CHANGES IN EMERGENCE PARAMETERS AS A RESULT OF TRANSPORTING STERILE QUEENSLAND FRUIT FLY *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE) PUPAE

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

[1]NSW Department of Primary Industries, Locked Bag 21, Orange NSW 2800, Australia
[2]NSW Department of Primary Industries, PO Box 1386, Bathurst NSW 2795, Australia
[3]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, PMB 8, Camden NSW 2570, Australia
Email: bernie.dominiak@dpi.nsw.gov.au

Summary

Emergence of sterile Queensland fruit fly was assessed at the Camden mass-production facility and at the Yanco release centre in New South Wales between November 2002 and January 2005. Pupal weight was positively and significantly related to the percentage emergence at both Camden and at Yanco. However, average emergence rates fell from 82.1% at Camden to 68.4% at Yanco. The date of larval hopping at Camden influenced percentage emergence. Pupal weights for the 4th, 5th and 6th days of larval hopping were more likely to significantly influence the percentage emergence of flies than it did on the first three days of hopping.

Keywords: *Bactrocera tryoni*, quality, transport, emergence, mass rearing

INTRODUCTION

The sterile insect technique (SIT) has been used on Queensland fruit fly (Qfly) *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) for several decades in New South Wales (NSW) (James 1992, Horwood and Keenan 1994, Dominiak *et al.* 2003a, b, Meats *et al.* 2003). Effort has focused on trialling different release techniques, assessing recapture rates in the field and quantifying insect quality parameters at the Camden production facility (Dominiak *et al.* 2002). The success of released sterile flies relies on their ability to reach sexual maturity, locate food and water and attain mating competitiveness with wild males and mating success with wild females. These parameters are likely to be linked to the initial quality of the pupae delivered from the production facility to the release centres. Some decline in quality between the production facility, post-irradiation, and the release centres has been suggested (James 1992) but not investigated. In this paper, we quantify changes in the emergence parameters (post-irradiation) due to transport procedures for each batch of pupae delivered from Camden to Yanco, NSW.

MATERIALS AND METHODS

At Camden, larval production over six days constituted the total complement of pupae irradiated each week. Each day's production (the day that larvae hop out of media trays known as the "hopping day") was collected separately and labelled with the date and hopping day number (eg. 1, 2, 3, etc.). Each day's production was then subjected to different temperature regimens to synchronise pupal development to two day pre-eclosion so that the entire weekly production could be irradiated on a single day. About 80,000 pupae were packed in a plastic bag, held inside a 2 L cardboard fruit juice carton. Five of these cartons were packed side by side into a long, heavy-duty cardboard box, and two of these boxes were placed in a foam box (57 cm long x 28 cm wide x 30 cm high). Each foam box contained 800 000 pupae.

Consignments were packed in plastic bags and sealed on Monday afternoon and kept at 17°C until Tuesday morning when they were taken by air-conditioned vehicle for irradiation. Irradiation (70-75 Gy) occurred in Sydney at the Australian Nuclear and Scientific Technology Organisation's Lucas Heights facility using a Cobalt-60 source. Consignments for both Camden and Yanco were treated identically until irradiation was complete.

After irradiation, which was usually completed by 4 pm, a sample of pupae from each hopping day was returned to Camden (about one hour's drive) where the bags were opened to break the anoxia state. Three replicate samples of 100 pupae from each hopping day were placed in petri dishes and held at 12:10 Light:Dark (one hour each to ramp up and ramp down) at 25±1°C and 65±5% relative humidity to emerge. Samples were assessed for the proportion of emerged and non-emerged pupae, and for viable (fully emerged with fully expanded wings) and deformed adults (fully emerged with partially expanded wings or with other defects).

For the Yanco samples, consignments were driven by courier for about one hour to the domestic air freight depot where they were kept in shade. Consignments were transported by air for about one hour to Narrandera arriving early on Wednesday morning. They were then transported by air-conditioned vehicle to Yanco and the anoxia seals broken about 9 am. As each delivery was received, a sample consisting of 100

pupae was taken from each hopping day and placed in a petri dish. Petri dishes were held under the same conditions as at Camden and pupae assessed for emergence and non-emergence. The non-emerged pupae were further separated into those which failed to break open the pupal case (reported as percentage failed) and those where the adults were stuck or partially contained by the pupal case (reported as percentage stuck). Of the fully emerged flies, assessments were made on the numbers of viable and deformed adults. These parameters were compared to the average pupal weight per hopping day assessed at Camden. Pupal weight was considered the key quality parameter (Dominiak *et al.* 2002). Data from 123 hopping days between November 2002 and January 2005 were analysed.

