

# FIELD CAGE RELEASE OF STERILE QUEENSLAND FRUIT FLY (*BACTROCERA TRYONI* (FROGGATT))

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## Summary

Field cages were trialed as an alternative, low resource method of releasing sterile fruit fly. For the whole trial, there was an average emergence of 51.77% from cages which included cages of sub-optimal loadings and a recapture rate of sterile males of 0.0677%. The field cage release system gave acceptable results (74.7% emergence) so long as the depth of pupae did not exceed 9 mm (approximately 0.8 million per cage). These results compare favourably with the results of other Qfly trials.

## INTRODUCTION

The use of sterilised fruit fly is a common practise in many countries to combat fruit fly attacks or outbreaks which cause the loss of millions of dollars to fruit industries. Although the control programs have a common objective, the release strategy of the Sterile Insect Technique (SIT) varies from one program to another.

Several broad classes of release strategies have been tried with both pupae and emerged adults; these include hatchery buckets; suspended platforms; cages; plastic bins and aerial releases. Ground releases of pupae or adult flies have evolved considerably and remain part of most release programs. They include various ways of introducing the flies or pupae into the field from the rearing facility. Pupal hatchery buckets referred to as 'Lanai' hatchery buckets were used extensively in Hawaii, in Japan (Iwahashi 1977), and in Los Angeles (Cunningham *et al.* 1980). Comparative studies on ground release and pupal hatchery methods showed that the ground release of adult flies resulted in the recovery of about twice as many flies as the bucket technique (Harris *et al.* 1975).

Most programs use a variety of strategies including pesticide sprays or male annihilation blocks, and commonly use aerial release of emerged adult flies. Airborne release of adult flies by helicopter was used in the Japanese islands (Anon 1987). The combined release of airborne and ground releases was practised in Argentina (Aruani *et al.* 1996) and in California (Penrose 1996).

Several release techniques have been used in Australian SIT programs with Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Qfly) and have given varying results. Andrewartha *et al.* (1967) used the platform release technique described by Monro and Osborn (1967) where release platforms were hung in trees with dense foliage above and below the

platform. The platforms were hung with a rain shield and on cotton soaked in dieldrin to prevent ant attacks on the unemerged pupae or freshly emerged adults.

Field cages were used to release emerged adults for the first three releases in Victoria (MacFarlane *et al.* 1987) before the adoption of the 45 L plastic bin release technique. Irradiated pupae are placed in commonly available 45 L plastic garbage bins and held for several days in controlled conditions until most flies emerge before release in the field. This latter method was also used at Wodonga (MacFarlane and Betlinski 1988).

Up to 600 plastic release bins were used in the 1990 control campaign in Western Australia (WA) (Fisher 1992). The lids of the bins were made of fly wire on a wooden frame to aid ventilation. Pupae were held up to 98 hours to allow maximum emergence of adults before being loaded into air-conditioned vehicles for transport to the release sites. At the release site, the lids were removed and only flying fruit flies were released. This program was centred in Perth, WA, with a specifically designed coolroom as the rearing facility. The SIT program for Adelaide, South Australia (SA), has used the specifically designed coolroom and the garbage bin release technique for many years (Perepelicia and Bailey 1993; Perepelicia *et al.* 1994; Reynolds *et al.* 1995; Jackman *et al.* 1996).

The plastic garbage bin release system has raised some problems where specifically designed coolrooms were not available. James (1992) found the relative humidity was 20% higher and temperature was 5°C higher inside bins which were held in a brick building equipped with a domestic air-conditioner, typical of facilities available in rural areas such as Cowra in New South Wales. Horwood and Keenan (1994) also reported that the relative humidity was 5–10% greater in the bins; and Duthie (unpublished data) reported humidity and transport

difficulties with the garbage bin technique in Trangie trials during 1996. The bin system usually has only a 30 cm hole in the lid for ventilation and this hole is sometimes partially covered by sponges which provide water to the adults. Humidity appears to increase over time due to increasing amounts of excreta as flies are held in bins to mature; this increased humidity combined with ammonia, also from excreta, results in premature and increasing mortality. Other characteristics of flies such as flightability and competitiveness are also likely to decrease. These problems are less likely to occur in mesh-sided holding cages where normal air flow or ventilation does not allow humidity to build up to damaging levels.

