Genetic control of *Lucilia cuprina*, past and prospects.

R.J. Mahon
CSIRO Entomology, PO Box 1700, Canberra, ACT, 2601.
Email: Rod.Mahon@ento.csiro.au

Summary

The Lucilia cuprina *genetic control program conducted by members of CSIRO Entomology is reviewed. A strategy for reviving interest in area-wide control of this pest is suggested through the development of mass-rearing abilities, leading to the application of SIRM to eradicate the pest from Tasmania. Genetic control methods could be integrated in the SIRM program if appropriate strains could be developed, particularly sexing strains. The potential exists to gain synergy with other SIRM programs within Australia if a multi-species SIRM facility capable of producing sterile* L. cuprina *and other pest species is constructed.*

Keywords

Genetic control, *Lucilia cuprina,* SIRM

Introduction

For over two decades, entomologists within CSIRO Entomology were involved in developing a novel means of controlling the Australian Sheep blowfly *Lucilia cuprina* by the release of genetically modified flies. The genetic control program and its associated ecological programs (Wardhaugh, 2001) (these proceedings) received financial backing from the Division, and considerable support from the then Meat Research Council and, in the latter years, Wool Development Research Council. The program had some spectacular successes, including the development of a variety of strains carrying chromosomal arrangements that had potential as genetic control agents. In addition, a trial on an island off the South Australian coast demonstrated that genetic control using chromosomal rearrangements could suppress a native population. Unfortunately, a major trial of genetic control on Flinders Island in Bass Strait encountered major logistical problems, which ultimately resulted in the closure of the program. I would like to provide a brief review of the program, and suggest a means to exploit the wealth of knowledge accumulated.

Genetic control of *Lucilia cuprina*

The impetus for the genetic control program on *L. cuprina* was the successful development of a proposal by Knipling (1955) for a novel means to control insect pests. The technique known as the Sterile Insect Release Method (SIRM), or Sterile Insect Technique, (SIT) was employed against the New World Screw-worm fly, *Cochliomyia hominovorax,* (NWSWF) and has successfully eradicated the pest from the USA and subsequently much of central America. While no technical impediment was known at that time (or is presently known) that would prevent the implementation of SIRM against *L. cuprina,* Waterhouse (1962) considered that the cost of SIRM to eradicate sheep blowfly from Australia would be prohibitive. The overriding factor considered by Waterhouse was the permanent distribution of the pest over half the continent, and extensive additional areas where its occurrence is sporadic. A calculation for Tasmania alone was also not favourable, as based on the cost of the NWSWF eradication program in Florida, Waterhouse estimated that eradication from Tasmania would be some 15 times the annual cost of the pest.

Alternatives to SIRM were sought for sheep blowfly control that would be more cost effective (Foster *et al*., 1972; Whitten and Foster, 1975; Whitten *et al*., 1977; Whitten, 1979). The search was stimulated by the propensity of *L. cuprina* to develop resistance to insecticides. Within a period of 10 years, resistance evolved to three separate groups of chemicals, cyclodienes (Shanahan, 1958), organophosphates (Shanahan, 1966), and carbamates (Shanahan, 1967). Thus, during the 60's and 70's, there emerged an understandable concern that soon there would be no effective method of protecting sheep against this pest.

Initial alternatives to SIRM centred on isolating and exploiting "transporting mechanisms". At the time, the best-known example of a transporting mechanism was meiotic drive as understood from studies of *segregation distorter* in *Drosophila melanogaster.* Heterozygotes carrying this factor produced sperm carrying a particular chromosome far more frequently than the 50% of occasions expected of heterozygotes. In theory, such a mechanism could be exploited by releasing flies expressing the transport mechanism that was linked to deleterious genes (say, genes restricting the inability to overwinter). The mechanism would ensure the spread of the genes throughout the native population before the conditional lethal condition was expressed. A suitable meiotic drive like *segregation distorter* that was potentially useful for genetic control was not found in *L. cuprina* (Foster and Whitten, 1991*)*, and later advances in understanding of the mechanism of *segregation distorter* made this mechanism less attractive than originally thought (Whitten, 1971; Whitten, 1985; Foster and Whitten, 1991). However, to aid the search for meiotic drive mechanisms, morphological mutants were accumulated. This collection provided the ability to synthesise chromosomally rearranged strains that offered more useful control genetic control strategies (Whitten *et al*., 1977; Whitten, 1985).

