THE EFFECT OF PUPAL SIZE ON THE ECLOSION OF QUEENSLAND FRUIT FLY *BACTROCERA TRYONI.*

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Summary

Mass rearing facilities are used to produce many different species of fruit fly around the world for use in the Sterile Insect Technique. Facilities use standard tests to assess the quality of the sterile flies produced and to optimise the quality of flies distributed for release. A pupal sorter was used to assess the relationship between pupal weight and eclosion of the Queensland fruit fly, *Bactrocera tryoni* (Froggatt) in four separate trials. Eclosion was significantly related to pupal size and to the day of larval hopping. The new genetic stock had significantly greater eclosion than the existing genetic colony. Sorting the pupae twice did not result in significantly different weights in each collection chute. Percentage eclosion ranged from 66.56% to 94.75% during the four trials.

Key words: insect mass production, insect quality parameters, pupal sorter,

INTRODUCTION

The Sterile Insect Technique (SIT), in which sterile males are released to disrupt reproduction of wild pest populations, was originally postulated by Knipling (1955). SIT is now deployed for numerous pest insects, including tephritid flies, tsetse flies, mosquitoes, and moths (Anon. 2009). There are currently many facilities around the world producing large quantities of many different insect species annually (Krafsur 1998). Most mass rearing facilities conduct standard quality control tests to monitor the product quality supplied to field release programs. Boller *et al.* (1981) published the first standard quality control tests to assess the quality of mass reared Mediterranean fruit fly (*Ceratitis capitata* Wiedemann). With various revisions for other species, these tests have largely been followed by mass rearing facilities throughout the world (FAO/IAEA/UDSA 1999, 2003).

High rates of eclosion are one of the desired outcomes from most production facilities. In house flies *Musca domestica* L, eclosion rates have been shown to rise as pupal size increases (Moreland and McLeod 1957). Krainacker *et al.* (1989) claimed a similar relationship between size and eclosion for *C. capitata* and *Dacus dorsalis* (Hendel). Vera *et al.* (2007) concluded that increasing pupal weights of *Anastrepha fraterculus* (Weidemann) over several years resulted in increasing rates of eclosion. Similar results have also been reported for Queensland fruit fly (Qfly) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). Using a pupal sorter, Fisher (1992) concluded that the mean pupal diameter provided more information about pupal size than pupal volume. Eclosion of small pupae was lower than for larger pupae.

Qfly is a significant threat to Australia's horticultural production (Yonow and Sutherst 1998). The Fruit Fly Exclusion Zone (FFEZ) has been established in the drier irrigated production areas of Victoria, South Australia and New South Wales (NSW) to regionally combat the fruit fly threat. Controls of commercial produce and the travelling public, along with public awareness campaigns, greatly reduce the number of incursions into the FFEZ (Dominiak and Coombes 2009). There are occasional incursions into the FFEZ and these populations are controlled by early detection, chemical measures, SIT and fruit destruction. SIT has been used for Qfly management since the 1960's in NSW (Monro and Osborne 1967; Andrewartha *et al.* 1967) with applications increasing during the early 1990's. There has been increased use of SIT for Qfly control (Horwood and Keenan 1994; Dominiak *et al.* 2003, 2011; Meats *et al.* 2003; Reynolds *et al.* 2010; Worsley *et al.* 2008). Many of the release methods developed in Australian are unique because of sub-optimal funding and long distance transport from the production facility to the release sites. Releases usually are in urban environments surrounded by dry rural environments (Dominiak *et al.* 2006).

The Elizabeth Macarthur Agricultural Institute (EMAI) mass rearing facility for Qfly is located at Camden (Sydney suburb), NSW, Australia and has been operating since 1996. While eclosion and pupal weight at EMAI has been measured weekly (Dominiak *et al.* 2008), the effects of pupal size on eclosion were not specifically tested. In this paper, we present results from four trials that utilised a pupal sorter to establish the relationship between pupal weight and adult eclosion.