Resources available for the eradication campaigns of Qfly in Western Australia (Fisher 1992), *B. papaya* in north Queensland, and *B. phillipinensis* in Northern Territory are enormous given trade implications. The plastic garbage bin system uses considerable resources in manpower and vehicles and requires a rearing facility, and appears to be well suited to eradication campaigns. Eradication usually achieves an obvious and measurable outcome and quarantine measures should prevent another incursion. However the circumstances are different for the Fruit Fly Exclusion Zone (FFEZ) in New South Wales, an area free from Qfly. FFEZ is subject to continuous incursion pressure from a northern population which cannot be eradicated. The program of suppression, not eradication, in the surrounding Risk Reduction Zone (RRZ) requires a low cost release strategy that uses less manpower and minimal specialised buildings.

This present study describes the field testing of and the efficiency of field cages that function as a hatchery compartment as well as release containers.

## METHODS

### *Release cages*

Each of the four field cages were constructed (1.8 m x 0.7 m wide x 1.2 m high) from tube steel frame and with 75% grade shade cloth as the outer covering. The base of each cage was made of wood for serviceability during moves to and from release sites; stainless steel trays with a side lip 30 mm high was fixed to the wooden base to hold the pupae. To prevent newly emerged adults from squeezing through the holes in the shade cloth, voleen material was inserted across the bottom and 20 cm up the sides. Additional cloth curtaining was hung inside the cage to provide standing room for flies; this afforded the same function as the newspaper in the garbage bin release mechanism. Flies were released by opening the top screen panel.

### *Fruit flies, stocking rates and release methods*

A temporary fruit fly production facility was established at the Biological and Chemical Research Institute, Rydalmere, in Sydney. Flies were produced to the pupal stage at about the two day pre-eclosion stage, covered in fluorescent dye (Nova Red, Arc Chrome, and Comet Blue), and packed in plastic bags or socks in two litre cardboard containers. Each bag was sealed after packing and each two litre container held about 80,000 pupae. Five two litre containers were packed in a cardboard box; two cardboard boxes were packed in one styrene foam box. Each styrene box contained about 0.8 million pupae. Pupa remained in these boxes in a 17°C room overnight and were transported in air-conditioned vehicles to the Australian Nuclear Science and Technology Organisation, Lucas Heights in Sydney, for irradiation. The average radiation dose was 72 Grays (Gy) (range 70–75). Consignments of pupae were air freighted overnight to Wagga Wagga each week, arriving about 36 to 48 hours after packing.

On arrival, pupae were placed in the bottom trays in the cages at different loadings with numbers ranging from 160,000 to 1,603,000 per cage. Cages loaded with pupae at 250,000 per cage resulted in a layer 3 mm deep; cage loadings of 400,000 resulted in the pupal depth of 5 mm. Pupa were allowed to emerge with sugar and water being provided to feed the newly emerged adults. The bulk of emergence occurred within three days of receiving the pupae. Flies were liberated from the cages in a number of different strategies but all cages had to be emptied and cleaned within a week to receive the next week's consignment of sterilised pupae.

Only four cages could be used for each week's release. This release strategy might be called 'high density low frequency' because a high number or high density of flies were released at a low frequency or at a small number of locations. By contrast, the garbage bin system might be called 'low density high frequency' because a low number of flies are released from a high number of locations. Releases were made near 'hot spots' determined on a weekly basis from monitoring traps. However the 'high density low frequency' method did not achieve a uniform distribution over a large area.

In order to achieve a better distribution near 'hot spots', releases were attempted from open cages while the vehicle was in motion. Even at the lowest speed, the wind caused by the forward motion of the vehicle was enough to cause the flies to 'hang on' to the outer or inner fabric. About five minutes after the vehicle had stopped, the flies flew out of the cage at temperatures above 20°C. Despite varied trials, flies were eventually released from stationary cages

usually under the shade of a tree, fruiting or otherwise.

At the end of each weekly cycle, the pupal layer was sampled using a film plastic canister (35 cc) and counts were made of empty pupal cases, partly emerged adults and unemerged pupae. It was assumed that empty pupal cases equated to emergence. Counts were also made of deformed adult flies and apparently normal adult flies in the pupal layer that failed to leave the cage.