Chromosomal rearrangements

Early attention was directed towards the synthesis of Y-autosome translocations. In the most valuable strain developed during the program (T(Y;5;3)23-3), males possessed a unique chromosomal element containing portions of chromosomes 3 and 5, together with a portion of the Y chromosome containing the male determining factor. The portions of autosomes included in the element, carried wild type alleles for recessive mutations (e.g. eye pigmentation) whereas the other copy of those regions of chromosomes (derived from the female parent) carried mutant alleles. Females of the strain are homozygous at the eye colour loci and are blind. Such females survive and reproduce in the laboratory, but blindness is lethal in the field. When released, males mating with a field female impart genetic load on the native population through two mechanisms. Firstly, as a consequence of the presence of the translocation, males have reduced fertility (only 45% of fertilised eggs will hatch). Secondly, males pass the mutations they carry to all surviving daughters and, in subsequent generations, these cause the elimination (through blindness) of a proportion of their descendents (mostly females) that are homozygous for the mutant alleles.

The isolation of compound chromosomes in *L. cuprina* (Foster *et al*., 1976) was one of the highlights of the program, and offered many potential benefits as agents of genetic control (Whitten *et al*., 1977). Compound chromosome elements (CC's) consist of a centromere attached to two copies of either the left or two copies of the right arms of a single chromosome. To be viable, a zygote must inherit from its parents, two compound elements, one consisting of right arms and one of left arms. Fertility of compound strains is low, as only approximately 25% of zygotes inherit one copy of both CC elements. However, the unique and appealing aspect of the CC strains is that hybrids between chromosomally normal individuals and CC individuals are all inviable. This situation presents opportunities to replace a native population with a tailor-made CC one. A CC population would resist immigration of wild types and could be genetically manipulated in a variety of ways to limit impact on the sheep industry (Whitten *et al*., 1977). Unfortunately, CC strains proved to be poorly competitive in the field (Whitten *et al*., 1977; Foster *et al*., 1985).

Competitiveness of released insects is determined by a complex mix of factors, including, the ability to survive under field conditions, the ability to locate a mate, and the capacity to successfully copulate in competition with other males. Studies on a variety of species have shown that released, mass-reared insects are almost invariably less competitive than wild males (Mahon, 1996). A reduction in competitiveness can be caused by either genetic or environmental factors, or a combination of the two. There are many opportunities where the artificial environment of mass rearing or subsequent handling of the adult could impact on the viability and fitness of the insect. As an example, a proportion of mass-reared insects might be undernourished, and therefore possess reduced energy resources that condemn them to a short lifespan in the field. Rapid genetic changes can occur during colonisation, and also as the colony adapts genetically to mass rearing conditions. The accumulated genetic changes through genetic drift and/or selection can result in insects that possess traits that while appropriate in the mass-rearing environment, are unsuitable or less favoured in the field.

To reduce the impact of genetic changes that may result in reduced competitiveness, the strain being mass reared can be regularly replaced with a "fresh", field-derived strain. For example the mass-rearing colony of New World Screw-worm Fly that supplies the release insects for the Central American SIRM program is replaced at 3-5 year intervals. Less simple solutions are available for genetically modified strains, however Y-autosome strains of *L. cuprina* could be regularly outcrossed to recently colonised wild-type strains. Outcrossing the CC strains was impossible, as they are incompatible with structurally normal strains. Foster (1982) overcame this problem through the use of an elaborate bridging system involving crossing CC individuals to a series of translocation strains. The bridging system achieved the same outcome for the CC strain that outcrossing achieved for the Y-autosome strain, namely the replacement of much of the genetic material with "fresh" wild type genetic material. Despite the employment of the bridging strains, the competitiveness of the CC strains remained low. CC's were also poorly competitive relative to the Y-autosome strain (Mahon, 1983) and focus of the genetic control program returned to Y-autosome strains.