MATERIALS AND METHODS

Study Insects

Qfly pupae were produced at the EMAI mass rearing facility located at Camden, NSW. Trials were conducted in the EMAI laboratories maintained at 25°C (± 1°C), 65% (± 5%) RH and a light:dark period of 14:10 with a 1h simulated dawn and dusk as the lights ramped up and down at the start and end of the light phase. The rooms also have a skylight (Deece *et al.* 2000). Full production details were described by Dominiak *et al.* (2008). Pupae were collected daily for three hopping days, starting from when the first larvae started to "hop" from the growing media. The first three hopping days represent the majority of pupae. Each day's collection was treated as a separate unit. All trials used pupae produced from various weekly production batches between 23 June 2009 and 21 April 2010.

Pupal sorter design

A pupal sorting machine was constructed based on data from Worley (*pers. comm*. 2007) and FAO/IAEA/USDA (2003). The machine consists of a variable speed electric motor driving a gearbox that turns two rollers by large rubber "o" ring belts (Fig. 1). Rollers are polished stainless steel cylinders, 912 mm long and 30 mm in diameter. The rollers diverge and rotate in opposite directions. The machine is on a 5º incline with 65mm height difference between upper and lower end of rollers. A hopper feeds pupae onto the rollers at the upper end (Fig. 2). The hopper was designed to allow a small number of pupae to pass onto the rotating rollers and pupae slowly move down slope away from the hopper. Pupae continued to form a blockage at the bottom of the hopper preventing the flow of pupae. A manual method was devised to feed pupae onto the rollers. Groups of approximately 10 pupae were placed on the rollers manually. Pupae quickly aligned their longitudinal axis with the roller axis. The movement of smooth rotating rollers and incline cause the pupae to slowly slide down the rollers until the pupal diameter is narrower than the gap between the rollers. Collection chutes are located below and along the length of the rollers to capture pupae of varying size. Pupae fall onto one of 15 collection chutes. After some initial experimentation, pairs of chutes were combined so that pupae fell into one of seven collection points and then into a collection container. The distance along the roller for each collection point is 109 mm. The 8th chute captured all material that did not fall through the gaps and this included pupae stuck together and debris; collections from this chute were not assessed. Despite the manual pupae feed, some pupae rode on top of others for a short distance. A separate trial was conducted to test whether the machine was consistently sorting the pupae according to size (Trial 4).

Figure 1. Dorsal view of the rubber drive mechanism and rollers

Figure 2. Lateral view of the sorter showing hopper and collection chutes.

Trials

Four trials were conducted. For Trial 1, the sorting machine was set with a roller gap of 1.900 mm and 2.032 mm apart at the narrowest (hopper end) and widest ends respectively: this is calculated as an angle of 0.0099°. For trials 2, 3 and 4, the machine was set with a roller gap of 1.715 mm and 2.197 mm apart at the narrowest and widest ends respectively: this is calculated as an angle of 0.0361° between the rollers. Each weekly batch was treated as a replicate. For Trial 1, evaluations were conducted for five weeks between 23 June 2009 and 30 July 2009. In Trial 2, evaluations were conducted for five weeks from 4 August 2009 and 31 December 2009. Trial 3 was conducted in the last two weeks of Trial 2 to compare the existing production colony (used in all the other trials) to the new genetic mother colony being developed. Trial 4 used the same initial methodology as Trial 2 to evaluate if the second sorting altered the total weight in each collection point. Evaluations were conducted for six weeks between 1 March 2010 and 21 April 2010. After each batch had been sorted, the pupae from each collection point was poured back into the sorter (one point at a time) for a second sorting. After the second sorting of all collection points was completed, the pupae were evaluated as in the other trials.