#### *Analysis of emergence data*

In this analysis, we examined a number of fly emergence variables that might be affected by the density of pupae in the release cages. *Failure counts* are defined to include all unemerged flies from pupae and non-viable flies that have emerged but failed to leave the pupal layer. *Bad pupae counts* counts are defined to include only unhatched pupae whereas *empty pupal counts* are pupal cases that have hatched. The analysis was conducted on both count data and their proportion to the sampling size. The results of the two approaches may provide different inferences because the sizes of unhatched pupae, empty pupal cases and non-viable adults are likely to be different when sampled with the plastic film canister. The analysis on count data ignores the total counts in the samples and hence, has an advantage of not being relied on any assumption of sample sizes. The analysis on the proportional data would, however, give prediction of the failure rate of the release loadings.

Count data (for partly emerged adults, unemerged pupae and empty pupal cases) were normalised by logarithmic transformation whereas their percentages (P%) over the sample sizes were normalised by logit transformation. A number of non-linear curves were tried to describe the relationship between count data and the loadings and the best fit was chosen by their highest contribution to the variance. Except for empty case counts, data logistic curves were selected to model the relationship :

$$Y = A + \frac{C}{1 + e^{\{-B(X-M)\}}}$$

where Y is log(count) or log{P/(1-P)}, X is the loadings, A is the lower limit, C is the difference

between lower and upper limits, B is the acceleration rate and M is the inflexion point (X value at the middle of ascending or descending part of the curve).

Empty case data were fitted with an exponential curve, that is:

$$Y = A + Be^{-kX}$$

where k is the exponential coefficient.

The parameters were estimated using GENSTAT 5 software (Genstat 5 Committee, 1987).

#### *Monitoring flies after release*

There were 58 Lindfield traps (cuelure and malathion fruit fly traps) in Wagga Wagga on a 400 m grid to monitor the wild and sterile Qfly population. All traps were cleared weekly. All trapped flies were examined under a binocular microscope with an ultra-violet filter in the light source. Flies were determined to be sterile if there were obvious traces of the florescent dyes adhering to body or ptilinum. Any flies without obvious dye adhering to the exterior were deemed to be wild flies. Given the large number of flies, both wild and sterile, cleared weekly, there were insufficient resources to do a detailed dissection of the ptilinum or the testes.

## RESULTS

#### *Emergence of flies*

The failure counts started to increase at loadings of 0.8 million and maintained the maximum level above 1.3 million. However as a ratio to sample sizes, the failure began to incline at the loadings of 0.5 million and levelled off at 0.8 million and above. Unlike total failure, the analyses of bad pupae were consistent between the two approaches where the response variables climbed at the loadings of 0.9 million. The responses in empty pupal cases, on the other hand, suggested that they declined linearly as the loadings increased whereas the ratio to sample size, the empty cases dropped at the loadings of 0.9 million.

The emergence failure increased as the loadings increased ( $P < 0.01$ ) with the variance accounting for being 61.27% (table 1). The numbers and percentage of bad or failed pupae was also positively related to the loading rate ( $P < 0.01$ ). On the other hand, the proportion of the empty pupal cases decreased as the loadings increased ( $P < 0.01$ ) (fig. 1).

**Table 1. Functional relationship between emergence failure, bad pupae, empty pupal cases, and cage loadings.**

Response	Transformation	Functions (X=loadings in millions)	R <sup>2</sup> (%)
<b>Emergence failure</b>	log (Y)	$5.51 + 0.50/\{1 + e^{\{-6.68(X - 0.89)\}}\}$	61.27
As % of sample size	log {P/(1-P)}	$0.62 + 2.86/\{1 + e^{\{-9.10(X - 0.61)\}}\}$	48.00
<b>Bad pupae</b>	log (Y)	$4.38 + 1.24/\{1 + e^{\{-17.57(X - 1.01)\}}\}$	65.72
As % of sample size	log {P/(1-P)}	$-1.36 + 2.35/\{1 + e^{\{-7.34(X - 1.01)\}}\}$	67.57
<b>Empty pupal cases</b>	log (Y)	$7.24 - 1.39e^{0.37X}$	56.50
As % of sample size	log {P/(1-P)}	$-0.78 + 1.90/\{1 + e^{\{19.8(X - 1.00)\}}\}$	72.99