Field trials of Y-autosome strains

From 1976 to 1990, Y-autosome translocations, (particularly a T(Y;5;3)) were employed in a series of field trials. Much was learned from each trial and the accumulated knowledge lead to progressively more successful outcomes. Mass rearing the translocation strain provided some surprises as undesirable genotypes appeared in the mass-reared colony and these rapidly increased in frequency. Crossover within male *L. cuprina* is limited, however the conditions experienced in mass rearing exposed an unsuspected flaw. Rare crossover events within the translocation element produced new chromosomal arrangements that proved to be more fertile that the original element. Given this advantage, these new elements rapidly replaced the desired chromosomal arrangement (Foster *et al*., 1980). In later trials, this problem was controlled through regular replacement of the mass reared colony with individuals from a carefully screened back-up colony. A more elegant genetic solution to the problem (a pericentric inversion) was later implemented (Foster *et al*., 1991).

The field trials of Y-autosomes revealed the sensitivity of the system to immigration. This problem is largely one of scale. Genetic control and SIRM (which is a special form of genetic control) are only effective when implemented on an area-wide basis, as in a large area, immigration is only significant at the margins, whereas, in smaller areas (such as our field trials) most of the release area could be influenced by immigration of wild type flies. A search was therefore made for a situation safe from immigration to permit the full expression of the Y-autosome system while using limited numbers of mass-reared insects. This led to a very successful field application of a T(Y;5;3) strain on Flinders Island, a small island off the South Australian coast in the Great Australian Bight. Releases began in the spring of 1985 when a vigorous population of *L. cuprina* was present, and terminated in autumn, 1986. At the time releases were discontinued, the *L. cuprina* population had declined to levels below normal means of detection. The following spring, no releases occurred, however in contrast to the previous year, the spring and early summer population was present at extremely low levels. Genetic analysis confirmed that the few spring survivors were constrained through the persistent genetic load carried over from the previous season. The population did not recover until autumn, 1987.

The success of the Flinders Island trial encouraged the program to proceed with a much larger challenge, namely the suppression and eradication of *L. cuprina* from the Furneaux islands in Bass Strait. This program suffered through a failure in mass rearing. It was estimated that approximately 10 million flies per week were required throughout the two seasons that releases were planned. Unfortunately, that level of output was rarely achieved, and the released insects failed to make a significant impact on the native population. The mass rearing problems were unexpected, as while mass rearing was always a challenging and smelly exercise, output during trials had previously been consistent and adequate. A simple "scale-up" of the rearing techniques was anticipated to produce the numbers required for Furneaux, however the "scale-up" proved to be far from simple, and a suite of novel problems were generated. Egg supply from the colony was inconsistent, which was compounded by a hitherto unknown fungus that infected the gut of the mass rearing colony, limiting their ability to absorb nutrients. In addition, mass rearing technologies were being developed while the trial was in progress. With the benefit of hindsight, expansion of our capacity to mass rear this species and strain should have preceded this much-expanded field program.

The failed Furneaux trial marked the end of the genetic control program in CSIRO Entomology. Currently, few, if any, of the genetically unique and diverse strains survive.

Sexing strains to aid SIRM

In the years since the termination of the sheep blowfly genetic control program, there has been limited interest in the use of classical genetic mechanisms in insect control. Two exceptions involve the search for, and improvement of, genetic sexing mechanisms for SIRM programs. There is renewed interest in producing a genetic sexing system for the New World Screw-worm Fly SIRM program (Kaiser P., Skoda S. *pers. comm*.). A genetic sexing system has been developed for the medfly, *Ceratitis capitata* and a Y-autosome translocation system is now widely used in mass rearing for SIRM programs. The sexing mechanism currently employed, enables females to be killed at an early embryo stage through a temperature sensitive lethal, reducing diet costs during mass rearing. In addition, an important advantage accrues through the release of males (rather than of mixed sexes) as irradiated sterile males prove to be far more effective if they are not "distracted" by the presence of large numbers of sterile co-reared females. It is interesting that the medfly experience in rearing Y-autosome translocations mimics the events in the sheep blowfly program. As could perhaps be predicted given the instability of Y-autosome strains of sheep blowfly when mass reared, recombination within the medfly translocation element occurred, necessitating the introduction of a series of "filters" and regular replacement of the mass rearing strain (Robinson *et al*. 1999; Fisher and Caceres, 2000). Again, in a similar fashion to the sheep blowfly program, a more permanent solution is being sought that involves suppression of recombination within the translocation element by the inclusion of an inversion (FAO/IAEA Annual report, 2000; Robinson, A.S. *pers. comm*.).

