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Prepared by:	**Rudolf Urech, Peter Green, Sri Muharsini, Romsyah Maryam, Geoff Brown, Philip Spradbery, Robert Tozer** **Department of Primary Industries and Fisheries, Queensland**
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Improvements to screw-worm fly traps and selection of optimal detection systems

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Abstract

Screw-worm fly is an aggressive, exotic parasite of warm-blooded animals. If an incursion into Australia occurs it needs to be detected rapidly for planned action such as control and eradication to be initiated. An improved screw-worm fly surveillance trap, LuciTrap with Bezzilure-2, was developed which attracts more screw-worm flies and less other flies than previous trapping systems. The sensitivity of fly trapping and herd inspections for the detection of screw-worm fly was determined. Optimal screw-worm fly surveillance in Australia should include fly trapping, commercial herd inspections for fly strike and identification of larvae found in wounds. Adoption of the new trap and real-time PCR screening of trap catches will improve screw-worm fly surveillance by providing earlier and more reliable detection of an incursion. Further recommendations to minimise the impact from a screw-worm fly incursion into Australia are to evaluate the efficacy of insecticides, to carry out additional research and development and to enhance Australian screw-worm fly expertise by collaborating with overseas scientists.

Executive Summary

Old World screw-worm fly myiasis, caused by the obligate myiasis blowfly *Chrysomya bezziana*, is considered a serious threat to Australia's livestock industries. Screw-worm fly is endemic across all northern neighbours of Australia, including PNG, Indonesia, Malaysia and the Philippines. So far, it has not become established in Australia. The total costs of an endemic screw-worm fly infestation for Australia have been estimated at $900M per annum. An uncontrolled incursion of screw-worm fly into Australia would threaten the survival of the northern cattle industry (direct production losses would be in the order of $500 million per year). Australia has a screw-worm fly preparedness strategy, including the AUSVETPLAN for screw-worm fly. Components include surveillance, a bio-economic model and sterile insect technology for the eradication of screw-worm flies. It is clearly understood that the earlier an incursion is detected, the less its likely impact. Detection of adult screw-worm fly relies on trapping and monitoring sentinel herds. The Northern Australian Quarantine Strategy and the Ports Surveillance Program currently undertake this task across northern Australia.

This project was undertaken with the aim of improving screw-worm fly surveillance in Australia. The specific objectives were to develop and evaluate an improved screw-worm fly trap, to assess the effectiveness of fly trapping and sentinel herd inspections for screw-worm fly detection and to formulate recommendations for an optimal screw-worm fly surveillance system in Australia. All objectives have been met in this project and results, conclusions and recommendations are summarised in the following paragraphs.

An improved trapping system for screw-worm fly has been developed and comprehensively tested. It consists of the commercially available LuciTrap with a new attractant mixture (Bezzilure-2). The modification of enlarging the fly entry holes in the LuciTrap and the use of a pest strip have been eliminated in the new system allowing the manufactured LuciTrap to be used. The attractant consists of two bottles (Bezzilure-2 A and Bezzilure-2 B) containing an aqueous sodium sulfide solution and a mixture of eight chemicals respectively. The attractants are contained in plastic bottles fitted with wicks which assist in releasing the attractants at a constant rate over a period of approximately two months. A roof to protect the LuciTrap from rain (150-250 mm above the trap) is retained to provide good quality flies for subsequent processing.

The LuciTrap with Bezzilure-2 (or similar attractants) caught, on average, 3.5 times more *C. bezziana* than the sticky trap with Swormlure. The LuciTrap/Bezzilure combination provides selectivity for *C. bezziana* against other *Chrysomya* spp. (average factors 9–12) including *C. megacephala* which is difficult to differentiate from *C. bezziana* using morphological criteria. The LuciTrap also discriminates with a factor of approximately 100 against *Hemipyrellia* spp. compared to the sticky trap. This selectivity is important to maximise the probability of detecting *C. bezziana* in trap catches and to shorten the time and/or reduce the cost of the subsequent screening for *C. bezziana* by real-time PCR or morphological examination.

The sensitivity of adult screw-worm fly trapping and livestock inspections for the detection of *C. bezziana* was determined in areas with low and high density screw-worm fly populations. Both methods detected screw-worm fly at both fly densities. To detect *C. bezziana* with 95% confidence in the low density area either 12 LuciTraps with Bezzilure need to be deployed for 14 days or 507 animals have to be inspected. At Jelai Gemas during the higher strike prevalence period, 2 LuciTraps for a 7-day period or 209 animals inspected, and during lower strike prevalence 3 LuciTraps for a 10-day period or the inspection of 954 animals were required to achieve detection with 95% probability.

LuciTraps with Bezzilure and inspections of livestock should both be used for screw-worm fly surveillance in Australia. The two tools are complementary and their usefulness depends on circumstances. Traps are a flexible, convenient and reliable detection tool and can be

strategically located and serviced as required. Inspection of cattle at routine musters is also useful and, with trained inspectors, a reliable tool for the detection of *C. bezziana*. However, mustering cattle solely for fly strike inspections would not be cost effective in most cases.

Optimal screw-worm fly surveillance programs should use an integration of available detection tools. Besides fly trapping and livestock inspections, larvae detected in wounds on animals or humans in Australia (and the Torres Strait) should be submitted to designated institutions for identification. Such submissions are currently rare and measures to redress this lack of larval submissions should be instigated. Limitations, such as the exclusion of larvae from blowfly strike on sheep, should be applied to larval submissions.

Recommendations for future action are:

1. Use LuciTrap with Bezzilure-2 for surveillance of adult screw-worm fly populations in Australia
2. Develop an integrated approach to screw-worm fly surveillance which includes:
    a. Fly trapping
    b. Livestock inspections
    c. Targeted larval submissions
3. Establish real-time PCR screening of adult and immature fly samples as the primary identification process
4. Test and register insecticides that can be used for wound treatment and prophylaxis in the event of a screw-worm fly incursion to Australia
5. Carry out further research and development work on screw-worm fly surveillance, particularly to prolong the period flies can be left in LuciTraps before being screened by real-time PCR
6. Consider the integration of the Australian screw-worm fly research and development into the 5-year International Atomic Energy Agency sponsored Coordinated Research Project on “Applying Population Genetics and GIS for Managing Livestock Insect Pests (D4.20.13)” (start date 2008)

Adoption of the above recommendations will minimise the likelihood of an undetected incursion of screw-worm fly into Australia and the subsequent serious economic and animal welfare impact that would ensue. However, the industry must be aware of the ongoing risk of a screw-worm fly incursion which has not been reduced by improvements to the surveillance program. Effective screw-worm fly surveillance must be maintained to minimise the impact to the Australian livestock industries from an incursion of this exotic and highly damaging pest. Retention of Australian expertise in screw-worm fly research and development, control and eradication would be facilitated by collaboration with other similar programs around the world under the IAEA sponsored project on managing livestock insect pests.

Contents

1 Background

The Old World screw-worm fly (SWF), *Chrysomya bezziana,* is an aggressive parasite of all warm-blooded animals, including humans. SWF is endemic across all northern neighbours of Australia, including PNG, Indonesia, Malaysia and the Philippines. So far, it has not become established in Australia. The total costs of an endemic SWF infestation to Australia have been estimated at $900M per annum (Spradbery 2002).

Australia has a SWF preparedness strategy, including the AUSVETPLAN for SWF (Animal Health Australia 2007). Components include surveillance, a bio-economic model and sterile insect technology for the eradication of screw-worm flies. It is clearly understood that the earlier an incursion is detected, the less its potential impact. Detection of adult SWF relies on trapping and monitoring sentinel herds. The Northern Australian Quarantine Strategy and the Ports Surveillance Program currently undertake this task across northern Australia.

Previous R&D work in our DPI&F based group has led to improvements in the trapping system, particularly in its specificity and capability to deliver good quality flies for identification (Urech *et al*. 2002). The substitution of the sticky trap/Swormlure combination with a modified LuciTrap and new attractants provided equal numbers of SWF but lower numbers of non-target flies (typically 10 to 100 fold decrease in total fly catch). The new attractants were from two distinct groups, one containing sulfide based components (eg dimethyl disulfide; this group included Swormlure) and the other group containing 2-mercaptoethanol (2-me, a key component of Lucilure, an attractant for the Australian sheep blowfly *Lucilia cuprina*). An attractant belonging to this second group was recommended for use in SWF surveillance traps at the conclusion of the previous project. The new attractant was named Bezzilure reflecting the target species' name. Additional attractant mixtures had been developed which were more powerful than Bezzilure in laboratory assays but they had never been tested in the field and thus could not be recommended for use. We were confident that a more powerful and possibly more selective attractant for SWF could be developed. Additional clues for the formulation of an optimal attractant mixture were to be obtained from collection and analyses of volatile chemicals originating from a SWF infested wound (Cork 1994).

It has been recently suggested that monitoring commercial cattle herds, with SWF infestation of natural wounds as the indicator for SWF presence, may be more sensitive than fly trapping for detecting a SWF incursion into Australia (Mahon pers. comm.). Although it is known that detection using artificially wounded cattle, with a deep X-shaped wound, is about 5 times more sensitive than one trap day [numbers of egg masses versus sticky trap with Swormlure, (Mahon *et al.* 2004; Spradbery 1994)], animal welfare considerations prevent the use of this approach in Australia. The sensitivity of using commercial herds with natural wounds for the detection of SWF is unknown and this information is vital for effective decision-making and allocating resources in SWF detection. We compared the sensitivity of these two methods for detecting SWF at low population density during this project.

The project was carried out by the DPI&F based Insect Chemical Ecology Group (leader: Dr Rudolf Urech), two Australian SWF experts (Dr Philip Spradbery and Mr Bob Tozer), the Parasitology Group at the Indonesian Research Institute for Veterinary Science (Bbalitvet) in Bogor, Indonesia (leader: Dr Sri Muharsini) and collaborators in Malaysia (leader: Mr Yuen Tack Kan).

2 Project Objectives

To improve the system for detecting SWF in Australia by:

1. Improving the SWF trapping system
2. Assessing the effectiveness of SWF detection systems (trapping versus sentinel herd)
3. Formulating recommendations for an optimal SWF detection system.

The recommendations are expected to describe components of an improved trapping system and to provide guidance for design and implementation of an optimal detection system in Australia. Adoption of these recommendations by the Australian Quarantine and Inspection Service would provide a better screw-worm fly surveillance program and earlier detection of this undesirable and, for the livestock industries, potentially disastrous exotic insect species.

3 Methodology

3.1 Attractant mixtures

The attractant mixtures were prepared from analytical or laboratory grade chemicals purchased from Sigma-Aldrich Pty Ltd (Castle Hill, NSW 2154). Each mixture was given a unique identification code, eg B10. The compositions of the mixtures are provided in Appendix 1, with quantities given in millilitres (ml) for liquids and grams (g) for solids. Sodium sulfide was technical grade flakes (60%) from Ajax (Auburn NSW 2144).

The attractants were contained in 30 ml high density polyethylene plastic bottles. The bottles were fitted with a cotton wick (dental roll) which was held in place by a custom-designed insert. The insert allows the pressure inside and outside the bottle to equalise and prevents spillage of contents if the bottle is knocked over after removal of the lid. The wick height can be adjusted from level to 25 mm above the bottle rim and this allows the evaporation rate of the attractant to be regulated. The wick dispensing system was developed for LuciTrap and bottles, inserts and wicks are available from Bioglobal Pty Ltd (Wacol Qld 4076).

Stability of attractants during storage was assessed in accelerated ageing studies at 50°C for 80 days.

3.2 Traps

The traps used were a sticky trap (Spradbery 1981), a standard and a modified LuciTrap® (Bioglobal Pty Ltd) and a wind-orienting trap (see Appendix 4). The LuciTrap modification included enlarged fly entry holes (6.5 mm) and a round, blue plastic roof (diameter about 500 mm) about 200-250 mm above the trap top) to protect trap contents from rain. A small piece of Scuttle Bug Pest Strip (18 pieces from one strip; 186 g/kg dichlorvos; Barmac Industries, Swanbank Qld 4306) was placed in the LuciTrap to prevent trapped flies from escaping. The entry holes in the commercial LuciTrap (5.5 mm) were enlarged by drilling them out with a 7.5 mm spade bit (due to the flexibility of plastic cones this resulted in entry holes with a diameter of 6.5 mm; standard wood/steel drill bits tend to tear the plastic material).

Dark and black LuciTrap buckets were also tested. The dark buckets were wrapped with a matt black cloth and black buckets were spray painted with black paint (gloss for room assay; matt for field trials).

3.3 Fly colony

A screw-worm fly colony was maintained at Bbalitvet to supply flies for the laboratory assays. The experimental details are provided in Sukarsih *et al*. (2000). During the project Waterlock was

replaced by a cellulose fibre for the preparation of the larval media (Chaudhury & Skoda 2007). For quality assurance, pupal weights, sex ratios and presence of parasitoids were regularly monitored. Flies of mixed sex (approximately 50:50), 4 to 8 days old were used for the laboratory assays.

3.4 Cage assay

3.4.1 Principle

Comparisons of the responses of screw-worm flies, *C. bezziana*, to two attractants in jar traps were made by offering the two treatments to a known number of flies in a cage in a choice situation. The numbers of flies, female and male, inside the jars at the end of a specified period (30 min) were used as the measure of attractancy.

3.4.2 Facility and materials

The cage assay for olfactory screening of *C. bezziana* was set up in a room on the ground floor of the Parasitology Department at Bbalitvet in Bogor. The room was 4 x 3.2 x 2.9 m and had external and internal windows on its narrower walls. All the windows were covered with brown paper to reduce variations in light conditions during the experiments. An exhaust fan was located in the centre of the external wall. A diagram of the cage assay room and experimental arrangements are provided in Figure 1.

Fluorescent lights were situated in the centre of the room (two tubes) and above the cages (single tube). The room was at ambient temperature and this was recorded at the start of each session. Fly cages (60x60x60 cm) made from steel frame and metal mesh (1 mm) were placed on a support board (positions marked) about 1 m from the ground and on a line perpendicular to the air flow created by the fan. Two cages were used concurrently (=session) and access was via a sleeve. Two hundred flies were used per cage and cages were used only once per day and then left to air overnight.

The devices for trapping the flies were wide-mouth glass jars (about 1L) to which a downward pointing mesh cone was fitted. The narrow cone opening was approximately 15 mm and this prevented the trapped flies from leaving the jar. The narrow openings of all cones were smooth and of equal size and shape. The cones were never in contact with the attractant bottles.

The attractant bottles were fitted with lids containing 8 mm holes to reduce the release rate of the attractants. The attractant bottles were placed in the glass jars, the cones fitted and 10 minutes allowed for the odour to fill the glass jar.

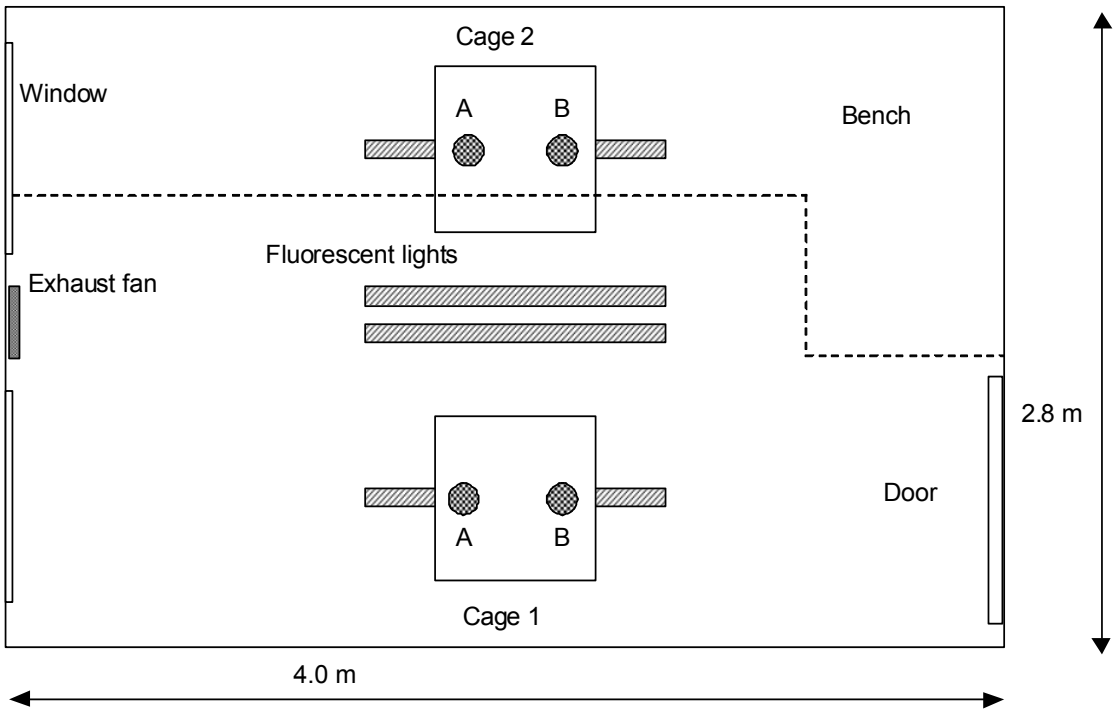

Figure 1: Diagram of cage assay room at Bbalitvet with two fly cages (1, 2) containing two jar traps (A, B) each.

3.4.3 Experimental procedure

For each assay a "Screw-worm Fly Cage Assay – Laboratory Form Template" with the number provided by a computer-generated random list of numbers 1 to 4 was selected. The template forms provided the positions of the treatments in the cages for all sessions of the assay. After the initial assignments, the treatment positions were swapped for every subsequent session. The glass jars were placed in cages on positions A and B (marked on support board) as indicated on laboratory form template. The exhaust fan was turned off and after a 10 min equilibration period, flies could be introduced into the cage. After 30 minutes, the glass jars were capped and removed from the cage. The exhaust fan was turned on and the flies in the jars were transferred to labelled plastic bags. The attractant bottles were put back into the same glass jars and the corresponding mesh cone fitted. The jars were placed into new cages in positions indicated on the Laboratory Form Template to start the next session. Six sessions were run for each assay (12 pair-wise comparisons). The flies caught in glass jars were sexed, counted and analysed (one-way ANOVA in randomised blocks of transformed (square root) fly counts). The results are presented as back-transformed mean number of flies caught with attractants 1 and 2 and the probability value for female, male and total fly numbers.

3.5 Room assay

3.5.1 Principle

Comparisons of the responses of screw-worm flies, *C. bezziana*, to two treatments (eg attractants or traps) were made by offering the two treatments to flies in a room in a choice situation. The treatments were placed equidistant from a fly release point on the upwind side of the room. The numbers of female and male flies caught on or in the traps during a specified period (eg 30 min), were used to measure the flies' responses.

3.5.2 Facility

The assay room was located in the Parasitology annexe building at Bbalitvet in Bogor. The room was 5.5x4.5x2.85 m with internal windows to the corridors on its shorter walls (Figure 2). All the windows were covered with brown paper to reduce variations in light conditions during the experiments. A screened entrance alcove prevented flies escaping from the room when an operator entered or left. The room (see below) was at ambient temperature and this was recorded at the start of each replicate.

A variable speed exhaust fan was located in the centre of one of the narrower walls. Two air inlet tubes were located in the opposite corners of the room, providing air which was sourced from above the roof of the building. All other doors and windows were sealed. Four fluorescent lights (capable of holding two fluorescent tubes each) were attached to the ceiling. Two targets were placed symmetrically near the air inlet tubes. The targets were presented on height adjustable stands, capable of taking horizontal and vertical platforms and other traps or targets.

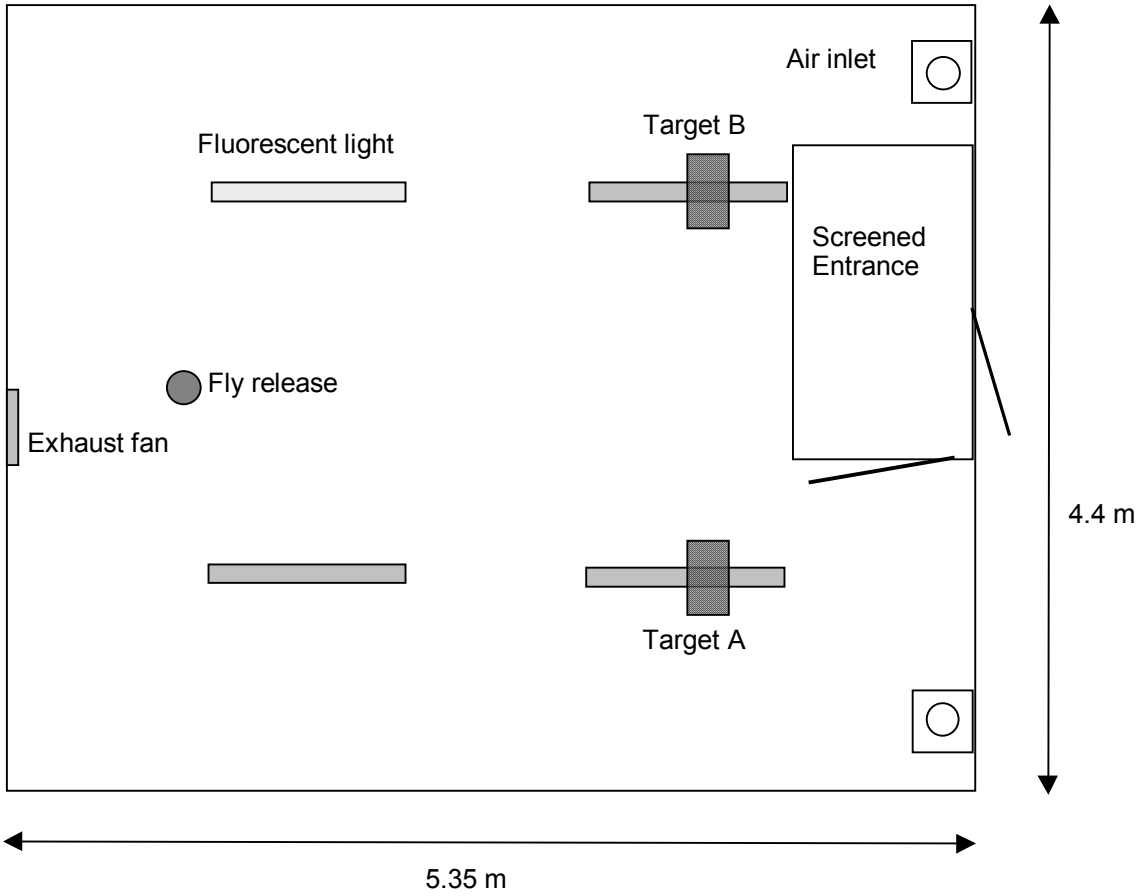

Figure 2: Schematic outline of room used for screw-worm fly assay.

3.5.3 Experimental Procedure

Standard assay conditions were: 45 minute replicates or 30 minutes if one or more sticky traps were used, fan off during replicate, fan on for 15 min after replicate 3 and 6, horizontal target with a half-size yellow sticky pad (Starkeys Products, Wangara WA 6065), 1.2 m above ground, 4 fluorescent tubes on, 8 replicates; wicks on attractants were level with bottle top on open (sticky) targets and 10 to 25 mm above bottle rim in LuciTraps. When LuciTraps were used in the room assay, two traps were used for each treatment. This allowed for one trap to equilibrate (attractant to saturate the air inside the bucket trap) for the subsequent replicate while the other trap was used in the assay. After fly removal the trap was immediately re-assembled and left to

equilibrate. Any variations from these standard conditions were noted on laboratory and result sheets.

Flies from one large cage (from approximately 1400 pupae) were released at the start of the assay and flies from a small cage (700 pupae) were released before replicate 4. The flies were released from a shallow tray on a stool below the fan and equidistant from the targets. They were immobilised by placing the fly cage into a freezer for less than one minute and then transferred onto a flat plastic tray. After allowing for recovery (10 minutes outside the assay room), the flies were released from the tray in the room by removing the gauze cover. At the end of each replicate, the caught flies were removed and the positions of the treatments swapped. The number of female and male flies were counted and analysed (paired samples t test, square root transformed counts). The results are presented as back-transformed mean number of flies caught on treatments 1 and 2, and the probability value for female, male and total fly numbers.

3.6 Investigation of screw-worm fly infested wound

3.6.1 Wound volatiles

Odours from a screw-worm fly infested wound on a Banteng cow were collected at Bbalitvet, Bogor, in March 2007. The artificial wound (Spradbery 1991) was behind the shoulder. Odours from the infested wound were collected on day 1, 3 and 6 post-infestation with screw-worm fly larvae.

For the duration of the odour collection, a modified stainless steel bowl was placed over the wound and held in place with a leather strap (see photograph in Appendix 4). The bowl was modified by drilling two holes on opposite sides and fitting these with collection tubes containing inert adsorbent (Tenax TA). A constant flow air pump was connected to the outlet tube, drawing air (60 ml/min) for 30 min from the outside through the inlet tube and then through the outlet tube. Wound odours were also collected with a solid phase microextraction (SPME) device. This was a syringe-like device, which has a fused silica fibre coated with a polymer designed to adsorb organic compounds. The SPME device was inserted through one hole in the steel bowl and the second hole was closed off (no air flow). Collection time was 20 min.

The Tenax tubes and SPME fibres were kept under cool conditions (refrigerator or esky), transported to Jakarta and analysed within a few days of collection. Desorption from Tenax was by solvent elution (diethyl ether) whereas the SPME was directly inserted into the gas chromatography (GC) injector. Analysis was by GC/mass spectrometry (MS) and the compounds were identified by comparison to a MS library (National Institute of Standards and Technology). Some peaks could only be tentatively identified (indicated by a question mark). The intensity of the MS peak is indicated through reconstituted ion current (mass spectrometer output).

3.6.2 Wound microbiology

3.6.2.1 Sample collection

Swabs were taken from the wound, before, and after a wound incision was made, then processed for bacteriological examination. The assays of total bacteria isolation and identification were conducted at the Bacteriology Laboratory of the Indonesian Research Centre for Veterinary Science (IRCVS). The swab samples were collected from cutaneous and subcutaneous areas of the wound by sterile cotton swabs on day 0, day 3 and day 6 of the SWF infestation.

3.6.2.2 Examination of bacteria

Bacteriological examination was conducted by counting total bacteria (Total Plate Count/TPC), isolation and identification. TPC was based on the method by Vanderzant and Stoesser (1992). Tubes containing 15 ml of Plate Count Agar (PCA) medium were placed in a water bath at 50°C

and allowed to cool before use. The swabs were extracted into 10 ml of buffer peptone-water (BPW) in 1:1; 1:10, 1:100 and 1:1000 dilutions, mixed by vortexing. Two Petri dishes were prepared for each dilution and 1 ml of BPW was poured into the dishes. One tube of PCA was added to each dish and homogenised by moving the dish gently six times in a clockwise circle. After standing for a few minutes the dishes were incubated at 37°C for 24-48 hours. Dishes with 25-250 colonies were used for TPC.

The identification of the bacteria was based on colony morphology, microscopic assessment and culture criteria. Furthermore, the bacteria were identified according to conventional methods for aerobic bacteria, *Proteus* spp. and anaerobic bacteria (Barrow & Feltham 1981; Holt 1994). Aerobic bacteria in the wounds were isolated and grown using BPW which was incubated at 37°C for 24 hours. The bacteria grown in BPW were inoculated on Eosin Methylene Blue (EMB) agar, Blood agar (4% agar) and Nutrient agar. Inoculates were then incubated at 37°C for 24 hours. Pure colonies were taken from culture, smeared on a cleaned glass slide and processed by Gram's staining. Morphological characteristics of the bacteria were observed microscopically. Biochemical tests such as the motility medium, TSI agar, oxidase, catalase, indole, LIA, aesculin, urease, KCN, gelatine liquefaction, methyl red, Voges Proskauer, Simmon's citrate and H_2S production were also carried-out to determine their specific chemical properties. The test of sugar fermentation properties included adonitol, glycerol, maltose, salicin, dulcitol, xylose, trehalose, arabinose, inositol, mannitol, sucrose, lactose, rhamnose and glucose (Barrow & Feltham 1981; Holt 1994).

Proteus spp were isolated from bacteria grown in BPW incubated at 37°C for 24 hours. The cultures (1 ml) were added to Manitol Selenit Cystein Broth (MSCB, 9 ml) and incubated at 37°C for 24 hours. The bacteria was sub-cultured on a Xylose Lysine Desoxycholate (XLD) agar plate and incubated at 37°C for 24 hours. The *Proteus* bacteria were confirmed using the same method as the aerobic bacteria.

The isolation and identification of spore-forming anaerobic bacteria were conducted by examining swabs heated at 75°C for 10 minutes prior to inoculation on Brain Infusion Broth (BHI). The cultures were incubated anaerobically at 37°C for several days, then sub-cultured on Blood agar (4% agar) and Nutrient agar, and incubated further at 37°C for 24 hours. Biochemical test were also carried-out to confirm their specific chemical properties including motility medium, TSI agar, oxidase, catalase, indole, aesculin, urease and gelatine liquefaction. For sugar fermentation properties sorbitol, glycerol, maltose, salicin, dulcitol, xylose, trehalose, mannitol, sucrose and lactose were used (Barrow & Feltham 1981; Holt 1994).

3.7 Field trials - trap improvement

3.7.1 Background

Field trials were carried out at Jelai Gemas, a Malaysian Department of Veterinary Services farm used for breeding improved cattle for Malaysia, by importing breeds from other countries. At the start of the trials in 2006, the farm carried about 600 head of Australian Brahman-cross breed (Droughtmaster) on mainly improved pasture. The farm was destocked in late 2006 and carried only 20 animals during January/February 2007. In March 2007, 1800 head of Chinese Yellow cattle were introduced with 1600 of these present during the field trials 2007/08. Farm employees were trained to carry out the trials by Australian team members.

The objective of these trials was to compare different treatments (lures, traps etc) under field conditions. The experimental design was a duplicated 4x4 Latin square, (4 treatments, 2 x 4 sites, 4 periods) which minimises site and period effects (Perry *et al*. 1980). Treatments were randomly allocated to sites.

3.7.2 Materials

The traps used in the field trials are described in the Traps section (see 3.2). The attractants were provided in plastic bottles with a wick dispenser. The top of the wick was level with the top of the bottle on sticky traps. Inside an enclosed trap, eg LuciTrap, the wick was pulled up (10-20 mm depending on attractant composition) using tweezers, to provide the same release of attractant as on sticky traps (this applies to all lures except A9 where the wick was always left level with the bottle top). At the end of an experiment the wick was carefully pushed back into the bottle until level and the lid put back on (if attractant was to be used again). The treatments consisted of a trap and an attractant (one or two bottles), eg LTM/A9 B110 the modified LuciTrap with two attractant bottles, A9 and B110.

3.7.3 Duplicated 4 x 4 Latin square field trials - Experimental procedure

Two groups of eight sites were selected and marked with numbers 1 to 8 and 9 to 16. The sites were at least 100 metres apart. The sites had similar microenvironments (eg with regard to vegetation, water, shade) which hopefully provided similar fly populations. This was tested by placing a standard treatment at all sites and collecting the flies over four time periods. Traps at sites which provided consistently low catches were shifted. These preliminary catches also gave an indication of how long the time period had to be in order to provide a reasonable catch. A catch of 10 *C. bezziana*, per period was desirable.

In each of these two groups, four treatments were allocated to the sites/periods using random allocations. These treatments needed to be placed at the sites indicated for period 1 at the start of the experiment and then moved to the subsequent site at each change-over. After the first time period elapsed the trapped flies were collected and the treatments changed over. The flies collected from non-sticky traps were placed in separate plastic containers and 70% ethanol added as a preservative while the sticky sheets were kept in a refrigerator (5–10^{o}C) until dispatched to Australia. The fly collection and treatment change-over procedure was repeated with a constant time period until the experiment was completed. At the end of the experiment the flies were sent to Australia with the appropriate documents.

Flies were identified to species level (*C. bezziana* were also sexed) and analysed separately for each fly species by ANOVA after square root transformation. Back-transformed means were reported on a one page summary sheet which also contained the following calculated values which are indicative of the performance of the treatment in the field: composition of trap catch (*C. bezziana* as a percentage of yellow-faced flies and of total catch); potency, the relative catch for each species compared to treatment 1 (often a standard) and selectivity for *C. bezziana* against other fly species (= potency *C. bezziana*/potency other species). To compile results from multiple comparisons between treatments, the average of the potency values was calculated and they were used to calculate the selectivity (these results are contained in Appendix 2).

3.7.4 Physiological age of trapped screw-worm fly

The physiological age of samples of female screw-worm flies was determined as described by Spradbery and Sands (1976).

3.8 Detection of screw-worm fly

3.8.1 Background

Fly catches in modified LuciTraps containing Bezzilure and the prevalence of screw-worm fly strike on animals were concurrently determined on several cattle properties. Two of these properties Matowai Maringu (MM) and UPT Kabaru were in Sumba, eastern Indonesia, and the third property was Jelai Gemas (see section 3.7). MM carried 100 Brahman cross cattle and Kabaru 300 Ongole cattle.

3.8.2 Sumba

The flytrap was a modified LuciTrap (LTM) with Bezzilure attractant and a pest strip. On each farm, four LTMs were attached to a post or tree, 1.5 m above ground and a roof (plastic saucer) was fixed 150 mm above the trap top. The traps were emptied fortnightly at the same time as cattle herd inspections were carried out. Flies were sent to Brisbane for identification and counting.

The numbers of screw-worm fly strikes and wounds in approximately 100 animals were determined fortnightly in a race where animals were inspected closely. The number of animals inspected and the number of strikes and wounds were recorded. Only open, moist wounds (small and large) suitable for screw-worm strikes were counted. These included cuts, scratches, bites, lesions (such as from flies), navels of new born calves, vulva damage from calving or wounds inflicted by routine management practices, such as branding, castration and dehorning. The nature, size and location of strikes and wounds were also recorded.

3.8.3 Jelai Gemas

Trap catches were obtained from LuciTrap/Bezzilure used in the trap improvement field trials. Trapping periods were either 7 or 10 days.

The numbers of screw-worm fly strikes were obtained from the management records at Jelai Gemas. During the period with Droughtmaster cattle, all animals were inspected twice weekly whereas the Yellow cattle were inspected once a week. The number of new strike cases for each month was recorded. All wounds were treated with malathion (Droughtmaster) or coumaphos (Yellow cattle). Wounds with maggots were cleaned and disinfected (hydrogen peroxide, iodine), larvae removed and treated with dichlophenthion/malathion/coumaphos and the animal was treated with injectable ivermectin (Ahmad 2002). To calculate the average point prevalence for each month, the number of monthly strikes was divided by the number of inspections per month (8 with Droughtmasters, 4 with Yellow cattle).

3.8.4 Sample size and sensitivity

Sample sizes were calculated using Epi Tools (AusVet Animal Health Services 2002). The sensitivity of the two detection methods was obtained by dividing the number of positive tests by the number of tests where either one or both tests were positive.

4 Results and Discussion

4.1 Laboratory bioassays

4.1.1 Introduction

A laboratory colony of *C. bezziana* was maintained at Bbalitvet (Sukarsih *et al*. 2000) to provide sufficient flies for testing their responses in laboratory assays. Flies were reared on a blood/milk powder/ egg powder diet and were 4-8 days old when used for assays. The colony produced approximately 10,000 flies per week with an average pupal weight of 37.2 mg.

Two bioassays, a cage and room assay developed during a previous project were used to evaluate the response of SWF to attractants and traps. The cage assay is the initial step in the assessment of attractant mixtures and the room assay can be used to evaluate both attractants and traps in a controlled environment closer to field conditions. Colony bred flies were exposed to different attractants, traps or targets and their responses determined. Choice type assays were utilised in which two treatments were presented to the flies concurrently. These assays

were quick, repeatable, statistically analysable and indicated preferences of screw-worm fly for attractants and/or traps.

