CONTAINER LOADINGS AND ECLOSION UNITS FOR STERILE INSECT TECHNIQUE PROGRAMS OF *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE)

O.L. Reynolds[1] and B.A. Orchard[2]

[1]EH Graham Centre for Agricultural Innovation (Industry & Investment New South Wales and Charles Sturt University), Woodbridge Road, Menangle, NSW 2568 Australia
[2] Industry & Investment New South Wales, Private Mail Bag, Wagga Wagga, NSW 2650 Australia
Email: olivia.reynolds@industry.nsw.gov.au

Summary

Management of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) may be achieved through the sterile insect technique (SIT). Plastic adult rearing containers (PARCs) are commonly used to rear and release sterile fruit flies; however these containers have not been optimised for *B. tryoni*. A laboratory study compared whether six different PARC loadings of pupae (180 g, 200 g, 230 g, 250 g, 280 g and 300 g) affected emergence and flight of sterile *B. tryoni*. Eclosion units utilised to rear the pupae to adults in the PARCs were compared in a laboratory study of emergence and flight and in a follow-on field study with respect to trap recapture rates of *B. tryoni*. Eclosion units tested included a i) wire grid, ii) mesh bag and iii) paper bags, and were compared with iv) no eclosion unit (control). PARC box loadings and eclosion units tested did not adversely affect emergence or flight of sterile *B. tryoni*. Trap recapture rates were the highest for control followed by the mesh bag, wire grid and paper bags. The value of these findings for utilising PARCs to release sterile *B .tryoni* are discussed.

Keywords: Queensland fruit fly, plastic adult rearing container (PARC), flight, cue-lure, trap recapture

INTRODUCTION

The Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), is the most significant pest of edible fruit in Australia. In response, a fruit fly exclusion zone (FFEZ), which encompasses some of the most valuable horticultural production areas in New South Wales (NSW), South Australia, and Victoria, was established. The sterile insect technique (SIT) is one of several management approaches employed to manage *B. tryoni* in the FFEZ. Plastic adult rearing containers (PARCs) are used for rearing flies for ground release of sterile fruit fly both internationally (Salvato *et al.* 2003) and nationally (M. Nolan pers. comm. 2009) and for chilled adult release internationally (Shelly *et al.* 2006). However, in Australia the use of PARCs has not been optimised for rearing and release of adult *B. tryoni*.

In this study we tested whether PARC loadings of pupae and eclosion units (enclosure or covering utilised to rear the pupae to adult in the PARC) affected emergence and flight of sterile *B. tryoni*. We also tested whether eclosion units influenced trap recapture rates of sterile *B. tryoni*. We discuss the results in terms of the use of modified PARCs for the release of *B. tryoni* as part of a sterile insect release program.

MATERIALS AND METHODS

Study insects

B. tryoni were obtained as dyed pupae from the Fruit Fly Production Facility at the Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia. Eight-day old pupae were irradiated (target dose range of 70-75 Gy) at the Australian Institute of Nuclear Science and Engineering (AINSE) facility, Lucas Heights, to render them sterile before they were transported overnight by road to the Wagga Wagga Agricultural Institute (WWAI) entomology laboratory in NSW. Insects were reared out in a growth room at the WWAI at 26±2°C, 65±15% RH and a light:dark period of 14:10, with a simulated dawn and dusk as the lights ramped up and down at the beginning and end of the light phase. Pupae were dyed (1 g dye per 100 g pupae) as described by Reynolds *et al*. (2010), with one of four fluorescent pigments; Fiesta FEX 1 Arc chrome, Strong magenta, Flame orange or Stellar green (Swada, 30-32 Kilkenny Court, Dandenong South, Victoria, Australia) to differentiate between treatments. Studies have shown that there is no effect of the dye colours used in this trial on fruit fly performance (Reynolds unpub. data, Smallridge unpub. data), however the four dyes were rotated through each of the treatments on separate release dates in the trap recapture study.