Genetic control using transgenic insects

The waning interest in classical genetic mechanisms for insect control contrasts to the intense interest in the development of new strategies made possible using molecular biological techniques (Handler and James, 2000). Important insect pests have been successfully transformed (eg the mosquito *Anopheles gambiae* and *A. stephensi*) (Miller *et al*., 1987; Catteruccia *et al*., 2000) and exciting ideas have been suggested to exploit these technologies in a beneficial manner; either to reduce numbers of the pest, or in the case of vectors, to manipulate their ability to act as vectors of pathogens such as dengue and malaria. However, none of these techniques have yet been employed, nor have they been field-tested. It is important to note that to apply almost all of these strategies, the release of large numbers of insects to bring about the desired outcome will be required. Thus mass-rearing technologies will be required to implement them.

SIRM

In contrast to the new genetic methods that would employ transformation, SIRM is a well-defined, simple and stable technique and is employed in many parts of the world. While expensive to implement, SIRM is particularly valuable in the protection of high value crops or where nil-tolerance to insect damage is permitted. The major success story remains the NWSWF program that has succeeded in eradicating the pest from USA, and has pushed the fly south through central America to Panama. The release of sterile flies is presently taking place in Panama and in Jamaica. As rearing costs for NWSWF are high, the successful development of a sexing system for this species could see the cost:benefit ratios improve, and that in turn, might bring about the a more rapid expansion of the eradication program into Caribbean islands and perhaps even into areas of South America.

Approaching the NWSWF program in significance, is the widespread implementation of SIRM programs aimed at the eradication or suppression of medfly. There are currently some 13 medfly factories scattered throughout the world, and their output, (sterile pupae) is sold and shipped between continents. SIRM is also used against insect groups other than Diptera, including the codling moth, *Cydia pomonella*, to protect apple and pear production in a valley in western Canada, and Pink bollworm, *Pectinophora gossypiella,* to prevent the incursion of this pest into certain valleys in California. A recent trial of SIRM against the Old World Screw-worm fly, *Chrysomya bezziana*, (OWSWF) in Malaysia, (Mahon, unpublished) reinforced the findings of an earlier trial in Papua New Guinea (Spradbery *et al*., 1989) in which the efficacy of this technique was demonstrated.

SIRM for Lucilia cuprina

Early assessments concluding that SIRM would be too expensive to use against the Australian sheep blowfly need review. In an analysis by King *et al*. (1992) the cost:benefit ratio of eradication using genetic control methods, seemed reasonable, particularly for eradication from Tasmania. Substituting SIRM for the genetic control strains (which are no longer available) should not significantly change the cost structure. However since King's analysis was performed, eradication costs and costs to the sheep industry associated with blowfly attack would have changed dramatically. Hence, it is perhaps an appropriate time to update the 1992 study. In addition to changes in costs there are now new tools that can assist SIRM eradication. Hopefully, models of sheep blowfly can be developed that will provide a powerful tool to aid the implementation of a rigorous IPM program (Wardhaugh, 2001) (these proceedings) during, and perhaps preceding, the eradication program. In this connection, a reduction of pre-SIRM fly densities might be achieved through the widespread and early-season use of persistent insecticides (such as CLiK®), which would enhance the ratio of released (sterile) males relative to the fertile (native) population.