4.1.2 Cage assay

More than 50 cage assays were conducted in the early stages of the project to screen potentially new attractants. After overcoming a few problems at the start, the cage assays were highly repeatable and provided statistically significant results even with small response differences between attractants. The cage assay results are given in Table 1. The attractants (treatments), back-transformed mean numbers of female, male and total screw-worm flies responding to both treatments and the corresponding probability (P) value are provided. The response in the initial experiments was lower than obtained in the previous project. However, the response was as good as or better than in the previous project after light levels in the fly colony room were lowered. This measure resulted in less activity in fly colony cages while the flies matured before being used and increased fly activity during the cage assays. It was also established that there was no cage or position bias (CA270606, CA290606). The response between replicates within an assay was more consistent than observed in the previous project. Consequently, response differences between treatments were statistically significant in many assays.

The finding from the previous project that Bezzilure (B110) elicited a greater SWF response than Swormlure-2 (B10) was confirmed. A range of attractants related to B110 (B105, B106, B107, B108, B130, B131, B132, B133, B135, B136) were all slightly, but significantly less attractive than B110. B134 was the only mixture which gave a higher (not significant) response than B110. Another group of 2-me based attractants (B48, B95, B96, B99) was also less attractive than B110. B110, selected from the previous project as Bezzilure, and B134 were the best performing attractants in the 2-me based group in the cage assay.

Table 1: Cage assay results (back-transformed means for treatments 1 & 2, probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
CA170506	B10	A9	10.3	0.5	<0.001	6.5	1.2	<0.001	17.1	1.7	<0.001
CA070606	B10	B110	3.5	8.4	<0.001	2.2	3.9	<0.001	5.8	12.4	<0.001
CA080606	B10	B110	2.8	9.2	<0.001	2.0	5.0	<0.001	4.9	14.3	<0.001
CA130606#	B110	B110	12.3	7.3	0.002	4.7	4.3	0.760	17.2	11.9	0.012
CA150606	B110	B110/A9	7.5	7.1	0.520	2.7	3.4	0.270	10.5	10.6	0.920
CA200606#	B110	B110	23.9	19.8	0.015	9.7	10.0	0.750	33.9	30.1	0.036
CA210606	B110	nil	26.4	0.1	<0.001	11.6	0	<0.001	38.2	0.1	<0.001
CA220606	B110	B130	16.3	13.3	0.013	9.9	10.7	0.498	26.3	24.3	0.024
CA270606	B110	B110	21.1	19.4	0.351	9.6	9.0	0.410	30.9	28.6	0.204
CA290606#	B110	B110	18.7	20.4	0.096	11.7	12.1	0.690	30.5	32.6	0.128
CA030706	B110	B120	23.4	22.9	0.613	9.9	12.2	0.015	33.5	35.1	0.050
CA040706	B120	B128	25.1	21.1	<0.001	13.7	14.3	0.589	38.9	35.4	0.005
CA050706	B128	B129	19.7	14.7	<0.001	14.4	9.2	0.050	34.2	24.0	<0.001
CA100706	B82	B85	15.9	4.4	<0.001	4.8	1.4	<0.001	20.9	5.9	<0.001
CA110706	B118	B120	27.8	32.2	0.013	11.8	11.1	0.575	39.8	43.5	0.017
CA120706	B82	B120	16.8	28.5	<0.001	9.8	12.5	0.002	26.6	41.1	<0.001
CA170706	B95	B96	21.6	18.5	0.006	12.2	9.1	<0.001	33.7	27.8	<0.001
CA180706	B105	B106	20.3	28.3	<0.001	11.0	14.9	0.005	31.4	43.5	<0.001
CA190706	B95	B99	18.9	20.2	0.078	9.7	11.0	0.158	28.6	31.4	0.042
CA240706	B107	B108	21.8	17.9	0.010	9.1	7.8	0.257	31.2	25.8	0.007
CA250706	B106	B107	29.4	27.1	0.030	9.8	11.4	0.249	39.4	38.7	0.619
CA260706	B107	B110	29.3	30.6	0.039	11.3	11.6	0.763	40.8	42.5	0.004
CA310706	B107	B130	30.1	28.2	0.006	11.5	13.2	0.084	42.1	41.7	0.658
CA010806	B110	B110/A9	31.9	28.9	0.004	11.8	9.5	0.005	43.8	38.6	<0.001
CA090806	B99	B110	26.5	31.1	<0.001	10.1	11.8	0.049	36.8	43.1	<0.001
CA100806	B110	B106	29.9	26.6	<0.001	11.4	9.7	0.071	41.4	36.4	<0.001
CA140806	B110	B120	28.0	27.8	0.826	11.3	11.3	0.971	39.3	39.2	0.903

Attractants belonging to the sulfide group are indicated by shading; # different batches of attractants used;

Table 1 (continued): Cage assay results (back-transformed means for treatments 1 & 2, probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
CA280806	B110	B120	29.3	31.6	0.021	11.9	11.0	0.450	41.3	43.0	0.026
CA300806[§]	B110/A9	B110/A9	20.9	21.8	0.354	10.3	9.3	0.482	31.6	31.4	0.821
CA250906	B95	B110	17.7	25.9	<0.001	6.2	8.1	0.031	24.1	34.3	<0.001
CA260906	B106	B110	19.9	27.1	<0.001	6.9	10.6	0.006	26.9	37.9	<0.001
CA270906	B105	B110	16.6	28.5	<0.001	5.2	9.9	<0.001	22.0	38.6	<0.001
CA021006	B48	B106	20.3	22.5	0.048	6.2	7.6	0.128	26.8	30.4	0.001
CA031006	B131	B110	19.4	29.0	<0.001	8.8	10.1	0.293	28.3	39.4	<0.001
CA041006	B132	B110	22.7	25.7	0.032	8.2	10.2	0.104	31.0	36.1	0.003
CA111006	B131	B132	18.8	21.9	0.001	7.4	7.8	0.717	26.3	29.9	<0.001
CA191006[#]	B110	B110	21.8	19.4	0.011	8.6	7.9	0.478	30.8	27.4	<0.001
CA161106	B110	B132	23.4	20.2	0.007	9.3	8.5	0.404	32.9	29.0	0.009
CA221106	B130	B132	20.1	23.4	0.019	8.0	9.3	0.271	28.3	33.0	<0.001
CA061206	B133	B134	24.4	21.5	0.008	11.4	8.3	<0.001	35.9	29.9	<0.001
CA071206	B107	B133	26.2	26.2	0.984	12.2	13.3	0.458	38.7	39.8	0.211
CA211206	B110	B133	30.3	26.6	0.001	10.8	7.7	<0.001	41.3	34.5	<0.001
CA061206	B133	B134	24.4	21.5	0.008	11.4	8.3	<0.001	35.9	29.9	<0.001
CA071206	B107	B133	26.2	26.2	0.984	12.2	13.3	0.458	38.7	39.8	0.211
CA211206	B110	B133	30.3	26.6	0.001	10.8	7.7	<0.001	41.3	34.5	<0.001
CA040107	B95	B133	22.2	29.7	<0.001	7.7	10.1	0.004	29.9	39.9	<0.001
CA110107	B132	B133	26.2	30.1	<0.001	8.6	11.9	0.002	34.9	42.1	<0.001
CA230107	B132	B133	29.0	27.7	0.336	9.7	12.3	0.034	38.8	40.5	0.154
CA300107[#]	B95	B95	31.0	28.5	0.022	11.8	12.7	0.374	43.0	41.3	0.113
CA130207	B110	B134	29.9	31.9	0.099	11.9	11.5	0.701	42.0	43.7	0.137
CA140207	B133	B134	30.2	29.0	0.339	10.1	10.5	0.775	40.6	39.6	0.249
CA021007	B110	B135	32.5	29.0	0.009	10.9	10.0	0.330	43.6	39.2	<0.001
CA031007	B110	B136	29.0	26.8	0.054	11.7	11.1	0.511	40.9	38.1	<0.001
CA081007	B135	B136	22.0	24.9	0.006	8.0	7.6	0.649	30.3	32.7	<0.001

Attractants belonging to the sulfide group are indicated by shading; [#] different batches of attractants used;
[§] A9: T1 without lid, T2 with lid (8 mm hole)

In cage assays with sulfide based attractants (Swormlure being one of them) B120 was a better attractant than B82, B85, B118, B128 and B129. In direct comparisons between B110 and B120, B120 elicited a slightly higher response than B110 in two assays and they were equally attractive in one assay.

The addition of a sodium sulfide solution (A9), which releases small amounts of hydrogen sulfide, to the B110 attractant did not improve the SWF response in the cage assay. Previous findings in field trials indicated that A9 was essential for good SWF catches in LuciTraps. A possible explanation for this apparent inconsistency could be that A9 is required for long range attractancy, a parameter not measurable within the confined space of a cage. This finding was further investigated in insectary assays and field trials.

4.1.3 Room assay

Evaluations of attractants, traps and trap characteristics were obtained in 130 room assays. The room assay results are given in Table 2 (presented in the same format as cage assay results). Initial low responses were improved by installing new fluorescent tubes in the assay room. The new tubes increased the fly activity in the room resulting in higher responses to the treatments. It was demonstrated that the attractants were responsible for the attractancy to sticky pads (low catches on sticky pads without attractants) and that there was no bias between the two target locations in the room.

Results from the major traps used in these assays, the sticky trap, the modified LuciTrap (LTM; fly entry hole size 6.5 mm) and the LuciTrap (LT; hole size 5.5 mm) are described in more detail below. In the room assay no pest strips were used in the LuciTraps.

4.1.3.1 Sticky trap

On the sticky trap, B138 was the best attractant in the 2-me group. B99 was similar and B95 inferior to B110. B110 was as effective as B120 confirming the findings from the cage assay that the 2-me and sulfide based attractants have similar potency for *C. bezziana*. B110 and B138 were significantly better than Swormlure (B10) on sticky traps. The addition of A9 to B110 on the sticky trap did not improve the trap catch. B110 was also much more attractive to *C. bezziana* than spent larval media, a known SWF attractant.

In the room assays, the sticky trap caught more *C. bezziana* than LTM or LT independent of the attractants used. This is to be expected as LuciTrap capture requires additional behavioural steps for the flies to enter the trap. To maintain adequate trap catches in assays where only LuciTraps are used the running time for the replicates was increased from 30 to 45 minutes.

Table 2: Room assay results (back-transformed means for Treatments 1 & 2, Probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
RA140606[§]	ST/B110	ST/B110	4.5	10.9	<0.001	3.5	3.5	0.950	8.1	14.5	<0.001
RA220606	ST/B110	ST/nil	5.8	1.1	<0.001	3.3	0.8	<0.001	9.2	1.9	<0.001
RA050706	ST/B110	ST/nil	18.0	4.2	0.003	14.3	5.4	0.002	32.8	9.6	0.001
RA030806	ST/B110	ST/nil	27.5	3.1	<0.001	15.9	4.6	<0.001	43.6	7.7	<0.001
RA150806	ST/B110	ST/B110	20.9	21.7	0.547	16.3	16.4	0.957	37.3	38.3	0.714
RA230806	ST/B120	ST/B120	37.2	38.9	0.577	24.4	20.8	0.199	61.8	59.7	0.637
RA240806	ST/B110	ST/B120	32.1	29.8	0.254	16.2	21.6	0.196	48.6	51.3	0.568
RA050906	ST/B110A9	ST/B110	24.3	25.4	0.552	12.4	12.5	0.986	37.0	38.1	0.648
RA060906	ST/B110	LTM/B110A9	25.7	12.6	<0.001	11.2	6.3	0.009	37.0	19.1	<0.001
RA070906	LTM/B110A9	LTM/B110A9	6.5	7.3	0.270	3.2	3.6	0.641	9.9	11.2	0.044
RA130906	ST/B110	ST/B110	12.0	12.3	0.834	6.8	6.7	0.970	18.9	19.1	0.896
RA140906	LTM/B110	LTM/B110A9	7.7	6.9	0.308	3.2	2.3	0.279	11.1	9.4	0.049
RA180906	LTM/B110	LTM/B130	3.7	2.9	0.555	2.5	0.9	0.011	6.4	4.2	0.109
RA190906	LTM/B120	LTM/B120A9	5.2	5.0	0.843	1.9	2.4	0.296	7.2	7.5	0.783
RA051006	LTM/B110	LTM/B96	1.8	2.3	0.209	1.3	1.3	0.953	3.2	3.8	0.156
RA091006	LTM/B95	LTM/B99	4.5	3.5	0.172	1.7	1.6	0.905	6.4	5.3	0.348
RA101006	LTM/B110	LTM/B106	4.2	2.4	0.017	2.6	0.8	<0.001	7.0	3.3	<0.001
RA181006[#]	ST/B110	ST/B110	18.4	15.4	0.012	8.1	7.3	0.610	26.8	22.8	0.015
RA131106	LTM/B110	LTM/B95	5.7	1.0	<0.001	2.1	0.9	0.027	8.0	2.1	<0.001
RA141106	LTM/B110	LTM/B99	8.4	3.6	0.009	4.3	2.4	0.061	12.7	6.2	0.006
RA151106	LTM/B110	LTM/B99							9.7	5.4	0.052
RA221106	LTM/B95	LTM/B95A9	1.1	1.6	0.351	0.4	0.5	0.807	1.6	2.2	0.173
RA231106	LTM/B110	LTM/B132	3.8	3.0	0.216	2.2	1.5	0.339	6.1	4.6	0.125
RA291106	LTM/B95	LTM/B95A9	1.2	1.7	0.276	1.1	0.4	0.143	2.6	2.2	0.237
RA041206	LTM/B110	LTM/B132	5.9	4.1	0.054	3.1	3.1	0.941	9.2	7.3	0.225
RA111206	LTM/B107	LTM/B133	8.1	9.7	0.150	3.0	3.3	0.675	11.2	13.3	0.013
RA121206	LTM/B133	LTM/B134	9.0	4.2	<0.001	2.6	1.8	0.110	11.7	6.1	<0.001
RA131206	LTM/B107	LTM/B110	6.6	8.3	0.338	3.1	5.0	0.141	9.8	13.5	0.179
RA201206	LTM/B110	LTM/B133	11.5	7.5	0.022	7.3	4.5	0.022	19.0	12.2	0.013

Attractants belonging to the sulfide group are indicated by shading; [§] different wicks used; [#] different batches of attractants used.

Table 2 (continued): Room assay results (back-transformed means for Treatments 1 & 2, Probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
RA010207	LTM/B95	LTM/B110	5.3	11.3	0.066	3.6	4.8	0.409	9.0	16.3	0.093
RA050207	ST/B95	ST/B110	19.8	28.2	0.002	17.8	16.8	0.626	37.9	45.3	0.017
RA060207	ST/B99	ST/B110	28.3	28.1	0.891	21.9	20.1	0.447	50.6	48.2	0.341
RA070207	ST/B95	ST/B99	17.2	20.4	0.157	9.2	11.2	0.520	26.9	32.0	0.199
RA080207	ST/B10	LTM/B110	15.6	18.7	0.472	16.5	6.3	0.001	32.7	25.1	0.045
RA120207	LTM/B133	LTM/B134	6.7	5.2	0.088	3.3	2.6	0.302	10.1	7.8	0.048
RA150207	LTM/B110	LTM/B134	15.4	7.7	0.002	4.7	4.7	0.976	20.3	12.6	<0.001
RA200207	LTM/B110	LTM/B110A9	15.4	15.5	0.968	8.4	7.3	0.387	24.0	22.9	0.535
RA070307	ST/B10	ST/B110	7.9	17.7	0.003	11.0	12.1	0.509	19.1	29.8	0.008
RA080307	ST/B110	LTM/B110	20.0	11.1	0.005	15.1	4.3	0.010	35.1	15.6	0.006
RA140307	ST/B10	LTM/B10	18.9	3.6	0.001	16.6	3.7	0.013	35.9	7.5	0.002
RA200307	ST/B10	LTM/B10	15.4	2.6	<0.001	15.1	2.3	<0.001	30.7	4.9	<0.001
RA210307	LTM/B110	LT/B110	24.4	11.8	0.010	10.5	7.0	0.005	35.0	18.9	0.002
RA220307	ST/B10	LT/B110	14.7	10.1	0.019	14.2	5.0	0.004	29.1	15.3	0.002
RA120407	LTM/B110A9	LT/B110A9	12.1	7.6	0.024	3.5	3.0	0.693	15.8	10.6	0.062
RA160407	LT/B110	LT/B110A9	13.3	13.4	0.859	4.1	5.0	0.279	17.4	18.6	0.162
RA170407	LTM/B110wl	LT/B110wup	13.3	10.1	0.062	4.1	4.4	0.671	17.6	14.6	0.138
RA180407	LTM/B110	LTM/B48	12.7	6.6	<0.001	4.8	2.8	0.018	17.6	9.4	<0.001
RA190407	LTM/B130	LTM/B132	10.7	7.1	0.006	3.6	3.2	0.638	14.6	10.4	0.001
RA300407	LTM/B110wl	LTM/B110wup	15.2	20.7	0.063	7.9	10.1	0.030	23.0	30.9	0.019
RA010507	LTM/B110	LTM/B110 x 2	19.4	17.9	0.192	7.4	8.1	0.438	26.8	26.0	0.302
RA090507	LTM/B110	LTM(dark)/B110	15.2	26.7	0.033	9.0	9.9	0.287	24.3	36.7	0.031
RA100507	LTM/B110	LTM/B110+water	15.8	17.3	0.311	6.0	6.2	0.876	21.9	23.8	0.283
RA140507	LTM/B110	LTM/swf media	28.2	2.7	<0.001	11.7	3.3	<0.001	40.0	6.3	<0.001
RA150507	LTM/B110	LTM/swf media/water	34.8	5.8	<0.001	14.8	4.1	0.002	49.8	10.1	<0.001
RA230507	LTM/B110	LTM(bdark)/B110	16.6	16.5	0.943	6.5	7.9	0.382	23.1	24.5	0.595
RA240507	LTM/B110	LTM(sdark)/B110	15.4	22.7	<0.001	8.6	10.8	0.211	24.1	33.8	<0.001

Attractants belonging to the sulfide group are indicated by shading; wl = wick level; wup = wick up 25 mm; swf media = used SWF larval feed; bdark = dark bucket base; sdark = dark bucket side

Table 2 (continued): Room assay results (back-transformed means for Treatments 1 & 2, Probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
RA280507	ST/B110	ST/swf media/water	34.8	12.0	0.001	18.2	5.9	<0.001	53.2	18.1	<0.001
RA310507	LTM/B110	LT(dark)/B110	15.2	19.3	0.032	7.3	7.4	0.943	22.5	27.0	0.032
RA040607	LTM(dark)/B110	LTM(sdark)/B110	16.9	15.4	0.259	6.6	6.1	0.675	23.6	21.7	0.034
RA130607	LTM(white)/B110	LTM(dark)/B110	12.7	18.0	<0.001	5.2	7.7	0.041	18.1	25.8	<0.001
RA140607	LTM/B110	LTM(white)/B110	12.5	13.1	0.424	4.9	6.0	0.152	17.5	19.1	0.125
RA180607	LTM(sdark)/B110	LTM(white)/B110	21.0	13.9	<0.001	8.0	5.5	0.003	29.1	19.7	<0.001
RA020707	LTM(black)/B110	LTM/B110	18.1	14.0	0.024	6.0	6.0	0.959	24.1	20.2	0.008
RA030707	LTM(black)/B110	LTM(dark)/B110	11.6	17.9	<0.001	4.1	5.5	0.244	15.8	23.6	<0.001
RA040707	LTM(black)/B110	LTM(dark)/B110A9	11.2	16.1	<0.001	2.6	4.8	<0.001	14.0	20.9	<0.001
RA050707	LTM(black)/B110	LTM(dark)/B120	12.9	10.2	<0.001	4.8	2.9	0.002	17.8	13.1	<0.001
RA160707	LTM(black)/B110	LTM(blk+dk)/B110	10.1	14.3	<0.001	3.4	4.7	0.094	13.6	19.0	<0.001
RA170707	LTM(dark)/B110	LTM(blk+dk)/B110	20.2	18.3	0.166	5.9	5.8	0.900	26.3	24.1	0.218
RA180707	LTM(black)/B110	LTM(blk+wht)/B110	16.2	11.2	<0.001	4.2	2.5	0.064	20.6	13.9	<0.001
RA300707	LTM(black)/B110	LTM/B120	19.0	7.7	<0.001	6.1	2.5	<0.001	25.1	10.2	<0.001
RA310707	LTM(black)/B110	LTM(black)/B110A9	12.2	11.0	0.227	5.5	4.6	0.056	17.8	15.7	0.060
RA010807	LTM/B110	LT(black)/B110	11.5	9.0	0.006	3.1	4.2	0.142	14.6	13.3	0.062
RA080807	LTM/B110	LTM/B120	16.5	7.9	<0.001	4.6	2.4	0.020	21.2	10.4	<0.001
RA130807	LTM(black)/B110	LTM(blk,no hol)/B110	13.2	13.4	0.809	5.0	5.0	0.943	18.3	18.4	0.901
RA140807	LTM(dark)/B110	LTM(blk,no hol)/B110	16.8	15.8	0.354	5.1	4.4	0.368	22.0	20.3	0.181
RA210807	LTM/B110	LTM(bdark)/B110	13.0	12.8	0.789	4.7	4.6	0.891	17.9	17.4	0.426
RA220807	LTM(black)/B110	LTM(dark,tight)/B110	16.9	18.6	0.197	5.2	6.9	0.125	22.3	25.5	0.013
RA230807	LTM(black)/B110	LT(black)/B110	14.5	10.5	<0.001	4.4	3.0	0.045	19.1	13.5	<0.001
RA270807	LTM/B110	LTM(bdark)/B110	19.8	19.0	0.546	6.4	6.9	0.647	26.3	26.0	0.727
RA290807	LTM(black)/B110	LTM(black)/B132	14.3	10.7	<0.001	3.0	2.9	0.971	17.4	13.7	0.003
RA300807	LTM(black)/B110	LTM(black)/B133	14.2	8.3	0.004	3.7	2.4	0.061	18.1	10.7	<0.001
RA030907	LTM(black)/B110	LTM(black)/B134	17.3	9.0	<0.001	3.0	4.0	0.251	20.4	13.1	<0.001
RA040907	ST/B10	LTblack/B110	19.9	11.5	<0.001	7.2	4.0	0.004	27.3	15.6	<0.001

Attractants belonging to the sulfide group are indicated by shading; swf media = used SWF larval feed; dark (dk) = dark bucket (cloth covered); sdark = dark bucket side; white = bucket covered with white paper; black (blk) = black painted bucket (gloss); blk+dk = black bucket covered with dark cloth; blk+white = black bucket covered with white paper; no hol = drain slots covered; bdark = dark bucket base

Table 2 (continued): Room assay results (back-transformed means for Treatments 1 & 2, Probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
RA050907	ST/B10	LTM(black)/B110	22.1	16.4	<0.001	6.1	3.4	0.024	28.3	20.1	<0.001
RA060907	ST/B110	LTMblack/B110	27.8	13.2	<0.001	9.0	2.8	0.005	37.0	16.1	<0.001
RA170907	ST/B10	LTM/B110	25.4	14.0	<0.001	6.4	3.8	0.019	31.8	17.8	<0.001
RA240907[#]	ST/B10	ST/B10	21.4	21.0	0.714	10.6	11.7	0.543	32.4	33.0	0.646
RA250907[#]	LTM/B10	LTM/B10	4.8	4.4	0.468	2.3	1.9	0.596	7.1	6.4	0.269
RA260907	ST/B10	LTM/B110	20.2	12.4	0.010	11.8	2.8	<0.001	32.2	15.2	<0.001
RA091007	LTM(black)/B110	LTM(black)/B135	12.6	8.7	<0.001	3.2	2.9	0.604	16.0	11.6	0.002
RA231007	LTM(black)/B110	LTM(black)/B136	12.7	12.6	0.927	4.1	3.2	0.381	17.0	16.1	0.412
RA241007	LTM(black)/B135	LTM(black)/B136	8.3	8.7	0.664	2.7	3.2	0.474	11.4	11.9	0.471
RA011107	LTM/B135	LTM/B136	8.6	7.0	0.148	2.6	2.7	0.817	11.2	10.0	0.088
RA051107	LTM/B110	LTM/B135	11.7	10.5	0.238	2.5	3.4	0.252	14.4	13.9	0.604
RA191107	LTM/B135	LTM/B133	7.3	8.8	0.214	4.3	2.7	0.106	11.7	11.9	0.815
RA201107	LTM(black)/B135	LTM(black)/B133	10.6	8.7	0.076	3.2	2.2	0.339	14.2	11.0	0.002
RA291107	LTM(black)/B110	LTM(black)/B130	13.6	11.7	0.027	2.7	4.7	0.014	16.4	16.7	0.589
RA031207	LTM(black)/B136	LTM(black)/B130	13.7	11.7	0.030	4.1	3.9	0.835	17.9	15.8	0.013
RA111207	LTM(black)/B137	LTM(black)/B135	12.5	7.8	<0.001	3.5	3.6	0.957	16.4	11.3	<0.001
RA121207	LTM(black)/B137	LTM(black)/B136	10.0	12.3	0.033	2.9	2.8	0.838	13.0	15.3	0.001
RA131207	LTM(black)/B138	LTM(black)/B135	8.5	11.9	0.003	3.3	3.1	0.828	12.1	15.0	<0.001
RA171207	LTM(black)/B138	LTM(black)/B136	8.9	11.9	0.005	2.9	2.4	0.396	12.0	14.5	<0.001
RA020108	LTM/B110	LTM/B136	12.0	13.7	0.101	4.0	4.1	0.894	16.0	17.8	0.003
RA030108	LTM(black)/B137	LTM(black)/B138	8.1	11.4	0.008	3.1	2.8	0.664	11.3	14.4	<0.001
RA070108	LTM(black)/B137	LTM(black)/B110	13.0	12.8	0.740	4.4	3.8	0.550	17.5	16.7	0.058
RA140108[#]	LTM(black)/B110	LTM(black)/B110	12.5	12.1	0.712	3.5	3.9	0.694	16.2	16.2	0.875
RA150108	LTM(black)/B110	LTM(black)/B138	11.6	12.5	0.316	5.0	5.0	0.962	16.8	17.6	0.194
RA160108	LTM(black)/B135	LTM(black)/B138	9.5	10.3	0.453	3.7	2.7	0.274	13.5	13.2	0.639
RA210108	LTM(black)/B137	LTM(black)/B107	9.5	7.1	0.062	3.3	2.6	0.571	13.1	9.9	<0.001
RA290108	LTM/B110	LTM/B137	13.8	8.3	<0.001	5.0	3.1	0.040	19.0	11.5	<0.001

Attractants belonging to the sulfide group are indicated by shading; [#] different batches of attractants used; black = black painted bucket (gloss)

Table 2 (continued): Room assay results (back-transformed means for Treatments 1 & 2, Probability values)

Assay ID	T1	T2	Catches (back-transformed)								
			Female			Male			Total		
			T1	T2	P	T1	T2	P	T1	T2	P
RA300108	LTM/B110	LTM/B138	12.8	11.7	0.195	3.2	4.3	0.408	16.2	16.3	0.978
RA310108	LTM(black)/B107	LTM(black)/B135	7.5	7.5	0.986	3.6	2.3	0.215	11.5	9.9	0.019
RA050208	LTM/B138	LTM/B136	12.4	10.6	0.100	4.4	2.6	0.175	17.0	13.4	<0.001
RA060208 #	LTM/B110	LTM/B110	11.4	11.6	0.863	4.5	3.3	0.308	16.1	15.2	0.141
RA110208	LTM(black)/B138	LTM(black)/B136	9.8	10.3	0.721	2.8	3.2	0.753	12.8	13.8	0.093
RA180208	LTM/B138	LTM/B135	13.0	11.2	0.207	3.8	3.8	0.934	17.0	15.3	0.012
RA190208	LTM/B138	LTM/B137	11.2	12.6	0.196	4.1	3.0	0.354	15.7	15.7	0.977
RA200208	LTM/B138	LTM/B133	12.6	11.1	0.380	4.7	3.3	0.044	17.6	14.5	0.043
RA250208	LTM(black)/B138	ST/B10	11.3	23.0	<0.001	3.5	14.2	<0.001	14.9	37.6	<0.001
RA260208	LTM/B138	ST/B10	12.5	20.6	0.005	4.2	14.9	<0.001	16.7	35.5	<0.001
RA040308	LTM(black)/B107	LTM(black)/B135	8.5	12.0	0.006	2.8	6.0	0.003	11.5	18.2	<0.001
RA050308	ST/B10	ST/B138	17.9	28.1	0.003	10.0	14.8	0.035	28.0	43.1	0.002
RA120308	ST/B110	ST/B138	18.6	23.5	0.054	9.8	11.9	0.188	28.4	35.5	0.049
RA130308	LTM/B136	LTM/B138	13.3	11.5	0.088	4.1	6.1	0.032	17.5	17.7	0.859
RA120508	ST/B110	ST/B138	4.8	4.8	0.872	3.5	3.8	0.135	5.9	6.1	0.008
RA130508	LTM/B136	LTM/B138	3.0	3.7	<0.001	2.0	2.1	0.601	3.6	4.2	<0.001

Attractants belonging to the sulfide group are indicated by shading; # different batches of attractants used; black = black painted bucket (gloss)

4.1.3.2 LuciTrap

The addition of A9 to B110, B120 or B95 in LTM did not improve the catch and in one case reduced it, while in LT a slight improvement (not significant) was observed. This is in agreement with the findings in the cage assays and the sticky trap in the room assays, but contradictory to previous findings in field trials. Further evaluations of the impact of sodium sulfide solution on trap catches were carried out in field tests.

A variety of 2-me based attractants, which were selected from the results of cage assays or previous field trials, were tested in the LTM in the room assay. B110 was a better attractant for *C. bezziana* than B107, B132, B133, B134 and B135. A series of attractants (B95, B96, B99, B107, B130, B132, B135 and B138) were equal to B110. B136 was significantly better than B110, however the response increased only by approximately 10%. B135, B136 and B138 were equally attractive when tested against each other. Similar results were obtained when a black LuciTrap was used. B107, B132, B133, B134 and B135 had lower catches than B110; B130, B136, B137 and B138 had similar catches to B110.

The addition of water to LTM/B110 did not alter the trap catch. Similar to the cage assay, spent screw-worm larval media (either pure or diluted 1:1 with water) had much lower catches than B110.

The impact of the quantity of attractant released on fly responses was also tested (RA170407, RA300407, RA010507). The release rate can be adjusted through altering the level of the wick in the dispensing system. When the release rate was reduced (by lowering the wick) the fly response decreased significantly in one experiment and slightly increased on one (not significant). Doubling the standard release rate did not increase the fly catch. These results indicated that we were using an appropriate release rate for the room assay.

A comparison between LTM (6.5 mm holes) and LT (5.5 mm) showed that LT caught only about half the flies of the LTM. This reduction was due to either reduced fly entry or lower attractant release through the smaller holes.

We investigated the use of a black instead of the standard translucent LuciTrap bucket. Initially a bucket wrapped with black cloth (=dark bucket) was used. The dark bucket had an increased catch (+50%) over the translucent bucket with LTM. A dark LT also had an increased catch over a translucent LTM (+20%). When only the sides of the bucket were covered with the cloth (clear bottom) the catch was also significantly better than the standard bucket but somewhat lower than the fully wrapped bucket. When only the bottom was covered with dark material, there was no improvement over the standard LTM. The black painted bucket also caught more flies than the translucent bucket but it was less efficient than the dark (wrapped) bucket. There were two obvious differences between the dark and black buckets. Firstly, the appearance is different (matt cloth versus gloss paint) and secondly the water drainage slots were covered and open in the dark and black buckets respectively. The black bucket with the slots covered was equal to the one with the open slots and was not as effective as the dark bucket.

There were two plausible causes for increased catches with black and dark buckets compared to translucent buckets: increased response of flies to the black bucket (visual stimuli) or a higher rate of fly entry through the cones into a black rather than a light transparent bucket. To differentiate these two causes we tested a white bucket (no light inside) against dark and black buckets and the white bucket had a lower catch than other buckets. Therefore, the dark/black buckets increase the orientation and approach of flies towards the LuciTrap in the room assay. This finding contrasts with earlier work where catches of *Lucilia cuprina* (Australian sheep blowfly) in LuciTraps with dark buckets were much lower than translucent buckets (Urech *et al*., unpublished) and it may offer a further mean of discriminating between *C. bezziana* and non-target flies. This hypothesis was tested in subsequent field trials.

In summary, the cage and room assays provided new findings with regard to attractant mixtures and trap characteristics. In both assays the inclusion of sodium sulfide solution (A9) did not improve the *C. bezziana* catches. Bezzilure (B110) was one of the best attractants for *C. bezziana* in both bioassays. There were a variety of other mixtures (B95, B96, B99, B107, B130, B132, B133, B134, B135, B136, B137 and B138) which had similar attractancy as B110 and they were evaluated in field trials where the selectivity against other fly species can be determined. In room assays, fly catches in LT were lower than in LTM. Black trap buckets increased the fly catch compared to the standard translucent buckets.

4.2 Investigation of a screw-worm fly infested wound

4.2.1 Background

A moist or seeping wound on warm-blooded animals or humans is a powerful attractant for screw-worm flies. However, a wound infested with screw-worm fly larvae is more attractive than a non-infested wound (Spradbery 1994). Screw-worm fly strikes are generally re-infested by screw-worm fly rather than other blowflies, in contrast to sheep blowfly strike where a succession of species generally occurs. A screw-worm infested wound is thus a potent and selective attractant for screw-worm fly and a good model for a synthetic attractant. Identification of volatile chemicals released from an infested wound could assist in the development of improved and more selective attractants for screw-worm fly.

The volatiles produced in an infested wound are believed to be produced mainly by bacteria. Although fly larvae will also produce volatile metabolites, their main influence may be to create conditions which favour certain bacteria. We initiated an analysis of the bacterial fauna in an infested wound over the time of larval development and odour collection.

4.2.2 Wound volatiles

Analyses of wounds infested with larvae of *C. bezziana* (Urech *et al.* 2002) and *Cochliomyia hominivorax* (New World screw-worm fly) (Cork 1994) by gas chromatography/mass spectrometry (GC/MS) have been reported. However, our GC/MS work was carried out in Australia with samples transported from Indonesia. In this project we collaborated with a chemistry laboratory in Jakarta, approximately a 2 h drive from Bbalitvet where the animal experiment was conducted. The short turn around of samples should provide better quality data.

Components identified by GC/MS using two volatile collection devices (solid phase microextraction [SPME] and adsorption/solvent desorption using Tenax) at day 6 from an artificial screw-worm strike on cattle are listed in Table 3. Components were being detected in samples of one or both odour collection systems. The two systems work on different principles and with different adsorbents resulting in variable capabilities of adsorbing different groups of chemicals.

