COMPARISON OF LOOSE AND BAGGED ECLOSION METHODS AT VARYING PUPAL DENSITIES FOR THE PRODUCTION OF ADULT STERILE QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT)(DIPTERA: TEPHRITIDAE)

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Summary

Holding dyed sterile pupae of Queensland fruit fly (*Bactrocera tryoni*) (Qfly) for adult eclosion loose in open tubs has produced variable results. We compared the effect of holding pupae loose and in mesh bags in tubs for a range of densities. Only at low density levels (10000 and 20000 per bag) was eclosion significantly higher for bagged, than loose pupae. Similarly eclosion of viable adults (those which successfully left the puparium) showed a similar trend, with differences between the methods only significant at densities ≤ 30000 pupae. We conclude that adult eclosion for the sterile Qfly could be optimised by reducing the density of pupae per rearing tub and by allowing flies to emerge from pupae held in mesh bags.

Keywords: *Bactrocera tryoni*, emergence systems, pupal density, field release, SIT

INTRODUCTION

The sterile insect technique (SIT) has been used against Queensland fruit fly (Qfly) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) for several decades in New South Wales (MacFarlane and Betlinski 1988, Horwood and Keenan 1994, Dominiak *et al.* 2000a, Meats *et al.* 2003) using flies produced from irradiated pupae. In New South Wales both adult and pupal releases have been used. Simply, adult release involves the emergence of flies from a layer of dyed pupae held in a tub for about two days pre-eclosion, under controlled temperature conditions, then releasing them into the environment. Pupal release, involves field placement of the dyed pupae in a vented container in the field to acclimatise (Meats 1973) from which they eclose and disperse. This method more closely mimics wild flies emerging from the soil, particularly when a substrate is used to cover the pupae. Dominiak *et al.* (2003a) reviewed the influence of covering pupae on fruit fly emergence. Substances trialled include sawdust (MacFarlane and Betlinski 1988), sand (Dominiak and Webster 1998, Dominiak *et al.* 2000a, b, 2003a) and both dry and moist vermiculite (Dominiak *et al.* 2000a, b, 2003a). Compared to uncovered (bare emergence) techniques, emergence rates from beneath most of these substrates, was improved (Dominiak *et al.* 2000b, 2003a).

Fruit flies emerge from the puparium using the ptilinum, a balloon-like structure that inflates and expands through a suture on the head capsule (CSIRO 1991). During normal emergence the soil is pushed in front of the fly as the ptilinum expands. The ptilinum is then anchored in the soil and as the fly retracts its body, it is withdrawn from the puparium. When the body is partially free the legs assist in the latter stage of emergence. Expansion and contraction of the ptilinum continues until the fly emerges from the soil. In nature successful eclosion is due to the anchoring of both the ptilinum and puparium in the soil. Without resistance being placed on the puparium, the adult is less likely to successfully emerge.

For sterile Qfly release programs, rearing and release methods have relied on pupae lying loose in the bottom of the holding container. Little or no resistance is applied to the pupal case and there is no provision of anchoring for the ptilinum. Loose pupae may expend more energy during emergence than those held firmly. Pupal depths in excess of 9 mm can reduce successful emergence (Dominiak *et al.* 1998). Covering pupae with sand or vermiculite requires additional labour and creates disposal issues when compared to a loose pupal holding method.

In 2000, to overcome perceived ventilation problems with deep bins (James 1992, Perepelicia *et al.* 1993, Horwood and Keenan 1994) and retain the ventilation advantages offered by cages (Dominiak *et al.* 1998, 2003b, Meats *et al.* 2003) plastic adult rearing containers (PARC system) were introduced. PARC boxes (54 x 38 x 26cm) were developed for the Mediterranean fruit fly SIT program (e.g. Dowell *et al.* 2005) where pupae are contained in paper bags to emerge.