SIRM requires a consistent supply of high-quality insects. As described above, the genetic control program encountered difficulties when "scaling-up" production of blowflies, albeit ones with unique features. It would be essential to improve mass rearing techniques for this species before the development of proposals to apply SIRM. Emphasis of the R&D program should be to develop protocols, equipment and rearing process to the stage where it becomes a reliable and sustainable routine. Small release trials can be employed to assess competitiveness of sterilised mass reared males. If a consistent supply of insects can be guaranteed, and if they have reasonable field fitness/competitiveness, there should be no technical impediments to the implementation of SIRM. While release technology is an important component of a SIRM program, appropriate equipment has been developed for the NWSWF and medfly programs, and is now commercially available.

It is clear that Tasmania would be the most appropriate initial target for eradication. *L. cuprina* introduced into Australia in the latter part of the 19th century took many years to cross from the mainland to Tasmania. Clearly, if the pest can be eradicated from the state, and if suitable quarantine measures are put in place, benefits from the eradication could be accrued for many years. While some strike will still occur through the activity of other blowfly species, it may well be of little economic consequence (King *et al*., 1992). If an eradication program was successful in Tasmania, an evaluation of the immense cost of a similar program on the mainland might then be undertaken.

In the event that an eradication program can be initiated, I would suggest that in the first instance, a simple SIRM program using sterilised wild-type males be used. While genetic methods have advantages (Foster *et al*., 1985; Foster, 1991) the strains are no longer available. However, opportunities should exist to substitute the wild type strains with a range of genetically modified strains, with the proviso that they can be reared successfully and prove competitive. As a first step, the potential savings in rearing costs associated with genetic sexing systems would be attractive. Further, I believe that given time and development, sophisticated strains like the female killing systems (Foster, 1991; Foster *et al*. 1992), or new applications produced through transformation of *L. cuprina*, will add to the efficacy of a control program. However, before these new strains could be utilised, they would require extensive testing to determine their competitiveness under field conditions.

The way forward

The specific steps that should be taken to revive genetic control/ SIRM for the control of *L. cuprina are:*

1. Re-evaluate the costs and benefits of eradication from Tasmania;
2. If the cost: benefit analysis is favourable, develop modern massrearing techniques;
3. A proof of concept eradication from an island;
4. If the island eradication is successful, proceed with eradication from Tasmania;

5. Evaluate and incorporate new strains (eg a “sexing” strain) if they can be demonstrated to advantageous.

Multi-species SIRM facility

A final comment should be made concerning opportunities for synergy of a SIRM program with the preparations for possible incursion of screw-worm fly into Australia. Like *L. cuprina*, OWSWF is a myiasis fly. It is present in tropical and sub-tropical regions surrounding the Indian Ocean and S.E. Asian countries as far south and east as Papua New Guinea. This pest would devastate significant components of our livestock industries if it ever became established in Australia (Anaman *et al*., 1993).

The Department of Agriculture Fisheries & Forestry, Australia (AFFA) has developed an emergency response strategy for a number of exotic diseases (Ausvetplan, 1996), that includes preparations for an incursion of OWSWF. SIRM is acknowledged as the only means OWSWF could be eradicated if it became established in this country. With CSIRO supplying the research capacity, and with industry backing (Meat and Livestock Australia Ltd.; Australian Wool Innovation Ltd.; Dairy Research and Development Corporation, and The Exotic Disease Preparedness Consultative Council), AFFA has recently completed an R&D program in Malaysia to enhance mass-rearing capability for OWSWF, (Mahon and Ahmad, 2000), and to conduct a SIRM trial (Mahon, unpublished data). The knowledge acquired in Malaysia, supplemented by examination of a variety of functioning insect mass rearing facilities in North and Central America, has been condensed into a design brief for a large, 300 million a week mass rearing facility suitable for construction in Australia. The timing of the construction is yet to be decided. Economic models developed by QDPI of an incursion of OWSWF and a SIRM response, (Anaman, *et al*., 1993) examined the merits of various options. A minimalist approach would be to do nothing until/if the pest is encountered, and then complete the design and construct the facility. Unfortunately that approach would mean that there would be a two to three-year delay before a SIRM response could begin. During that period, dispersal by adult flies, or as myiases on stock or feral animals would ensure dispersal of the pest and the economic damage would be significant. At the other end of the scale, a facility could be built immediately and mothballed until required. Between these extremes are options that would reduce the lag-time between discovery of the pest and a SIRM response. Unfortunately, all but the minimalist approach requires expenditure prior to an incursion.