Table 3: Components, GC retention time (RT) and MS total ion current abundance from solid phase microextraction (SPME) and adsorption/solvent desorption with Tenax

Component	SPME		Tenax	
	RT (min)	Abundance	RT (min)	Abundance
Dimethyl disulfide			3.17	l
Dimethyl trisulfide ?			10.02	t
Acetic acid	12.03	h	11.97	h
Butanediol ?			13.29	l
Propanoic acid	13.40	m		
Hexadacane	14.06	l		
Benzonitrile ?	14.11	l		
Butyrolactone ?	14.46	m		
Butanoic acid	14.64	l		
Heptadecane	15.38	l	15.38	l
Pentanoic acid	15.99	l	15.97	l
Dipentyl disulfide			16.12	l
Octadecane	16.56	l	16.56	l
Hexanoic acid	17.19	l	17.19	t
Benzyl alcohol ?			17.48	t
Dimethylsulfone	17.77	m		
Benzothiazole ?			18.24	t
Phenol	18.78	t	18.78	t
Octanoic acid ?	19.18	t		
4-Methylphenol	19.36	t		
Nonanoic acid ?	19.86	t		
Methyl hexadecanoate			20.15	l
Decanoic acid	20.49	t		
Methyl octadecanoate			21.23	l
2-methylthiobenzothiazole ?			21.31	t
Benzoic acid	21.37	l		
Indole	21.42	m	21.42	t
Dodecanoic acid	21.54	m		
1-Octadecene ?			21.60	m
Ethyl linoleate ?			21.71	l
C13 acid	21.99	t		
9-octadecenenitrile ?			22.15	m
Tetradecanoic acid	22.43	m		
Pentadecanoic acid	22.87	l		
Hexadecanoic acid	23.38	h	23.38	m
9-Hexadecenoic acid	23.58	l		
Octadecanoic acid	24.71	m	24.70	l
Octadecenoic acid	24.97	m		
9-Octadecenamide			28.37	m

Abundance (TIC): h = high (>50%); m = medium (10-50%); l = low (3-10%); t = trace (<3%);
? = tentative identification

The most common components detected with both systems were organic acids, starting with acetic acid and including most homologues to octadecanoic (C18) acid. These acids were much more prominent in this experiment than both previously reported analyses of wound components (Cork 1994; Urech *et al.* 2002). It is not known whether this high acid content is due to the shorter sample turnover time or due to differences in odour production from the wound. Several of these organic acids are already included in most candidate attractant mixtures. Increasing the concentration of various acids in the synthetic attractant did not result in increased fly responses or trap catches. It was not possible within this project to test acids with longer carbon chains (more than 6) for their effect on screw-worm fly response.

Many of the other components detected in wound volatiles are also present in synthetic attractants tested in this and the previous project. Dimethyl disulfide and dimethyl trisulfide were part of the sulfide based attractants but they were substituted by 2-mercaptoethanol which provided better selectivity for screw-worm flies against other flies. Phenol and 4-methylphenol (p-cresol) were also extensively tested. Indole is also an integral part of the synthetic attractants. However, there is also a range of components, which were only tentatively identified or which cannot be purchased from chemical suppliers and it was beyond the scope of this project to investigate their impact on the attractants' efficiency.

Although the synthetic attractants include many of the components emitted from infested wounds, a fundamental difference between the two odour sources remains. Screw-worm fly infested wounds attract mainly gravid female flies which are ready to deposit their eggs. LuciTraps and sticky traps baited with synthetic attractants attract and catch also mainly female flies, but in the early egg development stages (see section 4.3.5). At this point of development, flies are searching for protein to develop their eggs. Thus, a fundamental difference remains between the information originating from wounds and synthetic attractant.

4.2.3 Wound microbiology

The TPC results indicated that no spore-forming anaerobic bacteria were found in the wound. All of the bacteria were aerobic bacteria. These findings correlated with isolations from myiatic wounds of sheep infested by *Wohlfahrtia magnifica* (Khoga *et al*. 2002). The total aerobic counts detected in cutaneous and subcutaneous swabs are presented in Table 4. The results show that the bacteria from cutaneous and subcutaneous swabs increased with time. In general, TPC from cutaneous swabs were higher than subcutaneous swabs.

Table 4. Total aerobic bacteria in cutaneous and subcutaneous swabs of an artificial myiatic wound

Time (days)	Swab origin	Total aerobic bacteria (CFU/ swab)
0	cutaneous	2
	subcutaneous	0
3	cutaneous	1.3×10^6
	subcutaneous	4.8×10^5
6	cutaneous	2.7×10^6
	subcutaneous	6.4×10^5

The species of bacteria identified from cutaneous and subcutaneous swabs are given in Table 5. On day 0, there were only two species identified from the cutaneous swab and none from the subcutaneous swab. On day 3, seven species of bacteria were identified in the cutaneous swab and three species from the subcutaneous swab. On day 6, there were seven and six species detected from cutaneous and subcutaneous swabs respectively.

Table 5. Species of bacteria identified from cutaneous and subcutaneous swabs of an artificial myiatic wound

Time (days)	Swab origin	Species of bacteria
0	cutaneous	1. *Bacillus firmus* 2. *Bacillus megaterium*
	subcutaneous	-
3	cutaneous	1. *Proteus stuarti* 2. *Citrobacter freundii* 3. *Citrobacter kasseri* 4. *Bacillus sp* Wolf and Barker Group I 5. *Bacillus stearotermophilus* 6. *Bacillus brevis* 7. *Bacillus megaterium.*
	subcutaneous	1. *Pseudomonas fluorescens* 2. *Pseudomonas* sp. 3. *Bacillus megaterium*
6	cutaneous	1. *B. megaterium* 2. *B. pantothenticus* 3. *Proteus stuarti* 4. *Citrobacter freundii* 5. *Citrobacter kasseri* 6. *B. coagulans* 7. *Bacillus sp* Wolf and Barker Group I
	subcutaneous	1. *Pseudomonas fluorescens* 2. *Pseudomonas* sp. 3. *Pseudomonas putida* 4. *B. megaterium* 5. *B. coagulans* 6. *Edwardsiella tarda*

Most of the detected bacteria were close relatives of species previously isolated from screw-worm fly infested wounds (Spradbery 1994) (and cited references). This author also demonstrated that 55% of female *C. bezziana* attracted to bacterial broth cultures were gravid. The identification of the major species of bacteria in screw-worm fly infested wounds could assist in developing synthetic attractants which more closely mimic wounds.

4.3 Field trials – Trap improvement

4.3.1 Background

Field trials carried out in areas with native screw-worm fly populations formed the final stage for assessing attractants and traps selected from the results obtained in the cage and room assays. The trial sites had to meet several criteria to enable efficient assessment of the devices:

1. Screw-worm fly population densities must be as high as possible, so as to minimise the length of the sampling period required to provide meaningful *C. bezziana* trap catches
2. Infrastructure and personnel to conduct reliable field tests must be available
3. Fly species and environmental conditions should resemble those present in northern Australia.

Malaysia was selected for field trials because it has adequate SWF populations for longer periods than other South-East Asian countries, such as Indonesia and Papua New Guinea, and a similar range of non-target flies as Australia. Malaysia experiences two monsoon seasons per year (major and minor) which help to sustain SWF populations. The commercial beef farm used for field trials during the previous project, Darabif, was no longer available as it had been converted to palm oil production. In consultation with the Malaysian Department of Veterinary

Services (Dr Vincent Ng) the Government farm Jelai Gemas in Negeri Sembilan was chosen as the best place for field trials. Jelai Gemas was used for breeding improved cattle for Malaysia, by importing Australian Brahman cross breeds. In 2006 when the field trials started, the farm carried about 600 head (Brahman cross) on mainly improved pasture. The farm was destocked in late 2006 and carried only 20 animals during January/February 2007. In March 2007, 1800 head of Chinese Yellow cattle were introduced with 1600 of these present during the field trials 2007/08.

Jelai Gemas had previously been used for SWF research and data on SWF populations was already available (Mahon *et al*. 2004). The Veterinarian in Charge, Dr Arman Bin Kison, approved the proposed collaboration, with farm staff carrying out trap services for an agreed remuneration. Mr Yuen Tack Kan, the former manager of Darabif, was employed to supervise the field work at Jelai Gemas. The rotational grazing practised at Jelai Gemas made trap placement more complicated, as traps should ideally be located near cattle in stable SWF populations.

4.3.2 Results

The field evaluation of attractants and traps was carried out with Latin square experiments (Perry *et al.* 1980). Eight treatments could be tested with 16 trap sites over four periods of either 7 or 10 days (depending on *C. bezziana* abundance). The results from the 4x4 Latin square experiments are summarised in Table 6 and complete results are provided in Appendix 2. Alongside the mean trap catch for each species, the potency (the relative catch for each species compared to a reference treatment) and the selectivity for *C. bezziana* against other fly species is provided. Potency and selectivity of the reference are equal to one.

The results are presented and discussed in various sections for modifications of attractant and trap characteristics. Results obtained in field trials carried out on a different farm in Malaysia during the previous project (1999-2002) are sometimes also included to obtain the best possible evaluation. However, such data is clearly identified, e.g. by the use of italic font in tables.

Preliminary trials (data not shown in Table 6) with the same trap and attractant in all trap sites (modified LuciTrap, B110 A9) indicated that there were adequate SWF populations in Latin square 1 area (trap sites 1-8) with a mean trap catch of 13.0 SWF per week, but not for LS 2 (mean 2.1 SWF). They also indicated considerable variation between sites (ranges of back-transformed mean *C. bezziana* catches were 2.1 - 44.2 and 0.5 - 7.7 for Latin square 1 & 2 respectively) and periods (7.0 - 22.3 and 0.7 - 3.9 respectively). Adjustments were made to the trap sites with the lowest catches. The difference observed between squares 1 & 2 in these preliminary trials was probably due to the low number of animals in the paddocks surrounding the second square. The square difference disappeared during subsequent experiments. Site and period differences remained throughout the trials and these were most likely due to the rotational grazing practised at Jelai Gemas, resulting in frequent changes in animal numbers around trap sites. This variability made it less likely for treatment differences to be significant.

Table 6: Mean trap catches, potency and selectivity values for various fly species from Latin square experiments in Malaysia

Trial	Trap	Attractant	Mean trap catch					Potency					Selectivity for C bez against			
ID			C bez	C meg	C ruf	Hemi	Sarc	C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
MA050706A#	ST	B10	7.3	13.7	36.3[a]	251[a]	27.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B110 A9	11.8	2.7	4.1[b]	9.8[b]	25.4	1.6	0.2	0.1	0.04	0.9	8.2	14	41	1.7
	LTM	B120 A9	9.6	7.1	36.1[a]	4.4[b]	28.1	1.3	0.5	1.0	0.02	1.0	2.6	1.3	76	1.3
	LTM	B130 A9	10.3	2.8	3.2[b]	4.9[b]	25.8	1.4	0.2	0.1	0.02	0.9	7.0	16	73	1.5
MA050706B	LTM	B120 A9	1.3	2.6	25.1	0.5	16.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B81 A9	4.7	3.2	9.5	0.6	16.0	3.7	1.2	0.4	1.3	1.0	3.0	9.6	2.7	3.8
	LTM	B82 A9	1.2	0.8	10.8	0.1	7.4	0.9	0.3	0.4	0.2	0.5	3.2	2.2	4.7	2.1
	LTM	B85 A9	1.9	0.4	6.4	0.1	9.0	1.5	0.1	0.3	0.2	0.5	11	5.7	7.3	2.7
MA020806A	ST	B10	1.4	4.5[b]	41.1[b]	66.4[a]	11.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B120 A9	6.1	208[a]	861[a]	0.5[b]	35.4	4.4	47	21	0.01	3.0	0.1	0.2	577	1.5
	LTM	B118 A9	5.6	40.7[b]	201[b]	0.7[b]	23.8	4.1	9.1	4.9	0.01	2.0	0.5	0.8	375	2.0
	LTM	B128 A9	8.7	27.9[b]	141[b]	0.9[b]	27.1	6.4	6.2	3.4	0.01	2.3	1.0	1.9	465	2.7
MA020806B#	LTM	B110 A9	2.4[b]	6.7	35.6	1.5	13.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B95 A9	10.5[a]	21.7	112	4.2	14.2	4.5	3.2	3.1	2.8	1.1	1.4	1.4	1.6	4.2
	LTM	B96 A9	8.3[a]	34.2	88.1	4.2	21.6	3.5	5.1	2.5	2.9	1.6	0.7	1.4	1.2	2.2
	LTM	B99 A9	9.0[a]	10.5	34.1	3.6	11.2	3.8	1.6	1.0	2.4	0.8	2.5	4.0	1.6	4.5
MA300806A	LTM	B110 A9	7.1[ab]	1.2	13.1	1.6	23.0[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B105 A9	2.4[b]	1.4	7.4	0.4	8.4[c]	0.3	1.2	0.6	0.3	0.4	0.3	0.6	1.2	0.9
	LTM	B107 A9	13.4[a]	3.2	9.6	0.4	20.6[ab]	1.9	2.7	0.7	0.3	0.9	0.7	2.6	7.7	2.1
	LTM	B108 A9	7.0[ab]	1.3	6.5	0.7	13.1[bc]	1.0	1.1	0.5	0.4	0.6	0.9	2.0	2.3	1.7
MA300806B	LTM	B120 A9	5.0	19.0	108[b]	0.1	13.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B101 A9	4.7	1.5	8.4[b]	0.3	24.0	1.0	0.1	0.1	2.9	1.8	12	12	0.3	0.5
	LTM	B123 A9	6.6	67.9	690[a]	0.1	19.7	1.3	3.6	6.4	1.0	1.5	0.4	0.2	1.3	0.9
	LTM	B130 A9	2.8	0.5	7.7[b]	0.5	10.2	0.6	0.03	0.1	4.7	0.8	21	8.0	0.1	0.7
MA011106B	ST	B10	2.0[b]	20.2	56.9	83.1[a]	12.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B95 A9	10.7[a]	9.3	36.1	3.5[b]	14.2	5.4	0.5	0.6	0.04	1.1	12	8.5	129	4.7
	LTM	B99 A9	13.5[a]	11.9	17.1	3.2[b]	16.8	6.8	0.6	0.3	0.04	1.3	12	23	175	5.1
	LTM	B110 A9	10.5[a]	7.2	33.5	2.0[b]	24.4	5.3	0.4	0.6	0.02	2.0	15	8.4	223	2.7

Table 6 (continued): Mean trap catches, potency and selectivity values for various fly species from Latin square experiments in Malaysia

Trial ID	Trap	Attractant	Mean trap catch					Potency					Selectivity for C bez against			
			C bez	C meg	C ruf	Hemi	Sarc	C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
MA291106B	LTM	B110 A9	1.5	3.4	3.8	7.8	15.5[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LT	B110 A9	2.5	5.3	6.0	6.8	8.5[b]	1.7	1.6	1.6	0.9	0.6	1.0	1.0	1.9	3.0
	LTM	B110	0.7	3.8	4.2	5.9	6.0[b]	0.4	1.1	1.1	0.8	0.4	0.4	0.4	0.6	1.1
	WOT	B110 A9	0.5	4.1	2.1	11.2	4.4[b]	0.3	1.2	0.5	1.4	0.3	0.3	0.6	0.2	1.2
MA140407A	LTM	B110 A9	1.6	30.7	73.9	2.6	10.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B99 A9	1.0	30.4	85.5	0.9	9.3	0.6	1.0	1.2	0.4	0.9	0.6	0.6	1.7	0.7
	LTM	B105 A9	1.0	30.9	71.0	0.7	5.7	0.6	1.0	1.0	0.3	0.5	0.6	0.7	2.4	1.2
	LTM	B107 A9	1.0	26.2	68.8	1.3	7.6	0.7	0.9	0.9	0.5	0.7	0.8	0.7	1.3	0.9
MA120507A	LTM	B110 A9	4.5	10.3	40.7	8.0	5.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B130 A9	4.4	2.5	19.2	6.2	4.6	1.0	0.2	0.5	0.8	0.8	4.1	2.1	1.3	1.2
	LTM	B131 A9	7.7	13.0	44.1	9.4	10.7	1.7	1.3	1.1	1.2	1.9	1.4	1.6	1.5	0.9
	LTM	B132 A9	6.1	6.0	23.1	5.5	6.2	1.4	0.6	0.6	0.7	1.1	2.3	2.4	2.0	1.3
MA090607A	LTM	B110 A9	10.4	7.4	36.5	18.0	10.5[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTMnps	B110 A9	5.8	3.6	9.6	9.4	3.7[b]	0.6	0.5	0.3	0.5	0.4	1.1	2.1	1.1	1.6
	LT	B110 A9	8.2	4.4	15.1	10.1	5.2[b]	0.8	0.6	0.4	0.6	0.5	1.3	1.9	1.4	1.6
	LTnps	B110 A9	6.3	3.4	12.1	3.8	3.3[b]	0.6	0.5	0.3	0.2	0.3	1.3	1.8	2.9	1.9
MA090607B	LTM	B110 A9	4.0	0.9	9.0	3.2	3.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B95 A9	2.6	0.5	2.0	3.7	1.9	0.6	0.6	0.2	1.2	0.6	1.1	2.9	0.6	1.2
	LTM	B96 A9	12.7	2.6	11.0	5.9	3.0	3.2	2.9	1.2	1.8	0.9	1.1	2.6	1.7	3.7
	LTM	B99 A9	3.8	1.3	3.8	4.0	2.2	1.0	1.5	0.4	1.3	0.6	0.7	2.3	0.8	1.5
MA070707A	LTM	B110 A9	4.2	1.5	10.1	3.0	2.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B110 A11	4.9	1.8	26.2	2.9	1.8	1.2	1.2	2.6	1.0	0.8	1.0	0.5	1.2	1.5
	LTMblk	B110 A9	1.9	2.8	11.3	1.6	1.1	0.5	1.8	1.1	0.5	0.5	0.3	0.4	0.8	1.0
	LTMblk	B110	4.0	2.9	11.9	4.1	4.5	1.0	2.0	1.2	1.4	1.9	0.5	0.8	0.7	0.5
MA070707B	LTM	B110 A9	1.1	1.8	4.1	0.5	0.6[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B107 A9	1.7	1.6	7.9	2.4	1.1[ab]	1.6	0.9	1.9	4.4	1.9	1.8	0.8	0.4	0.8
	LTM	B133 A9	1.5	1.5	7.2	2.3	0.5[b]	1.4	0.8	1.8	4.3	0.9	1.6	0.8	0.3	1.6
	LTM	B134 A9	2.8	4.6	18.2	1.8	3.8[a]	2.5	2.5	4.4	3.3	6.3	1.0	0.6	0.8	0.4

Table 6 (continued): Mean trap catches, potency and selectivity values for various fly species from Latin square experiments in Malaysia

Trial	Trap	Attractant	Mean trap catch					Potency					Selectivity for C bez against			
ID			C bez	C meg	C ruf	Hemi	Sarc	C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
MA160807A	LTM	B110 A9	6.4	1.3[b]	8.7[ab]	5.9[b]	1.8[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LT	B110 A9	6.3	0.1[b]	3.0[bc]	3.4[b]	2.1[b]	1.0	0.1	0.3	0.6	1.2	13	2.9	1.7	0.8
	LTblk	B110 A9	1.2	0.1[b]	1.3[c]	1.6[b]	0.7[b]	0.2	0.1	0.2	0.3	0.4	2.5	1.2	0.7	0.5
	ST	B10	3.4	8.3[a]	14.6[a]	110[a]	75.7[a]	0.5	6.4	1.7	19	42	0.1	0.3	0.03	0.01
MA160807B	LTM	B99 A9	10.2[a]	2.5	2.9	3.6	4.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B46 A9	1.4[b]	0.7	2.7	1.2	6.3	0.1	0.3	0.9	0.3	1.4	0.5	0.2	0.4	0.1
	LTM	B100 A9	1.7[b]	1.0	1.9	1.3	4.1	0.2	0.4	0.7	0.4	0.9	0.4	0.3	0.5	0.2
	LTM	B101 A9	2.7[b]	1.8	4.9	1.1	6.5	0.3	0.7	1.7	0.3	1.4	0.4	0.2	0.9	0.2
MA300907A[#]	ST	B10	1.0	9.2	39.4	64.8[a]	19.5[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B130 A9	4.0	2.0	29.7	3.7[b]	1.4[b]	4.0	0.2	0.8	0.1	0.1	18	5.2	69	55
	LTM	B133 A9	2.8	0.5	11.3	1.3[b]	4.3[ab]	2.8	0.1	0.3	0.02	0.2	54	9.8	146	13
MA030208A	LTM	B110 A9	0.2	0.1	2.3	0.5	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B110Δ A9	0.3	0	1.7	0.6	1.1	1.2	-	0.7	1.2	6.4	-	1.7	1.1	0.2
	LTM	B99 A9	0.2	0.2	0.9	1.1	0.4	1.0	2.1	0.4	2.2	2.4	0.5	2.7	0.5	0.4
	LTM	B99Δ A9	0.4	0	3.0	1.3	0.3	1.8	-	1.3	2.6	1.9	-	1.4	0.7	0.9
MA030208B	LTM	B110 A9	0.4	0.2	5.2	0.9	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTM	B110 A11	0.4	0.2	9.8	1.5	0.8	1.0	1.0	1.9	1.7	1.3	1.0	0.5	0.6	0.8
	LTM	B110	0	0.1	3.8	0.4	0.7	-	0.6	0.7	0.5	1.1	-	-	-	-
	LTM	B134 A9	0.3	0.2	4.5	0.6	0.5	0.9	1.2	0.9	0.7	0.8	0.7	1.0	1.2	1.1
MA1100508A	LTM	B110 A9	3.4	1.8	3.8	`3.0	2.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	LTMnps	B110 A9	3.0	0.3	2.4	2.1	2.8	0.9	0.2	0.6	0.7	1.3	5.0	1.4	1.3	0.7
	LT	B110 A9	2.0	0.4	0.8	2.0	3.5	0.6	0.2	0.2	0.7	1.6	2.8	2.9	0.8	0.4
	LTnps	B110 A9	2.2	0.6	1.2	2.2	2.9	0.6	0.3	0.3	0.7	1.3	1.9	2.1	0.9	0.5

ST = sticky trap; LTM = modified LuciTrap; LT = LuciTrap; WOT = wind-orienting trap (with LuciTrap top); nps = no pest strip; blk = black bucket;
[#] missing trap catch(es); C bez = *C. bezziana*; C meg = *C. megacephala*; C ruf = *C. rufifacies*; Hemi = *Hemipyrellia*; Sarc = Sarcophagids;
[Δ] = heat treated (50°C, 80 days); attractants belonging to the sulfide group are indicated by shading;
trap catches with different superscripts differ significantly ($P < 0.05$) within trial and column

4.3.3 Evaluation of attractants

A variety of 2-me based attractants closely related to B110 were evaluated in field trials often in multiple comparisons. The best performance indicators for such multiple comparisons are the potency and selectivity values. In Table 7 these values and the number of replicates they were derived from are presented for attractants B95, B96 and B99. The first block of data was obtained in the 2006-08 trials; the second block (italics) from the previous field trials 2000-02 and the last block (bold) is the average of all trials.

Table 7: Potency and selectivity values for B95, B96, B99 compared to B110 (LTM, +A9)

Attractant	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
B95 [A]	3	2.0	1.7	1.5	1.9	0.8	1.2	1.4	1.1	2.7
B96 [A]	2	3.4	4.0*	1.9	2.4	1.3	0.8	1.8	1.4	2.7
B99 [A]	5	1.5	1.6	0.7	1.6	1.1	1.0	2.2	1.0	1.4
B95 [B]	*2*	*2.3*	*3.2*	*6.9*	*2.7*	*2.3*	*0.7*	*0.3*	*0.9*	*1.0*
B96 [B]	*1*	*6.0*	*21.9*	*9.1*	*-*	*2.6*	*0.3*	*0.7*	*-*	*2.3*
B99 [B]	*2*	*2.3*	*7.4*	*4.8*	*4.2*	*2.4*	*0.3*	*0.5*	*0.6*	*1.0*
B95 [C]	**5**	**2.2**	**2.3**	**3.6**	**2.2**	**1.4**	**0.9**	**0.6**	**1.0**	**1.6**
B96 [C]	**3**	**4.2**	**10.0**	**4.3**	**2.4**	**1.7**	**0.4**	**1.0**	**1.8**	**2.5**
B99 [C]	**7**	**1.8**	**3.2**	**1.9**	**2.0**	**1.4**	**0.5**	**0.9**	**0.9**	**1.2**

[A] 2006-08; [B] 2000-02; [C] all trials; * Potency value significantly (P<0.05) different from B110 (=1)

Across all trials, B95, B96 and B99 caught 2.2, 4.2 and 1.8 times as many *C. bezziana* as B110. They also caught more of the other flies and were somewhat less selective for *C. bezziana* than B110 against the other species with the exception of Sarcophagids. The only average potency value, which differed significantly from B110 was the B96 potency for *C. megacephala* in the recent trials. Most values were reasonably consistent between the recent and early trials with a few exceptions: The single trial with B96 in the 2000-02 trials gave unusually high potency values for most fly species and the potency of B99 for *C. rufifacies* compared to B110 was lower in the recent but higher in the early field trials. All three attractant mixtures performed on average better than B110 in catching *C. bezziana.*

Another group of attractants had further variations on Bezzilure: B105 lacked phenol, B107 included acetic acid and B108 had no phenol and half the indole. The field assessments over all trials showed that B107 and B108 were better attractants and B105 an inferior attractant for *C. bezziana* than B110 (Table 8). B108 had better selectivity for *C. bezziana* against the other species than B107.

Table 8: Potency and selectivity values for B105, B107 and B108 compared to B110 (LTM, +A9)

Attractant	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
B105 [A]	2	0.45	1.1	0.8	0.3	0.45	0.4	0.6	1.5	1.0
B107 [A]	3	1.4	1.5	1.2	1.7	1.2	0.9	1.2	0.8	1.2
B108 [A]	1	1.0	1.1	0.5	0.4	0.6	0.9	2.0	2.3	1.7
B105 [B]	*2*	*0.8*	*0.6*	*0.7*	*0.5*	*0.6*	*1.3*	*1.1*	*1.5*	*1.3*
B107 [B]	*1*	*2.9*	*4.3*	*1.4*	*-*	*1.5*	*0.7*	*2.1*	*-*	*1.9*
B108 [B]	*1*	*1.8*	*0.8*	*0.9*	*-*	*1.2*	*2.4*	*2.2*	*-*	*1.6*
B105 [C]	**4**	**0.6**	**0.9**	**0.8**	**0.4**	**0.5**	**0.7**	**0.8**	**1.5**	**1.2**
B107 [C]	**4**	**1.8**	**2.2**	**1.2**	**1.7**	**1.3**	**0.8**	**1.5**	**1.0**	**1.4**
B108 [C]	**2**	**1.4**	**0.9**	**0.7**	**0.4**	**0.9**	**1.5**	**2.1**	**3.6**	**1.6**

[A] 2006-08; [B] 2000-02; [C] all trials

A third group of B110 related attractants included B130 (50% less indole), B131 (50% less acids), B132 (2-me doubled), B133 (2-me increased, acetic acid added, phenol omitted) and B134 (B133 with increased alcohols). The results from their evaluations against B110 are given in Table 9 (only one or two comparisons). B131 to B134 all caught more *C. bezziana* than B110 and the selectivity of B130 to B132 was also better than B110.

Table 9: Potency and selectivity values for B130 to B134 compared to B110 (LTM, +A9)

Attractant	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
B130 [A]	2	0.94	0.62	0.64	0.65	0.91	1.5	1.5	1.4	1.0
B131 [A]	1	1.7	1.3	1.1	1.2	1.9	1.4	1.6	1.5	0.9
B132 [A]	1	1.4	0.6	0.6	0.7	1.1	2.3	2.4	2.0	1.3
B133 [A]	1	1.4	0.8	1.8	4.3	0.9	1.6	0.8	0.3	1.6
B134 [A]	2	1.7	1.9	2.7	2.0	3.6	0.9	0.6	0.9	0.5

[A] 2006-08;

Work in the previous project had demonstrated that an aqueous solution of sodium sulfide, which produces small amounts of hydrogen sulfide, must be present to obtain good *C. bezziana* catches with LuciTrap. Since then the B attractant has been improved and we retested this observation (Table 10). In the translucent LuciTrap the removal of A9 resulted in a 60% reduction of the *C. bezziana* catch. In the second of two experiments (with generally low fly catches) there were no *C. bezziana* in the traps without A9. In one experiment with black trap buckets the removal of A9 doubled the *C. bezziana* catch. It is not known if there is an interaction between the colour of the bucket and presence/absence of A9 or if this apparently contradictory result was obtained by chance.

Table 10: Potency and selectivity values for B110 compared to A9 B110 (LTM)

Attractant	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
B110 [A]	2	0.4 [C]	0.85	0.9	0.65	0.75	0.5	0.4	0.6	0.5
B110 [B]	1	2.1	1.0	1.1	2.6	4.1	2.0	2.0	0.8	0.5

[A] LTM; [B] LTM black; [C] only one replicate (*C. bezziana* catch on B110 was zero)

Trials were also run with sulfide based attractants (B81, B82, B85, B118, B120, B123, B128) which were more closely related to Swormlure (MA050706A, MA050706B, MA020806A, MA300806B). These attractants in LuciTrap caught a similar number of *C. bezziana* as Bezzilure and more *C. bezziana* than the sticky trap with Swormlure. However, unlike Bezzilure, they caught similar or higher numbers of other *Chrysomya* spp. than the sticky trap. The 2-me based attractants have selectivity factors for *C. bezziana* against other *Chrysomya* spp. of about 10,

compared to 1 for sulfide based attractants. Independently of the attractant, the LuciTrap discriminated well against *Hemipyrellia* spp. with an approximate selectivity factor of 100. About the same numbers of Sarcophagids are trapped with LuciTrap with both attractant groups and the sticky trap with Swormlure. Within the sulfide group of attractants B81 and B128 appear to attract the highest numbers of *C. bezziana*. The lower selectivity of the sulfide based attractants for *C. bezziana* makes them a lesser choice as an attractant for use in SWF surveillance traps.

Two attractants (B99, B110) were subjected to an accelerated ageing study (10 weeks at 50°C) to assess their performance after storage. The *C. bezziana* potency after the ageing process was the same as for fresh mixtures, although the catches were low in this experiment. This result confirmed similar observations in laboratory assays during the previous project and indicates that these and probably similar mixtures can be stored for reasonable periods without loss of activity.

4.3.4 Evaluation of traps

The catch of *C. bezziana* in the room assay was increased when the standard translucent buckets were replaced by black buckets. Follow-up room experiments indicated that this increase was due to a better response towards the black trap rather than enhanced fly entry into a dark space. If this observation was to translate to the field, a better catch could be expected. However, two experiments with black buckets, one with LTM and one with LT, showed a reduction of 50% and 80% in the *C. bezziana* catch compared to the standard trap respectively (Table 6, MA070707A, MA160807A). This obvious difference in responses between the room assay and the field may arise from flies using different visual cues indoors and outside (artificial and natural light).

The currently used SWF surveillance trap is a modified LuciTrap with enlarged fly entry holes and incorporates a piece of a commercial pest strip (releasing the insecticide dichlorvos) to increase retention of flies and facilitate collection of flies from the trap. Although the entry holes are cone-shaped to minimise the loss of trapped flies they do not provide 100% retention. Several trials were conducted to evaluate the impact of the hole size and the insecticide on trap catches. The results are summarised in Figure 3.

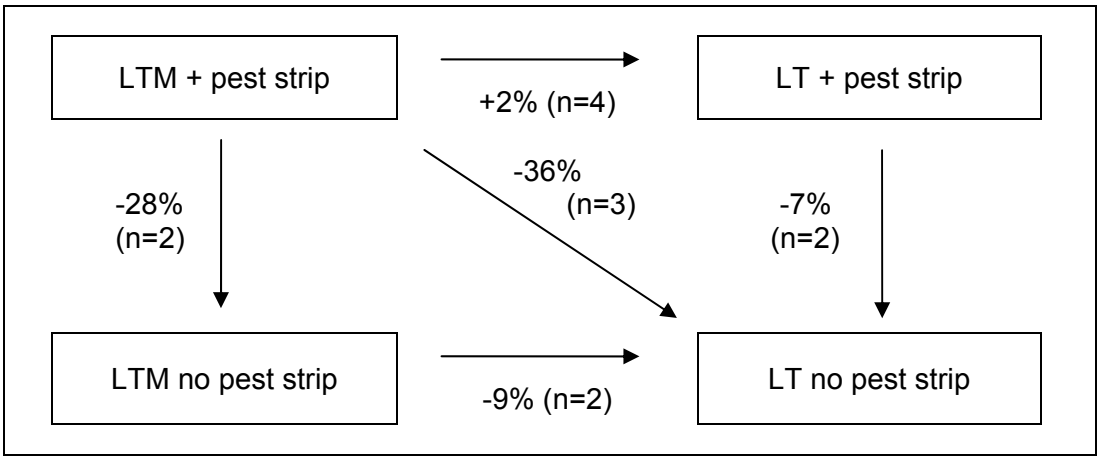

Figure 3: Changes in *C. bezziana* catches in LTM and LT with and without pest strips (number of replicates in brackets)

Reducing the fly entry hole size from 6.5 mm (LTM) to 5.5 mm (LT) resulted in no change in the *C. bezziana* catch over 4 experiments (range 0.6-1.7, mean 1.02). It appears that *C. bezziana* enter equally into LTM and LT. Removal of the pest strip from LTM and LT resulted in a 28% and 7% reduction in the *C. bezziana* catch respectively. It was expected that the impact of the insecticide would be lower in LT than LTM, as smaller holes should provide higher retention of flies when the insecticide is omitted. In three direct comparisons between LTM with and LT without a pest strip a 36% reduction in the *C. bezziana* catch in the LT was observed.

Nine direct comparisons between the sticky trap with Swormlure-2 (B10) and the modified LuciTrap with Bezzilure (B110) or closely related attractants were conducted during this project to solidly define the relation between the old and new trapping systems. Another ten similar comparisons had been conducted during the previous project. The results from these comparisons are presented in Table 11.

Table 11: Potency and selectivity values for LTM/Bezzilure (or closely related attractant) compared to the sticky trap /Swormlure-2

Year	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
2006-08	9	3.5*	0.3*	0.4*	0.04*	0.7	12.9	8.6	86.4	4.7
2000-02	*10*	*1.0*	*0.3**	*0.3**	*0.01**	*0.6**	*3.5*	*3.0*	*109.5*	*1.7*
Total	**19**	**2.2***	**0.3***	**0.4***	**0.02***	**0.7***	**7.8**	**5.9**	**91.1**	**3.3**

* Potency values are significantly (P<0.05) different from ST/Swormlure-2 (=1)

The average potency values for all fly species except *C. bezziana* were very close in the current and previous project. This indicates that in the field the LuciTrap/Bezzilure system consistently catches more *C. bezziana* and less of all non-target flies than the sticky trap/Swormlure combination. The observed difference in the potency value of *C. bezziana* between current and previous trials is likely due to the improvement in the attractants used for the comparisons. The attractants used for the comparisons in this project have been further optimised for catching *C. bezziana* with the new LTM/Bezzilure catching 3.5 times as many *C. bezziana* as the sticky trap. In the previous project both trapping systems caught the same numbers of *C. bezziana.* The LTM caught 30% to 40% of the other *Chrysomya* species, only a few percent of *Hemipyrellia* and 70% of Sarcophagids that were trapped on the sticky trap. The impressive improvement in the LTM system is clearly demonstrated by comparing fly numbers in trap catches containing one *C. bezziana* (Table 12). On the sticky trap there are 64 other flies for each *C. bezziana* whereas in the LTM only 4 other flies are present. The abundance of *C. bezziana* has increased from 1.5% in the sticky trap to 20% in the LTM. Particularly useful for surveillance trapping is the reduction in the other yellow faced fly, *C. megacephala*, by a factor of more than ten. The most common fly on the sticky trap *Hemipyrellia* spp. (59% of trap catch) is almost completely absent from the LuciTrap catch. The increased potency and selectivity for *C. bezziana* of the LuciTrap over the sticky trap has great advantages when used as a surveillance trap. Smaller trap catches take less time to sort and the increased percentage of *C. bezziana* makes it easier and faster to find the target fly.

Table 12: Trap composition for LTM/Bezzilure and sticky trap/Swormlure containing one *C. bezziana*

Trap	Number of flies					
	C bez	C meg	C ruf	Hemi	Sarc	Total
ST/Swormlure[A]	1.0	3.7	12.5	38.1	9.7	65.0
LTM/Bezzilure[B]	1.0	0.3	1.4	0.4	1.9	5.0

[A] Average trap composition 2006/08 trials;
[B] Composition calculated using 2006-08 potency factors (Table 11)

A previously described wind-orienting trap was also tested (MA291106). *Chrysomya bezziana* catches in the wind-orienting trap were 30% of those in the LuciTrap. However, fly catches were low due to inclement weather during this trial and, except for Sarcophagids, treatment differences were not significant.