Rearing protocol

According to treatment, for both trials pupae were placed in a translucent 46L PARC (Silverlock MH 0110, colour "natural", 645 mm x 413 mm x 275 mm high), with a 43 cm x 20 cm, 1 mm mesh on the lid and a 15 cm x 10 cm mesh on each length (side) of the container for ventilation. Resting space was provided by wedging cardboard dividers (approximately 160 cm in height), two running lengthways and five across the width of the container, to sit just above the pupal bed. Eighteen sugar cubes were placed on the base of each release container and a block of agar containing a mixture of sugar and water (5 g $100mL^{-1}$) (A. Jessup

unpub. data 2004) was placed on top of the mesh covered lid. For the pupal load trial (trial 1), the treatments were 180 g, 200 g, 230 g, 250 g, 280 g and 300 g; each replicated three times. For the eclosion unit trial (trial 2), the treatments were i) adults emerged under a wire grid (2 x 4 mm hole width,), ii) adults emerged in a mesh bag (2 x 2 mm hole width), iii) adults emerged in paper bags and iv) adults emerged with no eclosion unit (control); each replicated four times.

Emergence and flight – pupal load and eclosion unit

The effect of pupal load and eclosion unit on percentage eclosion and flight of adult sterile *B. tryoni* was determined in separate trials, but followed the same general method. After all emerged adult *B. tryoni* had been released (only for eclosion unit was trap recapture also recorded – see below), each PARC was taken back to the laboratory and sampled by mixing the remaining empty pupal cases, un-emerged pupae, partly emerged adults, deformed and non-deformed dead fruit flies ('*B. tryoni* debris') to ensure even distribution across the box as described in Reynolds *et al.* (2010). Briefly, each box was divided into eight equal segments, and 0.15 g of *B. tryoni* debris was then weighed from each segment, and the number of each component of the debris was recorded.

Emergence

The percentage emergence per release box was estimated based on the summed *B. tryoni* debris totalling 1.8 g for each release method. Emergence was defined as the percentage of *B. tryoni* which fully emerged, irrespective of whether they were deformed or flew, per release method.

Flight

Flight 'Fliers' was defined as the percentage of *B. tryoni* that eclosed per PARC and flew or left the box. The percentage of fliers was estimated based on the emergence samples described in the previous section, and calculated as ((total emergence - non-fliers)/total emergence) x 100. Non-fliers were defined as those *B. tryoni* that eclosed but never flew from the box, including both deformed and non-deformed adult fruit flies.

Trap recapture – eclosion unit

A trapping grid was established in the urban area of Wagga Wagga, NSW (35° 70' S, 147° 22' E), comprising 20 cue-lure and malathion baited Lynfield traps, spaced 400 m apart in a 5 x 4 grid. Four release sites, spaced 400 m apart (and 200 m from the nearest trap), were located centrally in the grid. At each release site four PARCs, each containing a different eclosion unit of sterile *B. tryoni* were released when the flies were two days of age. There were four release periods, release 1 (October 2008), 2 (December 2008), 3 (January 2009) and 4 (March 2009) and within each release period there were 6 consecutive weeks of recapture data collected, although some weeks were excluded from the analyses as flies were not trapped during those weeks.

Captured *B. tryoni* were collected, rinsed in 700 g L^{-1} alcohol and dried before being sent to the Orange Agricultural Institute, Orange, NSW, Australia where the dye colour observed in the ptilinal fissure (Norris 1957, Steiner 1965) was recorded for each fruit fly (see Reynolds *et al.* (2010) for method).

Analysis

ASREML-R release 2 (Butler *et al.* 2007) was used to fit linear mixed models to the logistic transformation (z) of percent emergence and percent fliers using the formulae given by McCullagh and Nelder (1989) and including weights (v^{-1}):

$$z = \ln\left(\frac{y + 0.5}{m - y + 0.5}\right)$$

where,

$$v = \mathrm{var}(z) = (y + 0.5)^{-1} + (m - y + 0.5)^{-1}$$

For percent emergence, y is the number of empty pupal cases and m is the total number of pupal cases while for percent fliers, y is the number of fliers and m is the number emerged. Treatments, pupal loading (trial 1) or [eclosion unit * release period] (trial 2) were fitted as fixed effects and replicate as a random effect (trial 1 and trial 2 (at each release period)).

Total fly count per trap was modelled using a generalised linear mixed model fitted in GenStat (2009) using the method of Schall (1991). Fixed effects included [eclosion unit * release period]. Random effects included trap, trap by treatment and trap on a release date. A logarithmic link function was used and an underlying Poisson distribution was assumed. In all models the significance of fixed effects was assessed according to Kenward and Roger (1997). The estimated Kenward and Roger (1997) denominator degrees of freedom are presented in the Results.

