PUPAL WEIGHT AS A KEY INDICATOR FOR QUALITY OF MASS PRODUCED ADULT QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE) IN 1997/1998

B.C. Dominiak[1], S. Sundaralingam[2], A.J. Jessup[3] and I.M. Barchia[2]

[1]NSW Agriculture, Locked Bag 21, Orange NSW, Australia 2800
[2]Elizabeth Macarthur Agricultural Institute, NSW Agriculture, PMB 8, Camden NSW, Australia 2570
[3] Horticultural Research and Advisory Station, NSW Agriculture, Locked Bag 26, Gosford NSW, Australia 2250
Email: bernie.dominiak@agric.nsw.gov.au

Summary

Quality parameters at the Camden sterile fruit fly production facility during the 1997/98 season were examined, particularly in their relationship with pupal weight. Percent emergence was positively related to pupal weight while lifespan was negatively related. There was no relationship between flight ability and pupal weight. The Flight Ability Index was adversely affected by irradiation. These results were compared with field reports and overseas information, which largely agreed with the facility data. The possible impact on sterile release programs is discussed.

Keywords: insect mass production, insect quality parameters, *Bactrocera*

INTRODUCTION

The Sterile Insect Technique (SIT) has been used in south-eastern Australia considerably in the last decade against Queensland fruit fly (QFF) *Bactrocera tryoni* (Froggatt). In 1995/96, a new fruit fly mass rearing facility was built at the Elizabeth Macarthur Agricultural Institute (EMAI) at Camden and began production in November 1996. Terras *et al.* (1997) reported the first year of operation and related facility processes in 1996/97.

The use of sterile insect release is a pesticide free method of fruit fly control. Wild fruit flies mate when males gather in a lek or mating site to attract females (Horng and Plant 1993). The sterile insect technique relies on sufficient sterile flies being released such that sterile males force wild males out of the lek site. The sterile males must then be able to successfully attract and mate with wild females. Such mating produces no viable offspring and the life cycle of wild flies is broken. The sterile males must have all the qualities of wild males except fertility. They must live long enough to become sexually mature, be able to fly to lek sites, be large enough to dislodge wild males and to attract and mate with females. Females usually only mate once; having mated with a sterile male, the female is unlikely to mate with a fertile wild male and therefore the first mating is critical to success. Much of the competitiveness of sterile flies is likely to depend on the quality of pupae supplied from the production facility into the field.

The Camden facility is subject to the usual fluctuations expected of rearing biological material such as; facility environmental control, damage due to manual handling, visual assessment, and reliable mechanical operation of equipment. In this paper, we report on quality parameters of the production during Camden in 1997/98 and examine these parameters' relationship with pupal weight.

MATERIALS AND METHODS

QFF production schedules are governed by demands from field programs. Insects produced were sampled and assessed from different stages in the production cycle. Due to the large volume of batch data (up to 52 weeks) and for ease of presentation, this information is summarised as monthly averages, however, analysis was conducted on weekly sample data. All the following parameters were measured in the quality control laboratory within the facility.

Pupal weight

Composite samples from the outgoing batches were taken for pupal weight measurement every week after irradiation. Three sub-samples of 100 pupae were counted out and weighed. Pupal weights for fertile (non irradiated) pupae are included for comparison in the observations (Table 1).

Emergence

Three sub-samples of 100 pupae were counted out into plastic petri dishes from irradiated and non-irradiated flies stocks. No food or water was provided. Once all the flies emerged and died they were counted and assessed as fully emerged, partly emerged or deformed flies. The remaining pupae that failed to emerge were classified as unemerged pupae. Failure to emerge include partly emerged, unemerged and deformed flies. Percentage emergence was the count of the fully emerged flies divided by the total

number of pupae in the sample expressed as a percentage. These figures were recorded for each batch and summarised into monthly average emergence figures in Table 2.