A concept that merits consideration and offers the benefit of a response capacity to OWSWF and other exotic insects would be to build a multi-species facility that can be readily adapted to target a range of exotic species (Anaman *et al*., 1993; Tweddle and Mahon, 2000). A SIRM facility for *Lucilia cuprina*, could fit well with the requirements of a facility that could be adapted to eradicate an incursion of OWSWF.

Acknowledgments

While the errors and omissions are all mine, this paper has benefited from critical review by friend and colleague, Keith Wardhaugh.

References

Anaman, K.A., Atzeni, M.G., Mayer, D.G., Stuart, M.A., Butler, D.G., Glanville, R.J., Walthall, J.C., and Douglas, I.C. (1993). Economic assessment of the expected producer losses and control strategies of a screwworm fly invasion of Australia. Queensland Department of Primary Industries, Project Report Series Q093016.

Ausvetplan (1996). The Australian Veterinary Emergency Plan Edition 2.0 Canberra. Department of Primary Industries and Energy.

Catteruccia F., Nolan, T., Loukeris, T.G., Blass, C., Savakis, C., Kafatos, F.C. and Crisanti, A. (2000). Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature*. **405:** 959-962.

FAO/IAEA (2000). Annual Report, FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria.

Fisher, K. and Caceres, C. (2000). A filter rearing system for mass reared sexing strains of Mediterranean fruit fly (Diptera: Tephritidae). *In:* Area-wide Control of Fruit Flies and Other Insect Pests, Ed. K.H. Tan, Pernerbit Universiti Sains Malaysia, Penang pp 543-550.

Foster, G.G. (1982). The use of bridging systems to increase genetic variability in compound chromosome strains for genetic control of Lucilia cuprina (Wiedemann). *Theor. Appl. Genet*. **63**: 295-305.

Foster, G.G. (1991) Simulation of genetic control. Homozygous-viable pericentric inversions in field-female killing systems. *Theor. Appl. Genet*. **82**: 368-378.

Foster, G.G. and Whitten, M.J (1991). Meiotic drive in *Lucilia cuprina* and chromosomal evolution. Am. *Nat*. **137**: 403-415.

Foster, G.G., Whitten, M.J., Prout, T. and Gill, R. (1972). Chromosome rearrangements for the control of insect pests. *Science* **176**: 875-880.

Foster, G.G., Whitten, M.J. and Konovalov. C. (1976). The synthesis of compound autosomes in the Australian sheep blowfly *Lucilia cuprina* . *Can.J. Genet.Cytol*. **18**: 169-177.

Foster, G.G, Madern, R.H. and Mills. A.T. (1980). Genetic instability in mass rearing colonies of a sex-linked translocation strain of *Lucilia cuprina* (wiedemann) (Diptera: Calliphoridae) during a field trial of genetic control. *Theor. Appl Genet*. **58**: 169-175.

Foster, G.G., Madern, R.H., Helman, R.A. and Reed, E.M. (1985). Field trial of a compound chromosome strain for genetic control of the sheep blowfly *Lucilia cuprina. Theor. Appl. Genet*. **70**:13-21.

Foster, G.G., Weller, G.L. and Bedo, D.G. (1991). Homozygous-viable pericentric inversions for genetic control of *Lucilia cuprina*. *Theor. Appl. Genet*. **82**: 681-689.

Foster, G,G. Weller, G.L., James, W.J., Paschalidis, K.M. and McKenzie, L.J. (1992) Advances in sheep blowfly genetic control in Australia. International Symposium on management of insect pests: Nuclear and related molecular and genetic techniques. Vienna, Austria, 1992. IAEA – SM-327/30.