RESULTS and DISCUSSION

In both trials, all sterile *B. tryoni* adults had visible dye on their ptilinum (R. Kerslake pers. comm. 2009). In laboratory comparisons (trial 1) of PARC loadings, there was no significant effect of pupal load on emergence (F=0.65, df=5, 11.1, P=0.670) (Table 1) or flight (F=1.34, df=5, 7.3, P=0.349) (Table 1). Based on the results of this study, pupal loadings ranging from 180 - 300 g per PARC achieved emergence and flight levels well above those recommended for *B. tryoni* used in SIT programs (FAO/IAEA/USDA 2003). Our flight test is equivalent to the FAO/IAEA/USDA (2003) 'Rate of Fliers' calculation.

Table 1. The effect of plastic adult rearing container (PARC) pupal loadings on emergence and flight of *Bactrocera tryoni*.

Pupal load (g)	Mean emergence ± SE (logit)	Emergence (%) (back-transformed mean)	Mean flight ± SE (logit)	Flight (%) (back-transformed mean)
180	2.21 ± 0.13a	90.5	1.87 ± 0.12a	87.0
200	2.14 ± 0.13a	89.8	1.91 ± 0.12a	87.5
230	1.99 ± 0.12a	88.4	1.75 ± 0.11a	85.5
250	2.41 ± 0.16a	91.8	2.11 ± 0.12a	89.5
280	2.29 ± 0.15a	91.2	1.86 ± 0.12a	86.9
300	2.21 ±0.14a	90.5	1.92 ±0.12a	87.6

Means in each column followed by the same letter do not differ significantly from each other (P>0.05) using least significant differences.

Table 2. The effect of plastic adult rearing container (PARC) eclosion units on emergence and flight of *Bactrocera tryoni*.

Pupal eclosion unit	Mean emergence ± SE (logit)	Emergence (%) (back-transformed mean)	Mean flight ± SE (logit)	Flight (%) (back-transformed mean)
No eclosion unit (control)	1.74 ± 0.09a	85.1	1.90 ± 0.15a	86.9
Mesh bag	1.99 ± 0.08a	87.8	1.86 ± 0.15a	86.6
Wire grid	1.90 ± 0.08a	87.0	1.60 ± 0.16a	83.3
Paper bag	1.72 ± 0.09a	84.8	1.72 ± 0.17a	84.8

Means in each column followed by the same letter do not differ significantly from each other (P>0.05) using least significant differences.

Table 3. The effect of plastic adult rearing container (PARC) eclosion units on the trap recapture rate of *Bactrocera tryoni*.

Pupal eclosion unit	Mean trap recapture ± SE (log_e)	Proportion (%) trap recapture (back-transformed)
No eclosion unit (control)	1.26 ± 0.44a	29.5
Mesh bag	1.08 ± 0.44ab	24.6
Wire grid	0.97 ± 0.44b	23.8
Paper bag	1.04 ± 0.44b	22.2

Means in each column followed by the same letter do not differ significantly from each other (P>0.05) using least significant differences.

In a separate study (trial 2), there was no significant effect of eclosion unit (F=2.54, df=3, 37.3, P=0.071; Table 2) or fly batch (F=3.77, df=3, 6.8, P=0.067) on the emergence of sterile *B. tryoni*. Similarly, there was no significant effect of eclosion unit (F=1.64, df=3, 36.8, P=0.197; Table 2) on the number of sterile *B. tryoni* capable of flight. However, there was a significant effect of fly batch on release period (F=13.12, df=3, 24.5, P<0.001), with more fliers reared at release 2 (91.52%) than either release period 1 (86.92%), 3 (78.51%) or 4 (81.98%). Release period 1 had more fliers than release 3. As the flies were reared in controlled temperature and humidity growth rooms we are unable to account for the difference in fliers for the different batches of flies, particularly since there was no difference in emergence between the batches.

Table 4. The effect of release period on trap recapture rate of *Bactrocera tryoni*.

Release period	Mean trap recapture ± SE (log_e)	Proportion (%) trap recapture (back-transformed)
1 (September 2008)	0.37 ± 0.50a	10.6
2 (October 2008)	1.26 ± 0.50bc	28.5
3 (January 2009)	0.81 ± 0.50ab	16.5
4 (March 2009)	1.80 ± 0.50c	44.4

Means in each column followed by the same letter do not differ significantly from each other (P>0.05) using least significant differences.