Flight ability and flight ability index

A minimum of two replicates of 100 pupae was tested for adult flight ability from irradiated and non-irradiated flies stocks respectively. The pupae were placed in a specially prepared 'flight ability container' which had been powdered lightly on the inside surface to prevent 'walkers' from escaping. Food, water and light source were placed at the furthermost point from emergence in the netting cage to encourage flight. Once emergence had ceased, the non-emerged pupae and flies inside of the flight ability cylinder that did not fly out of the flight ability container were counted to calculate the percentage fliers. The percentage fliers or the flight ability was calculated by dividing the number of flies that flew out of the chamber by the total number of emerged flies and expressed as a percentage. This test was performed for sterile and for wild flies.

The 'flight ability index' was calculated by dividing the percentage fliers of sterile flies by the percentage fliers of fertile flies. This is a measure of the flight ability of sterile flies relative to the wild or 'normal' flies. Given the possible adverse effects of irradiation, this index is usually below 100%. Results are summarised in Table 3.

Lifespan assessment

Lifespan 50 (LS50) is defined as the number of days until 50% of flies had died. Tests were performed each week on flies under controlled environment conditions to ensure that irradiated flies maintain a satisfactory lifespan (LS50) of at least 45 days. Two replicates of 100 flies (more than 12–24 hrs old) were collected and kept in a wire framed netting cage with irradiated and non irradiated flies stocks kept separately. Food and water were available *ad lib* and were replenished on a regular basis. The number of live flies was determined on a daily basis until 50% of the flies had died (LS 50). Figures for each week are summarised as monthly averages in Table 4.

Australian field data results for average emergence, average lifespan and flight ability are compared in Table 5. Results from the production facility are compared with these field results and with overseas data in the discussion.

Statistical analysis

The comparison between sterile and fertile groups in emergence and flight ability was tested using a generalized linear model with binomial errors on weekly data (McCullagh and Nelder 1989). A logit (log odd) function was used to link the fly type parameters with the observed data. The same method of analysis was used to relate pupal weight to emergence or flight ability. LS50 data were analysed using an analysis of variance to compare the types (sterile and wild) of flies (Table 6, Table 7).

RESULTS

Pupal weight

The average pupal weight was above 8 mg, except in April 98 (Table 1). Previous production from the Gosford facility has shown that 8 mg is the minimum acceptable weight with 10 mg considered as an ideal weight. There were no significant differences between sterilised and fertile pupal weights (($F_{1,9}$ = 0.55; P>0.05), Table 6).

Emergence

The emergence of sterile pupae ranged from 60-82% (Table 2, Figure 1). The emergence of the fertile type is significantly higher than that of the sterile types (P<0.01; Table 6). Pupal weight had a positive effect on emergence, with a significant rate of increase for the sterile type (P<0.05: Table 7). There was no interaction between pupal weight and fly types (P>0.05) indicating that the rate of increase in emergence by the sterile type is not significantly different from that of wild flies.

Flight ability and flight ability index

Flight ability was not related to pupal weight (P>0.05; Table 7, Figure 2) but there was difference between sterile and wild flies (P<0.01; Table 6) with sterile flies getting 89.2% compared with 91.9% for wild flies. The flight ability index (ratio of percent flight ability for sterile flies to that of the fertile type) was 97.04 (SE=0.78). This indicates the preparation and irradiation processes do reduce the ability of the insect to fly. There was no interaction between pupal weight and fly types (P>0.05) indicating that pupal weight was not a good predictor for flight ability in either group.

Lifespan

Lifespan reached by half of the sample (LS50) was negatively related to pupal weight (P<0.01; Table 7, Figure 3). The negative slope (-4.75) for the sterile type suggests the fly's LS50 decreases as pupal weight increases. This implies that we need to determine an optimum pupal weight in order to

Table 1. Average monthly pupal weights (mg) of irradiated (sterile) and non-irradiated (fertile) pupae (1997/98).

Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Fertile	9.0	9.7	9.9	10.1	9.6	9.9	9.5	8.5	7.2	8.8
Sterile	9.2	9.6	10.1	10.1	9.9	9.9	9.6	8.3	7.0	8.9

Table 2. Monthly percentage adult emergence for irradiated (sterile) and non-irradiated (fertile) flies (1997/98).

Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Fertile	71.2	79.1	77.0	82.2	77.9	81.0	85.3	77.3	80.3	73.4
Sterile	59.3	64.1	81.7	76.5	72.9	67.3	76.8	71.8	69.2	67.4

Table 3. Average monthly flight ability indices for fertile and sterile adults (1997/98).

Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Fertile	89.7	90.3	93.2	95.3	92.3	91.4	90.7	92.5	91.5	91.4
Sterile	75.8	83.5	91.9	90.3	91.2	88.1	91.5	90.2	91.7	88.6
Flight Ability Index	84.1	92.7	98.6	95.6	97.9	96.8	101.0	97.6	100.7	97.0

Table 4. Average monthly lifespan (LS 50) for irradiated and non-irradiated flies (1997/98).

Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
Fertile	82	71	56	52	43	38	47	65	59
Sterile	61	49	43	48	31	40	49	56	58

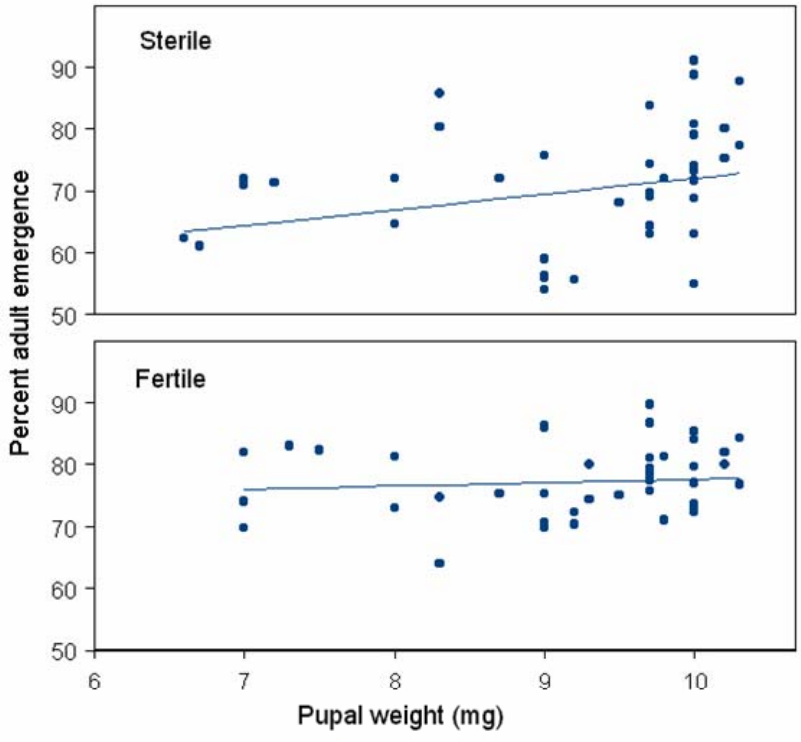

Figure 1. Adult emergence plotted against pupal weight for sterile and wild flies.

Table 5. Comparison of average (range in brackets) pupal weight, emergence, lifespan and flight ability from earlier release programs of irradiated QFF.

Average pupal weight (mg)	Average emergence (%)	Average lifespan (days)	Average flight ability (%)	Reference
9.66 (7.19 – 11.92)	67 (64.3 – 74.0)	30.81	87.2	Perepelicia *et al.* (1994)
10 (8.5 – 11.23)	62.17 (36.2 – 81.0)	39.2	91.87	Reynolds *et al.* (1995)
10 (8.55 – 11.8)	72.25 (67.7 – 75.3)	47.25	87.23	Jackman *et al.* (1996)
10 (8.8 – 10.2)	70 (46 – 83)	41	87.4	Perepelicia *et al.* (1997)

Table 6. Fly type means and F-test for emergence, flight ability, flight index, longevity pupal weight and residual fertility. Significance is denoted by "*" and "" for 5% and 1% significance level respectively. NS means no significant difference at 5% level.**

Fly types	Emergence		Flight ability		Flight index	Longevity	Pupal weight
	Logit	%	Logit	%	%	Days	mg
Fertile	1.26±0.07	77.93	2.43±0.08	91.9	-	55.84±0.12	9.20±0.16
Sterile	0.90±0.06	71.03	2.11±0.07	89.2	97.04±0.78	47.50±2.04	9.20±0.16
F-value	20.12**		15.20**			10.32**	0.55 NS