4.3.5 Physiological age of trapped screw-worm fly

During ovary development, the oocytes of female screw-worm flies pass through a number of different, definable stages (II to X) which indicate physiological age (Spradbery & Sands 1976).

The rates of development are temperature-dependant (Spradbery *et al.* 1991) and influenced by the ingestion of protein (Spradbery & Schweizer 1979). Although screw-worm flies are generally autogenous insects (not requiring external protein in the adult diet to mature their eggs), access to protein results in proportionally more eggs being developed and at a faster rate (Spradbery & Schweizer 1981). Smaller adult flies are more protein dependant than large ones.

In an earlier study using Swormlure-baited sticky traps in Papua New Guinea, the proportion of females in different ovarian cycles was 41%, 50% and 9% in cycles 1, 2 and 3 respectively (Spradbery & Vogt 1993). In that study, the proportion of females at different ovary stages of development (II-X) was determined (see Table 13).

In the present study, a small sample of females was dissected to determine ovary stage although their desiccated state after storage in ethanol did not permit determination of different ovary cycles and many samples (21%) were too dry to determine stage of development. Nevertheless, dissection of flies attracted to the new Bezzilure baits provide some indication of the physiological status of females attracted to the new lure (Table 13).

Table 13. Proportion at various ovary stages of screw-worm flies, *C. bezziana,* trapped by LuciTrap/Bezzilure and sticky trap/Swormlure

Ovary stage	LuciTrap/Bezzilure		Sticky trap/Swormlure [A]	
	Numbers	Percent	Numbers	Percent
II-III	37	49.3	132	22.2
IV	4	5.3	71	11.9
V	13	17.3	153	25.7
VI	17	22.7	64	10.8
VII	2	2.7	85	14.3
VIII	1	1.3	45	7.5
IX	0	0	11	1.8
X	1	1.3	34	5.7
Undetermined	20	-		

[A] Spradbery and Vogt 1993

The early stages of ovary development coincide with the period when females are seeking protein as a supplement for ovary growth and to maximise the number of oocytes matured. A comparison of stages II-V for the two batches shows that they are very similar with 72% of Bezzilure- trapped flies being in these stages compared with 60% in the Swormlure study. The proportion of gravid females attracted to the lures was low with 1.3% to Bezzilure and 5.7% to Swormlure, indicating that the chemically-defined lures are not acting as a 'host-finding factor'. It is thus fair to assume that the new synthetic attractant, like its predecessor, is acting primarily as a protein food source for foraging screw-worm fly females.

4.3.6 Summary

Several attractant mixtures gave equal or better *C. bezziana* trap catches than Bezzilure when used in the LuciTrap. Mixtures based on 2-mercaptoethanol gave better results than sulfide based attractants because they show increased selectivity for *C. bezziana* against non-target flies. The omission of the sodium sulfide solution from Bezzilure reduced the *C. bezziana* catch by over 50%. The selection of an optimal attractant mixture for use in surveillance traps will be described in a separate section.

The use of black rather than translucent trap buckets reduced the *C. bezziana* LuciTrap catch in the field trials. The commercial LuciTrap with smaller fly entry holes caught the same number of *C. bezziana* as the modified LuciTrap. However, removing the pest strip from the LuciTrap with larger and smaller holes did reduce the catch. A wind-orienting trap was less efficient in catching *C. bezziana* than the LuciTrap.

In multiple comparisons in the field the LuciTrap with Bezzilure caught an average 3.5 times more *C. bezziana* than the sticky trap with Swormlure. The LuciTrap/Bezzilure combination provides selectivity for *C. bezziana* against other *Chrysomya* spp. (average factors 9–12) including the yellow-faced *C. megacephala* which is difficult to differentiate from *C. bezziana* on morphological criteria. The LuciTrap also discriminates with about a factor of 100 against *Hemipyrellia* spp. compared to the sticky trap. The selectivity against other *Chrysomya* spp. is not achieved when sulfide based attractants are used in the LuciTrap. This selectivity is important to maximise the probability of detecting *C. bezziana* in trap catches and to reduce the resources required to process trap catches either by morphological inspection or real-time PCR assay.

4.4 Detection of screw-worm fly

4.4.1 Background

Several tools for the detection of a SWF incursion into Australia are available. These include tools for detecting adult flies (traps, screw-worm strike), larvae removed from animals or humans or, less likely, egg masses in wounds. All these systems need a method to collect and then identify the samples. It is in the best interest of Australia to use optimal detection systems, i.e. tools which are capable of detecting SWF at low density and which are cost effective. We investigated the sensitivity of two tools for the detection of adult flies, trapping and inspections of animals for screw-worm fly strike.

Although it is known that detection using artificially wounded cattle, with a deep X-shaped wound, is about 5 times more sensitive than one trap day [numbers of egg masses versus catches on sticky trap with Swormlure (Mahon *et al.* 2004; Spradbery 1994)]. Animal welfare considerations prevent the use of artificially wounded animals in Australia. It has been suggested that monitoring commercial cattle herds, with SWF infestation of natural wounds as the indicator for SWF presence, may be used instead of artificially wounded sentinel animals. Alternatively, the newly-developed screw-worm fly trap, which can be used over extended periods, is available as an improved fly trapping tool. We have investigated the sensitivity of these tools in concurrent trapping and animal inspections in two locations with endemic screw-worm fly populations. The first location was Sumba (eastern Indonesia) where SWF populations are low and environmental conditions are similar to northern Australia. The second location was Jelai Gemas (Malaysia) with higher SWF populations in a high rainfall and improved pasture environment. We also collected data on wounding in extensively grazed cattle in northern Australia to verify the applicability of the overseas data to Australia.

4.4.2 Sumba

The fly trapping and animal inspections commenced in Sumba in June 2007 and continued to the end of October 2007 when they were suspended due to dry weather and absence of screw-worm flies. Monitoring was resumed in February 2008 after the monsoonal rainfall and continued to June 2008. The number of *C. bezziana,* blowflies and other flies caught in LuciTraps on two farms (Matowai Maringu and Kabaru) are provided in Table 14. As expected, the abundance of *C. bezziana* was low in Sumba. A total of 23 and 29 *C. bezziana* were caught on Matowai Maringu and Kabaru respectively. Over the same period 492 and 1370 other blowflies or 942 and 1993 total flies were trapped. Thus, only 1.8% of the flies trapped in LuciTrap/Bezzilure were *C. bezziana.*

Table 14: Fly catches from LTM/Bezzilure at Matowai Maringu (MM) and Kabaru

Date	Number of flies [A]					
	C. bezziana		Blowflies		All flies	
	MM	Kabaru	MM	Kabaru	MM	Kabaru
10/07/2007	0	6	0	8	7	13
24/07/2007	0	0	0	0	19	6
11/08/2007	0	4	1	5	15	21
26/08/2007	0	0	1	4	7	7
10/09/2007	1	1	1	12	8	18
23/09/2007	0	1	1	19	5	21
12/10/2007	0	1	17	331	19	334
26/10/2007	0	1	24	47	24	47
26/02/2008	4	1	7	5	50	34
12/03/2008	2	0	9	3	53	34
27/03/2008	10	8	17	356	124	483
12/04/2008	0	3	93	335	110	475
27/04/2008	3	1	300	194	364	230
12/05/2008	2	2	13	17	90	102
27/05/2008	1	0	7	33	21	141
6/06/2008	0	0	1	1	26	27
Total	**23**	**29**	**492**	**1370**	**942**	**1993**

[A] 4 LTM, 14-day trapping period

From 16 collections (= dates), 7 (44%) at MM and 11 (69%) at Kabaru contained *C. bezziana* providing a positive result for the detection of SWF. Twenty-one percent and 25% of the individual trap catches at MM and Kabaru respectively contained *C. bezziana.*

The results from the fortnightly herd inspections are given in Table 15. Over the whole inspection period, there were 13 screw-worm fly strikes at MM and one strike at Kabaru. On both farms there were plenty of wounds suitable for a fly strike with average wounding rates of 11.1% and 12.5% at MM and Kabaru respectively. Wounds were consistently present at both farms during the experiment. A break down by wound types showed that there were about the same abundance of cuts (3.6 and 4.0%) and brand wounds (2.2% and 2.7%) on MM and Kabaru; MM had a higher incidence of lesions from ticks (4.8%) than Kabaru (2.9%) but the latter had more lesions from *Hippobosca* sp. flies (5.5%) than MM (2.4%). Other wounds accounted for less than 0.2% at both farms. SWF strikes were found on 6 (38%) and on 1 (6.3%) of the 16 animal herd inspections on MM and Kabaru respectively, with an average number of strikes per inspection of 0.81 and 0.063.

Table 15: Wound and strike prevalence at Matowai Maringu (MM) and Kabaru

Date	No. animals		Strikes		Wounds		Strikes (%)		Wounds (%)	
	MM	Kabaru	MM	Kabaru	MM	Kabaru	MM	Kabaru	MM	Kabaru
26/06/2007	113	84	4	0	10	9	3.5	0.0	8.8	10.7
10/07/2007	110	64	3	0	5	4	2.7	0.0	4.5	6.3
24/07/2007	111	54	3	0	6	11	2.7	0.0	5.4	20.4
26/08/2007	51	53	0	0	5	8	0.0	0.0	9.8	15.1
10/09/2007	55	50	0	0	8	8	0.0	0.0	14.5	16.0
23/09/2007	59	74	0	0	8	8	0.0	0.0	13.6	10.8
12/10/2007	54	65	0	0	7	12	0.0	0.0	13.0	18.5
26/10/2007	56	85	0	0	8	7	0.0	0.0	14.3	8.2
12/02/2008	72	88	0	0	10	9	0.0	0.0	13.9	10.2
26/02/2008	69	64	0	0	7	10	0.0	0.0	10.1	15.6
12/03/2008	71	64	0	0	6	8	0.0	0.0	8.5	12.5
27/03/2008	80	85	1	1	11	11	1.3	1.2	13.8	12.9
12/04/2008	66	96	1	0	11	9	1.5	0.0	16.7	9.4
27/04/2008	71	83	1	0	10	10	1.4	0.0	14.1	12.0
12/05/2008	95	72	0	0	8	8	0.0	0.0	8.4	11.1
27/05/2008	78	73	0	0	7	7	0.0	0.0	9.0	9.6
Total	**1211**	**1154**	**13**	**1**	**127**	**139**	**0.8**	**0.1**	**11.1**	**12.5**

The LuciTrap and animals with suitable wounds are both potential indicators for the presence of screw-worm flies. With the exception of March and April 2008, there appeared to be little correlation between the positive results from the traps and the animal inspections. In addition, MM which had 13 out of 14 strikes had fewer *C. bezziana* in the traps than Kabaru. MM is an open farm with little vegetation but a few living fences (rows of 3 to 5 m high trees) whereas Kabaru has large areas of thick and tall rain forest interspersed between cleared paddocks. It is known that the paddock to dense forest interface provides higher trap catches of *C. bezziana* than open paddocks or dense forests, probably because this is a favoured resting place for flies. This hypothesis may be supported by the fact the Kabaru traps also gave higher catches of other flies than the traps at MM. Another possible reason, particularly for the incongruity between fly catches and strike prevalence on the two farms, is the difference in cattle breed. Although, both cattle breeds are *Bos indicus* derived, the Ongole breed at Kabaru may possess a higher resistance to ticks and other ectoparasites than the Brahman cross cattle at MM. There were fewer tick lesions on the animals at Kabaru (2.9%) than MM (4.8%), but the reverse was true for *Hippobosca* sp. fly lesions (5.5% and 2.4% respectively).

A summary of the results from the animal inspections and fly trapping for both farms and totals over the whole experiment are shown in Table 16. Sizes of the samples required to detect screw-worm fly with a given probability were calculated from the prevalence or proportion values (AusVet Animal Health Services 2002) and shown in Table 17. To detect SWF with 95% confidence at the average abundance of the two Sumba farms, the inspection of 507 animals or the setting of 12 LuciTraps with Bezzilure over a 14-day period was required. Both systems were capable of detecting SWF at low density.

A direct comparison of the number of positive and negative results obtained from the two tests on both farms is provided in Table 18 (only values used from dates when trap catches and herd inspections were carried out). At MM, on two occasions both tests were positive, on five occasions the trap was positive and the animal inspection negative, on three occasions the traps were negative and the inspection positive and on 4 occasions both were negative. At Kabaru, the traps gave a positive result on 11 occasions and on only one of these was fly strike detected. From these correlations, relative sensitivity for each test can be calculated. Sensitivity (or the true positive rate) reflects the proportion of sample times when SWF are present that the method returns a positive result. Fly trapping using four LuciTraps/Bezzilure over a fortnight was more

sensitive than herd inspection (75 animals) at both farms and the average sensitivity was 0.85 and 0.30 for fly traps and herd inspection respectively.

Table 16: Summary of results from animal inspections and fly trapping in Sumba

Animal inspections	MM	Kabaru	Total
No of animals	1211	1154	
No of wound	127	139	
No of strikes	13	1	
Prevalence of strike	0.0107	0.0009	0.0059
Fly trapping			
No of collection dates	16	16	
No of trap collections	63	64	
No of *C. bezziana*	23	29	
C. bezziana/collection	1.44	1.81	1.63
C. bezziana/trap	0.365	0.453	0.409
C. bezziana/trap/day	0.026	0.032	0.029
No of positive dates	7	11	
Proportion of positive dates	0.44	0.69	0.56
No of positive traps	13	16	
Proportion of positive traps	0.21	0.25	0.23

Table 17: Sample sizes required for the detection of screw-worm fly by animal inspections and fly trapping in Sumba

Method	Prevalence	Sample size at various levels of confidence				
		50%	80%	90%	95%	99%
Fly strike	0.0059	118	272	390	507	779
Trap [A]	0.23	3	7	9	12	18

[A] LuciTrap/Bezzilure, 14-day trapping period

Table 18: Number of herd inspections and fly trap collections which gave positive or negative results and sensitivity of these tests for detecting *C. bezziana*

		MM Fly strike [B]		Kabaru Fly strike [B]		Total
		+	-	+	-	
Trap [A]	+	2	5	1	9	
	-	3	4	0	4	
Sensitivity	Strike	0.50		0.10		0.30
	Trap	0.70		1.00		0.85

[A] 4x LuciTrap/Bezzilure, 14-day trapping period; [B] Inspection of 75 animals in race

4.4.3 Jelai Gemas

Trap catches and strike prevalence were also obtained at Jelai Gemas which has a higher SWF population. Trap catches were derived from the attractant and trap evaluation experiments and strike data were recorded for standard farm management. Historic data (1996-2001) on strike prevalence and trap catches at Jelai Gemas was also available. Over this period there were 1000-3000 Droughtmaster cattle on the farm and the average monthly proportion of animals with strikes was 0.028 (Mahon pers. comm.). This value was obtained from two inspections of the whole herd per week. Average catches of female *C. bezziana* on sticky traps with Swormlure over the same period was 0.54 flies per day on the farm and 0.05-0.08 flies off the farm.

The monthly number of *C. bezziana* per LuciTrap/Bezzilure and the number of monthly strikes during our experiments are shown in Figure 4. Trap catches and strike prevalence were higher

than in Sumba. As there were no zero values for traps or animals, both sampling methods had 100% sensitivity at this location. The low values from December 2006 to February 2007 were observed when the farm was destocked and very few animals remained. Trap catches and strike numbers are tightly correlated over time. Although about three times more cattle (of a different breed) were introduced to Jelai Gemas in March 2007, the number of strikes was lower than during the previous year. The average strike rate dropped from 1.4% (June to November 2006) to 0.26% (April 2007 to March 2008). This reduction in strike prevalence is due either to lower SWF susceptibility of the Chinese Yellow cattle compared to the Droughtmaster breed or to a change in the insecticide used for treatment of wounds. After the arrival of Yellow cattle, malathion was replaced by coumaphos for wound treatment. Coumpahos is considered one of the better treatments for protection against screw-worm fly strike and will last for up to 14 days (James *et al.* 2006). Because of this marked difference in strike prevalence we kept these two groups separate for the calculation of sample sizes.

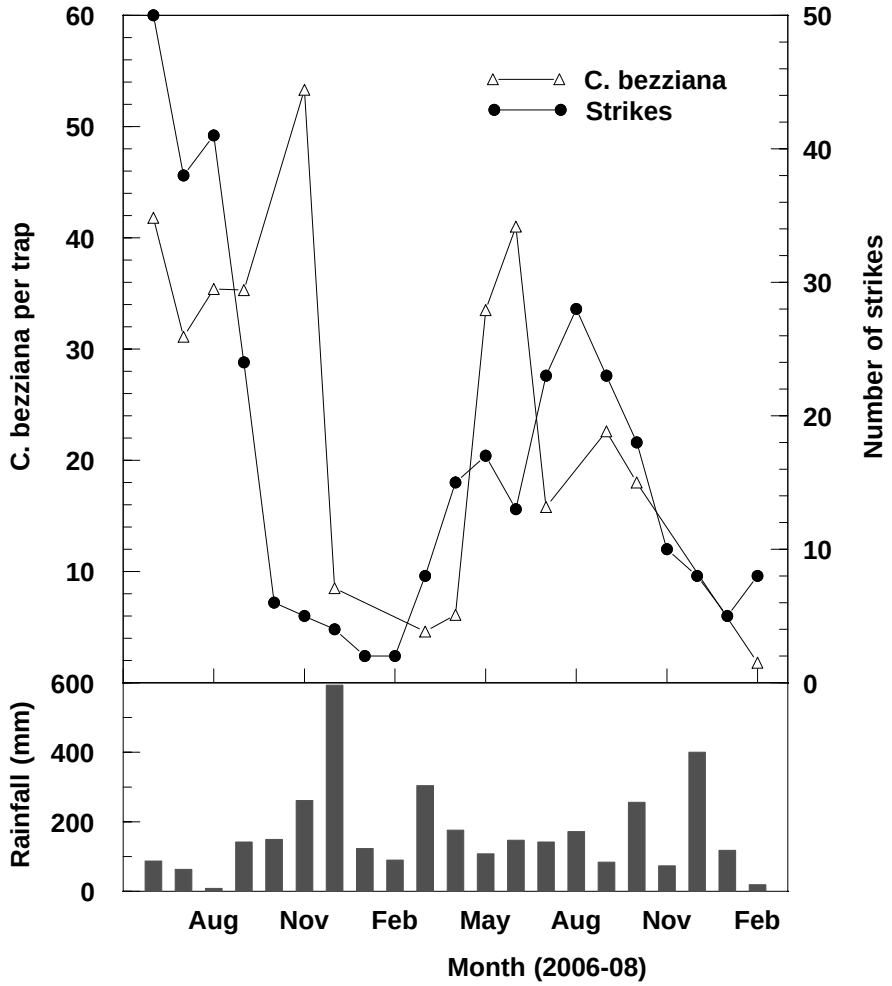

Figure 4: Monthly rainfall, *C. bezziana* catches (LTM/Bezzilure) and strikes on Jelai Gemas from June 2006 to February 2008

A summary of strike prevalence and trap catches at Jelai Gemas are given in Table 19. Because of the difference in strike prevalence before and after the introduction of Yellow cattle these two periods (June to November 2006 and April 2007 to March 2008) were treated separately. In the first period with the higher strike prevalence (0.014) 209 animals had to be inspected or two LuciTraps opened for 7 days to detect SWF at Jelai Gemas with 95% confidence. During the period of low strike prevalence (0.0026) 954 animals had to be inspected or three LuciTraps needed to be opened for 10 days. The traps were more sensitive in detecting screw-worm flies at Jelai Gemas than in Sumba with 85% and 66% of the traps containing *C. bezziana* during the first and second period respectively. The strike prevalence at Jelai Gemas was also higher than

Sumba during the first period but only half of the Sumba prevalence during the second period. Therefore, only two or three traps were required but 209 and 954 animals have to be inspected to detect screw-worm fly with 95% certainty during the first and second period respectively. One possible explanation for the difference between Sumba and Jelai Gemas is that only new cases of strike are recorded at Jelai Gemas whereas all strikes were counted in Sumba. The strikes are treated with an insecticide and disinfectant at both locations but some strikes may remain active to the next inspection.

Table 19: Summary of animal inspections and fly trapping at Jelai Gemas and sample sizes required for the detection of screw-worm fly (population size: 4000 for strikes, large (infinite) for traps)

Unit	Prevalence	Sample size at various levels of confidence				
		50%	80%	90%	95%	99%
Strikes (1996-2001) [A]	0.0035	194	435	607	771	1122
Strikes Jun – Nov 2006 [B]	0.0140	50	114	162	209	316
Strikes Apr 2007 – Mar 2008 [B]	0.0026	245	545	756	954	1369
C bezziana 1996-2001 outside farm [C]	0.05-0.08					
C bezziana 1996-2001 inside farm [C]	0.54					
C bezziana Jun – Nov 06 [C]	1.4					
C bezziana Apr07 – Mar08 [C]	0.62					
Positive traps 1996-2001 outside [D]	0.06	12	27	38	49	75
Positive traps Jun – Nov 06 [E]	0.85	1	1	2	2	3
Positive traps Apr07 – Mar08 [F]	0.66	1	2	3	3	5

[A] 8 inspections per month; [B] 4 inspections per month ; [C] per trap per day;
[D] 1-day trapping; [E] 7-day trapping; [F] 10-day trapping

4.4.4 Prevalence of wounds on cattle in Northern Australia

The prevalence of wounds on cattle in northern Australia was required to ascertain if the comparisons between the two detection methods in Indonesia and Malaysia were valid for Australia. It was essential for an extrapolation of the findings to Australia that the proportion of animals susceptible to screw-worm fly strike was similar. Screw-worm fly strikes are initiated on open and moist wounds; therefore the prevalence of wounds was a good indicator of susceptible animals. It was assumed that screw-worm fly would respond similarly to the LuciTrap/Bezzilure trapping system in Australia as in Malaysia and Indonesia.

The proportion of beef cattle with open wounds caused by management and natural causes was obtained by Anaman *et al.* (1993) from 21 questionnaires completed by extension officers in Queensland, New South Wales and South Australia. The average proportion of cattle with open wounds per week in regions with extensive grazing was 4.9%, 8.9% and 15.8% for steers, cows and calves respectively (Anaman *et al.* 1993).

A survey form was distributed to determine the number and type of wounds on cattle in northern Queensland and the Northern Territory (NT). From the small return (2 surveys from NT, total 1000 animals), the point prevalence of wounds was approximately 2% in both herds. The main types of wounds were sarcoids and barbed wire damage. In addition, one inspected herd (approximately 100 head) had a 10% prevalence of buffalo fly lesions.

The proportion of animals with wounds will vary over time and the two surveys likely reflect period and point prevalence. The presence or absence of buffalo fly lesions (caused by *Stephanofilaria*) can change the susceptibility to screw-worm fly considerably with buffalo fly lesions reported on up to 98% of animals (10% raw and weeping) (Sutherst *et al.* 2006). However, the available wound prevalence values for (northern) Australia are roughly equivalent to or lower than the Indonesian wounding rates. This means that the same or higher numbers of

animals need to be inspected in Australia compared to Indonesia in order to maintain screw-worm fly detection parity with fly traps.

4.5 Optimal Screw-worm fly surveillance trapping system

4.5.1 Background

An optimal screw-worm surveillance trapping system is sensitive and selective for *C. bezziana.* Other considerations in the selection of the system are its availability, cost, ease of use and acceptability to users (i.e. toxicity, smell). In this section we define such an optimal detection system using current information and provide a rationale for the selection.

The currently used screw-worm fly trapping system consists of a modified LuciTrap (diameter of fly entry hole 6.5 mm), Bezzilure (two attractant mixtures A9 and B110), a piece of a commercial pest strip releasing the volatile insecticide dichlorvos and a roof to prevent entry of rain. The LuciTrap is commercially available from Bioglobal Pty Ltd and is modified by enlarging the existing fly entry holes from 5.5 mm to 6.5 mm. Modifications of the trapping system have primarily been assessed against the existing system.

4.5.2 Screw-worm fly trap

The currently used trap incorporates a piece of commercial pest strip to increase retention of flies and to facilitate collection (live flies can escape when traps are serviced). Although the fly entry holes are cone-shaped to prevent the loss of trapped flies they do not provide 100% retention. It was expected that the impact of a pest strip on the trap catch was dependent on the size of the fly entry hole and therefore, the unmodified and modified LuciTrap were tested with and without the pest strip (see section 4.3.4). The unmodified LuciTrap caught 2% more *C. bezziana* than the modified trap (both with pest strip), indicating that the smaller hole size did not reduce fly entry into the trap. Removal of the pest strip from the modified and unmodified LuciTrap resulted in a 28% and 7% reduction in the *C. bezziana* catch respectively, indicating that the insecticide increased the catch. A 36% reduction in the *C. bezziana* catch was observed when moving from the modified LuciTrap with pest strip to the unmodified trap without pest strip.

The commercial pest strips releasing dichlorvos have been removed from sale in several countries due to concerns of human exposure to the insecticide. This could also happen in Australia at some future time. There is no equivalent replacement insecticide and most replacement products rely primarily on repellency rather than toxicity to insects. Such products are obviously not suitable for inclusion in a trapping system. It may be prudent to select a screw-worm fly surveillance trap which does not rely on the availability of these pest strips.

The field trial results demonstrated that the loss of *C. bezziana* is lower from traps with smaller than with larger entry holes when no pest strip is present. Fly entry through the smaller holes appeared to be at least equivalent to entry through larger holes. Therefore, **it is recommended that the commercial LuciTrap without a pest strip is used as the surveillance trap for screw-worm fly in Australia**. The change from modified to unmodified LuciTrap will lower the expected *C. bezziana* catch by about one third, but it eliminates the need for pest strips and allows the use of a commercial product without modifications. The change to the commercial trap also eliminates the modification of the LuciTrap which required the enlargement of more than 50 entry holes with an appropriate drill bit. However, it may be advantageous to continue using the pest strip in the commercial trap while it is available. Particularly, when using short trapping periods (e.g. less than 2 weeks) a considerable proportion of the trapped flies may still be alive and could escape on collection. No other insecticides should be used to kill the flies as many are known repellents and could reduce trap catches. Agents which are acceptable to immobilise flies while they are transferred to containers are carbon dioxide or low temperature but both are inconvenient for field use.

The only alternative trap tested in this project was the wind-orienting trap. It was the best performer of a much bigger selection of traps screened in the previous project. The wind-orienting trap caught only 30% of *C. bezziana* caught in the LuciTrap and is much less user friendly to set up and service. For trapping *C. bezziana* the wind-orienting trap is inferior to the LuciTrap.

In field trials, the catch of *C. bezziana* in LuciTraps with black buckets was half or less of the traps with translucent buckets. Although this result is opposite to the results in the room assays, where black buckets performed better, the currently used translucent buckets should be retained.

During a transition period the currently-used modified LuciTrap and the newly recommended commercial LuciTrap may be in circulation. For this reason, it is recommended that all existing modified LuciTraps are clearly marked “LTM” on the yellow bracket with a permanent marker pen. The modified LuciTrap can still be used, but in this case it is recommended that a piece of pest strip is also used.

4.5.3 Screw-worm fly attractant

The current attractant for *C. bezziana,* Bezzilure, is made up of two immiscible solutions presented in two bottles (Bezzilure A = A9; Bezzilure B = B110). Bezzilure A is an aqueous solution of sodium sulfide which releases a small amount of hydrogen sulfide (rotten egg gas) when exposed to air. Bezzilure B is a mixture of organic chemicals such as short fatty acids, alcohols and aromatic components. Results form the previous project had indicated that both mixtures had to be present for optimum attraction of *C. bezziana*. During this project we tested the impact of variations to the chemical composition of the mixtures on the catch of *C. bezziana* and other flies in laboratory and field trials. This information and other criteria listed in 4.4.1 are used to select an optimal attractant for a screw-worm fly surveillance trap.

The removal of Bezzilure A from the combination resulted in a 60% reduction in the *C. bezziana* catch in one experiment. In the second experiment there were nil *C. bezziana* when only Bezzilure B was present and a small number of *C. bezziana* when Bezzilure A and B were present. It appears that the retention of Bezzilure A is essential for good attraction of *C. bezziana*. Sodium sulfide is an inexpensive chemical and the aqueous solution of this salt is easily prepared.

Several experiments confirmed that 2-mercaptoethanol based attractants were superior to mixtures based on organic sulfides, i.e. dimethyl disulfide. The former group attracted as many or more *C. bezziana* and fewer non-target flies than the latter group. Many variations of 2-mercaptoethanol based attractants, including the addition of other components, the omission of components and variations in their relative concentrations, were tested during this project (see section 4.3.3). The potency and selectivity values for the best performing attractant mixtures are given in Table 20. All attractants in a modified LuciTrap caught more *C. bezziana* than the current Bezzilure with factors ranging from 1.4 to 4.2. The selectivity of most mixtures for *C. bezziana* against other fly species was in the same range as the current Bezzilure. B131 and B132 appear to have better selectivity against most other flies. Although mixtures B131 to 134 performed well and could be candidates for an improved Bezzilure, currently their use cannot be recommended because of the limited field data available (only 1 or 2 replicates). The other four mixtures are good candidates for an optimal *C. bezziana* attractant with B96 the best performer with regard to potency.

Table 20: Potency and selectivity values for selected attractants compared to B110 (LTM, +A9)

Attractant	Reps.	Potency					Selectivity for *C. bezziana* against			
		C bez	C meg	C ruf	Hemi	Sarc	C meg	C ruf	Hemi	Sarc
B95	5	2.2	2.3	3.6	2.2	1.4	0.9	0.6	1.0	1.6
B96	3	4.2	10.0	4.3	2.4	1.7	0.4	1.0	1.8	2.5
B99	7	1.8	3.2	1.9	2.0	1.4	0.5	0.9	0.9	1.2
B107	4	1.8	2.2	1.2	1.7	1.3	0.8	1.5	1.0	1.4
B131	1	1.7	1.3	1.1	1.2	1.9	1.4	1.6	1.5	0.9
B132	1	1.4	0.6	0.6	0.7	1.1	2.3	2.4	2.0	1.3
B133	1	1.4	0.8	1.8	4.3	0.9	1.6	0.8	0.3	1.6
B134	2	1.7	1.9	2.7	2.0	3.6	0.9	0.6	0.9	0.5

The chemical composition of these candidate mixtures is provided in Table 21. With 11 components, B95 contains more chemicals than the other attractants. This increase is not reflected in its performance and it can be eliminated from the pool of favourites. Compared to B110, all other mixtures have acetic acid added and phenol removed. This is an advantage as acetic acid is inexpensive and non-toxic whereas phenol is toxic and corrosive. B99 has the least components with the two alcohols also absent. B96 has one extra component, acetone which is also inexpensive.

Table 21: Chemical composition of selected attractant mixtures

Component	B95	B96	B99	B107	B110
Acetic acid (ml)	1.20	1.20	1.50	2.00	
Butyric acid (ml)	1.60	1.60	2.00	4.00	4.00
Valeric acid (ml)	1.20	1.20	1.50	4.00	4.00
Benzoic acid (g)	0.33				
Indole (g)	0.33	0.33	1.00	1.00	1.00
Phenol (g)	0.33				0.10
Cresol (g)	0.33				
iso-Butanol (ml)	0.84	0.84		0.84	0.84
sec-Butanol (ml)	1.20	1.20		1.20	1.20
Acetone (ml)	0.75	0.75			
2-Mercaptoethanol (ml)	2.80	2.80	5.00	2.00	2.00
Number of components	11	8	5	7	7

Combining the performance data from the field trials and the chemical composition it is concluded that B96 is the optimal attractant mixture for a screw-worm fly surveillance trap. The savings which could be made by using the 5-component B99 do not justify an approximately 50% reduction in the *C. bezziana* catch. In addition, the selectivity of B96 for *C. bezziana* is superior to the other candidates.

The recommended attractant for screw-worm fly surveillance trapping is a two bottle system consisting of A9 and B96. To avoid confusion with the previous Bezzilure and to keep in step with the principle used for Swormlure, the new attractant is named **Bezzilure-2.** The two bottles will thus be labelled Bezzilure-2 A (=A9) and Bezzilure-2 B (=B96) respectively. Both bottles have to be concurrently placed inside the LuciTrap for effective *C. bezziana* trapping.

The recipe for preparing Bezzilure-2 is given in Table 22. Bezzilure should only be prepared in facilities which have staff trained in handling smelly and toxic components and are appropriately equipped (fume cupboard). For the preparation of Bezzilure-2 A, sodium sulfide is added in portions to 800 ml of water (preferably deionised or distilled) with efficient stirring. The solution is allowed to cool to room temperature and made up to 1 litre with water. A fine precipitate may form which is allowed to settle before transferring into bottles. For Bezzilure-2 B, the alcohols and

acetone are mixed first, followed by indole and the acids. 2-Mercaptoethanol is added last to minimise odour release during the preparation. Both solutions should be prepared in a fume hood. Laboratory or technical grade components can be utilised.

Table 22: Recipe for Bezzilure-2 A and Bezzilure-2 B (1 litre each)

Attractant	Components	Quantity
Bezzilure-2 A	Sodium sulfide (tech. flakes)	200 g
	Water	approx. 1 L
Bezzilure-2 B	Acetic acid	124 ml
	Butyric acid	165 ml
	Valeric acid	124 ml
	Indole	34 g
	iso-Butanol	124 ml
	sec-Butanol	87 ml
	Acetone	77 ml
	2-Mercaptoethanol	289 ml

Bezzilure-2 A and Bezzilure-2 B should be dispensed in 50 ml high density polyethylene bottles. Wicks are put in place using a specific insert (bottles, inserts and wicks are available from Bioglobal Pty Ltd, Wacol Qld 4076). When placed in the trap the wick of bottle A should be level with the bottle top (as supplied by manufacturer) whereas the wick in bottle B needs to be pulled up 10 to 20 mm above the bottle rim. The attractants will last about 8 weeks in warm (maximum day temperature 30-34°C) weather. However, the loss of attractant can vary and is accelerated by high temperature, wind and low humidity. The height of the wick can be adjusted to correct for environmental conditions. Both bottles should be changed at the same time when one of them (normally bottle B) has less than 20% of its content or if it will not last to the next service.

Although accelerated ageing studies have not been carried out for B96, it is reasonable to assume that B96 will behave similarly to the closely related B99 and B110 which showed no loss of activity during such studies (see Table 6).

Material and Safety Data Sheets (MSDS) for Bezzilure-2 A and Bezzilure-2 B have been produced and are provided in Appendix 3. The MSDS differ only slightly from the previous Bezzilure sheets.

4.5.4 Processing of trap catches

It is recommended that the newly developed real-time PCR test for detecting *C. bezziana* in bulk trap catches (Morgan *et al.* 2008) is used in the Australian screw-worm fly surveillance program. This test appeared to be 100% specific for *C. bezziana* with samples of up to 1000 flies. We suggest that the real-time PCR test is more sensitive and reliable than the currently used morphological examination by field and laboratory staff. It would also provide considerable savings in time used for processing trap samples.

The most efficient process for detecting *C. bezziana* depends on the size of the trap samples. Small samples could be processed either by morphological examination or real-time PCR test, whereas for large samples the real-time PCR test would be far superior. A number of 1000-fly aliquots could be screened from large catches to obtain the required probability of detection. Small trap catches could be pooled to make their screening by real-time PCR cost effective. When the real-time PCR test is used, it is also recommended that 50% of (pooled) trap catches be retained for confirmation through morphological examination by an expert entomologist (and possibly subsequent re-screening by real-time PCR) in case of a positive PCR test result.

The use of the real-time PCR test for detecting *C. bezziana* has one limitation with the trapping period being restricted to 10 days or less in order to maintain 100% sensitivity. The LuciTrap/Bezzilure system runs for 60 days without service and thus increases the probability of catching *C. bezziana.* It would be useful to investigate the currently unknown cause of the decline in real-time PCR test response and hopefully extend the allowable trapping period to at least partially match the capacity of the trapping system.