Trap recapture rates from trial 2 revealed a significant effect of eclosion unit (F= 8.98, df=3, 2.99, P=0.035; Table 3) with higher recaptures rates evident for the control compared to the paper bag and wire grid; the mesh bag did not differ significantly from any of the eclosion units or the control. Therefore, under controlled conditions *B. tryoni* pupae do not require any eclosion unit to optimise trap recapture rates. Paper bags, often used in chilled adult release, recaptured the lowest number of flies, probably as not all flies capable of flight were observed to leave the bags. While this is still a suitable eclosion unit to use for chilled adult release, which requires ease of separation of the pupal debris from the adult flies, there is clearly potential to optimise this method. There was also a significant effect of release period on trap recapture rate (F= 15.06, df=3,5.02, P=0.011; Table 4) with lower recapture rates evident for release 1 compared with release period 2 and 4; release period 3 had lower recaptures than 4 but release 2 and 4 do not differ significantly. This is expected as release environment and climate are known to affect trap recapture rates of fruit flies (Weldon and Meats 2010) and varied for each release period (data not shown).

Collectively, these results indicate that PARCs are suitable as release containers, as described above, at any *B. tryoni* pupal loading in the range of 180 - 300 g, with no eclosion unit required. Paper bags, used commonly for chilled adult release, require improvement to maximise the number of adult *B. tryoni* that leave the bag.

ACKNOWLEDGMENTS

Thanks to Vincent van der Rijt and Michael Stout for providing technical assistance, Rosy Kerslake and Michelle Rossetto for identifying assorted pigmented sterile adult *B. tryoni* and Geoff Gurr for providing comments on the manuscript. This project was facilitated by Horticulture Australia Ltd. in partnership with industry.

REFERENCES

Butler, D., Cullis, B.R., Gilmour, A.R. and Gogel, B.J. (2007). Analysis of mixed models for S language environments. AS-Reml-R reference manual: Release 2.00, Training series QE02001. The State of Queensland, Department of Primary Industries and Fisheries.

Food and Agriculture Organisation of the United Nations (FAO), International Atomic Energy Agency (IAEA) & United States Department of Agriculture (USDA). (2003). *Product quality control and shipping procedures for sterile mass-reared tephritid fruit flies. Version 5.* IAEA, Vienna, Austria.

GenStat (2009). Eleventh Edition version 11.1.0.1575, VSN International Limited.

Kenward, M.G. and Roger, J.H. (1997). The precision of fixed effects estimates from restricted maximum likelihood. *Biometrics* **53**: 983-997.

McCullagh, P. and Nelder, J.A. (1989). Binary data. In: Cox, D.R, Hinkley, D.V., Rubin, D., and Silverman, B.W. (Eds.) Generalized Linear Models: second edition, chapter 4 (pp.98-135). Chapman and Hall, London.

Norris, K.R. (1957). A method of marking Calliphoridae (Diptera) during emergence from the puparium. *Nature* **180**: 1002.

Reynolds, O.L., Dominiak, B.C. and Orchard, B.A. (2010). Pupal release of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) in the sterile insect technique: seasonal variation in eclosion and flight. *Australian Journal of Entomology* **49**: 150-159.

Salvato, M., Hart, G., Holler, T. and Roland, T. (2003). Release of sterile Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae), using an automated ground release vehicle. *Biocontrol Science and Technology* **13**: 111-117.

Shelly, T.E., Holler, T.C. and Stewart, J.L. (2006). Mating competitiveness of mass-reared males of the Mediterranean fruit fly (Diptera: Tephritidae) from eclosion towers. *Florida Entomologist* **89**: 380-387.

Steiner, L.F. (1965). A rapid method for identifying dye-marked fruit flies. *Journal of Economic Entomology* **58**: 374-375.

Schall, R. (2001). Estimation in generalized linear models with random effects. *Biometrika* **78**: 719-727.

Weldon, C. and Meats, A. (2010). Dispersal of mass-reared sterile, laboratory-domesticated and wild male Queensland fruit flies. *Journal of Applied Entomology* **134**: 16-25.