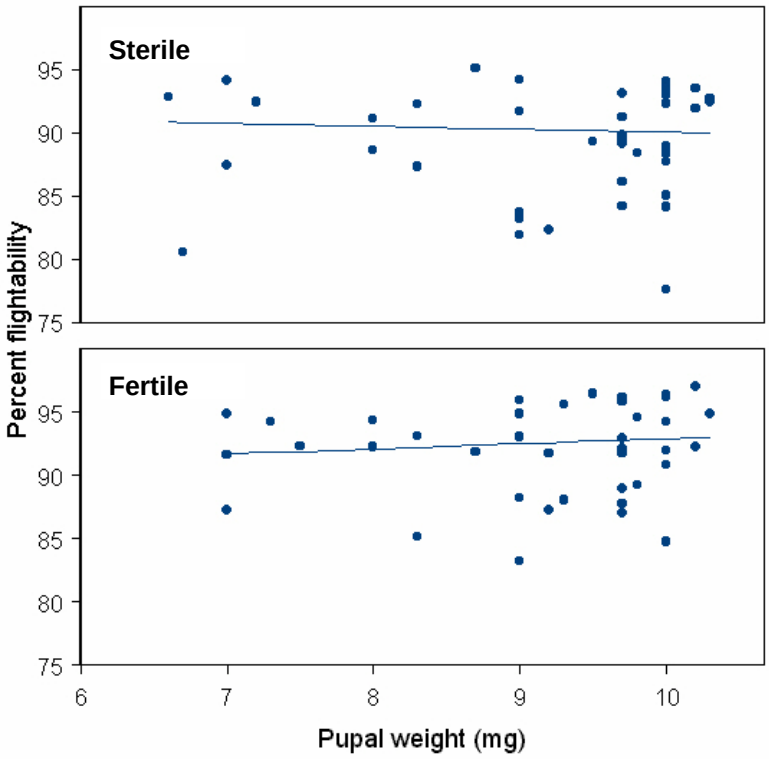

Figure 2. Flight ability of wild and sterile flies plotted against pupal weight.

types were significantly different (P<0.01) with wild flies having average median lifespan of 56 days compared with 48 days for the sterile type (Table 6). Table 4 shows that LS50 of both sterile and fertile adults appeared to be related to season: LS50 decreased during summer though LS50 in most cases remained above 40 days. No interaction was found between pupal weight and fly types (P>0.05) indicating that the wild and sterile flies were equally influenced by pupal weight at similar rate (Table 7).

Table 7. Regression coefficients and F-values given by GLM and ANOVA. Significance is denoted by "*"and ""for 5% and 1% significance level respectively. NS means not significant at 5% level.**

Variables	Fly types	Regression		F-values from analysis of Deviance		
		Intercept	Slope	Pupal weight	Fly types	Interaction
Emergence	Fertile	0.72±0.64	0.06±0.07	4.13*	15.78**	0.35NS
	Sterile	-0.14±0.53	0.11±0.06*			
Flight ability	Fertile	2.26±0.75	0.02±0.08	0.07NS	9.76	0.01NS
	Sterile	1.98±0.60	0.02±0.06			
Longevity	Fertile	109.5±19.3	-5.80±2.08**	14.36**	8.60**	0.15NS
	Sterile	91.7±16.3	-4.75±1.76**			