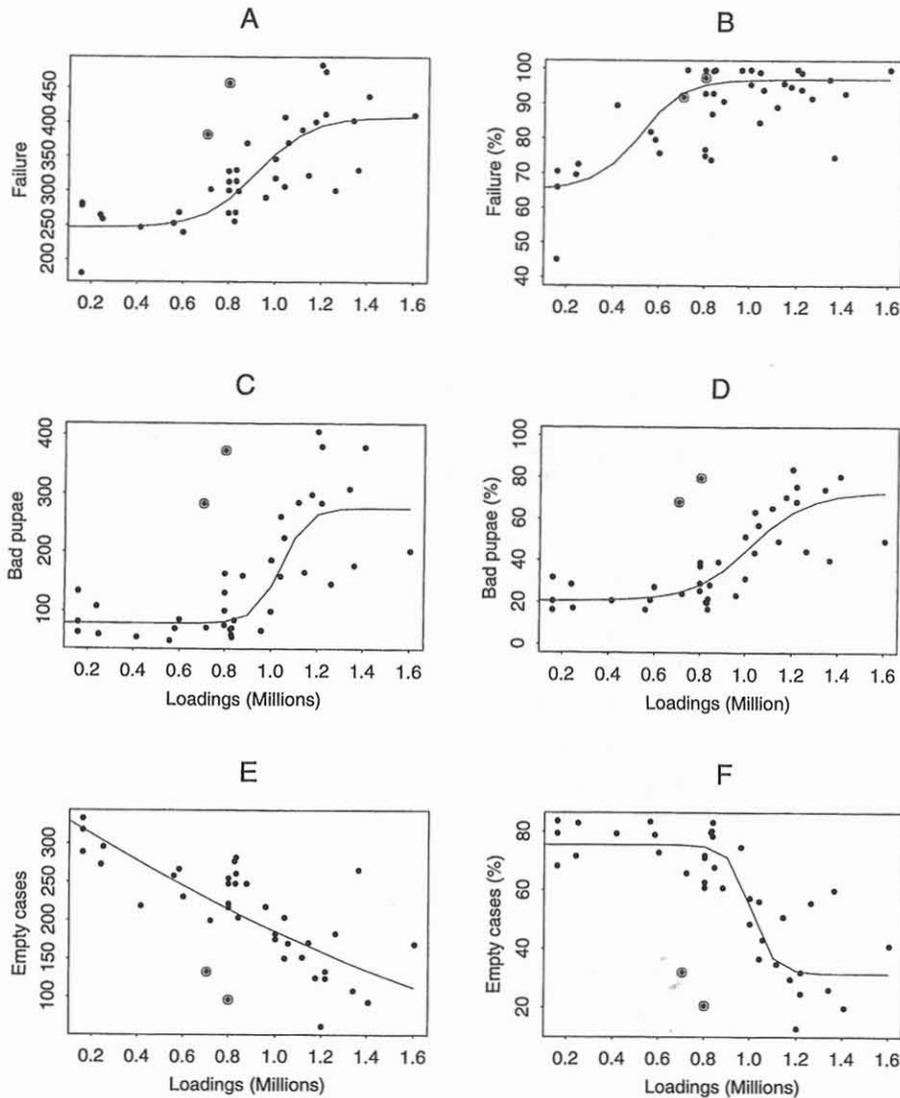


Figure 1. Logistic curves of Emergence Failure(A), Percentage Emergence Failure (B), Bad Pupae (C), Percentage Bad Pupae (D), Percentage Empty Pupal Cases, (F) and Exponential curve of Empty Pupal Cases (E) on the total loadings (in millions). Dots with a circle were outliers and excluded from modelling.

The optimal loading for our cage design appears to be 800,000 pupae (see figure 1-F). This is the highest loading (corresponding to a depth of pupae of 9 mm) that could be used without adversely affecting emergence rate. As the loading increased above 800,000, the rate and total pupal mortality increased. There was a decrease in adults from the cage litter as the pupal loading increased, in agreement with a corresponding decrease in empty pupal cases. A possible explanation for these results is that as the depth of the pupal layer increased, temperature within the layer increased to lethal levels due to respiration. Alternatively, oxygen deprivation may also have been a factor. This appears to have resulted in fewer adults emerging from the pupal cases as the depth of the pupal layer increased. If this explanation is correct,

there is an optimum depth of pupae on a solid metal tray floor. Alternatively, the floor should have ventilation holes to allow air circulation and dissipate heat to thereby potentially minimise pupal mortality.

It would appear that a depth of about 6 mm or so is the threshold for increased mortality in this system (figure 1-A). Terras (unpublished data) reported similar responses to pupal layer depths in the production facility which supplied the sterilised pupae.