The aim of this trial was to determine if using re-usable/washable mesh bags for adult eclosion was more efficient than the currently used method of loose pupae lying on the floor of the emergence tubs. The

pupal bag provides resistance at eclosion, aids in the emergence of the adult from the puparium, simplifies waste disposal and assists with the removal of excess dye from the flies. We measured this by comparing the emergence of *B. tryoni* flies from uniform mesh bags at six pupal densities against current practice. We reasoned that optimal emergence from both pupal release systems would be determined by the pupal load within a release container.

MATERIALS AND METHODS

Site description and source of flies

The study was conducted in PARC boxes at the Qfly emergence facility at the Yanco Agricultural Institute, New South Wales. The facility was maintained at 26±2ºC, 70±20% RH with a L12:D12 light regimen. Pupae were obtained from the culture maintained at the Elizabeth Macarthur Agriculture Institute, Camden and dyed with 4g of fluorescent pigment (Fiesta, Astral Pink 1) per 40000 pupae (or 1L). Pupae were irradiated 2-3 days prior to adult eclosion with a standard dose of 71.3-73.8 Gy of gamma radiation from a cobalt-60 source at Lucas Heights.

Adult eclosion

Pupae were sent to Yanco by air and road transport arriving within 24h of leaving Lucas Heights. For the trial the holding systems were: (a) loose holding method - pupae free in the bottom of the PARC box and (b) bag holding method - pupae held in a mesh bag and lying in the PARC box. Each method was replicated four times for each of six pupal loadings (10000, 20000, 30000, 40000, 50000 and 60000 pupae per box) on three occasions one week apart (batches), commencing 22 November, 2005. The mesh bags (21 x 30 cm) were manufactured from knitted Sarlon™ shade cloth (75% shading), with an exposed surface area approximately 31% of that of the PARC boxes. PARC boxes, without food or water, were held for seven days prior to sub-sampling to ensure maximum pupal emergence and the death of all flies.

Sub-sampling of flies

After seven days the contents of each pupal bag was poured into the tub, and then the tub for both bagged and loose treatments were sampled in the same way. The contents of each box for each holding system were separated into flies and pupal cases. Adults and pupae were then randomly sampled, using a set volume, to yield approximately 500 individuals per replicate. The flies were then classified as either viable (fully emerged and apparently capable of flight) or deformed (wings not expanded or damaged and incapable of flight). Pupae were classified as either completely emerged (empty pupal cases), partially emerged (split pupal cases with adult partially encased) or as not emerged. The sum of the partially and not emerged pupae is equivalent to the complementary variable for fully emerged pupae. For each replicate the proportion of potentially releasable adults was calculated as the product of the proportion of fully emerged pupal cases and flies apparently capable of flight.

Statistical analysis

The binary traits (viable flies, completely emerged pupae, partially emerged pupae, not emerged pupae) were analysed separately. A generalized linear mixed model (GLMM) was fitted to the data. The experimental structure was fitted as random effects and the treatments as fixed effects. The symbolic representation of the model is as follows:

Logit(p) = fixed(method + density + method.density) + random(batch + batch.method + batch.density + batch.replicate.method + batch.method.density + batch.replicate.density + error)

where the error term is the four factor interaction whose variance is used to scale the binomial error variance for over-dispersion. The GLMM parameters were estimated using the method of Schall (1991) and the statistical analysis performed using the ASReml software package (Gilmour *et. al*. 2006).

RESULTS

The proportion of fully emerged pupae was significantly ($P<0.01$) affected by the pupal release method. The bag holding method produced a predicted mean of 85% emergence as opposed to 79% for the loose method (Table 1). The proportions of fully emerged pupae were independent of pupal density ($P>0.05$) and no significant interaction between release method and pupal number per PARC box was found ($P>0.05$). Partially emerged pupae were affected by the pupal release system ($P<0.05$) and the pupal density ($P<0.01$). The bag system, as opposed to the loose method, significantly reduced the level of partial emergence from 7% to 6% (although this is probably of no field importance). The proportion of not-emerged pupae was significantly ($P<0.05$) affected by the release system with 13% of pupae failing to partly or completely emerge in the loose method, as opposed to 9% for the bag method.