Pupal weight and eclosion

Assessments were conducted on pupal weight and eclosion. Weights for the total sample and for each collection point were measured in grams. To estimate pupal weight, one hundred randomly selected pupae from each collection point were weighed to estimate average pupal weight. Eclosion rates of each of the separated samples were assessed by sealing three replicates of 100 randomly selected pupae inside plastic lidded petri dishes, secured with two rubber bands. Flies were allowed to emerge and die at the EMAI laboratory and then petri dishes sent to Orange Agricultural Institute for eclosion assessment. Full eclosion was indicated by the adults completely leaving the pupal case. Percentage eclosion was calculated by dividing the number of fully eclosed adults by the total number of pupae in the sample.

Statistical analysis

For all trials, the proportion fully eclosing for weight of pupae and hopping day was fitted using a generalised linear model with a binomial distribution and a logit link function. For Trial 1, 2 and 4, the model was:

Percentage Full Eclosion = f(weight of 100 pupae * week * hopping day and their interactions)

For Trial 3, the model was:

Percentage Full Eclosion = f(weight of 100 pupae * week * hopping day* colony and their interactions).

Non significant terms were dropped from the final model.

The weights ranged from 0.12 to 1.36 grams. For trials 1, 2 and 3, the results are presented in five equally spaced weights within this weight range. Only the relationship of pupal weight to collection point was tested using linear regression. In Trial 4, the weight range was 0.19 to 1.89 g and the results are presented in seven equally spaced weights. The weight in each collection point before and after the second sorting was compared using a t-test. All analyses were conducted using GenStat software (Payne *et al.* 2010).

RESULTS

Collection points were used to obtain a range of weights. The weights varied within the collection points from week to week and five or seven weight ranges were used to provide ecolsion percentages. A trend of increasing pupal weight and increasing eclosion was observed in all of the trials conducted. In trial 1, pupal eclosion was significantly related to the main effects of weight of 100 pupae, week and hopping day ($\chi^2_{7,\ 307}$=25.66, P=<0.001). No interaction terms were significant. Percentage pupal eclosion was skewed (mean 89.43%: range 44% to 100%). Table 1 shows the predicted eclosion percentage for each of three larval hopping days. The weight per 100 pupae was significantly related to collection point: Weight=0.905+0.03(±0.001) collection point (R^2=73.0%). In trial 2, all main and interaction terms were significant ($\chi^2_{19,\ 284}$=81.08, P=<0.001). Table 1 shows the predicted eclosion percentage (mean 69.93%: range 4% to 97%) for weights from 0.8 g/100 to 1.2 g/100 for each of three hopping days. In trial 3, all main and interaction terms were significant ($\chi^2_{23,\ 228}$=81.82, P=<0.001). Table 2 shows the predicted eclosion percentage (mean 74.60%: range 9% to 97%) for weights from 0.8 g/100 to 1.2 g/100 for the existing and new genetic colony. In trial 3, there was no significant difference (P=0.87) in weight of pupae in each collection chute before and after the second sorting. In trial 4, all main effects and two way interactions were significant ($\chi^2_{31,\ 304}$=84.51, P=<0.001). Table 1 shows the predicted eclosion percentage for weights from 0.8 g/100 to 1.4 g/100 for each of three hopping days.

Table 1. The percent eclosion for different pupal weights of Queensland fruit flies over three hopping days (standard error in brackets).