4.6 Optimal screw-worm fly detection system

There are a range of tools available for detecting a screw-worm fly incursion into Australia. Adult flies can be detected with traps and immature screw-worm (larvae) by inspections of livestock, companion animals, wildlife, feral animals and humans which are all potential hosts for screw-worm fly. Further possibilities which are currently not available include a serological test for screw-worm antibodies in animals and the use of specifically trained detector dogs. All tools in the first group involve obtaining samples followed by confirmation of *C. bezziana* presence.

Knowledge about the sensitivity and specificity of various detection tools will assist in the design and implementation of surveillance programs. In this project we compared the sensitivity of trapping and livestock inspections for the detection of *C. bezziana.* This comparison was carried out in two locations with different prevalence of screw-worm fly and environmental conditions. The first comparison on the Indonesia island of Sumba was in an area of lower and seasonal rainfall and low screw-worm fly populations, resembling environmental conditions encountered in northern Australia. The second comparison was at Jelai Gemas in Negeri Sembilan Province in Malaysia with relatively high rainfall and fly populations. Fly trapping and inspections of cattle in a race provided positive results for the presence of screw-worm fly in both locations. From the number of positive traps or animals the sample sizes required to detect screw-worm fly were calculated. To detect *C. bezziana* with 95% confidence in Sumba either 12 LuciTraps with Bezzilure need to be deployed for 14 days or 507 animals have to be inspected. At Jelai Gemas during the higher strike prevalence period 2 LuciTraps for a 7-day period or 209 animals inspected, and with lower strike prevalence 3 LuciTraps for a 10-day period or the inspection of 954 animals were required.

The LuciTrap is expected to work equally well in Australia, in catching C. bezziana, as in Indonesia or Malaysia. The study was carried out with the modified LuciTrap and Bezzilure and a higher sensitivity can be expected for the new system which is more effective in catching screw-worm fly. The number of animals to be inspected in Australia should be the same or higher than in Sumba; as the prevalence of wounds suitable for screw-worm fly infestation can be considerably lower in Australia (wounding rate in Malaysia is not known). Both detection tools have very different characteristics and requirements and these are briefly discussed in the following paragraphs.

For *C. bezziana* trapping, LuciTraps and Bezzilure need to be available and the traps need to be set up and serviced. One advantage of traps is that they can be set up at high risk locations and can be used in areas where there is no livestock (e.g. livestock free zones in northern Queensland). There may be alternative hosts in these areas which are not readily surveyable. The timing of trap setting and trapping period can be chosen to optimise *C. bezziana* detection and if desired can be carried out continuously. Detection of trapped *C. bezziana* is 100% specific with the new real-time PCR test. Fly trapping is a flexible, convenient and reliable tool for surveillance of adult populations.

Inspection of managed livestock is also a viable means of detecting screw-worm fly. These inspections should be conducted in a race with two trained observers present. Inspections could possibly be carried out in small yards, however with the risk of missing covert fly strikes. If strikes are detected, larvae need to be extracted and dispatched to an expert for identification. It is also possible to confirm the presence of *C. bezziana* with DNA based tests. However, access to

livestock in northern Australia is restricted or impossible for extended periods. Particularly during the wet season, when screw-worm fly populations are expected to increase, access to livestock is very restricted. Generally, livestock in northern Australia is only mustered twice a year to apply various management processes, to harvest for export or transfer for finishing. Such musters provide a good but infrequent opportunity to inspect livestock for fly strike. These inspections for fly strike need to be carried out at the location of muster and not at the port or abattoir because the animals most likely infested with fly strike have probably been previously drafted off. Inspection of animals with wounds from management processes (castration, branding, dehorning) or shortly after calving (vulva, navel) may also provide an indicator for *C. bezziana* presence at the site of activity.

LuciTraps with Bezzilure and inspections of livestock should be used for screw-worm fly surveillance in Australia. The two tools are complementary and their usefulness depends on circumstances. Traps are a flexible, convenient and reliable detection tool and they can be strategically located and serviced as required. Inspection of cattle at routine musters is also a useful, and with trained inspectors, reliable tool for the detection of *C. bezziana*. However, mustering cattle solely for fly strike inspections is in most cases not cost effective.

Optimal screw-worm fly surveillance programs should use an integration of available detection tools. Besides fly trapping and livestock inspections, **larvae detected in wounds on animals or humans in Australia** (and the Torres Strait) **should be submitted to designated institutions for identification**. Submission of larvae from all warm-blooded hosts with the exception of sheep (to avoid numerous submissions from sheep blowfly strike) should be targeted. Old and New World screw-worm fly are national notifiable animal diseases. In spite of several attempts to secure submissions of larvae from veterinary and medical practices, abattoirs and the general public such submissions are rare. The provision of sampling kits and awareness campaigns have limited and short-lived impact on the number of submissions. The increase in submissions following such campaigns indicates that larval infestations are found but not submitted in normal circumstances. Measures to redress this lack of larval submissions should be instigated.

5 Success in Achieving Objectives

All three objectives were fully met within this project. An improved trapping system for screw-worm fly has been developed and recommended for use in the Australian surveillance program. The trapping system, LuciTrap with Bezzilure-2 attractant, catches more screw-worm flies than either the sticky trap with Swormlure or the LuciTrap/Bezzilure. The new system is also more selective for the target fly as it discriminates against other fly species with factors ranging from nine to one hundred. The need to enlarge fly entry holes in the commercial LuciTrap and the use of pest strips for fly retention have also been eliminated.

The sensitivity for detecting screw-worm fly has been determined for fly traps and inspections of cattle herds for fly strike. Both are capable of detecting screw-worm fly at low population density. Four LuciTraps with Bezzilure deployed for a fortnight were more sensitive than an inspection of about 80 animals in a race. However, the two systems are complimentary and both should be used for screw-worm fly surveillance in Australia.

Recommendations have been provided to optimise Australian screw-worm fly surveillance activities. The LuciTrap/Bezzilure-2 system should be used in conjunction with the newly developed real-time PCR test for screw-worm fly in bulk trap catches. This combination will provide a more effective, more reliable and convenient surveillance tool. In addition to the fly traps and inspections of livestock herds for strike, larvae obtained from wounds on animals and humans should also be used as an indicator for the presence of screw-worm flies.

Adoption of these recommendations would provide a better screw-worm fly surveillance program and earlier detection of this undesirable and, for the livestock industries, disastrous exotic insect pest.

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

The Old World screw-worm fly is an aggressive parasite of all warm-blooded animals, including humans. Screw-worm fly is endemic across all northern neighbours of Australia, including PNG, East Timor, Indonesia, Malaysia and the Philippines, and could arrive in Australia through migration of flies, particularly across the islands of the Torres Strait, or through movements of animals or humans or on board livestock vessels. So far, it has not become established in Australia. The total costs of an endemic screw-worm fly infestation to Australia have been estimated at $900M per annum (Spradbery 2002). The livestock industry in Australia would be severely hampered by an incursion of screw-worm fly.

Australia has a screw-worm fly preparedness strategy, including the AUSVETPLAN for screw-worm fly. Components include surveillance, a bio-economic model and sterile insect technology for the eradication of incursions. It is clearly understood that the earlier an incursion is detected, the less its potential impact. It is expected that livestock in screw-worm fly infested areas and in buffer zones will have to be treated regularly with insecticides preventing the establishment of fly strikes. Monitoring of screw-worm fly relies on adult trapping and monitoring of myiasis in sentinel herds. The Northern Australia Quarantine Strategy and the Ports Surveillance Program currently undertake this task across northern Australia and at seaports respectively.

Detection of screw-worm fly is the first and critical step in the Australian screw-worm fly preparedness strategy. Early detection will lessen the impact of a screw-worm fly incursion into Australia and the cost of eradication. Early detection of adult screw-worm fly can be achieved with better traps and the effective use of other detection systems.

Improvements in screw-worm fly surveillance can be achieved immediately with the adoption of the improved surveillance trap (LuciTrap/Bezzilure-2) and the real-time PCR screening test for trap catches. The new system has a higher sensitivity for detecting screw-worm fly, resulting in the detection of screw-worm fly at lower population densities. Earlier detection will minimise the fly's dispersion area and result in earlier intervention and lower cost for containment and eradication.

Further improvement in surveillance can be achieved by implementation of the recommended integrated use of screw-worm fly detection tools. These tools include fly traps, inspection of livestock herds for fly strike and submission of larvae removed from wounds on animals and humans. A combination of these complimentary tools will further enhance the probability of detecting a screw-worm fly incursion into Australia.

The meat and livestock industry will profit immediately if the outcomes and recommendations from this project are implemented. Such an implementation will provide an immediate and ongoing benefit to the livestock industry as it increases the probability for, and shortens the delay to, a detection of a screw-worm fly incursion. This will reduce the impact on the industry from such an incursion.

However, the industry must be aware of the ongoing risk of such an incursion, which has not been reduced by improvements to the surveillance program. Effective screw-worm fly surveillance must be maintained to minimise the impact to the Australian livestock industries from an incursion of this exotic and highly damaging pest.

7 Conclusions and Recommendations

7.1 Conclusions

7.1.1 Screw-worm fly surveillance trapping system

An improved trapping system for the Old World screw-worm fly, *C. bezziana,* has been developed. It consists of the commercially available LuciTrap with a new attractant mixture (Bezzilure-2). The modification of enlarging the fly entry holes in the LuciTrap and the use of a pest strip, have been eliminated in the new system. The attractant consists of two bottles (Bezzilure-2 A and Bezzilure-2 B) containing an aqueous salt solution and a mixture of chemicals respectively. The attractants are contained in plastic bottles which are directly attached to the trap platform after removing the lids. The bottles contain a wick which assists in releasing the attractant at a constant rate over a period of approximately two months (50 ml attractant). The rate of attractant release can be adjusted by changing the length of the exposed wick. The trap is easy to use and service and can be attached to posts or trees. A roof to protect the LuciTrap from rain (150-250 mm above the trap) is retained to provide good quality flies for subsequent processing.

The LuciTrap with Bezzilure (or similar attractants including Bezzilure-2) caught an average 3.5 times more *C. bezziana* than the sticky trap with Swormlure. The LuciTrap/Bezzilure combination provides selectivity for *C. bezziana* against other *Chrysomya* spp. (average factors 9–12) including the yellow-faced *C. megacephala* which is difficult to differentiate from *C. bezziana* on morphological criteria. The LuciTrap also discriminates with a factor of approximately 100 against *Hemipyrellia* spp. compared to the sticky trap. This selectivity is important to maximise the probability of detecting *C. bezziana* in trap catches and to shorten the time and/or reduce the cost for the subsequent screening for *C. bezziana* by real-time PCR or morphological examination.

The newly developed real-time PCR test for detecting *C. bezziana* in bulk trap catches (Morgan *et al.* 2008) appears to be 100% specific for *C. bezziana* with samples of up to 1000 flies. It is most likely more sensitive and reliable than the currently used morphological examination and should be adopted by the Australian screw-worm fly surveillance program. A protocol, optimising the cost benefit ratio, should be devised for processing trap catches, including pooling of small catches and testing aliquots from large catches. Fifty percent of (pooled) trap catches should be retained for confirmation through morphological examination (and possibly subsequent re-screening by real-time PCR) in case of a positive real-time PCR test result.

7.1.2 Screw-worm fly detection system

The sensitivity of adult screw-worm fly trapping and livestock inspections for the detection of *C. bezziana* was determined in areas with low and high density screw-worm fly populations. Both methods were capable of detecting screw-worm fly at both fly densities. To detect *C. bezziana* with 95% confidence in Sumba (low density area) either 12 LuciTraps with Bezzilure need to be deployed for 14 days or 507 animals have to be inspected. At Jelai Gemas during the higher strike prevalence period 2 LuciTraps for a 7-day period or 209 animals inspected, and with lower strike prevalence 3 LuciTraps for a 10-day period or the inspection of 954 animals were required to achieve detection with 95% certainty.

Both LuciTraps with Bezzilure and inspections of livestock should be used for screw-worm fly surveillance in Australia. The two tools are complementary and their usefulness depends on circumstances. Traps are a flexible, convenient and reliable detection tool and they can be strategically located and serviced as required. Inspection of cattle at routine musters is also a useful, and with trained inspectors, reliable tool for the detection of *C. bezziana*. However, mustering cattle solely for fly strike inspections is in most cases not cost effective.

Optimal screw-worm fly surveillance uses an integration of available detection tools. Besides fly trapping and livestock inspections, all larvae detected in wounds on animals or humans in Australia (and the Torres Strait) should be submitted to designated institutions for identification. However, such submissions are rare and measures to redress this lack of larval submissions should be instigated.

7.2 Recommendations

As a result of this project research we recommend that:

1. LuciTrap with Bezzilure-2 be used for surveillance of adult screw-worm fly populations in Australia
2. An integrated approach to screw-worm fly surveillance be developed which includes
    a. Fly trapping
    b. Livestock inspections
    c. Targeted larval submissions
3. Real-time PCR screening of adult and immature samples be established as the primary process
4. Insecticides that can be used for wound treatment and prophylaxis in the event of a screw-worm fly incursion to Australia should be tested and possibly registered
5. Further research and development work on screw-worm fly surveillance be carried out, particularly on prolonging the period flies can be left in LuciTraps before being screened by real-time PCR.
6. Integration of Australian screw-worm fly research and development into the 5-year IAEA sponsored Coordinated Research Project on “Applying Population Genetics and GIS for Managing Livestock Insect Pests (D4.20.13)” (start date 2008) should be considered.

The LuciTrap/Bezzilure-2 is more effective in catching screw-worm flies than either the sticky trap with Swormlure or the LuciTrap/Bezzilure. This system is also more selective for the target fly as, for each trapped screw-worm fly, the LuciTrap contains four other flies compared to 64 other flies on the sticky trap. The need to enlarge fly entry holes in the commercial LuciTrap and the use of pest strips for fly retention has been eliminated. A roof above the trap was retained to provide good quality flies.

The detection of a screw-worm fly incursion into Australia can be optimised with an integrated use of several detection tools. Effective tools include LuciTrap/Bezzilure-2 for adult flies, livestock inspections for the presence of screw-worm fly strike and larval submission from veterinary and medical institutions. These detection tools are complimentary and their combined application in screw-worm fly surveillance will maximise the probability of detecting an incursion.

The newly developed real-time PCR test for detecting *C. bezziana* offers great advantages for screening bulk trap catches. Its adoption into the screw-worm fly surveillance program will increase the probability of detecting screw-worm fly in trap catches. The test can also be used for screening larvae collected from wounds on animals or humans.

Insecticides play a pivotal role in minimising the dispersion of flies and assist in suppression of populations prior to eradication with sterile fly releases (Garcia *et al.* 2007). A recent review of insecticides available in Australia against screw-worm fly concluded that only a few products were currently available and that several newer and probably more effective products should be tested against screw-worm fly and possibly registered (James *et al.* 2006).
Further improvements to screw-worm fly surveillance could be achieved through research and development work. It would be particularly useful to overcome the limitation of the real-time PCR test of restricting the trapping period of *C. bezziana* to 10 days. The LuciTrap/Bezzilure system can be operated unattended for 60 days or more. It would be useful to investigate the currently

unknown cause of the decline in real-time PCR test response and hopefully extend the allowable trapping period to at least partially match the capacity of the trapping system.

The International Atomic Energy Agency (IAEA) initiated a 5-year coordinated research project on area-wide control of livestock insect pests. The target species include Old World screw-worm fly, New World screw-worm fly and tsetse fly. This provides an opportunity for Australia to be involved in research and development of new means to control screw-worm fly. Interaction with scientists working on similar pests across the world would enhance the Australian expertise. It would also provide the opportunity to rejuvenate collaboration on screw-worm fly with our South-East Asia neighbours, where screw-worm fly is endemic. Indonesia and Malaysia have expressed interest for such collaboration in preliminary discussions.

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10 Appendices

10.1 Appendix 1 – Composition of attractant mixtures

Chemicals	Attractant code													
	A9	A11	B10	B48	B81	B82	B85	B95	B96	B99	B105	B106	B107	B108	B110
Acetic acid (ml)			1.20		1.20	1.20	1.20	1.20	1.20	1.50			2.00		
Propanoic acid (ml)															
Butyric acid (ml)			1.60	8.00	1.60	1.60	1.60	1.60	1.60	2.00	4.00	8.00	4.00	4.00	4.00
Valeric acid (ml)			1.20		1.20	1.20	1.20	1.20	1.20	1.50	4.00		4.00	4.00	4.00
Benzoic acid (g)			0.33					0.33							
Indole (g)			0.33	1.00	0.33	0.33	0.33	0.33	0.33	1.00	1.00	1.00	1.00	0.50	1.00
Phenol (g)			0.33					0.33							0.10
Cresol (g)			0.33					0.33							
iso-Butanol (ml)			0.84		0.84	0.84	0.84	0.84	0.84		0.84	0.84	0.84	0.84	0.84
sec-Butanol (ml)			1.20		1.20	1.20	1.20	1.20	1.20		1.20	1.20	1.20	1.20	1.20
Acetone (ml)			0.75		0.75			0.75	0.75						
2-Mercaptoethanol (ml)				2.00				2.80	2.80	5.00	2.00	2.00	2.00	2.00	2.00
Dimethyl sulfide (ml) (DMS)															
Dimethyl disulfide (ml) (DMDS)			1.30		0.65	0.65	1.30								
Dimethyl trisulfide (ml) (DMTS)					0.13	0.13	0.26								
Sodium Sulfide (g)	2.00	1.00													
Water (ml)	10.00	10.00													

Chemicals	Attractant code														
	B110	B118	B120	B123	B128	B129	B130	B131	B132	B133	B134	B135	B136	B137	B138
Acetic acid (ml)		1.20		1.20	1.20	1.20				2.00	2.00	2.00	2.00	4.00	
Propanoic acid (ml)			4.00		4.00								2.00		2.00
Butyric acid (ml)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Valeric acid (ml)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Benzoic acid (g)															
Indole (g)	1.00	1.00	1.00	1.00	1.00	1.00	0.5	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Phenol (g)	0.10	0.10	0.10	0.10	0.10	0.50	0.10	0.10	0.10			0.10	0.10	0.10	0.10
Cresol (g)				0.10		0.10									
iso-Butanol (ml)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	2.50	0.84	0.84	0.84	0.84
sec-Butanol (ml)	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	3.60	1.20	1.20	1.20	1.20
Acetone (ml)															
2-Mercaptoethanol (ml)	2.00						2.00	2.00	4.00	10.00	10.00	2.00	2.00	2.00	2.00
Dimethyl sulfide (ml) (DMS)		0.50	0.50	0.50		0.50									
Dimethyl disulfide (ml) (DMDS)		0.65	0.65	0.65	0.65	0.65									
Dimethyl trisulfide (ml) (DMTS)		0.13	0.13	0.13	0.13	0.13									
Sodium Sulfide (g)															
Water (ml)															

10.2 Appendix 2 – Results of Field Trials – Trap Improvement

Screwworm fly field trials - 4x4 LS results

Trial ID: MA050706A

T1 = ST-W B10

T2 = LTM B110 A9

T3 = LTM B120 A9

T4 = LTM B130 A9

Comments:

	wick	lure loss
B10	0 mm	22 ml
B110	20 mm	18 ml?
B120	10 mm	21 ml
B130	20 mm	16 ml
A9	0 mm	5 ml

Several containers have conflicting lables (in and out)
Assigned with reasonable confidence
ST-W one missing value

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.83	3.22	2.52	3.59	3.33	3.46	2.82	3.43	3.13	2.71	3.69	3.20
C. bezziana	males	1.18	1.49	1.34	0.71	1.13	0.92	0.93	0.97	0.95	0.71	1.25	0.98
C. bezziana	total	2.05	3.53	2.79	3.59	3.42	3.51	2.87	3.48	3.18	2.71	3.86	3.29
C. mega/saf	total	3.59	3.95	3.77	2.17	1.41	1.79	2.10	3.41	2.75	1.57	2.05	1.81
C. rufifacies	total	4.86	7.27	6.07	2.31	1.98	2.15	5.12	6.99	6.05	1.39	2.45	1.92
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	18.01	13.69	15.85	3.59	2.84	3.21	2.48	1.93	2.20	2.59	2.06	2.32
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	5.69	4.87	5.28	5.86	4.33	5.09	5.29	5.41	5.35	4.43	5.82	5.13

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.83	9.87	5.87	12.39	10.56	11.45	7.46	11.27	9.27	6.87	13.09	9.75
C. bezziana	males	0.90	1.71	1.28	0.00	0.77	0.34	0.36	0.43	0.39	0.00	1.05	0.45
C. bezziana	total	3.71	11.93	7.28	12.39	11.20	11.79	7.73	11.64	9.59	6.87	14.38	10.30
C. mega/saf	total	12.42	15.10	13.73	4.23	1.48	2.71	3.91	11.10	7.08	1.96	3.72	2.78
C. rufifacies	total	23.16	52.31	36.30 a	4.82	3.43	4.10 b	25.70	48.32	36.14 a	1.42	5.48	3.17 b
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	323.75	186.86	250.66 a	12.35	7.55	9.82 b	5.63	3.23	4.36 b	6.19	3.74	4.89 b
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	31.88	23.24	27.39	33.83	18.22	25.44	27.53	28.76	28.14	19.08	33.42	25.77
Yellow faces				21.01			14.49			16.67			13.08
Totals				335.35			53.85			85.31			46.91

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	34.6%	81.3%	57.5%	78.7%
C. bezziana /Total catch	2.2%	21.9%	11.2%	22.0%
Yellow faces/Total catch	6.3%	26.9%	19.5%	27.9%
C. bezziana fem/Total *C. bezziana*	80.6%	97.2%	96.6%	94.6%
C. mega/saf /Total catch	4.1%	5.0%	8.3%	5.9%
C. rufifaces /Total catch	10.8%	7.6%	42.4%	6.8%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	74.7%	18.2%	5.1%	10.4%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	8.2%	47.2%	33.0%	54.9%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.95	1.58	1.66
C. bezziana	males	1.00	0.27	0.31	0.35
C. bezziana	total	1.00	1.62	1.32	1.41
C. mega/saf	total	1.00	0.20	0.52	0.20
C. rufifacies	total	1.00	0.11	1.00	0.09
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.04	0.02	0.02
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.93	1.03	0.94

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	8.21	2.56	6.99
C. bezziana/C. rufifaces	1.00	14.33	1.32	16.19
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	41.34	75.82	72.50
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	1.74	1.28	1.50

Screwworm fly field trials - 4x4 LS results

Trial ID: MA050706B

T1 = LTM B120 A9

T2 = LTM B81 A9

T3 = LTM B82 A9

T4 = LTM B85 A9

Comments:

	wick	lure loss
B120	15 mm	24 ml
B81	10 mm	25 ml
B82	10 mm	24 ml
B85	7 mm	25 ml
A9	0 mm	5 ml

One container no date
Assigned with high confidence
B81, B82 & B85 at 10-20% on 3rd collection, empty at 4th coll.
Some wicks lowered

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.84	1.74	1.29	2.16	2.38	2.27	1.06	1.56	1.31	1.17	1.92	1.55
C. bezziana	males	0.71	1.06	0.88	0.71	0.84	0.77	0.71	0.71	0.71	0.71	0.71	0.71
C. bezziana	total	0.84	1.85	1.34	2.16	2.42	2.29	1.06	1.56	1.31	1.17	1.92	1.55
C. mega/saf	total	1.19	2.34	1.77	2.41	1.43	1.92	0.97	1.27	1.12	0.71	1.14	0.93
C. rufifacies	total	3.35	6.77	5.06	4.38	1.96	3.17	0.84	5.88	3.36	1.55	3.70	2.62
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	0.84	1.14	0.99	1.30	0.84	1.07	0.84	0.71	0.77	0.71	0.84	0.77
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	2.83	5.42	4.12	4.72	3.40	4.06	1.77	3.86	2.82	2.26	3.89	3.08

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.20	2.51	1.15	4.16	5.18	4.66	0.61	1.94	1.21	0.86	3.20	1.89
C. bezziana	males	0.00	0.63	0.28	0.00	0.20	0.10	0.00	0.00	0.00	0.00	0.00	0.00
C. bezziana	total	0.20	2.91	1.30	4.16	5.36	4.74	0.61	1.94	1.21	0.86	3.20	1.89
C. mega/saf	total	0.92	4.98	2.62	5.30	1.54	3.18	0.43	1.12	0.75	0.00	0.81	0.36
C. rufifacies	total	10.74	45.29	25.10	18.64	3.35	9.54	0.20	34.06	10.78	1.89	13.20	6.39
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.20	0.81	0.48	1.19	0.20	0.64	0.20	0.00	0.10	0.00	0.20	0.10
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	7.51	28.84	16.50	21.80	11.05	15.99	2.64	14.43	7.44	4.59	14.66	8.96
Yellow faces				3.91			7.92			1.97			2.25
Totals				46.00			34.09			20.28			17.68

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	33.2%	59.9%	61.7%	84.1%
C. bezziana /Total catch	2.8%	13.9%	6.0%	10.7%
Yellow faces/Total catch	8.5%	23.2%	9.7%	12.7%
C. bezziana fem/Total *C. bezziana*	88.9%	98.3%	100.0%	100.0%
C. mega/saf /Total catch	5.7%	9.3%	3.7%	2.0%
C. rufifaces /Total catch	54.6%	28.0%	53.1%	36.1%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	1.0%	1.9%	0.5%	0.5%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	35.9%	46.9%	36.7%	50.6%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	4.04	1.05	1.64
C. bezziana	males	1.00	0.34	0.00	0.00
C. bezziana	total	1.00	3.65	0.93	1.46
C. mega/saf	total	1.00	1.22	0.29	0.14
C. rufifacies	total	1.00	0.38	0.43	0.25
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	1.34	0.20	0.20
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.97	0.45	0.54

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	3.00	3.24	10.65
C. bezziana/C. rufifaces	1.00	9.61	2.18	5.72
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	2.73	4.68	7.28
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	3.77	2.07	2.68

Screwworm fly field trials - 4x4 LS results

Trial ID: MA020806A

T1 = ST-W B10

T2 = LTM B120 A9

T3 = LTM B118 A9

T4 = LTM B128 A9

Comments:

	wick	lure loss
B118	15 mm	22 ml
B120	15 mm	23 ml
B128	15 mm	20 ml
B10	0 mm	25 ml
A9	0 mm	10 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.26	1.41	1.33	2.13	2.79	2.46	1.86	2.90	2.38	2.31	3.56	2.93
C. bezziana	males	0.71	0.84	0.77	0.71	1.22	0.96	0.84	1.17	1.00	1.10	0.97	1.03
C. bezziana	total	1.26	1.48	1.37	2.13	3.00	2.56	1.92	3.03	2.47	2.47	3.61	3.04
C. mega/saf	total	2.63	1.83	2.23	6.28	22.63	14.46	3.08	9.75	6.42	2.31	8.34	5.33
C. rufifacies	total	7.28	5.62	6.45	15.57	43.12	29.34	9.15	19.23	14.19	5.95	17.87	11.91
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	9.42	6.94	8.18	0.71	1.30	1.00	0.97	1.25	1.11	0.71	1.67	1.19
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	4.38	2.61	3.49	6.05	5.93	5.99	4.57	5.29	4.93	4.97	5.55	5.26

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.08	1.49	1.28	4.02	7.26	5.53	2.96	7.93	5.17	4.81	12.18	8.10
C. bezziana	males	0.00	0.20	0.10	0.00	0.98	0.43	0.20	0.86	0.50	0.70	0.43	0.56
C. bezziana	total	1.08	1.69	1.37	4.02	8.49	6.07	3.17	8.67	5.61	5.61	12.52	8.74
C. mega/saf	total	6.44	2.84	4.48 b	38.99	511.53	208.48 a	8.99	94.54	40.65 b	4.82	69.12	27.86 b
C. rufifacies	total	52.48	31.12	41.12 b	241.86	1858.66	860.51 a	83.30	369.14	200.86 b	34.90	318.87	141.35 b
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	88.27	47.64	66.41 a	0.00	1.19	0.51 b	0.43	1.05	0.72 b	0.00	2.28	0.91 b
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	18.64	6.31	11.70	36.13	34.69	35.40	20.38	27.46	23.79	24.17	30.26	27.13
Yellow faces				5.85			214.54			46.26			36.60
Totals				125.08			1110.97			271.63			205.98

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	23.5%	2.8%	12.1%	23.9%
C. bezziana /Total catch	1.1%	0.5%	2.1%	4.2%
Yellow faces/Total catch	4.7%	19.3%	17.0%	17.8%
C. bezziana fem/Total *C. bezziana*	92.9%	91.2%	92.1%	92.7%
C. mega/saf /Total catch	3.6%	18.8%	15.0%	13.5%
C. rufifaces /Total catch	32.9%	77.5%	73.9%	68.6%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	53.1%	0.0%	0.3%	0.4%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	9.4%	3.2%	8.8%	13.2%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	4.33	4.05	6.35
C. bezziana	males	1.00	4.43	5.25	5.87
C. bezziana	total	1.00	4.42	4.08	6.36
C. mega/saf	total	1.00	46.56	9.08	6.22
C. rufifacies	total	1.00	20.93	4.89	3.44
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.01	0.01	0.01
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	3.03	2.03	2.32

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	0.09	0.45	1.02
C. bezziana/C. rufifaces	1.00	0.21	0.84	1.85
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	577.36	374.93	464.79
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	1.46	2.01	2.74

Screwworm fly field trials - 4x4 LS results

Trial ID: MA020806B

T1 = LTM B110 A9

T2 = LTM B95 A9

T3 = LTM B96 A9

T4 = LTM B99 A9

Comments:

	Wicks	Lure loss
B110	20 mm	16 ml
B95	15 mm	17 ml
B96	15 mm	24 ml
B99	15 mm	16 ml
A9	0 mm	10 ml

Missing value for B110, site 16, 23/8/06

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.84	1.48	1.66	2.80	3.68	3.24	3.14	2.76	2.95	3.22	2.78	3.00
C. bezziana	males	0.84	0.75	0.79	0.93	0.84	0.88	0.84	0.71	0.77	1.14	0.71	0.93
C. bezziana	total	1.89	1.49	1.69	2.90	3.72	3.31	3.16	2.76	2.96	3.37	2.78	3.08
C. mega/saf	total	3.77	1.61	2.69	5.78	3.64	4.71	7.46	4.32	5.89	4.46	2.18	3.32
C. rufifacies	total	7.79	4.23	6.01	12.06	9.11	10.58	11.12	7.71	9.42	7.33	4.44	5.88
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.25	1.57	1.41	2.06	2.27	2.17	2.27	2.08	2.18	2.33	1.72	2.02
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	4.01	3.42	3.71	3.07	4.59	3.83	4.61	4.78	4.70	3.21	3.63	3.42

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.90	1.68	2.25 b	7.36	13.01	10.00 a	9.34	7.12	8.20 a	9.86	7.25	8.51 a
C. bezziana	males	0.20	0.06	0.13	0.36	0.20	0.28	0.20	0.00	0.10	0.81	0.00	0.36
C. bezziana	total	3.06	1.72	2.35 b	7.93	13.30	10.45 a	9.50	7.12	8.27 a	10.88	7.25	8.97 a
C. mega/saf	total	13.71	2.10	6.74	32.87	12.74	21.67	55.11	18.14	34.16	19.36	4.27	10.53
C. rufifacies	total	60.22	17.41	35.64	144.82	82.55	111.52	123.04	59.01	88.14	53.26	19.17	34.12
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	1.05	1.95	1.48	3.74	4.66	4.19	4.66	3.82	4.23	4.91	2.46	3.59
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	15.55	11.21	13.29	8.90	20.55	14.15	20.78	22.34	21.55	9.82	12.68	11.20
Yellow faces				9.09			32.11			42.42			19.50
Totals				59.51			161.97			156.35			68.42

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	25.8%	32.5%	19.5%	46.0%
C. bezziana /Total catch	3.9%	6.5%	5.3%	13.1%
Yellow faces/Total catch	15.3%	19.8%	27.1%	28.5%
C. bezziana fem/Total *C. bezziana*	95.9%	95.7%	99.1%	94.8%
C. mega/saf /Total catch	11.3%	13.4%	21.8%	15.4%
C. rufifaces /Total catch	59.9%	68.9%	56.4%	49.9%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	2.5%	2.6%	2.7%	5.3%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	22.3%	8.7%	13.8%	16.4%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	4.44	3.64	3.78
C. bezziana	males	1.00	2.14	0.74	2.77
C. bezziana	total	1.00	4.45	3.52	3.82
C. mega/saf	total	1.00	3.21	5.07	1.56
C. rufifacies	total	1.00	3.13	2.47	0.96
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	2.84	2.86	2.43
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.06	1.62	0.84

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.38	0.69	2.45
C. bezziana/C. rufifaces	1.00	1.42	1.42	3.99
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.57	1.23	1.57
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	4.18	2.17	4.53

Screwworm fly field trials - 4x4 LS results

Trial ID: MA300806A

T1 = LTM B110 A9

T2 = LTM B105 A9

T3 = LTM B107 A9

T4 = LTM B108 A9

Comments:

	wicks	lure lost
B105	15 mm	10 ml
B107	15 mm	13 ml
B108	15 mm	11 ml
B110	15 mm	12 ml
A9	0 mm	18 ml

Animals moved out of farm; 5/9 P14; 18/9 P12; 19/9 P6; 21/9 P1(
22/9 P6,7,9; 25/9 P14; 26/9 P15

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.50	2.91	2.70	2.24	1.14	1.69	3.21	3.95	3.58	1.81	3.59	2.70
C. bezziana	males	0.93	0.93	0.93	0.71	0.71	0.71	1.13	1.28	1.20	0.84	0.84	0.84
C. bezziana	total	2.56	2.96	2.76	2.24	1.14	1.69	3.38	4.09	3.73	1.85	3.62	2.73
C. mega/saf	total	1.18	1.41	1.30	1.52	1.22	1.37	1.92	1.94	1.93	1.06	1.60	1.33
C. rufifacies	total	3.02	4.36	3.69	2.59	3.04	2.81	2.02	4.33	3.18	1.52	3.76	2.64
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.58	1.32	1.45	1.22	0.71	0.96	0.93	0.97	0.95	0.97	1.22	1.09
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	5.21	4.49	4.85	3.31	2.64	2.98	4.14	5.05	4.60	2.83	4.56	3.69

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	5.73	7.98	6.81 ab	4.50	0.81	2.36 b	9.79	15.12	12.32 a	2.77	12.40	6.79 ab
C. bezziana	males	0.36	0.36	0.36 ab	0.00	0.00	0.00 b	0.77	1.14	0.95 a	0.20	0.20	0.20 b
C. bezziana	total	6.04	8.27	7.12 ab	4.50	0.81	2.36 b	10.91	16.21	13.44 a	2.93	12.58	6.97 ab
C. mega/saf	total	0.90	1.48	1.18	1.81	0.98	1.37	3.19	3.25	3.22	0.61	2.06	1.26
C. rufifacies	total	8.60	18.50	13.09	6.18	8.74	7.41	3.60	18.23	9.59	1.80	13.62	6.45
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	1.99	1.24	1.60	0.98	0.00	0.43	0.36	0.43	0.39	0.43	0.98	0.69
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	26.60	19.63	22.98 a	10.44	6.49	8.35 c	16.66	24.99	20.61 ab	7.51	20.26	13.14 bc
Yellow faces				8.30			3.73			16.66			8.24
Totals				45.97			19.91			47.25			28.51

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	85.8%	63.2%	80.7%	84.7%
C. bezziana /Total catch	15.5%	11.8%	28.4%	24.5%
Yellow faces/Total catch	18.0%	18.7%	35.2%	28.9%
C. bezziana fem/Total *C. bezziana*	95.7%	100.0%	91.7%	97.4%
C. mega/saf /Total catch	2.6%	6.9%	6.8%	4.4%
C. rufifaces /Total catch	28.5%	37.2%	20.3%	22.6%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	3.5%	2.1%	0.8%	2.4%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	50.0%	41.9%	43.6%	46.1%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.35	1.81	1.00
C. bezziana	males	1.00	0.00	2.65	0.56
C. bezziana	total	1.00	0.33	1.89	0.98
C. mega/saf	total	1.00	1.16	2.73	1.07
C. rufifacies	total	1.00	0.57	0.73	0.49
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.27	0.25	0.43
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.36	0.90	0.57