The proportion of viable adults produced was significantly and inversely affected by the pupal density and significantly interacted with the release method (Tables 1 and 2). The bagged releases, despite higher initial numbers of viable adults at the lower densities, showed a faster rate of decline with increased pupal loading than the loose release method. The predicted overall proportion of adults capable of flight for each pupal release system and loading ranged from 52 - 83% (Table 2). A higher proportion of flies are capable of emerging at the lower pupal loadings for both release systems.

Table 1. F-statistics of fixed terms, variance components of random terms and predicted means of release methods and pupal densities on fully emerged, partially emerged, failed to emerge and emerged adults. Standard errors of difference (SED) and Least Significant Differences (LSD) at 5% are provided for the release methods and densities. Values in the same subset of numbers followed by the same letter are not significantly different.

Source of variation	Fully emerged pupae	Partially emerged pupae	Not emerged pupae	Emerged adults
Release method	7.50 **	4.91 *	5.35 *	1.33 NS
Density	2.18 NS	6.80 **	1.34 NS	12.01 **
Method.density	1.08 NS	1.45 NS	1.23 NS	5.07 **
Methods		Predicted means (Logit and retransformed)		
Loose	1.35a 0.79	-2.57a 0.07	-1.92a 0.13	1.05 0.74
Bag	1.70b 0.85	-2.80b 0.06	-2.28b 0.09	1.65 0.84
SED	0.12	0.08	0.15	0.38
LSD at 5%	0.23	0.16	0.29	0.74
Densities		Predicted means (Logit and retransformed)		
10 000	1.90a 0.87	-2.99a 0.05	-2.46a 0.08	
20 000	1.74ab 0.85	-2.98ab 0.05	-2.24ab 0.10	
30 000	1.66abc 0.84	-2.85abc 0.05	-2.18ab 0.10	
40 000	1.34bc 0.79	-2.62cd 0.07	-1.88ab 0.13	
50 000	1.21c 0.77	-2.47d 0.08	-1.76b 0.15	
60 000	1.31c 0.79	-2.21e 0.10	-2.08ab 0.11	
SED	0.26	0.17	0.30	
LSD at 5%	0.51	0.33	0.59	

* and ** denotes the 5% and 1% level of significance

DISCUSSION

In the Qfly sterile insect release program, apart from quality issues (Parker 2005), the factors of greatest interest is the proportion of pupae that fully emerge and the overall proportion of viable adults produced. The loose emergence system resulted in significantly fewer completely emerged adults but had no effect on the proportion of viable adults. Density had no significant effect on adult emergence but significantly affected the proportion of viable flies produced. The combination of release method and pupal loading resulted in the loose method, even at the lowest density of 10000 pupae per tub, producing a similar overall proportion of potentially releasable adults (69%) as the bagged method at 40000 per tub. This is well below the estimated 83% potentially releasable adults for bags with a pupal loading of 10000.

A more rapid and greater reduction in the proportion of potentially releasable adult flies occurred with increasing density for the bagged than for the loose release method. For the loose method the number of viable adults is only significantly different at the 10000 and 60000 pupal loadings, suggesting the surface area available for the flies to escape the pupal mass, as opposed to the depth of pupae in the box, is the determining factor for successful emergence.

While there is evidence to indicate that the use of a pupal bag significantly enhances the emergence of flies, at densities of up to 30000, the bags used in the current study are inappropriate for routine use. The upper surface of the bag lying in the PARC box (approximately 30% of the floor area) had an estimated 12740 holes available for flies to escape through.

Table 2. Predicted means (Logit and re-transformed) for fully emerged pupae and viable flies (including Standard Errors of difference (SED) and least significant difference (LSD) at 5%) for testing treatments within densities and the percentage of potentially releasable adults from the PARC boxes. Asterisks indicate significant difference between treatments within corresponding densities.