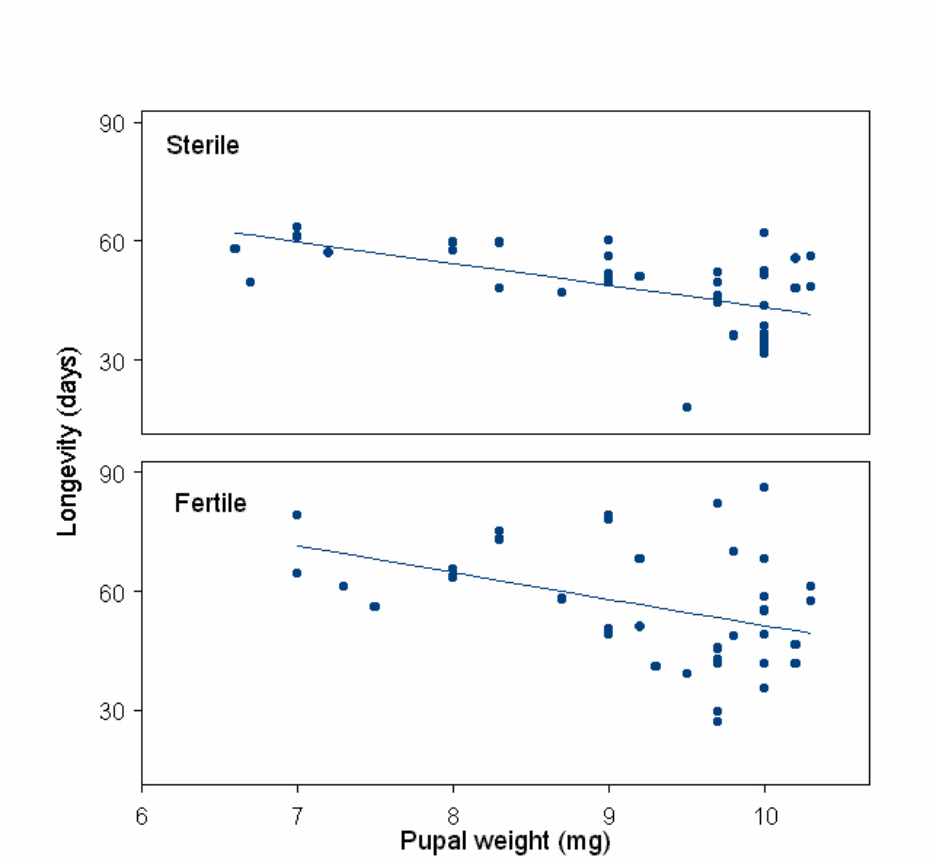

Figure 3. Lifespan (longevity) of wild and sterile flies plotted against pupal weight.

DISCUSSION

Pupal weight

Higher pupal weights were correlated to increased adult emergence; there would appear to be value in maintaining higher pupal weights. However high pupal weights were negatively related to lifespan and short lifespan is a disadvantage in SIT release programs. There needs to be a balance between a fly which is large enough to hold territory in a lek site but last long enough to compete for a mating. However, patterns of short lived adults in summer may be a natural pattern and if so, facility managers need to be aware of these patterns and not attempt to compensate for perceived changes in quality of produced pupae.

The Gosford fruit fly laboratory has found that the average wild QFF has an average pupal weight 10.5mg with a range of 9-13mg. Observations indicate that as pupal weight changes so do many measured performance parameters, some positively and some negatively.

In South Australia, Reynolds *et al.* (1995) noted that 52.78% of delivered pupae were above 10 mg, compared with 38.15% in 1994 (Perepelicia *et al.* 1994) and only 18% in 1993 (Perepelicia *et al.* 1993). This subsequently increased to 66.1% (Jackman *et al.* 1996).

Emergence

There was a significant relationship between pupal weight and emergence of both non-irradiated and irradiated pupae. Irradiation results in a slightly lower but not significantly different emergence. There was no interaction between sterile and wild flies indicating that emergence of both were equally influenced by pupal weight. There is no need to increase pupal weight to counter the effects of irradiation. However in a sterile release program, higher emergence results in more sterile flies in the field to minimise the chances of a fertile mating.

These comments are largely supported by field reports from South Australia. Irradiation caused a slight decrease of average emergence (Perepelicia *et al.* 1994). Reynolds *et al.* (1995) noted the average emergence for non-irradiated pupae was 71.53% compared with 62.17% for irradiated pupae. This increased to 80% for non-irradiated pupae and 72.25% for irradiated pupae (Jackman *et al.* 1996) and to 83% and 70% (Perepelicia *et al.* 1997). The overall emergence appears to increase as the average weight increased (Table 5).

Flight ability and flight ability index

As SIT adult flies have to fly to lek sites to compete with the wild flies, adult flight ability is also an important parameter in determining the field performance of flies produced. The higher the percentage of fliers, the greater the potential of emerged sterile flies to be effective in the field. However flight ability appeared to be unrelated to the major events in the factory or irradiation processes. There was no interaction between lines indicating that the influence of changes in pupal weight of wild and sterile flies were equal with respect to flight ability.