Pupal weight (g) for 100 pupae	Hopping days		
	Day 1	Day 2	Day 3
Trial 1			
0.8	86.85 (0.85)	84.00 (0.95)	81.06 (0.11)
0.9	89.46 (0.48)	87.10 (0.52)	84.62 (0.62)
1.0	91.60 (0.28)	89.67 (0.30)	87.61 (0.34)
1.1	93.34 (0.27)	91.77 (0.32)	90.09 (0.34)
1.2	94.75 (0.33)	93.48 (0.39)	92.12 (0.44)
Trial 2			
0.8	77.65 (0.65)	72.07 (0.80)	70.63 (0.79)
0.9	79.33 (0.48)	73.33 (0.60)	71.82 (0.60)
1.0	80.85 (0 .40)	74.50 (0.47)	72.96 (0.48)
1.1	82.24 (0.39)	75.57 (0.45)	74.03 (0.47)
1.2	83.49 (0.45)	76.54 (0.53)	75.05 (0.54)
Trial 4			
0.8	86.92 (0.73)	80.71 (0.91)	66.58 (1.68)
0.9	88.24 (0.47)	82.24 (0.62)	67.53 (1.22)
1.0	89.37 (0.32)	83.65 (0.41)	68.41 (0.87)
1.1	90.31 (0.30)	84.95 (0.34)	69.21 (0.81)
1.2	91.09 (0.34)	86.14 (0.41)	69.94 (1.11)
1.3	91.73 (0.40)	87.23 (0.54)	70.60 (1.58)
1.4	92.25 (0.46)	88.22 (0.66)	71.18 (2.11)

Table 2. The percent eclosion for different pupal weights of Queensland fruit flies from existing and new genetic stock (standard error in brackets).

Pupal weight for 100 pupae (Trial 3)	Stock	
	New stock	Existing stock
0.8	75.52 (1.19)	70.50 (0.55)
0.9	76.44 (0.87)	70.72 (0.49)
1.0	77.33 (0.59)	70.93 (0.45)
1.1	78.20 (0.40)	71.15 (0.43)
1.2	79.04 (0.41)	71.36 (0.45)

DISCUSSION

This study confirms previous studies that demonstrated that heavier pupal weights are positively linked to increasing eclosion (Dominiak *et al.* 2002, 2007a). However, at higher average pupal weights, size may be less important. Whereas these two studies examined eclosion from pupae weighing 9.2 and 9.4mg respectively, a later study of pupae weighing 10.0-12.3 mg did not find this effect (Dominiak *et al.* 2007b). Furthermore, studies of the effects of marker dye (Dominiak *et al.* 2010) and irradiation (Dominiak *et al.* 2008) also failed to find a significant effect of pupal size on eclosion.

Adult *B. tryoni* eclosion was highest on the first larval hopping day and declined on each subsequent day. Cambell *et al.* (2009) and Dominiak *et al.* (2007b) both claimed that larval hopping day had no significant effect on eclosion when tested for the first two and three hopping days respectively. However, there was a highly significant relationship between eclosion and pupal weight during the fourth to sixth hopping days (Dominiak *et al.* 2007b).

In Test 1, using a narrow angle between the rollers, the interaction terms were not significant. For the other trials, the angle between the rollers was increased and all interaction terms were significant. We infer that increasing the angle between the rollers decreases the discrimination. At EMAI, the genetic stock is fully replaced approximately every two years. Trial 3 demonstrated that the new genetic colony had between 5 to 8% greater eclosion compared with the existing production colony. Sorting the pupae for a second time did not significantly alter the weight of pupae in collection points. We conclude that sorting pupae once is adequate.

The sorter may have merit in removing the smaller pupae from consignments destined for field releases. However, the throughput of the current sorting machine was small and if sorting pupae was desirable, a larger device would have to be manufactured to handle the large production volumes. The operational need for a sorter is likely to be negated by altering facility processes (decreasing the number of eggs deposited in each larval tray) to ensure larger larvae and hence larger pupae are produced. Alternatively the pupal sorter could be used as a quick check on a pupal sample without having to wait for eclosion testing to be completed. Larval competition is regarded as a stress that produces small adults with smaller energy resources for metamorphous (Zolubas and Byers 1995). Any residual energy reserves are then used for eclosion and survival; small individuals were unlikely to survive to find food. Moraiti *et al.* (2012) found that larger body size was associated with prolonged dormancy for up to one year in *Rhagoletis cerasi*. Longer dormancy was considered to be an adaptive advantage under irregular seasons and unpredictable resource availability during climate change. The results of this and previous trials support the principle that, for Qfly pupae, "bigger is better" (Dominiak *et al.* 2002, 2007a, 2008).

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