Handler A.M. and James A.A. (2000)"Insect Transgenesis: Methods and applications." CRC Press LLC Florida USA

Knippling, E. F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males, *J. Econ. Ent*. **48**, 459-462.

King, R.L., Pauley, J.R. and McShane, R.N. (1992). An investigation of the economic feasibility of the CSIRO's autocidal techniques for the genetic control of the sheep blowfly in Australia. Department of Primary Industries and Fisheries, Tasmania.

Mahon, R.J. (1983) The competitiveness of strains of potential use in the genetic control of the sheep blowfly. In: Sheep Blowfly and Flystrike in sheep. Department of Agriculture, New South Wales, Sydney . pp 268-273.

Mahon R.J. (1996). Frequency dependent competitiveness and the sterile insect release method. In: Frontiers of Population Ecology. Eds Floyd, R.B., Sheppard, A.W. and De Barro, P.J. CSIRO Publishing, Melb. pp 561-572.

Mahon R.J. and Ahmad, H. (2000). Mass rearing the Old World screw-worm Fly *Chrysomya bezziana*. *In:* Area-wide Control of Fruit Flies and Other Insect Pests, Ed K.H. Tan, Pernerbit Universiti Sains Malaysia, Penang pp 325-328.

Miller, L.H., Sakai, R.K., Romans, P., Gwadz, R.W., Kantoff, P. and Coon, H.G., (1987). Stable integration and expression of a bacterial gene in the mosquito, *Anopheles gambiae. Science*, **237**: 779-781.

Robinson A.S. Franz, G. and Fisher, K. (1999) Genetic sexing in the medfly, Ceratitis capita: Development, mass rearing and field application. *Trends in Entomology* **2**: 81-104.

Shanahan, G. J. (1958). Resistance to dieldrin and Aldrin in *Lucilia cuprina* Wied. *J. Instit. Agric. Sci*. **24** 157.

Shanahan, G. J. (1966). Development of a changed response in *Lucilia cuprina* (Wied.) to organophosphorus insecticides in New South Wales. *Bull. Ent. Res*. **57**: 93-100.

Shanahan, G. J. (1967). The sheep blowfly's tolerance to insecticides. *Agric. Gaz. N.S.W.* **78**: 444-445.

Spradbery, J.P., Tozer, R.S., Robb, J.M. and Cassels, P. (1989). The screw-worm fly *Chrysomya bezziana* Villeneuve (Diptera: Calliphoridae) in a sterile insect release trial in Papua New Guinea. *Res. Pop. Ecol.* **31**: 353-366.

Tweddle N., and Mahon, R.J. (2000). Insurance against an Old World Screw-worm fly invasion of Australia. *In:* Area-wide Control of Fruit Flies and Other Insect Pests, Ed K.H. Tan, Pernerbit Universiti Sains Malaysia, Penang. Pp 95-99.

Wardaugh, K.G. (2001). The biology of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) - an update. (*these proceedings*).

Waterhouse, D. F. (1962). Insect control by radiation sterilization in Australia. *J. Appl. Rad. Isot*. **13**: 435-439.

Whitten, M.J. (1971). Insect control by genetic manipulation of natural populations. *Science*. **173**: 628-684.

Whitten, M.J. (1979). The use of genetically selected strains for pest replacement or suppression. In Genetics in Relation to Insect Management eds Hoy,M.A and McElvoy. Pp 31-40. The Rockefeller Foundation.

Whitten, M.J. (1985).The conceptual basis for genetic control. *Comp. Insect Phys. Biochem. Pharm*. **12**: 465-528.

Whitten M.J. and Foster, G.G. (1975). Genetical methods of pest control. *Ann. Rev. of Entmol*. **20**: 461-476.

Whitten, M.J., Foster, G.G., Vogt, W.G., Kitching, T.L. Woodburn, T.L. and Konovalov, C. (1977). Current status of genetic control of the Australian Sheep Blowfly, *Lucilia cuprina* (Wedemann) (Diptera: Calliphoridae). Proc.XV Int. Cong. Entomology. Washington DC, 1976. pp 129-139.