These findings appear to be supported by the results of Australian field reports. In previous programs, flight ability was little different (Perepelicia *et al.* 1994) for irradiated (87.17%) and non-irradiated (90.78%). Reynolds *et al.* (1995) reported a smaller difference of 91.87% and 92.73% respectively, compared with 87.24% and 94.78% (Jackman *et al.* 1996). The difference declined to 87.4% and 88.55 respectively (Perepelicia *et al.* 1997).

Radiation appears to have no effect on flight ability, which is different to the other measured parameters. There was also very little yearly fluctuation in this characteristic, again which is quite different to other characteristics. These field indications agree with the findings for the production facility reported above.

However the flight ability index (a similar but different way of calculating flight ability), which is a further calculation on the original flight ability measurements, was significantly different. Flight ability is based on the number of fliers per 100 pupae while the flight ability index is based on the number of sterile fliers per normally-eclosed adults. The significant differences between the fertile and sterile flies indicate that irradiation does exert an influence, presumably by lowering pupal weight after irradiation (Table 3).

Lifespan

Lifespan is critical for the success of a SIT program, as sterile flies have to survive long enough to mature sexually and mate with wild flies. The lifespan of sterile flies was shortest in December and January (Table 4) when pupal weights were the highest. This may be a natural pattern or a result of the summer heat and humidity and its affect on the facility air-conditioning.

If lifespan is linked to pupal weights, it may be possible to monitor lifespan by monitoring pupal

weight with regular monthly checks. The amount of time taken to monitor pupal weight is considerably less than the time taken to monitor lifespan and there would be considerable savings in the procedure.

There appears to be some support with observations in the field. Tests need to be conducted to check for factors that may affect lifespan and whether they are related to the biology of the insect or to production facility processes.

Previous field observations do not clearly support our findings regarding decreased lifespan associated with increased weight. The steady increase in pupal weight (Table 5) is linked generally with increasing lifespan, except in the last year (Perepelicia *et al.* 1997). There could be several explanations for this observation. In the assessment of lifespan at the facility, the amount of travel is considerably less than with delivery to Adelaide and this may affect the results from those reports. There is no attempt to incorporate the weather (i.e. variation between seasons) during these releases or seasonal variation (summer releases compared with autumn release programs). This area requires further research to clarify the relationship between lifespan and pupal weight in the field.

Australian field observations suggest that lifespan is reduced following irradiation. Irradiation was reported to cause a reduction in lifespan from 36.14 days (non-irradiated) to 30.81 days (irradiated) (Perepelicia *et al.* 1994). This improved to 55.6 days and 39.3 days respectively (Reynolds *et al.* 1995) however the difference between the two groups increased. This difference increased again to 74.5 days and 47.25 days respectively (Jackman *et al.* 1996) however the overall performance increased for both groups, but decreased to 56 days and 41 days respectively (Perepelicia *et al.* 1997).

In summary, pupal weight appears to be the most critical parameter to measure as it appears to be positively linked to adult emergence and inversely linked to lifespan. Monitoring pupal weight on a batch basis should continue however more time consuming measurements of other parameters could be reduced to monthly operations.

The issue of "bigger is better" is supported by several Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) programs in which size rather than weight is measured. Orozco and Lopez (1993) noted that body size was an important factor in the success of sterile males, since larger males achieved almost half of all matings. They felt that sterile males should be larger than wild males. Even within the laboratory culture, larger males were more competitive than smaller males.

Male size during initial matings appeared to have a significant effect on the length of time females are refractive to subsequent matings with larger males producing a longer refractive period than smaller males (Bloem *et al.* 1993). They also reported that larger males mate more rapidly, frequently and for a longer time than smaller males.

Krainacker *et al.* (1988) reported that larger size was linked to higher eclosion rates and higher egg production. Higher pupal weight was also noted as an important quality parameter contributing to competitiveness and flight propensity (Sharp *et al.* 1983; Churchill-Stanland *et al.* 1986).

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