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	0.28	0.69	0.92
C. bezziana/C. rufifaces	1.00	0.59	2.58	1.99
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.24	7.65	2.27
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	0.91	2.10	1.71

Screwworm fly field trials - 4x4 LS results

Trial ID: MA300806B

T1 = LTM B120 A9

T2 = LTM B101 A9

T3 = LTM B123 A9

T4 = LTM B130 A9

Comments:

	Wicks	Lure loss
B101	20	14 ml
B120	10	14 ml
B123	10	13 ml
B130	20	13 ml
A9	0	10 ml

Animals out 5/9 D13; 12/9 P4

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.51	2.11	2.31	2.12	2.41	2.27	2.14	3.15	2.64	1.40	2.08	1.74
C. bezziana	males	0.71	0.93	0.82	0.93	0.71	0.82	0.71	0.93	0.82	0.97	0.71	0.84
C. bezziana	total	2.51	2.17	2.34	2.17	2.41	2.29	2.14	3.19	2.67	1.56	2.08	1.82
C. mega/saf	total	7.36	1.47	4.41	1.47	1.34	1.40	6.41	10.13	8.27	1.10	0.93	1.01
C. rufifacies	total	16.55	4.30	10.43	3.55	2.40	2.98	25.62	26.92	26.27	3.65	2.07	2.86
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	0.71	0.84	0.77	1.06	0.71	0.88	0.71	0.84	0.77	0.84	1.12	0.98
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	4.41	2.95	3.68	4.82	5.08	4.95	4.54	4.45	4.50	3.64	2.90	3.27

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	5.82	3.96	4.85	4.01	5.31	4.64	4.07	9.42	6.49	1.47	3.81	2.53
C. bezziana	males	0.00	0.36	0.17	0.36	0.00	0.17	0.00	0.36	0.17	0.43	0.00	0.20
C. bezziana	total	5.82	4.20	4.98	4.21	5.31	4.74	4.07	9.70	6.60	1.95	3.81	2.81
C. mega/saf	total	53.64	1.65	18.97	1.65	1.31	1.47	40.54	102.14	67.86	0.70	0.36	0.52
C. rufifacies	total	273.50	18.01	108.22 b	12.10	5.26	8.35 b	655.68	724.29	689.56 a	12.82	3.79	7.68 b
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.00	0.20	0.10	0.61	0.00	0.28	0.00	0.20	0.10	0.20	0.75	0.45
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	18.96	8.18	13.04	22.74	25.32	24.01	20.13	19.29	19.71	12.76	7.92	10.21
Yellow faces				23.94			6.22			74.46			3.33
Totals				145.29			38.85			783.83			21.67

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	20.8%	76.3%	8.9%	84.4%
C. bezziana /Total catch	3.4%	12.2%	0.8%	13.0%
Yellow faces/Total catch	16.5%	16.0%	9.5%	15.4%
C. bezziana fem/Total *C. bezziana*	97.5%	97.8%	98.2%	89.9%
C. mega/saf /Total catch	13.1%	3.8%	8.7%	2.4%
C. rufifaces /Total catch	74.5%	21.5%	88.0%	35.4%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	0.1%	0.7%	0.0%	2.1%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	9.0%	61.8%	2.5%	47.1%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.96	1.34	0.52
C. bezziana	males	1.00	1.00	1.00	1.21
C. bezziana	total	1.00	0.95	1.33	0.57
C. mega/saf	total	1.00	0.08	3.58	0.03
C. rufifacies	total	1.00	0.08	6.37	0.07
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	2.88	1.00	4.74
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.84	1.51	0.78

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	12.29	0.37	20.61
C. bezziana/C. rufifaces	1.00	12.36	0.21	7.97
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	0.33	1.33	0.12
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	0.52	0.88	0.72

Screwworm fly field trials - 4x4 LS results

Trial ID: MA011106Bcorr

Comments:

T1 = ST B10

T2 = LTM B95 A9

T3 = LTM B99 A9

T4 = LTM B110 A9

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.70	1.26	1.48	3.85	2.80	3.32	3.27	4.03	3.65	3.57	2.93	3.25
C. bezziana	males	1.04	0.84	0.94	0.82	0.84	0.83	0.98	1.20	1.09	0.95	0.93	0.94
C. bezziana	total	1.83	1.32	1.58	3.87	2.83	3.35	3.34	4.15	3.74	3.64	2.98	3.31
C. mega/saf	total	4.40	4.70	4.55	3.77	2.49	3.13	2.70	4.33	3.51	2.49	3.07	2.78
C. rufifacies	total	7.25	7.91	7.58	7.84	4.25	6.05	3.61	4.77	4.19	5.57	6.43	6.00
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	8.94	9.34	9.14	1.96	2.03	1.99	1.83	2.03	1.93	1.75	1.39	1.57
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	3.38	3.83	3.61	4.21	3.47	3.84	3.23	5.08	4.15	4.45	5.53	4.99

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.37	1.09	1.68 b	14.31	7.32	10.54 a	10.19	15.71	12.81 a	12.25	8.07	10.06 a
C. bezziana	males	0.59	0.20	0.38	0.18	0.20	0.19	0.46	0.94	0.69	0.41	0.36	0.38
C. bezziana	total	2.85	1.25	1.99 b	14.47	7.52	10.72 a	10.62	16.72	13.51 a	12.72	8.40	10.46 a
C. mega/saf	total	18.87	21.63	20.22	13.73	5.68	9.28	6.77	18.27	11.85	5.68	8.92	7.21
C. rufifacies	total	52.02	62.08	56.94	60.97	17.57	36.05	12.52	22.25	17.05	30.57	40.83	35.51
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	79.46	86.72	83.06 a	3.32	3.64	3.48 b	2.84	3.62	3.22 b	2.56	1.43	1.96 b
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	10.94	14.17	12.50	17.19	11.55	14.24	9.92	25.31	16.76	19.29	30.10	24.41
Yellow faces				22.21			20.01			25.36			17.67
Totals				174.71			73.77			62.38			79.55

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	8.9%	53.6%	53.3%	59.2%
C. bezziana /Total catch	1.1%	14.5%	21.7%	13.1%
Yellow faces/Total catch	12.7%	27.1%	40.6%	22.2%
C. bezziana fem/Total *C. bezziana*	84.8%	98.3%	94.8%	96.2%
C. mega/saf /Total catch	11.6%	12.6%	19.0%	9.1%
C. rufifaces /Total catch	32.6%	48.9%	27.3%	44.6%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	47.5%	4.7%	5.2%	2.5%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	7.2%	19.3%	26.9%	30.7%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	6.25	7.60	5.97
C. bezziana	males	1.00	0.49	1.81	1.00
C. bezziana	total	1.00	5.40	6.80	5.26
C. mega/saf	total	1.00	0.46	0.59	0.36
C. rufifacies	total	1.00	0.63	0.30	0.62
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.04	0.04	0.02
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.14	1.34	1.95

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	11.75	11.60	14.76
C. bezziana/C. rufifaces	1.00	8.52	22.71	8.44
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	128.95	175.33	222.80
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	4.74	5.07	2.70

Screwworm fly field trials - 4x4 LS results

Trial ID: MA291106B

T1 = LTM B110 A9

T2 = LT B110 A9

T3 = LTM B110

T4 = WOT-LT B110 A9

Comments:	wicks	lure loss
LTM B110	20	8 ml
LT B110	20	7.5 ml
LTM B110	20	8 ml
WOT B110	2	10 ml
LTM A9	0	5 ml
LT A9	0	5 ml
WOT A9	0	5 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.19	1.65	1.42	1.68	1.54	1.61	0.97	1.13	1.05	1.06	0.84	0.95
C. bezziana	males	0.71	0.71	0.71	0.93	1.00	0.96	0.71	0.84	0.77	0.84	0.71	0.77
C. bezziana	total	1.19	1.65	1.42	1.76	1.71	1.73	0.97	1.19	1.08	1.18	0.84	1.01
C. mega/saf	total	1.52	2.43	1.97	2.30	2.54	2.42	2.36	1.76	2.06	1.69	2.61	2.15
C. rufifacies	total	1.70	2.46	2.08	2.21	2.90	2.56	2.08	2.26	2.17	1.12	2.09	1.60
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	2.80	2.97	2.88	2.70	2.71	2.71	2.88	2.19	2.54	3.94	2.90	3.42
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	3.37	4.62	4.00	2.39	3.61	3.00	1.83	3.28	2.55	2.39	2.02	2.20

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.92	2.24	1.52 ab	2.32	1.86	2.09 a	0.43	0.77	0.60 b	0.61	0.20	0.39 b
C. bezziana	males	0.00	0.00	0.00	0.36	0.50	0.43	0.00	0.20	0.10	0.20	0.00	0.10
C. bezziana	total	0.92	2.24	1.52	2.60	2.41	2.51	0.43	0.92	0.66	0.90	0.20	0.52
C. mega/saf	total	1.80	5.39	3.39	4.77	5.95	5.34	5.08	2.59	3.75	2.35	6.29	4.11
C. rufifacies	total	2.38	5.54	3.81	4.36	7.93	6.03	3.82	4.59	4.20	0.75	3.86	2.07
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	7.33	8.31	7.82	6.78	6.85	6.82	7.79	4.31	5.94	15.03	7.90	11.20
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	10.86	20.85	15.47 a	5.21	12.52	8.49 b	2.84	10.23	6.01 b	5.20	3.58	4.36 b
Yellow faces				4.91			7.85			4.41			4.63
Totals				32.01			29.19			20.55			22.25

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	31.0%	31.9%	15.0%	11.2%
C. bezziana /Total catch	4.8%	8.6%	3.2%	2.3%
Yellow faces/Total catch	15.3%	26.9%	21.5%	20.8%
C. bezziana fem/Total *C. bezziana*	100.0%	83.2%	90.1%	75.9%
C. mega/saf /Total catch	10.6%	18.3%	18.2%	18.5%
C. rufifaces /Total catch	11.9%	20.7%	20.4%	9.3%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	24.4%	23.4%	28.9%	50.3%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	48.3%	29.1%	29.2%	19.6%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.37	0.39	0.26
C. bezziana	males	DIV 0	DIV 0	DIV 0	DIV 0
C. bezziana	total	1.00	1.65	0.43	0.34
C. mega/saf	total	1.00	1.58	1.11	1.21
C. rufifacies	total	1.00	1.58	1.10	0.54
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.87	0.76	1.43
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.55	0.39	0.28

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.04	0.39	0.28
C. bezziana/C. rufifaces	1.00	1.04	0.40	0.63
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.89	0.57	0.24
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	3.00	1.12	1.21

Screwworm fly field trials - 4x4 LS results

Trial ID: MA140407A

T1 = LTM B110 A9

T2 = LTM B99 A9

T3 = LTM B105 A9

T4 = LTM B107 A9

Comments:

	wick	lure loss
B110	20 mm	15 ml
B99	15 mm	14 ml
B105	20 mm	14 ml
B107	15 mm	13 ml
A9	0 mm	5 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.22	1.56	1.39	1.06	1.39	1.22	0.97	1.39	1.18	1.27	1.13	1.20
C. bezziana	males	0.71	0.84	0.77	0.71	0.71	0.71	0.71	0.84	0.77	0.71	0.84	0.77
C. bezziana	total	1.22	1.65	1.43	1.06	1.39	1.22	0.97	1.46	1.21	1.27	1.19	1.23
C. mega/saf	total	3.94	7.23	5.59	5.47	5.64	5.56	4.59	6.61	5.60	3.87	6.47	5.17
C. rufifacies	total	7.13	10.12	8.62	8.55	10.00	9.28	8.22	8.69	8.46	6.95	9.70	8.32
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.41	2.09	1.75	1.28	1.12	1.20	0.97	1.19	1.08	1.06	1.61	1.33
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	2.84	3.84	3.34	2.83	3.44	3.13	2.42	2.57	2.50	2.64	3.07	2.85

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.98	1.93	1.43	0.61	1.42	0.99	0.43	1.42	0.88	1.12	0.77	0.94
C. bezziana	males	0.00	0.20	0.10	0.00	0.00	0.00	0.00	0.20	0.10	0.00	0.20	0.10
C. bezziana	total	0.98	2.22	1.55	0.61	1.42	0.99	0.43	1.63	0.97	1.12	0.92	1.02
C. mega/saf	total	15.06	51.73	30.69	29.43	31.35	30.39	20.59	43.21	30.88	14.44	41.35	26.20
C. rufifacies	total	50.35	101.83	73.86	72.60	99.50	85.53	67.12	75.05	71.04	47.77	93.49	68.76
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	1.49	3.86	2.56	1.14	0.75	0.94	0.43	0.92	0.66	0.61	2.09	1.28
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	7.58	14.23	10.66	7.50	11.30	9.31	5.35	6.12	5.73	6.46	8.91	7.63
Yellow faces				32.25			31.38			31.85			27.22
Totals				119.31			127.15			109.28			104.88

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	4.8%	3.2%	3.0%	3.7%
C. bezziana /Total catch	1.3%	0.8%	0.9%	1.0%
Yellow faces/Total catch	27.0%	24.7%	29.1%	25.9%
C. bezziana fem/Total *C. bezziana*	91.8%	100.0%	91.1%	92.4%
C. mega/saf /Total catch	25.7%	23.9%	28.3%	25.0%
C. rufifaces /Total catch	61.9%	67.3%	65.0%	65.6%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	2.1%	0.7%	0.6%	1.2%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	8.9%	7.3%	5.2%	7.3%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.69	0.62	0.66
C. bezziana	males	1.00	0.00	1.00	1.00
C. bezziana	total	1.00	0.64	0.62	0.66
C. mega/saf	total	1.00	0.99	1.01	0.85
C. rufifacies	total	1.00	1.16	0.96	0.93
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.37	0.26	0.50
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.87	0.54	0.72

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	0.64	0.62	0.77
C. bezziana/C. rufifaces	1.00	0.55	0.65	0.70
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.74	2.41	1.31
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	0.73	1.16	0.91

Screwworm fly field trials - 4x4 LS results

Trial ID: MA120507a

T1 = LTM / B110 A9

T2 = LTM / B130 A9

T3 = LTM / B131 A9

T4 = LTM / B132 A9

Comments:

	wicks	lure lost
B110	20 mm	15 ml
B130	20 mm	14 ml
B131	20 mm	15 ml
B132	20 mm	14 ml
A9	0 mm	5 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.87	2.48	2.18	2.02	2.37	2.20	2.52	2.89	2.70	3.42	1.68	2.55
C. bezziana	males	0.71	1.13	0.92	0.71	0.93	0.82	1.19	1.14	1.17	0.84	0.71	0.77
C. bezziana	total	1.87	2.59	2.23	2.02	2.42	2.22	2.65	3.09	2.87	3.44	1.68	2.56
C. mega/saf	total	1.81	4.76	3.29	1.52	1.96	1.74	4.13	3.21	3.67	2.90	2.18	2.54
C. rufifacies	total	4.43	8.41	6.42	3.61	5.27	4.44	6.09	7.28	6.68	5.85	3.86	4.86
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	2.23	3.61	2.92	1.80	3.36	2.58	3.29	3.02	3.15	2.95	1.93	2.44
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	1.76	3.21	2.48	1.48	3.02	2.25	3.24	3.45	3.35	2.99	2.18	2.58

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	3.00	5.67	4.24	3.59	5.12	4.33	5.83	7.86	6.81	11.19	2.33	6.01
C. bezziana	males	0.00	0.77	0.34	0.00	0.36	0.17	0.92	0.81	0.86	0.20	0.00	0.10
C. bezziana	total	3.00	6.22	4.48	3.59	5.38	4.44	6.53	9.05	7.74	11.36	2.33	6.07
C. mega/saf	total	2.78	22.19	10.31	1.80	3.34	2.52	16.56	9.82	12.98	7.90	4.27	5.96
C. rufifacies	total	19.13	70.19	40.70	12.53	27.27	19.21	36.56	52.43	44.14	33.75	14.42	23.10
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	4.45	12.53	8.01	2.73	10.80	6.16	10.30	8.60	9.44	8.20	3.23	5.45
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	2.58	9.82	5.67	1.68	8.60	4.55	10.00	11.43	10.70	8.42	4.24	6.16
Yellow faces				14.79			6.96			20.71			12.03
Totals				69.18			36.88			84.99			46.74

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	30.3%	63.8%	37.4%	50.5%
C. bezziana /Total catch	6.5%	12.0%	9.1%	13.0%
Yellow faces/Total catch	21.4%	18.9%	24.4%	25.7%
C. bezziana fem/Total *C. bezziana*	94.6%	97.4%	88.0%	99.0%
C. mega/saf /Total catch	14.9%	6.8%	15.3%	12.7%
C. rufifaces /Total catch	58.8%	52.1%	51.9%	49.4%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	11.6%	16.7%	11.1%	11.7%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	8.2%	12.3%	12.6%	13.2%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.02	1.61	1.42
C. bezziana	males	1.00	0.49	2.53	0.28
C. bezziana	total	1.00	0.99	1.73	1.35
C. mega/saf	total	1.00	0.24	1.26	0.58
C. rufifacies	total	1.00	0.47	1.08	0.57
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.77	1.18	0.68
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.80	1.89	1.09

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	4.05	1.37	2.34
C. bezziana/C. rufifaces	1.00	2.10	1.59	2.39
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.29	1.47	1.99
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	1.23	0.91	1.25

Screwworm fly field trials - 4x4 LS results

Trial ID: MA090607a

T1 = LTM B110 A9

T2 = LTMnps B110 A9

T3 = LT B110 A9

T4 = LTnps B110 A9

Comments:	Wicks	Lure loss
LTM B110	20	13 ml
LTMnps B110	20	13 ml
LT B110	20	13 ml
LTnps B110	20	13 ml
A9	0	7 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	3.23	3.29	3.26	1.72	3.29	2.50	2.94	2.79	2.87	2.26	2.95	2.60
C. bezziana	males	0.97	0.84	0.90	0.71	0.84	0.77	0.93	1.06	0.99	0.71	0.84	0.77
C. bezziana	total	3.28	3.31	3.30	1.72	3.30	2.51	3.01	2.88	2.94	2.26	2.97	2.61
C. mega/saf	total	2.57	3.05	2.81	1.12	2.95	2.03	1.56	2.84	2.20	1.19	2.77	1.98
C. rufifacies	total	5.06	7.10	6.08	2.23	4.12	3.18	2.60	5.30	3.95	1.35	5.75	3.55
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	3.77	4.82	4.30	2.03	4.27	3.15	2.53	4.00	3.26	1.38	2.76	2.07
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	2.89	3.75	3.32	1.10	3.01	2.05	1.85	2.94	2.39	1.43	2.49	1.96

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	9.94	10.31	10.12	2.44	10.30	5.76	8.16	7.31	7.73	4.59	8.21	6.28
C. bezziana	males	0.43	0.20	0.31 ab	0.00	0.20	0.10 b	0.36	0.61	0.48 a	0.00	0.20	0.10 b
C. bezziana	total	10.26	10.47	10.37	2.44	10.42	5.80	8.53	7.79	8.16	4.59	8.31	6.33
C. mega/saf	total	6.09	8.81	7.39	0.75	8.19	3.63	1.95	7.58	4.36	0.92	7.17	3.42
C. rufifacies	total	25.11	49.97	36.50	4.46	16.48	9.58	6.27	27.59	15.11	1.31	32.60	12.10
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	13.71	22.74	17.96	3.62	17.76	9.43	5.89	15.48	10.14	1.39	7.10	3.77
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	7.85	13.59	10.54 a	0.70	8.55	3.71 b	2.92	8.12	5.22 b	1.55	5.68	3.34 b
Yellow faces				17.76			9.43			12.52			9.74
Totals				82.75			32.15			42.99			28.96

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	58.4%	61.5%	65.2%	64.9%
C. bezziana /Total catch	12.5%	18.0%	19.0%	21.9%
Yellow faces/Total catch	21.5%	29.3%	29.1%	33.7%
C. bezziana fem/Total *C. bezziana*	97.6%	99.2%	94.7%	99.3%
C. mega/saf /Total catch	8.9%	11.3%	10.1%	11.8%
C. rufifaces /Total catch	44.1%	29.8%	35.1%	41.8%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	21.7%	29.3%	23.6%	13.0%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	12.7%	11.5%	12.1%	11.5%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.57	0.76	0.62
C. bezziana	males	1.00	0.31	1.54	0.31
C. bezziana	total	1.00	0.56	0.79	0.61
C. mega/saf	total	1.00	0.49	0.59	0.46
C. rufifacies	total	1.00	0.26	0.41	0.33
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.53	0.56	0.21
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.35	0.50	0.32

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.14	1.33	1.32
C. bezziana/C. rufifaces	1.00	2.13	1.90	1.84
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.07	1.39	2.91
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	1.59	1.59	1.92

Screwworm fly field trials - 4x4 LS results

Trial ID: MA090607b

T1 = LTM / B110 A9

T2 = LTM / B95 A9

T3 = LTM / B96 A9

T4 = LTM / B99 A9

Comments:

	Wicks	Lure loss
B110	20	13 ml
B95	15	15 ml
B96	10	14 ml
B99	20	13 ml
A9	0	5 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.44	2.72	2.08	1.10	2.18	1.64	1.63	5.17	3.40	1.29	2.79	2.04
C. bezziana	males	0.71	0.93	0.82	0.84	1.06	0.95	0.71	2.17	1.44	0.71	0.93	0.82
C. bezziana	total	1.44	2.80	2.12	1.18	2.31	1.75	1.63	5.62	3.63	1.29	2.84	2.07
C. mega/saf	total	1.34	1.00	1.17	0.97	1.06	1.01	1.10	2.40	1.75	0.71	1.95	1.33
C. rufifacies	total	2.52	3.64	3.08	1.54	1.59	1.57	2.76	4.03	3.39	1.30	2.83	2.07
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.53	2.32	1.92	2.34	1.74	2.04	2.20	2.84	2.52	1.34	2.92	2.13
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	1.60	2.38	1.99	1.27	1.84	1.56	1.26	2.47	1.86	1.34	1.95	1.64

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.56	6.89	3.81	0.70	4.26	2.19	2.17	26.23	11.07	1.16	7.30	3.67
C. bezziana	males	0.00	0.36	0.17	0.20	0.61	0.39	0.00	4.21	1.57	0.00	0.36	0.17
C. bezziana	total	1.56	7.33	3.98	0.90	4.82	2.55	2.17	31.10	12.66	1.16	7.57	3.76
C. mega/saf	total	1.31	0.50	0.87	0.43	0.61	0.52	0.70	5.26	2.55	0.00	3.29	1.26
C. rufifacies	total	5.87	12.71	8.98	1.88	2.02	1.95	7.10	15.74	11.02	1.18	7.53	3.76
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	1.83	4.89	3.20	4.99	2.53	3.67	4.35	7.55	5.85	1.28	8.01	4.02
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	2.06	5.18	3.47	1.12	2.88	1.92	1.08	5.61	2.97	1.28	3.29	2.20
Yellow faces				4.85			3.07			15.21			5.03
Totals				20.50			10.61			35.05			15.01

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	82.0%	83.0%	83.2%	74.9%
C. bezziana /Total catch	19.4%	24.0%	36.1%	25.1%
Yellow faces/Total catch	23.7%	28.9%	43.4%	33.5%
C. bezziana fem/Total *C. bezziana*	95.7%	85.9%	87.4%	97.4%
C. mega/saf /Total catch	4.2%	4.9%	7.3%	8.4%
C. rufifaces /Total catch	43.8%	18.4%	31.4%	25.1%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	15.6%	34.6%	16.7%	26.8%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	16.9%	18.1%	8.5%	14.6%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.57	2.90	0.96
C. bezziana	males	1.00	2.38	9.47	1.00
C. bezziana	total	1.00	0.64	3.18	0.95
C. mega/saf	total	1.00	0.60	2.93	1.45
C. rufifacies	total	1.00	0.22	1.23	0.42
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	1.15	1.83	1.26
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.55	0.86	0.63

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.07	1.09	0.65
C. bezziana/C. rufifaces	1.00	2.94	2.59	2.26
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	0.56	1.74	0.75
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	1.15	3.71	1.49

Screwworm fly field trials - 4x4 LS results

Trial ID: MA070707a

T1 = LTM / B110 A9

T2 = LTM / B110 A11

T3 = LTM black / B110 A9

T4 = LTM black / B110

Comments:	Wicks	Lure loss
LTM B110 (with A9)	20	23 ml
LTM B110 (with A11)	20	23 ml
LTM black B110 (with A9)	20	23 ml
LTM black B110	20	23 ml
A9	0	16 ml
LTM black A9	0	20 ml
A11	0	13 ml

LTM black = bucket painted matt black

10 day trapping periods

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.32	2.90	2.11	2.53	1.97	2.25	1.86	1.19	1.53	2.49	1.71	2.10
C. bezziana	males	0.71	1.06	0.88	0.93	0.93	0.93	0.93	0.71	0.82	0.71	0.93	0.82
C. bezziana	total	1.32	3.03	2.18	2.58	2.06	2.32	1.92	1.19	1.56	2.49	1.76	2.13
C. mega/saf	total	1.32	1.51	1.41	1.86	1.17	1.52	1.75	1.85	1.80	1.68	2.02	1.85
C. rufifacies	total	2.21	4.32	3.26	5.94	4.39	5.17	3.11	3.76	3.43	2.20	4.83	3.52
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.06	2.69	1.87	1.83	1.85	1.84	1.63	1.28	1.46	1.89	2.40	2.14
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	1.54	1.86	1.70	1.60	1.43	1.52	1.38	1.13	1.25	2.31	2.14	2.22

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.24	7.88	3.94	5.88	3.37	4.55	2.97	0.92	1.83	5.69	2.41	3.89
C. bezziana	males	0.00	0.61	0.28	0.36	0.36	0.36	0.36	0.00	0.17	0.00	0.36	0.17
C. bezziana	total	1.24	8.70	4.23	6.15	3.74	4.88	3.19	0.92	1.92	5.69	2.61	4.02
C. mega/saf	total	1.24	1.77	1.50	2.97	0.86	1.80	2.58	2.94	2.75	2.31	3.59	2.92
C. rufifacies	total	4.37	18.15	10.14	34.82	18.78	26.20	9.14	13.65	11.29	4.34	22.86	11.86
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.61	6.73	3.00	2.84	2.90	2.87	2.16	1.14	1.62	3.06	5.24	4.08
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	1.87	2.94	2.38	2.07	1.54	1.80	1.40	0.77	1.07	4.82	4.08	4.45
Yellow faces				5.73			6.68			4.68			6.94
Totals				21.25			37.54			18.65			27.33

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	73.9%	73.1%	41.1%	57.9%
C. bezziana /Total catch	19.9%	13.0%	10.3%	14.7%
Yellow faces/Total catch	27.0%	17.8%	25.1%	25.4%
C. bezziana fem/Total *C. bezziana*	93.0%	93.3%	95.3%	97.0%
C. mega/saf /Total catch	7.0%	4.8%	14.8%	10.7%
C. rufifaces /Total catch	47.7%	69.8%	60.5%	43.4%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	14.1%	7.6%	8.7%	14.9%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	11.2%	4.8%	5.7%	16.3%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.15	0.46	0.99
C. bezziana	males	1.00	1.29	0.60	0.60
C. bezziana	total	1.00	1.15	0.45	0.95
C. mega/saf	total	1.00	1.20	1.84	1.95
C. rufifacies	total	1.00	2.58	1.11	1.17
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.96	0.54	1.36
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.75	0.45	1.87

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	0.96	0.25	0.49
C. bezziana/C. rufifaces	1.00	0.45	0.41	0.81
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.20	0.84	0.70
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	1.53	1.01	0.51

Screwworm fly field trials - 4x4 LS results

Trial ID: MA070707b

T1 = LTM / A9 B110

T2 = LTM / A9 B107

T3 = LTM / A9 B133

T4 = LTM / A9 B134

Comments:

	Wicks	Lure loss
B110	20	22 ml
B107	20	24 ml
B133	20	24 ml
B134	20	24 ml
A9	0	19 ml

10 day trapping periods

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.40	1.13	1.27	1.19	1.63	1.41	0.97	1.80	1.38	1.60	1.97	1.79
C. bezziana	males	0.71	0.71	0.71	0.84	0.84	0.84	0.71	0.84	0.77	0.71	0.84	0.77
C. bezziana	total	1.40	1.13	1.27	1.32	1.68	1.50	0.97	1.86	1.41	1.60	2.02	1.81
C. mega/saf	total	1.10	1.94	1.52	1.10	1.79	1.44	1.26	1.59	1.42	0.84	3.67	2.26
C. rufifacies	total	1.22	3.08	2.15	2.81	2.97	2.89	2.71	2.83	2.77	2.12	6.53	4.32
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	0.71	1.34	1.02	1.81	1.59	1.70	1.26	2.12	1.69	1.26	1.77	1.51
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	0.97	1.13	1.05	1.14	1.42	1.28	1.06	0.97	1.01	1.83	2.31	2.07

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.47	0.77	1.10	0.92	2.14	1.48	0.43	2.73	1.41	2.06	3.39	2.69
C. bezziana	males	0.00	0.00	0.00	0.20	0.20	0.20	0.00	0.20	0.10	0.00	0.20	0.10
C. bezziana	total	1.47	0.77	1.10	1.24	2.31	1.74	0.43	2.96	1.50	2.06	3.60	2.78
C. mega/saf	total	0.70	3.26	1.80	0.70	2.69	1.57	1.08	2.02	1.52	0.20	12.99	4.59
C. rufifacies	total	0.98	8.96	4.11	7.37	8.34	7.85	6.87	7.50	7.18	3.98	42.11	18.18
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.00	1.28	0.54	2.77	2.04	2.39	1.08	3.97	2.34	1.08	2.62	1.79
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	0.43	0.77	0.60 b	0.81	1.51	1.14 ab	0.61	0.43	0.52 b	2.85	4.82	3.78 a
Yellow faces				2.90			3.32			3.02			7.37
Totals				8.15			14.70			13.06			31.11

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	37.9%	52.6%	49.6%	37.8%
C. bezziana /Total catch	13.5%	11.9%	11.5%	8.9%
Yellow faces/Total catch	35.6%	22.6%	23.1%	23.7%
C. bezziana fem/Total *C. bezziana*	100.0%	85.0%	94.0%	96.6%
C. mega/saf /Total catch	22.1%	10.7%	11.7%	14.7%
C. rufifaces /Total catch	50.4%	53.4%	55.0%	58.4%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	6.7%	16.3%	17.9%	5.7%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	7.3%	7.8%	4.0%	12.2%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.35	1.28	2.44
C. bezziana	males	DIV 0	DIV 0	DIV 0	DIV 0
C. bezziana	total	1.00	1.59	1.36	2.53
C. mega/saf	total	1.00	0.87	0.84	2.54
C. rufifacies	total	1.00	1.91	1.75	4.43
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	4.41	4.32	3.29
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.91	0.87	6.34

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.82	1.61	1.00
C. bezziana/C. rufifaces	1.00	0.83	0.78	0.57
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	0.36	0.31	0.77
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	0.83	1.56	0.40

Screwworm fly field trials - 4x4 LS results

Trial ID: MA160807a

T1 = LTM / B110 A9

T2 = LT / B110 A9

T3 = LTbl / B110 A9

T4 = ST-W / B10

Comments:	Wicks	Lure loss
LTM B110	15	22 ml
LT B110	15	22 ml
LTbl B110	15	22 ml
B10	0	21 ml
A9	0	18 ml

10 day trapping periods

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	2.73	2.27	2.50	2.26	2.72	2.49	1.41	1.19	1.30	2.31	1.27	1.79
C. bezziana	males	0.71	1.18	0.95	0.93	1.19	1.06	0.71	0.71	0.71	0.97	1.06	1.01
C. bezziana	total	2.73	2.52	2.63	2.36	2.86	2.61	1.41	1.19	1.30	2.38	1.56	1.97
C. mega/saf	total	0.97	1.71	1.34	0.84	0.71	0.77	0.84	0.71	0.77	3.22	2.70	2.96
C. rufifacies	total	3.17	2.89	3.03	1.65	2.09	1.87	1.57	1.13	1.35	5.62	2.16	3.89
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.82	3.23	2.52	1.84	2.11	1.97	1.65	1.28	1.46	13.21	7.83	10.52
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	2.04	1.00	1.52	2.19	1.06	1.62	0.97	1.23	1.10	6.38	11.08	8.73

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	6.95	4.65	5.75	4.59	6.88	5.69	1.49	0.92	1.19	4.82	1.12	2.70
C. bezziana	males	0.00	0.90	0.39	0.36	0.92	0.62	0.00	0.00	0.00	0.43	0.61	0.52
C. bezziana	total	6.95	5.86	6.39	5.05	7.65	6.29	1.49	0.92	1.19	5.16	1.95	3.39
C. mega/saf	total	0.43	2.41	1.28 b	0.20	0.00	0.10 b	0.20	0.00	0.10 b	9.86	6.78	8.25 a
C. rufifacies	total	9.52	7.84	8.66 ab	2.21	3.85	2.98 bc	1.96	0.77	1.32 c	31.12	4.16	14.64 a
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	2.79	9.90	5.85 b	2.87	3.95	3.39 b	2.22	1.14	1.64 b	174.06	60.78	110.17 a
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	3.65	0.50	1.80 b	4.31	0.61	2.14 b	0.43	1.00	0.70 b	40.22	122.18	75.68 a
Yellow faces				7.68			6.38			1.28			11.64
Totals				23.99			14.89			4.94			212.13

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	83.3%	98.5%	92.5%	29.1%
C. bezziana /Total catch	26.6%	42.2%	24.0%	1.6%
Yellow faces/Total catch	32.0%	42.9%	26.0%	5.5%
C. bezziana fem/Total *C. bezziana*	89.9%	90.4%	100.0%	79.8%
C. mega/saf /Total catch	5.4%	0.6%	1.9%	3.9%
C. rufifaces /Total catch	36.1%	20.0%	26.6%	6.9%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	24.4%	22.8%	33.2%	51.9%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	7.5%	14.4%	14.1%	35.7%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.99	0.21	0.47
C. bezziana	males	1.00	1.57	0.00	1.32
C. bezziana	total	1.00	0.98	0.19	0.53
C. mega/saf	total	1.00	0.07	0.07	6.42
C. rufifacies	total	1.00	0.34	0.15	1.69
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.58	0.28	18.83
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.18	0.39	41.94

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	13.17	2.49	0.08
C. bezziana/C. rufifaces	1.00	2.86	1.22	0.31
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.70	0.66	0.03
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	0.83	0.48	0.01

Screwworm fly field trials - 4x4 LS results

Trial ID: MA160807b

T1 = LTM / A9 B46

T2 = LTM / A9 B99

T3 = LTM / A9 B100

T4 = LTM / A9 B101

Comments:

	Wicks	Lure loss
B46	15	21 ml
B99	15	21 ml
B100	15	23 ml
B101	15	21 ml
A9	0	9 ml

10 day trapping periods

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.97	1.81	1.39	2.68	3.58	3.13	0.84	2.02	1.43	1.47	2.06	1.76
C. bezziana	males	0.71	0.71	0.71	1.47	0.71	1.09	0.84	0.71	0.77	0.71	0.84	0.77
C. bezziana	total	0.97	1.81	1.39	2.97	3.58	3.27	0.93	2.02	1.47	1.47	2.10	1.78
C. mega/saf	total	1.06	1.13	1.09	0.97	2.48	1.72	0.84	1.63	1.23	1.00	2.05	1.53
C. rufifacies	total	1.46	2.11	1.79	1.13	2.54	1.84	1.44	1.68	1.56	1.34	3.31	2.33
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.14	1.46	1.30	1.50	2.55	2.02	1.51	1.18	1.35	0.84	1.68	1.26
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	2.58	2.63	2.61	2.05	2.47	2.26	2.28	1.98	2.13	2.06	3.22	2.64

