cyromazine	Cyromazine	Larvae	Manure treatment

[#] Mode of action classification for insecticides

Table 27 contains active constituents which are registered for fly control in feedlots, animal facilities, farm buildings or agricultural buildings and are constituents in currently available products. The corresponding products, which are all restricted to non-animal use, can be found on Infopest (2008) or the PUBCRIS database on APVMA website (http://services.apvma.gov.au/PubcrisWebClient/welcome.do). Guidelines for safe and effective use of agricultural and veterinary chemicals are available form the DPI&F website http://www.dpi.qld.gov.au/cps/rde/dpi/hs.xsl/4790_4906_ENA_HTML.htm.

7.3.3.5 Fly population monitoring

Fly population monitoring is an important element of an IPM program. Such a program can provide information on the identity of the problem species and on fluctuations in fly populations. It can provide an early warning for anticipated fly waves before adult fly populations escalate. Fly control is more effective if monitoring is implemented before fly numbers increase.

To keep track of fly population fluctuations and to assess the effectiveness of actions, population monitoring needs to be carried out on a regular and systematic basis. The monitoring system and the site, timing and duration of the monitoring have to remain constant. The results should be assessed immediately after the monitoring period and recorded. Records on a graph will facilitate recognising trends in fly populations.

Several monitoring systems for fly populations are available. Monitoring of adult flies can be achieved by using sticky sheets or traps, or structured observations of fly resting sites or animal behaviour. The extent of fly breeding can be established through inspections of major fly breeding sites. Monitoring immature fly populations will give an earlier indication of increases in fly populations than adult monitoring.

- Sticky sheets will retain flies landing on the sheet. Preferably, they should be placed on vertical surfaces, e.g. walls or posts, near preferred fly resting sites. They should be kept away from excessive dust which renders the sticky surface ineffective. The species and number of flies caught on the sticky sheet over a fixed time can be determined. The exposure time of the sticky sheet must be chosen to avoid saturation with flies (1 to 7 days may be appropriate). Identification of the major feedlot flies can be achieved using "tips&tools FL14: Feedlot flies – identifying the problem and some solutions" and a magnifying glass. Sticky sheets are commercially available (e.g. from Starkey Products, 46 Achievement Way, Wangara WA 6065). Alternatively, smaller sticky surfaces, such as fly tapes or fly ribbons could be used for a less accurate fly monitor.
- The Alsynite trap selectively attracts stable flies, however it also works well as a sticky trap for house flies. The cylindrical Alsynite panel strongly reflects UV light (from the sun) which makes it attractive for stable flies and for this reason should only be used in open areas. A transparent sticky sheet is wrapped around the cylinder to catch landing flies. The Alsynite trap is also commercially available from the USA (Olson Products, Medina, Ohio 44258 USA; http://www.olsonproducts.com/bite/bite.html).
- Fly counts on preferred fly resting sites, such as fence railings, feed bunks, walls or other sites where flies usually congregate can also be used as a rough indicator for fly populations. This method is less accurate because counts may fluctuate depending on time of day, weather conditions and other variables. It may also be difficult or impossible to identify fly species.
- Our results have shown that there is strong correlation between animal movements and the number of adult flies in the feedlot. The frequency of tail swishes, ear flicks and head tosses can be used to gauge house fly and bush fly populations. Likewise, the number of leg stomps correlated well with the stable fly populations. Counts of these movements over a specified time (e.g.1 min) on several animals (e.g. 5 to 10) can provide an estimate of prevailing fly populations.
- Larvae are the most appropriate indicator for immature fly populations. Inspection of manure at major fly breeding sites such as under the pen fence lines, the hospital area, drains, sedimentation pond, silage and wet manure stock piles can provide a measure of immature fly populations. At each site, manure needs to be turned over and examined at several locations and a larval rating or estimate assigned to each site. House fly and stable fly larvae can be distinguished using "tips&tools FL14: Feedlot flies – identifying the problem and some solutions" and a magnifying glass.

To improve the consistency of the results, fly monitoring should preferably be carried out by a single operator. Some of the monitoring systems are more subjective than others and only a single operator can deliver useful results.

The optimal solution for feedlots would be to contract out fly control, or at least fly population monitoring, to specialists. Such specialists would have knowledge of fly populations and integrated fly control. They would gather information on the identity and location of fly problems (scouting) and assist with the selection of or application of suitable treatments and the assessment of their effectiveness. Contracting out of pest control to experts is a common

practice in horticultural industries. With a trend to larger feedlots in Australia, such contracts are more likely to eventuate.

7.4 Recommendations

It is recommended that:

1. Feedlot managers design and implement an integrated pest management (IPM) program for nuisance flies, including the application of the following elements:
   a. Feedlot design
   b. Manure management
   c. Fly population monitoring
   d. Biological control
   e. Use of insecticides
2. Fungal biopesticides be developed into a tool for fly control in intensive animal facilities (formulation, efficacy, registration)
3. The use of parasitic wasps and fungal biopesticides for fly control beyond feedlots should be investigated to maximise their commercial potential in Australia.

Fly populations on feedlots are complex systems influenced by many factors. The planning and implementation of fly control must start long before flies are a serious problem. An integrated pest management (IPM) program for nuisance flies in cattle feedlots has been provided, utilising benign control tools, such as biological agents. The program contains information on flies and their natural enemies and provides advice on feedlot deign, manure management, the use of biological agents and as a last resort insecticides. IPM programs need to be tailored to particular situations to provide optimal fly control.

We have demonstrated that fungal biopesticides infect and kill flies in feedlots and their use would provide another biological tool for fly control. To develop fungal biopesticides into commercial fly control agents, additional work on formulations and efficacy needs to be completed and they need to be registered with the AVPMA.

Parasitic wasps and fungal biopesticides can be used as biological agents to manage nuisance flies. There is interest from Australian companies to produce and market both agents. However, the market in feedlot fly control may not be big enough to sustain commercial productions. There are several related industries which have similar fly problems and could benefit from using these new biological agents. An expansion into other livestock industries such as dairy and poultry, the horse industries, abattoirs and council refuse stations should be investigated.

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10 Appendices

10.1 Appendix 1 - *Spalangia endius* - nuisance fly parasite production (Dan Papacek, Bugs for Bugs)

10.2 Report on the commercial potential of M16 in feedlots (Chris Fraser, Becker Underwood)