#### Trapping males

The total number of released males in table 2 is based on the assumption that empty pupal cases equated to emergence however it is clearly an overestimate. The sampling also identified non viable flies and normal

adults which failed to leave the cage. In the subsample, these adults occupy more space than an equivalent number of pupae because of the space that legs and wings take up. However there appeared to be no plausible way to relate the non viable adults to the emergence rate; sometimes the total number of non viable adults exceeded the empty pupal counts in some samples for particular cage loadings. The released numbers in table 2 therefore identify the number of pupae which left the pupal bed but overestimate the number of winged adult males which left the cage. This being the case, the recapture rate is an underestimate however it could not be estimated by how much.

The recovery (recapture) rates of sterile flies are one gauge of successful release. High recovery rates may be expected if pupal quality is high and if the release method is efficient. However recovery may also be influenced by climate after release of flies, and it is likely to be influenced by the density of cuelure traps per hectare. In the Wagga trials, a maximum of 9,296,050 sterile males were released with 0.0677%

**Table 2. Total number of sterile males released and caught in traps at Wagga, 1996.**

Month	Release numbers	Recapture numbers
February	2,338,400	821
March	3,460,910	3,850
April	3,497,360	995
May		626
June		0
July		1
August		1
		6,294 (0.0677%)

of sterile males being recovered in Lindfield traps (using cuelure) on a 400 metre grid.

Flies were marked with three fluorescent dyes during different phases of the program. Comet Blue was found to be preened off or otherwise lost from the exterior of flies and marked flies were often without dye three weeks after release. Nova Red and Arc Chrome was used during the rest of the program.

**Table 3. Emergence rates of irradiated Queensland fruit fly from this study and other Australian release programs. The release techniques and the number of pupae per release container are provided. The estimated number of release containers required to release 1,000,000 pupae and the estimated number of release containers required to release one million adults are calculated (based on emergence rates) to show the relative efficiency of different release programs.**

Location	Emergence average (%)	Release method	Loadings	Number of release containers required to release one million		Reference
				Pupae	Adults	
Perth, WA	60.7	bin	About 25,000	91	150	Fisher 1992
Cowra, NSW (Oct-Dec)	53	bin	up to 40,000	25	47	James 1992 <sup>1</sup>
Cowra, NSW (Feb-Apr)	31.4	bin	up to 40,000	25	80	James 1992 <sup>1</sup>
Cowra, NSW (Apr-May)	72.7	bin	up to 40,000	25	34	James 1992
Adelaide, SA	62	bin	15,000	67	108	Perepelicia & Bailey 1993
Griffith, NSW	59	bin	About 20,000	50	85	Horwood & Keenan 1994
Adelaide, SA	70	bin	15,000	67	96	Perepelicia <i>et al.</i> 1994
Adelaide, SA	60	bin	15,000	67	112	Reynolds <i>et al.</i> 1995
Adelaide, SA	67.8	bin	15,000	67	99	Jackman <i>et al.</i> 1996 <sup>2</sup>
Adelaide, SA	68.4	bin	15,000	67	98	Jackman <i>et al.</i> 1996 <sup>2</sup>
Adelaide (Glenside), SA	86.3	bin	15,000	67	78	Jackman <i>et al.</i> 1996 <sup>3</sup>
Adelaide (Moana), SA	89.05	bin	15,000	67	75	Jackman <i>et al.</i> 1996 <sup>3</sup>
Wagga Wagga, NSW	77.7	cage	0-500,000	2	2.6	This study
	68.2	cage	500,000-750,000	1.3	1.9	This study
	68.1	cage	750,000-999,999	1	1.4	This study
	39.8	cage	> 999,999	<1	2.5	This study
Young, NSW	82.3	sand bed	800,000	1.25	1.5	Dominiak & Webster (1998)

<sup>1</sup> This study had initial problems with mortality due to transport problems.

<sup>2</sup> These flies were reared at the Gosford Horticulture Research and Advisory Station.

<sup>3</sup> These flies were reared at Biological and Chemical Research Institute, Rydalmere.

The comparison of emergence rates from this and other Australian programs is given in table 3. The relative efficiency of these programs is also calculated by comparing the estimated number of release containers needed to deploy one million pupae and to release one million adults (pupae corrected for emergence rates). The cage technique in this study and the bed technique (Dominiak and Webster 1998) used considerable fewer containers compared with the bin system.