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.43	2.77	1.42 b	6.68	12.29	9.28 a	0.20	3.59	1.54 b	1.65	3.76	2.61 b
C. bezziana	males	0.00	0.00	0.00	1.65	0.00	0.68	0.20	0.00	0.10	0.00	0.20	0.10
C. bezziana	total	0.43	2.77	1.42 b	8.30	12.29	10.20 a	0.36	3.59	1.67 b	1.65	3.91	2.68 b
C. mega/saf	total	0.61	0.77	0.69	0.43	5.66	2.47	0.20	2.16	1.02	0.50	3.72	1.83
C. rufifacies	total	1.63	3.97	2.69	0.77	5.97	2.87	1.56	2.31	1.92	1.30	10.48	4.91
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.81	1.63	1.19	1.74	5.98	3.59	1.77	0.90	1.31	0.20	2.31	1.08
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	6.14	6.44	6.29	3.72	5.62	4.63	4.71	3.43	4.05	3.74	9.85	6.46
Yellow faces				2.11			12.67			2.70			4.51
Totals				12.28			23.76			9.97			16.96

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	67.3%	80.5%	62.1%	59.4%
C. bezziana /Total catch	11.6%	42.9%	16.8%	15.8%
Yellow faces/Total catch	17.2%	53.3%	27.0%	26.6%
C. bezziana fem/Total *C. bezziana*	100.0%	91.0%	92.2%	97.5%
C. mega/saf /Total catch	5.6%	10.4%	10.3%	10.8%
C. rufifaces /Total catch	21.9%	12.1%	19.2%	29.0%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	9.7%	15.1%	13.2%	6.4%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	51.2%	19.5%	40.6%	38.1%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	6.53	1.09	1.84
C. bezziana	males	DIV 0	DIV 0	DIV 0	DIV 0
C. bezziana	total	1.00	7.18	1.18	1.89
C. mega/saf	total	1.00	3.58	1.48	2.65
C. rufifacies	total	1.00	1.07	0.71	1.83
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	3.01	1.10	0.90
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	0.74	0.64	1.03

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	2.00	0.79	0.71
C. bezziana/C. rufifaces	1.00	6.72	1.65	1.03
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	2.39	1.07	2.09
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	9.75	1.83	1.83

Screwworm fly field trials - 4x4 LS results

Trial ID: MA300907a

T1 = ST-W B10

T2 = LTM B81 A9

T3 = LTM B130 A9

T4 = LTM B133 A9

Comments:

	wicks	lure lost	
B10	0 mm	51 ml	
B81	5 mm	30 ml	30% left after 1st collection
B130	15 mm	23 ml	
B133	15 mm	23 ml	
A9	0 mm	15 ml	

B81 - missing 6 values (lure evaporated)
B10 - 3 missing values

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.24	0.92	1.08				1.29	2.88	2.08	1.27	2.28	1.78
C. bezziana	males	0.97	0.79	0.88				0.84	0.71	0.77	0.71	0.93	0.82
C. bezziana	total	1.35	1.11	1.23				1.35	2.88	2.11	1.27	2.37	1.82
C. mega/saf	total	3.11	3.13	3.12				0.84	2.32	1.58	0.84	1.14	0.99
C. rufifacies	total	5.61	7.03	6.32				1.72	9.27	5.50	2.12	4.74	3.43
C. varipes	total	0.71	0.71	0.71				0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	6.80	9.36	8.08				1.59	2.51	2.05	1.17	1.47	1.32
Lucilia	total	0.71	0.71	0.71				0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	3.46	5.48	4.47				1.45	1.31	1.38	2.25	2.15	2.20

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.04	0.35	0.67				1.16	7.78	3.84	1.12	4.70	2.66
C. bezziana	males	0.43	0.12	0.27				0.20	0.00	0.10	0.00	0.36	0.17
C. bezziana	total	1.31	0.72	1.01				1.33	7.78	3.97	1.12	5.10	2.81
C. mega/saf	total	9.17	9.29	9.23				0.20	4.86	1.98	0.20	0.81	0.48
C. rufifacies	total	30.92	48.96	39.43				2.45	85.49	29.70	4.00	21.94	11.26
C. varipes	total	0.00	0.00	0.00				0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	45.73	87.17	64.80 a				2.04	5.78	3.70 b	0.86	1.67	1.25 b
Lucilia	total	0.00	0.00	0.00				0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	11.49	29.54	19.49 a				1.60	1.23	1.41 b	4.57	4.10	4.34 ab
Yellow faces				10.24						5.95			3.29
Totals				133.96						40.76			20.13

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	9.8%		66.7%	85.4%
C. bezziana /Total catch	0.8%		9.7%	14.0%
Yellow faces/Total catch	7.6%		14.6%	16.4%
C. bezziana fem/Total *C. bezziana*	66.7%		96.7%	94.5%
C. mega/saf /Total catch	6.9%		4.9%	2.4%
C. rufifaces /Total catch	29.4%		72.9%	55.9%
C. varipes /Total catch	0.0%		0.0%	0.0%
Hemipyrellia /Total catch	48.4%		9.1%	6.2%
Lucilia /Total catch	0.0%		0.0%	0.0%
Sarcophagids/Total catch	14.5%		3.5%	21.5%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00		5.72	3.96
C. bezziana	males	1.00		0.36	0.62
C. bezziana	total	1.00		3.95	2.80
C. mega/saf	total	1.00		0.21	0.05
C. rufifacies	total	1.00		0.75	0.29
C. varipes	total	DIV 0		DIV 0	DIV 0
Hemipyrellia	total	1.00		0.06	0.02
Lucilia	total	DIV 0		DIV 0	DIV 0
Sarcophagids	total	1.00		0.07	0.22

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00		18.37	53.80
C. bezziana/C. rufifaces	1.00		5.24	9.80
C. bezziana/C. varipes	N/A		N/A	N/A
C. bezziana/ Hemipyrellia	1.00		69.08	145.58
C. bezziana/Lucilia	N/A		N/A	N/A
C. bezziana/Sarcophagids	1.00		54.67	12.57

Screwworm fly field trials - 4x4 LS results

Trial ID: MA030208a

T1 = LTM / B110 A9

T2 = LTM / B110heat A9

T3 = LTM / B99 A9

T4 = LTM / B99heat A9

Comments: 10 day trapping periods

	Wicks	Lure loss
B110	20	22 ml
B110 heat	20	22 ml
B99	20	23 ml
B99 heat	20	21 ml
A9	0	11 ml

heat = lures were stored at 50C for 11 weeks before testing

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.71	0.84	0.77	0.93	0.71	0.82	0.71	1.00	0.85	1.19	0.71	0.95
C. bezziana	males	0.71	0.93	0.82	0.71	0.84	0.77	0.71	0.71	0.71	0.71	0.71	0.71
C. bezziana	total	0.71	1.00	0.85	0.93	0.84	0.88	0.71	1.00	0.85	1.19	0.71	0.95
C. mega/saf	total	0.71	0.84	0.77	0.71	0.71	0.71	0.84	0.84	0.84	0.71	0.71	0.71
C. rufifacies	total	1.45	1.91	1.68	1.06	1.90	1.48	0.84	1.51	1.17	1.52	2.21	1.86
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	0.71	1.30	1.00	1.12	0.97	1.04	1.14	1.41	1.28	1.34	1.35	1.34
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	0.71	0.93	0.82	1.79	0.71	1.25	0.84	1.06	0.95	0.97	0.84	0.90

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.00	0.20	0.10	0.36	0.00	0.17	0.00	0.50	0.23	0.92	0.00	0.40
C. bezziana	males	0.00	0.36	0.17	0.00	0.20	0.10	0.00	0.00	0.00	0.00	0.00	0.00
C. bezziana	total	0.00	0.50	0.23	0.36	0.20	0.28	0.00	0.50	0.23	0.92	0.00	0.40
C. mega/saf	total	0.00	0.20	0.10	0.00	0.00	0.00	0.20	0.20	0.20	0.00	0.00	0.00
C. rufifacies	total	1.60	3.15	2.32	0.61	3.11	1.68	0.20	1.77	0.87	1.81	4.36	2.97
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.00	1.19	0.51	0.75	0.43	0.58	0.81	1.48	1.13	1.30	1.31	1.30
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	0.00	0.36	0.17	2.71	0.00	1.06	0.20	0.61	0.39	0.43	0.20	0.31
Yellow faces				0.32			0.28			0.43			0.40
Totals				3.32			3.60			2.82			4.98

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	70.3%	100.0%	53.2%	100.0%
C. bezziana /Total catch	6.9%	7.7%	8.1%	8.0%
Yellow faces/Total catch	9.7%	7.7%	15.2%	8.0%
C. bezziana fem/Total *C. bezziana*	42.2%	60.1%	100.0%	100.0%
C. mega/saf /Total catch	2.9%	0.0%	7.1%	0.0%
C. rufifaces /Total catch	70.0%	46.7%	30.9%	59.5%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	15.3%	16.2%	40.0%	26.2%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	5.0%	29.5%	14.0%	6.3%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.73	2.37	4.17
C. bezziana	males	1.00	0.58	0.00	0.00
C. bezziana	total	1.00	1.21	1.00	1.76
C. mega/saf	total	1.00	0.00	2.09	0.00
C. rufifacies	total	1.00	0.72	0.38	1.28
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	1.15	2.22	2.57
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	6.41	2.38	1.88

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	#DIV/0!	0.48	-1118.78
C. bezziana/C. rufifaces	1.00	1.68	2.67	1.38
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.06	0.45	0.69
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/Sarcophagids	1.00	0.19	0.42	0.94

Screwworm fly field trials - 4x4 LS results

Trial ID: MA030208b

T1 = LTM / B110 A9

T2 = LTM / B110 A11

T3 = LTM / B110

T4 = LTM / B134 A9

Comments: 10 day trapping periods

	Wicks	Lure loss
B110 (A9)	20	21 ml
B110 (A11)	20	23 ml
B110	20	21 ml
B134	20	23 ml
A9	0	10 ml
A11	0	12 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.71	1.19	0.95	0.84	1.06	0.95	0.71	0.71	0.71	0.84	1.00	0.92
C. bezziana	males	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
C. bezziana	total	0.71	1.19	0.95	0.84	1.06	0.95	0.71	0.71	0.71	0.84	1.00	0.92
C. mega/saf	total	0.71	0.93	0.82	0.71	0.93	0.82	0.71	0.84	0.77	0.71	0.97	0.84
C. rufifacies	total	1.34	3.44	2.39	2.20	4.21	3.20	1.35	2.79	2.07	1.70	2.79	2.24
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	0.71	1.63	1.17	1.06	1.73	1.40	1.06	0.84	0.95	0.71	1.41	1.06
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	0.84	1.28	1.06	1.00	1.30	1.15	0.84	1.32	1.08	0.97	1.00	0.98

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	0.00	0.92	0.40	0.20	0.63	0.40	0.00	0.00	0.00	0.20	0.50	0.34
C. bezziana	males	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C. bezziana	total	0.00	0.92	0.40	0.20	0.63	0.40	0.00	0.00	0.00	0.20	0.50	0.34
C. mega/saf	total	0.00	0.36	0.17	0.00	0.36	0.17	0.00	0.20	0.10	0.00	0.43	0.20
C. rufifacies	total	1.28	11.32	5.20	4.32	17.24	9.76	1.33	7.30	3.79	2.38	7.29	4.54
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	0.00	2.14	0.86	0.63	2.50	1.45	0.63	0.20	0.40	0.00	1.49	0.62
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	0.20	1.14	0.62	0.50	1.18	0.82	0.20	1.24	0.66	0.43	0.50	0.46
Yellow faces				0.57			0.57			0.10			0.54
Totals				7.24			12.59			4.95			6.16

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	70.7%	70.7%	0.0%	63.0%
C. bezziana /Total catch	5.5%	3.2%	0.0%	5.5%
Yellow faces/Total catch	7.8%	4.5%	1.9%	8.8%
C. bezziana fem/Total *C. bezziana*	100.0%	100.0%	#DIV/0!	100.0%
C. mega/saf /Total catch	2.3%	1.3%	1.9%	3.3%
C. rufifaces /Total catch	71.8%	77.5%	76.6%	73.6%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	11.9%	11.5%	8.1%	10.1%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	8.6%	6.5%	13.4%	7.5%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	1.00	0.00	0.85
C. bezziana	males	DIV 0	DIV 0	DIV 0	DIV 0
C. bezziana	total	1.00	1.00	0.00	0.85
C. mega/saf	total	1.00	1.00	0.58	1.21
C. rufifacies	total	1.00	1.88	0.73	0.87
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	1.69	0.47	0.72
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.32	1.07	0.75

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	1.00	0.00	0.70
C. bezziana/C. rufifaces	1.00	0.53	0.00	0.98
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	0.59	0.00	1.18
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	0.76	0.00	1.14

Screwworm fly field trials - 4x4 LS results

Trial ID: MA110508a

T1 = LTM B110 A9

T2 = LTMnps B110 A9

T3 = LT B110 A9

T4 = LTnps B110 A9

Comments:	wick	lure loss
LTM B110	20 mm	23 ml
LTMnps B110	20 mm	23 ml
LT B110	20 mm	26 ml
LTnps B110	20 mm	21 ml
A9	0 mm	6 ml

Transformed (square root) mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	1.93	2.00	1.96	2.19	1.54	1.86	1.55	1.59	1.57	1.75	1.47	1.61
C. bezziana	males	0.71	0.84	0.77	0.84	0.71	0.77	0.71	0.71	0.71	0.84	0.71	0.77
C. bezziana	total	1.93	2.04	1.98	2.22	1.54	1.88	1.55	1.59	1.57	1.80	1.47	1.63
C. mega/saf	total	1.19	1.82	1.51	0.84	0.97	0.90	1.14	0.71	0.93	1.26	0.84	1.05
C. rufifacies	total	1.76	2.37	2.06	1.25	2.17	1.71	1.25	1.00	1.12	1.51	1.06	1.28
C. varipes	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Hemipyrellia	total	1.65	2.08	1.86	1.00	2.23	1.61	1.67	1.52	1.59	2.36	0.93	1.64
Lucilia	total	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71	0.71
Sarcophagids	total	1.41	1.89	1.65	1.96	1.69	1.83	2.12	1.89	2.00	2.67	1.00	1.83

Back transformed mean fly catches

Fly Species		T1			T2			T3			T4		
		S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2	S1	S2	S1+S2
C. bezziana	female	3.21	3.50	3.35	4.27	1.86	2.96	1.91	2.04	1.98	2.56	1.66	2.09
C. bezziana	males	0.00	0.20	0.10	0.20	0.00	0.10	0.00	0.00	0.00	0.20	0.00	0.10
C. bezziana	total	3.21	3.67	3.44	4.42	1.86	3.03	1.91	2.04	1.98	2.73	1.66	2.17
C. mega/saf	total	0.92	2.81	1.77	0.20	0.43	0.31	0.81	0.00	0.36	1.09	0.20	0.60
C. rufifacies	total	2.60	5.09	3.76	1.05	4.22	2.42	1.05	0.50	0.76	1.77	0.63	1.15
C. varipes	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hemipyrellia	total	2.22	3.81	2.97	0.50	4.46	2.10	2.28	1.80	2.03	5.07	0.36	2.20
Lucilia	total	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sarcophagids	total	1.49	3.06	2.21	3.35	2.37	2.84	3.97	3.08	3.51	6.63	0.50	2.86
Yellow faces				5.20			3.34			2.33			2.76
Totals				14.14			10.70			8.64			8.98

Calculated Values

Catch Composition	T1	T2	T3	T4
C. bezziana /Yellow faces	66.1%	90.7%	84.7%	78.4%
C. bezziana /Total catch	24.3%	28.3%	22.9%	24.1%
Yellow faces/Total catch	36.8%	31.2%	27.0%	30.8%
C. bezziana fem/Total *C. bezziana*	97.6%	97.9%	100.0%	96.6%
C. mega/saf /Total catch	12.5%	2.9%	4.1%	6.7%
C. rufifaces /Total catch	26.6%	22.6%	8.8%	12.8%
C. varipes /Total catch	0.0%	0.0%	0.0%	0.0%
Hemipyrellia /Total catch	21.0%	19.6%	23.5%	24.5%
Lucilia /Total catch	0.0%	0.0%	0.0%	0.0%
Sarcophagids/Total catch	15.6%	26.5%	40.6%	31.9%

Potency		T1	T2	T3	T4
C. bezziana	female	1.00	0.88	0.59	0.62
C. bezziana	males	1.00	1.00	0.00	1.00
C. bezziana	total	1.00	0.88	0.58	0.63
C. mega/saf	total	1.00	0.18	0.20	0.34
C. rufifacies	total	1.00	0.64	0.20	0.31
C. varipes	total	DIV 0	DIV 0	DIV 0	DIV 0
Hemipyrellia	total	1.00	0.71	0.68	0.74
Lucilia	total	DIV 0	DIV 0	DIV 0	DIV 0
Sarcophagids	total	1.00	1.28	1.59	1.29

Selectivity	T1	T2	T3	T4
C. bezziana/C. mega/saf	1.00	4.99	2.84	1.86
C. bezziana/C. rufifaces	1.00	1.37	2.85	2.06
C. bezziana/C. varipes	N/A	N/A	N/A	N/A
C. bezziana/ Hemipyrellia	1.00	1.25	0.84	0.85
C. bezziana/Lucilia	N/A	N/A	N/A	N/A
C. bezziana/ Sarcophagids	1.00	0.69	0.36	0.49

10.3 Appendix 3 – MSDS Bezzilure-2 A&B

BEZZILURE-2 A

Chemwatch Material Safety Data Sheet
Issue Date: 8-Jul-2008
NC317ECP

CHEMWATCH 15-9353
Version No:2.0
CD 2008/2 Page 1 of 12

Section 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME
BEZZILURE-2 A

PROPER SHIPPING NAME
CORROSIVE LIQUID, N.O.S.
(contains sodium sulfide)

PRODUCT USE
Blowfly attractant.

SUPPLIER
Company: Department of Primary Industries
Address:
665 Fairfield Road
Yeerongpilly
QLD, 4105
AUS
Telephone: 13 25 23
Fax: +61 7 3404 6900

Section 2 - HAZARDS IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE
HAZARDOUS SUBSTANCE. DANGEROUS GOODS. According to the Criteria of NOHSC, and the ADG Code.

POISONS SCHEDULE
S5

RISK	SAFETY
Harmful if swallowed.	Keep locked up.
Contact with acids liberates toxic gas.	Do not breathe gas/ fumes/ vapour/ spray.
Causes burns.	Avoid contact with eyes.
Risk of serious damage to eyes.	Wear suitable protective clothing.
Very toxic to aquatic organisms.	Use only in well ventilated areas.
	Keep container in a well ventilated place.
	To clean the floor and all objects contaminated by this material use water.
	Keep container tightly closed.
	This material and its container must be disposed of in a safe way.
	Take off immediately all contaminated clothing.
	In case of accident or if you feel unwell IMMEDIATELY contact Doctor or Poisons Information Centre (show label if possible).
	Use appropriate container to avoid environment contamination.
	Avoid release to the environment. Refer to special instructions/ safety data sheets.

continued...

BEZZILURE-2 A

This material and its container must be disposed of as hazardous waste.

Section 3 - COMPOSITION / INFORMATION ON INGREDIENTS

NAME	CAS RN	%
sodium sulfide	1313-82-2	10-30
water	7732-18-5	>60
slowly releases hydrogen sulfide	7783-06-4	NotSpec^

NOTE: Manufacturer has supplied full ingredient information to allow CHEMWATCH assessment.

Section 4 - FIRST AID MEASURES

SWALLOWED

- For advice, contact a Poisons Information Centre or a doctor at once.
- Urgent hospital treatment is likely to be needed.
- If swallowed do NOT induce vomiting.
- If vomiting occurs, lean patient forward or place on left side (head-down position, if possible) to maintain open airway and prevent aspiration.
- Observe the patient carefully.
- Never give liquid to a person showing signs of being sleepy or with reduced awareness; i.e. becoming unconscious.
- Give water to rinse out mouth, then provide liquid slowly and as much as casualty can comfortably drink.
- Transport to hospital or doctor without delay.

EYE

If this product comes in contact with the eyes:

- Immediately hold eyelids apart and flush the eye continuously with running water.
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids.
- Continue flushing until advised to stop by the Poisons Information Centre or a doctor, or for at least 15 minutes.
- Transport to hospital or doctor without delay.
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN

If skin contact occurs:

- Immediately remove all contaminated clothing, including footwear.
- Flush skin and hair with running water (and soap if available).
- Seek medical attention in event of irritation.

INHALED

- If fumes or combustion products are inhaled remove from contaminated area.
- Lay patient down. Keep warm and rested.
- Prostheses such as false teeth, which may block airway, should be removed, where possible, prior to initiating first aid procedures.
- Apply artificial respiration if not breathing, preferably with a demand valve resuscitator, bag-valve mask device, or pocket mask as trained. Perform CPR if necessary.
- Transport to hospital, or doctor.

If hydrogen sulfide gas is present do not attempt to rescue victim without proper breathing protection.

continued...

NOTES TO PHYSICIAN

Treat symptomatically.
For exposures involving sulfides and hydrogen sulfide (including gastric acid decomposition products of alkaline sulfides):

· Hydrogen sulfide anion produces its major toxic effect through inhibition of cytochrome oxidases.
· Symptoms include profuse salivation, nausea, vomiting and diarrhea. Central nervous effects may include giddiness, headache, vertigo, amnesia, confusion and unconsciousness. Tachypnoea, palpitations, tachycardia, arrhythmia, sweating, weakness and muscle cramps may also indicate overexposure.
Treatment involves:

· If respirations are depressed, application of artificial respiration, administration of oxygen (continue after spontaneous breathing is established).
· For severe poisonings administer amyl nitrite and sodium nitrite (as for cyanide poisoning) but omit sodium thiosulfate injection.
· Atropine sulfate (0.6 mg intramuscularly) may contribute symptomatic relief.
· Conjunctivitis may be relieved by installation of 1 drop of olive-oil in each eye and sometimes by 3 drops of epinephrine solution (1:1000) at frequent intervals.
Occasionally local anesthetics and hot and cold compresses are necessary to control pain.
· Antibiotics at first hint of pulmonary infection.
[Gosselin etal, Clinical Toxicology of Commercial Products]

Hydrogen sulfide is metabolised by oxidation to sulfate, methylation and reaction with metallic ion- or disulfide containing proteins (principally cytochrome c oxidase). This latter reaction is associated with aerobic, cellular respiration and is largely responsible for the toxic effects.

Section 5 - FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

· Water spray or fog.
· Foam.
· Dry chemical powder.
· BCF (where regulations permit).
· Carbon dioxide.

FIRE FIGHTING

· Alert Fire Brigade and tell them location and nature of hazard.
· Wear full body protective clothing with breathing apparatus.
· Prevent, by any means available, spillage from entering drains or water course.
· Use fire fighting procedures suitable for surrounding area.
· Do not approach containers suspected to be hot.
· Cool fire exposed containers with water spray from a protected location.
· If safe to do so, remove containers from path of fire.
· Equipment should be thoroughly decontaminated after use.

When any large container (including road and rail tankers) is involved in a fire, consider evacuation by 800 metres in all directions.

FIRE/EXPLOSION HAZARD

· Non combustible.
· Not considered to be a significant fire risk.
· Expansion or decomposition on heating may lead to violent rupture of containers.
· Decomposes on heating and may produce toxic/ irritating fumes.
· May emit acrid smoke.
May emit corrosive fumes.

continued...

Decomposition may produce toxic fumes of: sulfur oxides (SOx), hydrogen sulfide (H2S).

FIRE INCOMPATIBILITY
- Avoid strong acids.
- Avoid reaction with oxidising agents.

HAZCHEM: 2X

Personal Protective Equipment
Gas tight chemical resistant suit.

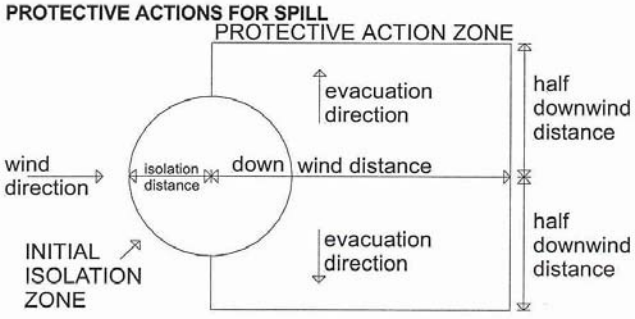
Section 6 - ACCIDENTAL RELEASE MEASURES

EMERGENCY PROCEDURES

MINOR SPILLS
- Clean up all spills immediately.
- Avoid breathing vapours and contact with skin and eyes.
- Control personal contact by using protective equipment.
- Contain and absorb spill with sand, earth, inert material or vermiculite.
- Wipe up.
- Place in a suitable labelled container for waste disposal.

MAJOR SPILLS
- Clear area of personnel and move upwind.
- Alert Fire Brigade and tell them location and nature of hazard.
- Wear full body protective clothing with breathing apparatus.
- Prevent, by any means available, spillage from entering drains or water course.
- Stop leak if safe to do so.
- Contain spill with sand, earth or vermiculite.
- Collect recoverable product into labelled containers for recycling.
- Neutralise/decontaminate residue.
- Collect solid residues and seal in labelled drums for disposal.
- Wash area and prevent runoff into drains.
- After clean up operations, decontaminate and launder all protective clothing and equipment before storing and re-using.
- If contamination of drains or waterways occurs, advise emergency services.

PROTECTIVE ACTIONS FOR SPILL



From IERG (Canada/Australia)
Isolation Distance 25 metres

continued...

Downwind Protection Distance	250 metres
IERG Number	37

FOOTNOTES

1 PROTECTIVE ACTION ZONE is defined as the area in which people are at risk of harmful exposure. This zone assumes that random changes in wind direction confines the vapour plume to an area within 30 degrees on either side of the predominant wind direction, resulting in a crosswind protective action distance equal to the downwind protective action distance.
2 PROTECTIVE ACTIONS should be initiated to the extent possible, beginning with those closest to the spill and working away from the site in the downwind direction. Within the protective action zone a level of vapour concentration may exist resulting in nearly all unprotected persons becoming incapacitated and unable to take protective action and/or incurring serious or irreversible health effects.
3 INITIAL ISOLATION ZONE is determined as an area, including upwind of the incident, within which a high probability of localised wind reversal may expose nearly all persons without appropriate protection to life-threatening concentrations of the material.
4 SMALL SPILLS involve a leaking package of 200 litres (55 US gallons) or less, such as a drum (jerrican or box with inner containers). Larger packages leaking less than 200 litres and compressed gas leaking from a small cylinder are also considered "small spills".
LARGE SPILLS involve many small leaking packages or a leaking package of greater than 200 litres, such as a cargo tank, portable tank or a "one-tonne" compressed gas cylinder.
5 Guide 154 is taken from the US DOT emergency response guide book.
6 IERG information is derived from CANUTEC - Transport Canada.

Personal Protective Equipment advice is contained in Section 8 of the MSDS.

Section 7 - HANDLING AND STORAGE

PROCEDURE FOR HANDLING

· DO NOT use aluminium, galvanised or tin-plated containers.
· Avoid all personal contact, including inhalation.
· Wear protective clothing when risk of exposure occurs.
· Use in a well-ventilated area.
· WARNING: To avoid violent reaction, ALWAYS add material to water and NEVER water to material.
· Avoid smoking, naked lights or ignition sources.
· Avoid contact with incompatible materials.
· When handling, DO NOT eat, drink or smoke.
· Keep containers securely sealed when not in use.
· Avoid physical damage to containers.
· Always wash hands with soap and water after handling.
· Work clothes should be laundered separately. Launder contaminated clothing before re-use.
· Use good occupational work practice.
· Observe manufacturer's storing and handling recommendations.
· Atmosphere should be regularly checked against established exposure standards to ensure safe working conditions are maintained.
· DO NOT allow clothing wet with material to stay in contact with skin.

SUITABLE CONTAINER

Plastic container.
· Check that containers are clearly labelled.
· Packaging as recommended by manufacturer.

STORAGE INCOMPATIBILITY

· Avoid strong acids.
· Contact with acids produces toxic fumes.

continued...

· Avoid reaction with oxidising agents.

STORAGE REQUIREMENTS

- Store in original containers.
- Keep containers securely sealed.
- Store in a cool, dry, well-ventilated area.
- Store away from incompatible materials and foodstuff containers.
- Protect containers against physical damage and check regularly for leaks.
- Observe manufacturer's storing and handling recommendations.

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS

Source	Material	TWA ppm	TWA mg/m³	STEL ppm	STEL mg/m³
Australia Exposure Standards	sodium sulfide (Inspirable dust (not otherwise classified))		10		
Australia Exposure Standards	sodium sulfide (Fluorides (as F))		2.5		
Australia Exposure Standards	hydrogen sulfide (Hydrogen sulphide)	10	14	15	21

The following materials had no OELs on our records
• water: CAS:7732- 18- 5

EMERGENCY EXPOSURE LIMITS

Material	Revised IDLH Value (mg/m3)	Revised IDLH Value (ppm)
sodium sulfide	250	

MATERIAL DATA

Not available. Refer to individual constituents.

INGREDIENT DATA

SODIUM SULFIDE:

It is the goal of the ACGIH (and other Agencies) to recommend TLVs (or their equivalent) for all substances for which there is evidence of health effects at airborne concentrations encountered in the workplace.

At this time no TLV has been established, even though this material may produce adverse health effects (as evidenced in animal experiments or clinical experience). Airborne concentrations must be maintained as low as is practically possible and occupational exposure must be kept to a minimum.

NOTE: The ACGIH occupational exposure standard for Particles Not Otherwise Specified (P.N.O.S) does NOT apply.

Sensory irritants are chemicals that produce temporary and undesirable side-effects on the eyes, nose or throat. Historically occupational exposure standards for these irritants have been based on observation of workers' responses to various airborne concentrations. Present day expectations require that nearly every individual should be protected against even minor sensory irritation and exposure standards are established using uncertainty factors or safety factors of 5 to 10 or more. On occasion animal no-observable-effect-levels (NOEL) are used to determine these limits where human results are unavailable. An additional approach, typically used by the TLV committee (USA) in determining respiratory standards for this group of chemicals, has been to assign ceiling values (TLV C) to rapidly acting irritants and to assign short-term exposure limits (TLV STELs) when the weight of evidence from irritation, bioaccumulation and other endpoints

continued...

combine to warrant such a limit. In contrast the MAK Commission (Germany) uses a five-category system based on intensive odour, local irritation, and elimination half-life. However this system is being replaced to be consistent with the European Union (EU) Scientific Committee for Occupational Exposure Limits (SCOEL); this is more closely allied to that of the USA.

OSHA (USA) concluded that exposure to sensory irritants can:

· cause inflammation
· cause increased susceptibility to other irritants and infectious agents
· lead to permanent injury or dysfunction
· permit greater absorption of hazardous substances and
· acclimate the worker to the irritant warning properties of these substances thus increasing the risk of overexposure.

WATER:

No exposure limits set by NOHSC or ACGIH.

PERSONAL PROTECTION

EYE

· Chemical goggles.
· Full face shield may be required for supplementary but never for primary protection of eyes
· Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lens or restrictions on use, should be created for each workplace or task. This should include a review of lens absorption and adsorption for the class of chemicals in use and an account of injury experience. Medical and first-aid personnel should be trained in their removal and suitable equipment should be readily available. In the event of chemical exposure, begin eye irrigation immediately and remove contact lens as soon as practicable. Lens should be removed at the first signs of eye redness or irritation - lens should be removed in a clean environment only after workers have washed hands thoroughly. [CDC NIOSH Current Intelligence Bulletin 59].

HANDS/FEET

· Butyl rubber gloves.
· Wear chemical protective gloves, eg. PVC.
· Wear safety footwear or safety gumboots, eg. Rubber.
· Neoprene rubber gloves.

OTHER

· Overalls.
· PVC Apron.
· PVC protective suit may be required if exposure severe.
· Eyewash unit.
· Ensure there is ready access to a safety shower.

RESPIRATOR

Selection of the Class and Type of respirator will depend upon the level of breathing zone contaminant and the chemical nature of the contaminant. Protection Factors (defined as the ratio of contaminant outside and inside the mask) may also be important.

Breathing Zone Level ppm (volume)	Maximum Protection Factor	Half- face Respirator	Full- Face Respirator
1000	10	- AUS P	-
1000	50	-	- AUS P
5000	50	Airline *	-
5000	100	-	- 2 P
10000	100	-	- 3 P
	100+		Airline**

continued...

BEZZILURE-2 A

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

* - Continuous Flow ** - Continuous-flow or positive pressure demand.

The local concentration of material, quantity and conditions of use determine the type of personal protective equipment required.
For further information consult site specific CHEMWATCH data (if available), or your Occupational Health and Safety Advisor.

ENGINEERING CONTROLS

Use in a well ventilated area, preferably outdoors.
General exhaust is adequate under normal operating conditions. Local exhaust ventilation may be required in special circumstances. If risk of overexposure exists, wear approved respirator. Supplied-air type respirator may be required in special circumstances. Correct fit is essential to ensure adequate protection. Provide adequate ventilation in warehouses and enclosed storage areas.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE

Straw yellow alkaline liquid; mixes with water. Rotten egg gas (hydrogen sulfide) smell.

PHYSICAL PROPERTIES

Mixes with water.
Corrosive.
Contact with acids liberates toxic gas.

Molecular Weight: Not Available	Boiling Range (℃): 100 approx.
Melting Range (℃): Not Available	Specific Gravity (water= 1): 1.1 approx
Solubility in water (g/L): Miscible	pH (as supplied): 10 approx
pH (1% solution): Not Available	Vapour Pressure (kPa): Not Available
Volatile Component (%vol): Not Available	Evaporation Rate: Not Available
Relative Vapour Density (air=1): Not Available	Flash Point (℃): Not A pplicable
Lower Explosive Limit (%): Not Applicable	Upper Explosive Limit (%): Not Applicable
Autoignition Temp (℃): Not Available	Decomposition Temp (℃): Not Available
State: LIQUID	Viscosity: Not Available

Section 10 - CHEMICAL STABILITY AND REACTIVITY INFORMATION

CONDITIONS CONTRIBUTING TO INSTABILITY

- Presence of incompatible materials.
- Product is considered stable.
- Hazardous polymerisation will not occur.

Section 11 - TOXICOLOGICAL INFORMATION

POTENTIAL HEALTH EFFECTS

ACUTE HEALTH EFFECTS

continued...

SWALLOWED
Considered an unlikely route of entry in commercial/industrial environments.
The material can produce chemical burns within the oral cavity and gastrointestinal tract following ingestion.
Ingestion may result in nausea, abdominal irritation, pain and vomiting.

EYE
The material can produce chemical burns to the eye following direct contact. Vapours or mists may be extremely irritating.
If applied to the eyes, this material causes severe eye damage.
The material may be irritating to the eye, with prolonged contact causing inflammation.
Repeated or prolonged exposure to irritants may produce conjunctivitis.

SKIN
The material can produce chemical burns following direct contact with the skin.
The material may cause skin irritation after prolonged or repeated exposure and may produce on contact skin redness, swelling, the production of vesicles, scaling and thickening of the skin.

INHALED
The material may produce respiratory tract irritation, and result in damage to the lung including reduced lung function.
Concentrate slowly releases highly irritant and toxic hydrogen sulfide gas.

CHRONIC HEALTH EFFECTS
Principal routes of exposure are by accidental skin and eye contact and by inhalation of vapours especially at higher temperatures.
Prolonged or repeated skin contact may cause drying with cracking, irritation and possible dermatitis following.
As with any chemical product, contact with unprotected bare skin; inhalation of vapour, mist or dust in work place atmosphere; or ingestion in any form, should be avoided by observing good occupational work practice.

TOXICITY AND IRRITATION
Not available. Refer to individual constituents.