Densities	Treatment	Fully emerged pupae	Viable adults	Potentially releasable adults (%)
10000	Loose	1.69 0.84	1.52 0.82	69
10000	Bagged	2.10* 0.89	2.56* 0.93	83
20000	Loose	1.45 0.81	1.07 0.74	60
20000	Bagged	2.03* 0.88	2.10* 0.89	79
30000	Loose	1.51 0.82	1.11 0.75	62
30000	Bagged	1.81 0.86	1.93* 0.87	75
40000	Loose	1.16 0.76	0.95 0.72	55
40000	Bagged	1.52 0.82	1.51 0.82	67
50000	Loose	1.03 0.74	0.89 0.71	52
50000	Bagged	1.39 0.80	1.01 0.73	59
60000	Loose	1.28 0.78	0.78 0.69	54
60000	Bagged	1.34 0.79	0.82 0.69	55
SED		0.28	0.35	
LSD at 5%		0.55	0.68	

Further examination of the bag material indicated there were two sizes of holes in the weave. It was estimated that half of the holes were less than the width of the head capsule of the escaping flies, based on the number of cells in the fabric containing entrapped flies in randomly assessed 3 x 3 cm squares of the mesh. Flies were observed during their attempt to pass through the mesh: in some cases, a leg and the head would go into different holes and flies appeared to make no effort to back up and try again. The design of any future mesh would need to eliminate this problem.

Despite the reduced surface area from which flies could escape, the greater unit density of pupae within the bags and the fact that 50% of the holes in the mesh inhibited the escape of flies, generally more viable adults were produced in the bag method for the same pupal loading. The superior result achieved using the bags suggest that factors such as the resistance imposed by the mesh on the pupae, significantly aided fly emergence.

Currently the production facility produces pupae of a mixed size and the average weight is an indicator of that size. Pupal weights continually change depending on many factors in the facility (Dominiak unpub. data) and the weather at Camden. Pupal weight was not recorded during initial evaluations as we were not aware that pupal size might change significantly or have profound impacts on our results. Some facilities overseas use a pupal sorter to deliver consignments of a uniform pupal size and weight. This approach may resolve the problems found in this study.

In an SIT program using pupal releases it is the number of the viable adults (adults capable of flight and dispersal that can mate successfully in the field) per unit of pupae, rather than the number of pupae emerging that determines success. From a production perspective identifying the causes and extent of pupal mortality and partial emergence is important to improving the release system. Pupae cost more than $1000 per million to produce and wastage or a low overall proportion of flies capable of flight comes at a financial cost. Our data using mesh bags suggests pupal loadings of 10000-30000 would seem to be ideal.

Transport stress associated with moving pupae from the production site to the emergence facility accounts for a 16.7% reduction in emerged flies (Dominiak *et al*. 2007). Optimising emergence parameters is the first step in a successful release program. Clearly, the

bagged holding method is superior to the loose method and the resistance imposed on emerging flies by the mesh fabric beneficial. Observations suggest that the Sarlon™ material used to construct the bags tested in this study was not appropriate. The fabric was too inflexible; holes were angular, uneven in size and hindered the escape of emerged adults. Softer, more flexible bags, made from a finer material with uniform weave and larger holes, warrant further investigation.

The emerged viable adults and the calculated overall proportion of flies capable of flight decreased with increasing pupal density. For the loose release method the results indicate little change in emerged viable adults until a tub loading in excess of 50000 pupae was reached. At higher pupal loadings parameters associated with the pupal bed (e.g. temperature, gaseous exchange) may come into play.

The results indicate that depending on pupal loading a bagged release system has potential. This trial suggests that expected emergence rates in excess of 80% are realistic with bagged releases. However, whether that translates into as flies capable of escaping from the tub, feeding and mating in the field has yet to be determined. This trial was an attempt to adapt an existing adult emergence method used in SIT worldwide, specifically for *B. tryoni* under Australian conditions. We conclude that optimal emergence will occur in bags containing about 30000 pupae within a PARC box.

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