### DISCUSSION

James (1992) reported that only minimal facilities and technical expertise were necessary for a successful SIT release program. The aim of the Wagga trials was to explore some of these minimum needs. Wagga Wagga is outside the Fruit Fly Exclusion Zone (FFEZ) which is managed to prevent fruit fly populations becoming established. The strategies inside the FFEZ are ultimately aimed at maintaining access to export markets sensitive to fruit fly infestation. Wagga Wagga is in the Risk Reduction Zone (RRZ) which is a zone about 80 kms wide surrounding the FFEZ. Fruit fly control activities in the RRZ are aimed at suppression of the wild population but must also counter regular reinvasion; the needs and resources of such a program might be quite different from an eradication program similar to that described by Fisher (1992).

#### *Cage loadings, pupal depths and emergence rates*

The emergence rate of flies for the entire trial was estimated as 51.77%. However in the broader subgroups, the estimated emergence rates for cage loadings of 0–500,000, 500–750,000, 750–999,999 and 1,000,000 pupae and above were 77.7% (range 68 to 84%), 68.2% (32 to 84%), 68.1% (20 to 83%) and 39.8% (13 to 65%) respectively. For cage loadings below 0.8 million, the emergence rate was 74.7%.

There are several possible parameters with which to compare this new release system with those previously trialed, e.g. emergence rate of pupae, and the recovery rate of sterile male flies that might indicate the relative vitality of released flies. The comparison of these parameters between this field release and other Australian programs is given in table 3.

The field cage release system gave acceptable results (around 75% emergence) so long as the depth of pupae did not exceed 9 mm (approximately 0.8 million per cage). These results compare favourably with the best results of other Qfly trials.

#### *Recapture rates of released flies compared with other Qfly trials*

Fletcher's (1974) work with fruit flies reared from infested fruit, marked, released and recaptured

perhaps offers the best achievable recapture rate as these male flies did not have to contend with the effects of irradiation. Fletcher's results suggest that a grid of traps spaced at 400 m would recapture approximately 8% of males *per week*. This figure however would be for an infinite grid. Where a finite grid is used as at Wagga Wagga, many flies will disperse beyond the grid. Meats (1998a) discussed the relative merits of different grid spacings and the interpretation of trapping results.

The recent series of releases in South Australia used the garbage bin release system with a monitoring grid with a spacing of 400 metres. Recoveries of sterile flies were recorded for the ten week release periods and therefore under-estimate the recovery rates compared with other reports which continued to record recoveries after releases had ceased. Recovery rates in South Australia are likely to be further diminished by the heavy pesticide baiting program which precedes release of sterile flies.

The recapture rates and related trapping grid spacing was reviewed by Dominiak and Webster (1998). When comparisons are made with other trials, allowance must be made for the size of the grid and trap spacing. Within these constraints, the recapture rate of 0.0677% at Wagga were lower than would be expected from some Qfly trials but higher than would be expected from some others. However, at Wagga, half the releases were from cages that had suboptimal loadings. Had optimal loadings been used throughout, the recapture rate would probably have been higher. Additionally the program at Wagga had a low priority for quantity and quality compared with other programs being conducted at the same time. These findings are supported by Meats (1998b) who calculated relatively poor estimates for mating expectation at maturity (25–33%) and daily survival decrement (65–77%) as a percentage of that expected of wild flies. Further research is required on ways of improving fly quality as indicated by the recapture rates.

The deployment of each container of flies or pupae takes time, manpower and other resources. The relative low requirement of manpower to release each million Qflies makes this cage method more attractive than the bin technique. Given the combination of reasonable emergence and recapture rates, the release system used at Wagga Wagga for Qfly suppression offers an alternative release system and is worthy of further testing. It offers a simple, low cost option for rural distribution of sterile fruit flies from centres where little infrastructure is available compared with larger cities. Future testing should continue to explore low cost release methods while endeavoring to increase emergence and recapture rates to ultimately release a more competitive adult male fly.

## ACKNOWLEDGMENT

The authors would like to thank Dr Alf Meats, University of Sydney, for his guidance in the early stages of this trial and for his constructive comments on the manuscript. Dr Colin Bower is thanked for his editorial comments. We also acknowledge the efforts of Neil Himsley for assistance on counting trap and cage samples and during the release and trapping work.

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