SODIUM SULFIDE:
unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances.

TOXICITY	IRRITATION
Oral (rat) LD50: 208 mg/kg	Nil Reported
Inhalation (Human) TCLo: 50 mg/m³/4h	
Intraperitoneal (Rat) LD50: 147 mg/kg	
Oral (Mouse) LD50: 205 mg/kg	

Asthma-like symptoms may continue for months or even years after exposure to the material ceases. This may be due to a non-allergenic condition known as reactive airways dysfunction syndrome (RADS) which can occur following exposure to high levels of highly irritating compound. Key criteria for the diagnosis of RADS include the absence of preceding respiratory disease, in a non-atopic individual, with abrupt onset of persistent asthma-like symptoms within minutes to hours of a documented exposure to the irritant. A reversible airflow pattern, on spirometry, with the presence of moderate to severe bronchial hyperreactivity on methacholine challenge testing and the lack of minimal lymphocytic inflammation, without eosinophilia, have also been included in the criteria for diagnosis of RADS. RADS (or asthma) following an irritating inhalation is an infrequent disorder with rates related to the concentration of and duration of exposure to the irritating substance. Industrial bronchitis, on the other hand, is a disorder that occurs as result of exposure due to high concentrations of irritating substance (often particulate in nature) and is completely reversible after exposure ceases. The disorder

continued...

is characterised by dyspnea, cough and mucus production.

WATER:
unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances.
No significant acute toxicological data identified in literature search.

Section 12 - ECOLOGICAL INFORMATION

Marine Pollutant:Not Determined
Prevent, by any means available, spillage from entering drains or water courses.
Do NOT allow product to come in contact with surface waters or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash-waters.
Wastes resulting from use of the product must be disposed of on site or at approved waste sites.
DO NOT discharge into sewer or waterways.
Refer to data for ingredients, which follows:

SODIUM SULFIDE:
Do NOT allow product to come in contact with surface waters or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash-waters.
Wastes resulting from use of the product must be disposed of on site or at approved waste sites.
Sulfide ion is very toxic to aquatic life, threshold concentration for fresh or saltwater fish is 0.5ppm. The product therefore is very toxic to aquatic life. The major decomposition product, hydrogen sulfide, is damaging to vegetation at 5ppm for 24 hours.
DO NOT discharge into sewer or waterways.
Sodium sulfide hydrated is predicted to have high mobility in soil, to be substantially biodegradable in water and substantially removed in biological treatment processes.
Fish LC50: L idus25 mg/l
Daphnia magna EC50 7.1 mg/l
Algal EC50: M. aeruginosa 8 mg/l
Protozoa EC50: E. sulcatum 14 mg/l

Section 13 - DISPOSAL CONSIDERATIONS

- Recycle wherever possible or consult manufacturer for recycling options.
- Consult State Land Waste Management Authority for disposal.
- Treat and neutralise at an effluent treatment plant.
- Recycle containers if possible, or dispose of in an authorised landfill.

Section 14 - TRANSPORTATION INFORMATION

Labels Required: CORROSIVE

continued...

HAZCHEM: 2X

UNDG:

Dangerous Goods Class:	8	Subrisk:	None
UN Number:	1760	Packing Group:	III

Shipping Name:CORROSIVE LIQUID, N.O.S.
(contains sodium sulfide)

Air Transport IATA:

ICAO/IATA Class:	8	ICAO/IATA Subrisk:	None
UN/ID Number:	1760	Packing Group:	III
Special provisions:	A3		

Shipping Name: CORROSIVE LIQUID, N.O.S. *

Maritime Transport IMDG:

IMDG Class:	8	IMDG Subrisk:	None
UN Number:	1760	Packing Group:	III
EMS Number:	F- A, S- B	Special provisions:	274
Limited Quantities:	None	Marine Pollutant:	Not Determined

Shipping Name: CORROSIVE LIQUID, N.O.S.

Section 15 - REGULATORY INFORMATION

POISONS SCHEDULE: S5

REGULATIONS

Bezzilure-2 A (CAS: None):
No regulations applicable

sodium sulfide (CAS: 1313-82-2) is found on the following regulatory lists;
Australia - Australian Capital Territory - Environment Protection Regulation: Pollutants entering waterways taken to cause environmental harm (IRRIG)
Australia - Australian Capital Territory Environment Protection Regulation Pollutants entering waterways - Agricultural uses (Stock)
Australia - Australian Capital Territory Environment Protection Regulation Pollutants entering waterways - Domestic water quality
Australia Exposure Standards
Australia Hazardous Substances
Australia High Volume Industrial Chemical List (HVICL)
Australia Inventory of Chemical Substances (AICS)
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Appendix E (Part 2)
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Appendix F (Part 3)
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Schedule 2
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Schedule 4
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Schedule 5
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Schedule 6
GESAMP/EHS Composite List of Hazard Profiles - Hazard evaluation of substances transported by ships
IMO IBC Code Chapter 17: Summary of minimum requirements
IMO MARPOL 73/78 (Annex II) - List of Noxious Liquid Substances Carried in Bulk
International Air Transport Association (IATA) Dangerous Goods Regulations
International Council of Chemical Associations (ICCA) - High Production Volume List
OECD Representative List of High Production Volume (HPV) Chemicals
The Australia Group Export Control List: Chemical Weapons Precursors
WHO Guidelines for Drinking-water Quality - Guideline values for chemicals that are of health significance in drinking-water

water (CAS: 7732-18-5) is found on the following regulatory lists;
Australia Inventory of Chemical Substances (AICS)
GESAMP/EHS Composite List of Hazard Profiles - Hazard evaluation of substances transported by ships
IMO IBC Code Chapter 18: List of products to which the Code does not apply
OECD Representative List of High Production Volume (HPV) Chemicals

Section 16 - OTHER INFORMATION

EXPOSURE STANDARD FOR MIXTURES

"Worst Case" computer-aided prediction of spray/ mist or fume/ dust components and concentration:

continued...

Composite Exposure Standard for Mixture (TWA) :100 mg/m³.

Classification of the preparation and its individual components has drawn on official and authoritative sources as well as independent review by the Chemwatch Classification committee using available literature references.
A list of reference resources used to assist the committee may be found at:
www.chemwatch.net/references.

The (M)SDS is a Hazard Communication tool and should be used to assist in the Risk Assessment. Many factors determine whether the reported Hazards are Risks in the workplace or other settings. Risks may be determined by reference to Exposures Scenarios. Scale of use, frequency of use and current or available engineering controls must be considered.

Issue Date: 8-Jul-2008
Print Date: 8-Jul-2008

BEZZILURE-2 B

Section 1 - CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

PRODUCT NAME
BEZZILURE-2 B

PROPER SHIPPING NAME
FLAMMABLE LIQUID, CORROSIVE, N.O.S.
(contains valeric acid and 2-butanol)

PRODUCT USE
Fly attractant for use in fly traps.

SUPPLIER
Company: Department of Primary Industries
Address:
665 Fairfield Road
Yeerongpilly
QLD, 4105
AUS
Telephone: 13 25 23
Fax: +61 7 3404 6900

Section 2 - HAZARDS IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE
HAZARDOUS SUBSTANCE. DANGEROUS GOODS. According to the Criteria of NOHSC, and the ADG Code.

POISONS SCHEDULE
S6

RISK
Flammable.
Harmful if swallowed.
Toxic in contact with skin.
Causes burns.
Risk of serious damage to eyes.

Harmful to aquatic organisms may cause long- term adverse effects in the aquatic environment.
HARMFUL - May cause lung damage if swallowed.
Vapours may cause drowsiness and dizziness.

SAFETY
Keep locked up.
Do not breathe gas/ fumes/ vapour/ spray.
Avoid contact with eyes.
Wear suitable protective clothing.
In case of insufficient ventilation wear suitable respiratory equipment.
Use only in well ventilated areas.

Keep container in a well ventilated place.

To clean the floor and all objects contaminated by this material use water and detergent.
Keep container tightly closed.
This material and its container must be disposed of in a safe way.
Keep away from food drink and animal feeding stuffs.
Take off immediately all contaminated clothing.
In case of accident or if you feel unwell IMMEDIATELY contact Doctor or Poisons Information Centre (show label if possible).

continued...

This material and its container must be disposed of as hazardous waste.
In case of accident by inhalation: remove casualty to fresh air and keep at rest.

Section 3 - COMPOSITION / INFORMATION ON INGREDIENTS

NAME	CAS RN	%
2- mercaptoethanol	60-24-2	30-60
acetic acid glacial	64-19-7	10-30
valeric acid	109-52-4	10-30
butyric acid	107-92-6	10-30
2- butanol	78-92-2	1-10
indole	120-72-9	1-10
isobutanol	78-83-1	1-10
acetone	67-64-1	1-10

NOTE: Manufacturer has supplied full ingredient information to allow CHEMWATCH assessment.

Section 4 - FIRST AID MEASURES

SWALLOWED

- For advice, contact a Poisons Information Centre or a doctor at once.
- Urgent hospital treatment is likely to be needed.
- If swallowed do NOT induce vomiting.
- If vomiting occurs, lean patient forward or place on left side (head-down position, if possible) to maintain open airway and prevent aspiration.
- Observe the patient carefully.
- Never give liquid to a person showing signs of being sleepy or with reduced awareness; i.e. becoming unconscious.
- Give water to rinse out mouth, then provide liquid slowly and as much as casualty can comfortably drink.
- Transport to hospital or doctor without delay.

EYE

If this product comes in contact with the eyes:

- Immediately hold eyelids apart and flush the eye continuously with running water.
- Ensure complete irrigation of the eye by keeping eyelids apart and away from eye and moving the eyelids by occasionally lifting the upper and lower lids.
- Continue flushing until advised to stop by the Poisons Information Centre or a doctor, or for at least 15 minutes.
- Transport to hospital or doctor without delay.
- Removal of contact lenses after an eye injury should only be undertaken by skilled personnel.

SKIN

If skin or hair contact occurs:

- Immediately flush body and clothes with large amounts of water, using safety shower if available.
- Quickly remove all contaminated clothing, including footwear.
- Wash skin and hair with running water. Continue flushing with water until advised to stop by the Poisons Information Centre.
- Transport to hospital, or doctor.

continued...

INHALED

· If fumes or combustion products are inhaled remove from contaminated area.
· Lay patient down. Keep warm and rested.
· Prostheses such as false teeth, which may block airway, should be removed, where possible, prior to initiating first aid procedures.
· Apply artificial respiration if not breathing, preferably with a demand valve resuscitator, bag-valve mask device, or pocket mask as trained. Perform CPR if necessary.
· Transport to hospital, or doctor.

NOTES TO PHYSICIAN

For acute or short term repeated exposures to strong acids:
· Airway problems may arise from laryngeal edema and inhalation exposure. Treat with 100% oxygen initially.
· Respiratory distress may require cricothyroidotomy if endotracheal intubation is contraindicated by excessive swelling
· Intravenous lines should be established immediately in all cases where there is evidence of circulatory compromise.
· Strong acids produce a coagulation necrosis characterised by formation of a coagulum (eschar) as a result of the dessicating action of the acid on proteins in specific tissues.

INGESTION:
· Immediate dilution (milk or water) within 30 minutes post ingestion is recommended.
· DO NOT attempt to neutralise the acid since exothermic reaction may extend the corrosive injury.
· Be careful to avoid further vomit since re-exposure of the mucosa to the acid is harmful. Limit fluids to one or two glasses in an adult.
· Charcoal has no place in acid management.
· Some authors suggest the use of lavage within 1 hour of ingestion.
SKIN:
· Skin lesions require copious saline irrigation. Treat chemical burns as thermal burns with non-adherent gauze and wrapping.
· Deep second-degree burns may benefit from topical silver sulfadiazine.
EYE:
· Eye injuries require retraction of the eyelids to ensure thorough irrigation of the conjuctival cul-de-sacs. Irrigation should last at least 20-30 minutes. DO NOT use neutralising agents or any other additives. Several litres of saline are required.
· Cycloplegic drops, (1% cyclopentolate for short-term use or 5% homatropine for longer term use) antibiotic drops, vasoconstrictive agents or artificial tears may be indicated dependent on the severity of the injury.
· Steroid eye drops should only be administered with the approval of a consulting ophthalmologist).
[Ellenhorn and Barceloux: Medical Toxicology].

Section 5 - FIRE FIGHTING MEASURES

EXTINGUISHING MEDIA

· Foam.
· Dry chemical powder.
· BCF (where regulations permit).
· Carbon dioxide.
· Water spray or fog - Large fires only.

FIRE FIGHTING

· Alert Fire Brigade and tell them location and nature of hazard.
· May be violently or explosively reactive.
· Wear full body protective clothing with breathing apparatus.

continued...

· Prevent, by any means available, spillage from entering drains or water course.
· If safe, switch off electrical equipment until vapour fire hazard removed.
· Use water delivered as a fine spray to control fire and cool adjacent area.
· Avoid spraying water onto liquid pools.
· DO NOT approach containers suspected to be hot.
· Cool fire exposed containers with water spray from a protected location.
· If safe to do so, remove containers from path of fire.
· Equipment should be thoroughly decontaminated after use.
When any large container (including road and rail tankers) is involved in a fire, consider evacuation by 1000 metres in all directions.

FIRE/EXPLOSION HAZARD
· Liquid and vapour are flammable.
· Moderate fire and explosion hazard when exposed to heat or flame.
· Vapour may travel a considerable distance to source of ignition.
· Acids may react with metals to produce hydrogen, a highly flammable and explosive gas.
· Heating may cause expansion or decomposition leading to violent rupture of rigid containers.
· May emit corrosive fumes.
Combustion products include: carbon dioxide (CO_2), carbon monoxide (CO), sulfur oxides (SOx), other pyrolysis products typical of burning organic material.
May emit poisonous fumes.
May emit corrosive fumes.

FIRE INCOMPATIBILITY
· Avoid contamination with oxidising agents i.e. nitrates, oxidising acids, chlorine bleaches, pool chlorine etc. as ignition may result.

HAZCHEM: 3W

Personal Protective Equipment
Gas tight chemical resistant suit.

Section 6 - ACCIDENTAL RELEASE MEASURES

EMERGENCY PROCEDURES

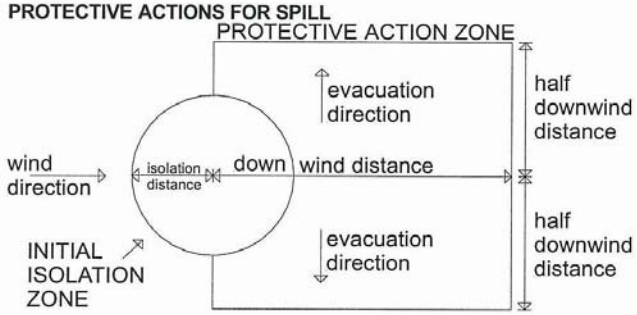
MINOR SPILLS
· Remove all ignition sources.
· Clean up all spills immediately.
· Avoid breathing vapours and contact with skin and eyes.
· Control personal contact by using protective equipment.
· Contain and absorb small quantities with vermiculite or other absorbent material.
· Wipe up.
· Collect residues in a flammable waste container.

MAJOR SPILLS
· Clear area of personnel and move upwind.
· Alert Fire Brigade and tell them location and nature of hazard.
· May be violently or explosively reactive.
· Wear full body protective clothing with breathing apparatus.
· Prevent, by any means available, spillage from entering drains or water course.
· No smoking, naked lights or ignition sources.
· Increase ventilation.
· Stop leak if safe to do so.
· Water spray or fog may be used to disperse vapour.
· Contain or absorb spill with sand, earth or vermiculite.

continued...

- Use only spark-free shovels and explosion proof equipment.
- Collect recoverable product into labelled containers for recycling.
- Collect solid residues and seal in labelled drums for disposal.
- Wash area and prevent runoff into drains.
- After clean up operations, decontaminate and launder all protective clothing and equipment before storing and re-using.
- If contamination of drains or waterways occurs, advise emergency services.

PROTECTIVE ACTIONS FOR SPILL



From IERG (Canada/Australia)	
Isolation Distance	50 metres
Downwind Protection Distance	300 metres
IERG Number	18

FOOTNOTES

1 PROTECTIVE ACTION ZONE is defined as the area in which people are at risk of harmful exposure. This zone assumes that random changes in wind direction confines the vapour plume to an area within 30 degrees on either side of the predominant wind direction, resulting in a crosswind protective action distance equal to the downwind protective action distance.

2 PROTECTIVE ACTIONS should be initiated to the extent possible, beginning with those closest to the spill and working away from the site in the downwind direction. Within the protective action zone a level of vapour concentration may exist resulting in nearly all unprotected persons becoming incapacitated and unable to take protective action and/or incurring serious or irreversible health effects.

3 INITIAL ISOLATION ZONE is determined as an area, including upwind of the incident, within which a high probability of localised wind reversal may expose nearly all persons without appropriate protection to life-threatening concentrations of the material.

4 SMALL SPILLS involve a leaking package of 200 litres (55 US gallons) or less, such as a drum (jerrican or box with inner containers). Larger packages leaking less than 200 litres and compressed gas leaking from a small cylinder are also considered "small spills".
LARGE SPILLS involve many small leaking packages or a leaking package of greater than 200 litres, such as a cargo tank, portable tank or a "one-tonne" compressed gas cylinder.

5 Guide 132 is taken from the US DOT emergency response guide book.

6 IERG information is derived from CANUTEC - Transport Canada.

EMERGENCY RESPONSE PLANNING GUIDELINES (ERPG)

The maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour WITHOUT experiencing or developing

life-threatening health effects is:
acetic acid glacial 250ppm

irreversible or other serious effects or symptoms which could impair an individual's ability to take protective action is:
acetic acid glacial 35ppm

continued...

other than mild, transient adverse effects without perceiving a clearly defined odour is:
acetic acid glacial 5ppm

American Industrial Hygiene Association (AIHA)

Ingredients considered according to the following cutoffs

Very Toxic (T+)	>= 0.1%	Toxic (T)	>= 3.0%
R50	>= 0.25%	Corrosive (C)	>= 5.0%
R51	>= 2.5%		
else	>= 10%		

where percentage is percentage of ingredient found in the mixture

Personal Protective Equipment advice is contained in Section 8 of the MSDS.

Section 7 - HANDLING AND STORAGE

PROCEDURE FOR HANDLING

- DO NOT allow clothing wet with material to stay in contact with skin.
- Avoid all personal contact, including inhalation.
- Wear protective clothing when risk of overexposure occurs.
- Use in a well-ventilated area.
- Prevent concentration in hollows and sumps.
- DO NOT enter confined spaces until atmosphere has been checked.
- Avoid smoking, naked lights or ignition sources.
- Avoid generation of static electricity.
- DO NOT use plastic buckets.
- Earth all lines and equipment.
- Use spark-free tools when handling.
- Avoid contact with incompatible materials.
- When handling, DO NOT eat, drink or smoke.
- Keep containers securely sealed when not in use.
- Avoid physical damage to containers.
- Always wash hands with soap and water after handling.
- Work clothes should be laundered separately.
- Use good occupational work practice.
- Observe manufacturer's storing and handling recommendations.
- Atmosphere should be regularly checked against established exposure standards to ensure safe working conditions.

SUITABLE CONTAINER

- Lined metal can. Lined metal drum. Lined metal safety cans.
- Packing as supplied and/or recommended by manufacturer.
- Plastic lining or containers may only be used if approved for flammable liquid (non-polar type).
- Check that containers are clearly labelled and free from leaks.
- Glass container is suitable for laboratory quantities.
- DO NOT use aluminium or galvanised containers.

STORAGE INCOMPATIBILITY

- Reacts with mild steel, galvanised steel / zinc producing hydrogen gas which may form an explosive mixture with air.
- Avoid reaction with oxidising agents, bases and strong reducing agents.
- Avoid strong bases.

continued...

STORAGE REQUIREMENTS

· Store in original containers in approved flammable liquid storage area.
· Store away from incompatible materials in a cool, dry, well-ventilated area.
· DO NOT store in pits, depressions, basements or areas where vapours may be trapped.
· No smoking, naked lights, heat or ignition sources.
· Storage areas should be clearly identified, well illuminated, clear of obstruction and accessible only to trained and authorised personnel - adequate security must be provided so that unauthorised personnel do not have access.
· Store according to applicable regulations for flammable materials for storage tanks, containers, piping, buildings, rooms, cabinets, allowable quantities and minimum storage distances.
· Use non-sparking ventilation systems, approved explosion proof equipment and intrinsically safe electrical systems.
· Have appropriate extinguishing capability in storage area (e.g. portable fire extinguishers - dry chemical, foam or carbon dioxide) and flammable gas detectors.
· Keep adsorbents for leaks and spills readily available.
· Protect containers against physical damage and check regularly for leaks.
· Observe manufacturer's storing and handling recommendations
In addition for tank storages (where appropriate):

· Store in grounded, properly designed and approved vessels and away from incompatible materials
· For bulk storages, consider use of floating roof or nitrogen blanketed vessels; where venting to atmosphere is possible, equip storage tank vents with flame arrestors; inspect tank vents during winter conditions for vapour/ ice build-up.
· Storage tanks should be above ground and diked to hold entire contents.

Section 8 - EXPOSURE CONTROLS / PERSONAL PROTECTION

EXPOSURE CONTROLS

Source	Material	TWA ppm	TWA mg/m³	STEL ppm	STEL mg/m³
Australia Exposure Standards	acetic acid glacial (Acetic acid)	10	25	15	37
Australia Exposure Standards	2- butanol (sec- Butyl alcohol)	100	303		
Australia Exposure Standards	indole (Inspirable dust (not otherwise classified))		10		
Australia Exposure Standards	isobutanol (Isobutyl alcohol)	50	152		
Australia Exposure Standards	acetone (Acetone)	500	1185	1000	2375

The following materials had no OELs on our records
• 2- mercaptoethanol: CAS:60- 24- 2
• valeric acid: CAS:109- 52- 4
• butyric acid: CAS:107- 92- 6

MATERIAL DATA

Not available. Refer to individual constituents.

INGREDIENT DATA

2-MERCAPTOETHANOL:
CEL TWA: 0.2 ppm; 6 mg/m3 (SKIN) (compare WEEL-TWA)

continued...

Threshold Recognition Value: 0.1 to 1.00 ppm
Exposure limits with "skin" notation indicate that vapour and liquid may be absorbed through intact skin. Absorption by skin may readily exceed vapour inhalation exposure. Symptoms for skin absorption are the same as for inhalation. Contact with eyes and mucous membranes may also contribute to overall exposure and may also invalidate the exposure standard.
2-mercaptoethanol has a highly offensive odour and is absorbed through the skin. The no-observed-adverse-effect-level (NOAEL) in rats exposed for 6-months to the vapour was 2 ppm (6 mg/m3).
The American Industrial Hygiene Association (AIHA) recommend a Workplace Environmental Exposure Level (WEEL) as providing an adequate margin of safety for employee health and to minimise complaints due to odour.

PERSONAL PROTECTION

EYE
· Chemical goggles.
· Full face shield may be required for supplementary but never for primary protection of eyes
· Contact lenses may pose a special hazard; soft contact lenses may absorb and concentrate irritants. A written policy document, describing the wearing of lens or restrictions on use, should be created for each workplace or task. This should include a review of lens absorption and adsorption for the class of chemicals in use and an account of injury experience. Medical and first-aid personnel should be trained in their removal and suitable equipment should be readily available. In the event of chemical exposure, begin eye irrigation immediately and remove contact lens as soon as practicable. Lens should be removed at the first signs of eye redness or irritation - lens should be removed in a clean environment only after workers have washed hands thoroughly. [CDC NIOSH Current Intelligence Bulletin 59].
· Safety glasses with side shields.

HANDS/FEET
· Wear chemical protective gloves, eg. PVC.
· Wear safety footwear or safety gumboots, eg. Rubber.
· When handling corrosive liquids, wear trousers or overalls outside of boots, to avoid spills entering boots.
NOTE:

· The material may produce skin sensitisation in predisposed individuals. Care must be taken, when removing gloves and other protective equipment, to avoid all possible skin contact.
· Contaminated leather items, such as shoes, belts and watch-bands should be removed and destroyed.

OTHER
· Overalls.
· PVC Apron.
· PVC protective suit may be required if exposure severe.
· Eyewash unit.
· Ensure there is ready access to a safety shower.
· Some plastic personal protective equipment (PPE) (e.g. gloves, aprons, overshoes) are not recommended as they may produce static electricity.

RESPIRATOR
Respiratory protection is required when ANY "Worst Case" vapour-phase concentration is exceeded (see Computer Prediction in "Exposure Standards").

Protection Factor (Min)	Half- Face Respirator	Full- Face Respirator

continued...

10 x ES	BAX- P- - AUS	-
	BAX- P- - PAPR- AUS	-
50 x ES	-	BAX- P- - AUS
	-	BAX- P- - PAPR- AUS
100 x ES	-	BAX- P- - 2
	-	BAX- P- - PAPR- 2

^ - Full-face.

The local concentration of material, quantity and conditions of use determine the type of personal protective equipment required.
For further information consult site specific CHEMWATCH data (if available), or your Occupational Health and Safety Advisor.

ENGINEERING CONTROLS

For flammable liquids and flammable gases, local exhaust ventilation or a process enclosure ventilation system may be required. Ventilation equipment should be explosion-resistant.

Section 9 - PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE

Pale yellow liquid with an unpleasant odour; slightly mixes with water. Gradually darkens to orange after a few months.

PHYSICAL PROPERTIES

Corrosive.
Acid.

Molecular Weight: Not Applicable	Boiling Range (℃): Not Available
Melting Range (℃): Not Available	Specific Gravity (water= 1): Not Available
Solubility in water (g/L): Partly Miscible	pH (as supplied): Not Available
pH (1% solution): Not Available	Vapour Pressure (kPa): Not Available
Volatile Component (%vol): Not Available	Evaporation Rate: Not Available
Relative Vapour Density (air=1): Not Available	Flash Point (℃): 53
Lower Explosive Limit (%): Not Available	Upper Explosive Limit (%): Not Available
Autoignition Temp (℃): Not Available	Decomposition Temp (℃): Not Available
State: LIQUID	Viscosity: Not Available

Section 10 - CHEMICAL STABILITY AND REACTIVITY INFORMATION

CONDITIONS CONTRIBUTING TO INSTABILITY

- Presence of incompatible materials.
- Product is considered stable.
- Hazardous polymerisation will not occur.

continued...

BEZZILURE-2 B

Section 11 - TOXICOLOGICAL INFORMATION

POTENTIAL HEALTH EFFECTS

ACUTE HEALTH EFFECTS

SWALLOWED
Accidental ingestion of the material may be harmful; animal experiments indicate that ingestion of less than 150 gram may be fatal or may produce serious damage to the health of the individual.
The material can produce chemical burns within the oral cavity and gastrointestinal tract following ingestion.
Ingestion of low-molecular organic acid solutions may produce spontaneous haemorrhaging, production of blood clots, gastrointestinal damage and narrowing of the oesophagus and stomach entry.

EYE
The material can produce chemical burns to the eye following direct contact. Vapours or mists may be extremely irritating.
If applied to the eyes, this material causes severe eye damage.
Solutions of low-molecular weight organic acids cause pain and injury to the eyes.

SKIN
Skin contact with the material may produce toxic effects; systemic effects may result following absorption.
The material can produce chemical burns following direct contact with the skin.
Open cuts, abraded or irritated skin should not be exposed to this material.
Toxic effects may result from skin absorption.

INHALED
Inhalation hazard is increased at higher temperatures.
Inhalation of vapours may cause drowsiness and dizziness. This may be accompanied by sleepiness, reduced alertness, loss of reflexes, lack of co-ordination, and vertigo.
Corrosive acids can cause irritation of the respiratory tract, with coughing, choking and mucous membrane damage. There may be dizziness, headache, nausea and weakness. Swelling of the lungs can occur, either immediately or after a delay; symptoms of this include chest tightness, shortness of breath, frothy phlegm and cyanosis. Lack of oxygen can cause death hours after onset.

CHRONIC HEALTH EFFECTS
Repeated or prolonged exposure to acids may result in the erosion of teeth, swelling and/or ulceration of mouth lining. Irritation of airways to lung, with cough, and inflammation of lung tissue often occurs. Chronic exposure may inflame the skin or conjunctiva.
There is limited evidence that, skin contact with this product is more likely to cause a sensitisation reaction in some persons compared to the general population.
Chronic exposure to mercaptans may result in damage to the lungs, kidneys and liver.
Chronic solvent inhalation exposures may result in nervous system impairment and liver and blood changes. [PATTYS].
As with any chemical product, contact with unprotected bare skin; inhalation of vapour, mist or dust in work place atmosphere; or ingestion in any form, should be avoided by observing good occupational work practice.

continued...

BEZZILURE-2 B

TOXICITY AND IRRITATION

Not available. Refer to individual constituents.

2-MERCAPTOETHANOL:
unless otherwise specified data extracted from RTECS - Register of Toxic Effects of Chemical Substances.

TOXICITY	IRRITATION
Oral (rat) LD50: 244 mg/kg	Skin (rabbit): 10 mg/24h (open)
Inhalation (mouse) LC50: 13200 mg/m³	Eye (rabbit): 1 mg - SEVERE
Dermal (rabbit) LD50: 150 mg/kg	

Tremors, convulsion, excitement, spasticity, respiratory depression recorded.

Section 12 - ECOLOGICAL INFORMATION

Marine Pollutant:Not Determined
Do NOT allow product to come in contact with surface waters or to intertidal areas below the mean high water mark. Do not contaminate water when cleaning equipment or disposing of equipment wash-waters.
Wastes resulting from use of the product must be disposed of on site or at approved waste sites.
DO NOT discharge into sewer or waterways.

Section 13 - DISPOSAL CONSIDERATIONS

· Containers may still present a chemical hazard/ danger when empty.
· Return to supplier for reuse/ recycling if possible.
Otherwise:
· If container can not be cleaned sufficiently well to ensure that residuals do not remain or if the container cannot be used to store the same product, then puncture containers, to prevent re-use, and bury at an authorised landfill.
· Where possible retain label warnings and MSDS and observe all notices pertaining to the product.
· Recycle wherever possible.
· Consult manufacturer for recycling options or consult local or regional waste management authority for disposal if no suitable treatment or disposal facility can be identified.
· Treat and neutralise at an approved treatment plant. Treatment should involve: Neutralisation with soda-ash or soda-lime followed by: Burial in a licenced land-fill or Incineration in a licenced apparatus
· Decontaminate empty containers with 5% aqueous sodium hydroxide or soda ash, followed by water. Observe all label safeguards until containers are cleaned and destroyed.

Section 14 - TRANSPORTATION INFORMATION

Labels Required: FLAMMABLE LIQUID,CORROSIVE
HAZCHEM: 3W

continued...

BEZZILURE-2 B

UNDG:

Dangerous Goods Class:	3	Subrisk:	8
UN Number:	2924	Packing Group:	III

Shipping Name:FLAMMABLE LIQUID, CORROSIVE, N.O.S.
(contains valeric acid and 2-butanol)

Air Transport IATA:

ICAO/IATA Class:	3	ICAO/IATA Subrisk:	8
UN/ID Number:	2924	Packing Group:	III
Special provisions:	A3		

Shipping Name: FLAMMABLE LIQUID, CORROSIVE, N.O.S. *

Maritime Transport IMDG:

IMDG Class:	3	IMDG Subrisk:	8
UN Number:	2924	Packing Group:	III
EMS Number:	F- E, S- C	Special provisions:	274
Limited Quantities:	None	Marine Pollutant:	Not Determined

Shipping Name: FLAMMABLE LIQUID, CORROSIVE, N.O.S.

Section 15 - REGULATORY INFORMATION

POISONS SCHEDULE: S6

REGULATIONS
Bezzilure-2 B (CAS: None):
No regulations applicable

2-mercaptoethanol (CAS: 60-24-2) is found on the following regulatory lists;
Australia Inventory of Chemical Substances (AICS)
Australia Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP) - Schedule 6
International Council of Chemical Associations (ICCA) - High Production Volume List
OECD Representative List of High Production Volume (HPV) Chemicals

Section 16 - OTHER INFORMATION

Denmark Advisory list for selfclassification of dangerous substances

Substance	CAS	Suggested codes
2- mercaptoethanol	60- 24- 2	Xn; R22 R43

INGREDIENTS WITH MULTIPLE CAS NUMBERS

Ingredient Name	CAS
2- butanol	78- 92- 2, 15892- 23- 6, 14898- 79- 4, 4221- 99- 2

EXPOSURE STANDARD FOR MIXTURES
"Worst Case" computer-aided prediction of vapour components/concentrations:
Composite Exposure Standard for Mixture (TWA) (mg/m3): 6 mg/m³
If the breathing zone concentration of ANY of the components listed below is exceeded,
"Worst Case" considerations deem the individual to be overexposed.
Component Breathing Zone ppm Breathing Zone mg/m3 Mixture Conc: (%).

Component	Breathing zone (ppm)	Breathing Zone (mg/m³)	Mixture Conc (%)
2-mercaptoethanol	0.20	6.0000	60.0

continued...

Classification of the preparation and its individual components has drawn on official and authoritative sources as well as independent review by the Chemwatch Classification committee using available literature references.
A list of reference resources used to assist the committee may be found at:
www.chemwatch.net/references.

The (M)SDS is a Hazard Communication tool and should be used to assist in the Risk Assessment. Many factors determine whether the reported Hazards are Risks in the workplace or other settings. Risks may be determined by reference to Exposures Scenarios. Scale of use, frequency of use and current or available engineering controls must be considered.

Issue Date: 8-Jul-2008
Print Date: 8-Jul-2008

10.4 Appendix 4 – Photographs

Bbalitvet Bogor, Indonesia

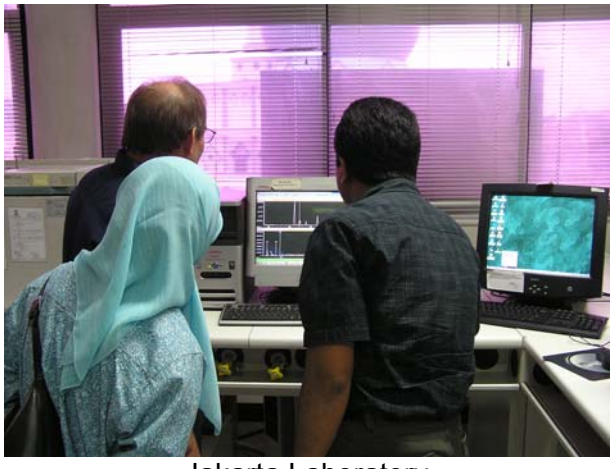
Cage assay

Bbalitvet group

Black LuciTrap

Dark LuciTrap

Wound odour collection



Jakarta Laboratory

Improvements to screw-worm fly traps and selection of optimal detection systems

Field trials Jelai Gemas, Malaysia

LuciTrap (modified)

Sticky trap

Trap service

LuciTrap/ Droughtmaster cattle

Screw-worm fly strike

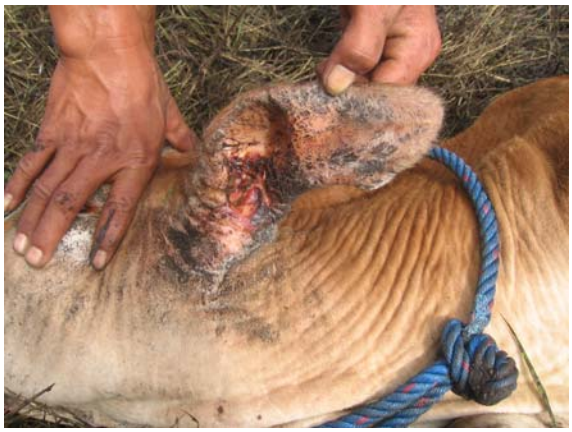
Jelai Gemas

Sumba and workshop

Matowai Maringu

Kabaru

Animal inspection



Screw-worm fly strike

LuciTrap at Kabaru

Screw-worm fly workshop June 2008