Emergence outcomes for the Camden and Yanco pupae were analysed using Pearson's correlation analysis. The relationship of pupal weight to viable emergence was compared between Camden and Yanco using a linear parallelism test (multiple linear regression) (Snedecore and Cochran 1967).

RESULTS

The average pupal weight was 9.36 mg (range 7.3-11.2). High pupal weight was significantly linked to high rates of emergence at both sites. Low pupal weight was a highly significant contributor to the failure of flies to emerge at both Yanco and Camden (Table 1). Pupal weight was negatively related to percentage deformed flies for Camden, but not for Yanco. It was also significantly but negatively correlated to percentage stuck and percentage failed categories for Yanco.

On average, the percentage emergence was 82.1% at Camden falling to 68.4% at Yanco. In the comparison of emergence categories between Camden and Yanco, there were significant relationships for the percentage emergence ($P<0.05$) and percentage not-emerged ($P<0.01$) but no significant relationship with the percentage deformed adults.

The relationship between percentage emergence at Camden and Yanco was tested. There was a highly significant, positive relationship ($P<0.01$) between pupal weight and emergence at both sites, and a highly significant difference ($P<0.01$) in emergence between the two sites. The non-significant interaction ($P=0.146$) between pupal weight and emergence at the two site indicates that pupal weight influenced fly emergence equally at Yanco as at Camden, even though emergence rates were significantly different at the two sites.

Results at both Camden and Yanco indicated that pupal weight was significantly and negatively linked to the percentage of pupae that failed to emerge on hopping days 4, 5, and 6. However there was no significant relationship between pupal weight and emergence success for larvae that had completed feeding by days 1, 2 and 3 (Table 2). Day 2 and 3 generally had the lowest number of significant correlations with the parameters assessed while day 6 had highly significant correlations with all parameters.

In the relationship between Camden and Yanco for each of these six days (Table 3), there was a significant correlation ($P<0.01$) for five of the six days for percentage emergence and also for percentage not-emerged but there was no significant relationship for percentage deformed adults for four of the six larval hopping days.

DISCUSSION

In order to gain further efficiencies in the effectiveness of the sterile fruit fly release program, it is important to identify as many of the factors as possible throughout the production, transport and release process that impact on the ultimate quality of the released flies. In this study, we have identified a notable decline of 16.7% in emergence of pupae between the production site at Camden and the release centre at Yanco. While there seems to be limited potential to increase emergence much above 82.1% at Camden, significant improvements in handling and transport between Camden and Yanco could minimise losses for the release program.

Pupal weight is a key quality indicator for the production facility because of the significant flow-on effect on fly emergence at both sites. The results found in the current study (average pupal weight of 9.36 mg) are supported by earlier work where similar pupal weights (average 9.66 mg) were recorded (Dominiak *et al.* 2002).

The correlations in Table 2 were generally uniform however minor variations occur and this may be due to the lack of replication for the Yanco data. There was a highly significant and positive relationship between pupal weight and emergence in the last three days of larval hopping. We assume that the general lack of significance between pupal weight and other parameters in the first three days indicates that pupal viability was inherently high in the first three days of hopping and weight was of less consequence. From the fourth day, the "bigger is better" principle (Dominiak *et al.* 2002) appears to have a positive influence on emergence. The 'non-emerged' category had a negative and highly significant relationship with pupal weight for the same days, again supporting the "bigger is better" principle. The results presented in Table 1

Table 1. Average values (range in brackets) and correlations between pupal weight and emergence and non-emerged pupae, and the percentage of deformed adults at Camden and Yanco.

Site	Variables	Average percentage (range in brackets)	Correlations with pupal weight
Camden	emergence	82.1 (4.3-97.3)	0.141*
	non-emerged	15.2 (1.0–94.7)	-0.293**
	deformed	2.6 (0-13.0)	-0.235**
Yanco	emergence	68.4 (3.0-95.0)	0.307**
	non-emerged	20.9 (2.0-97.0)	-0.298**
	deformed	8.3 (0-24.0)	-0.083
	stuck	3.7 (0-24.0)	-0.159*
	failed	17.2 (0-97.0)	-0.277**

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

Table 2. Correlation of pupal weight with emergence and non-emergence of pupae, and the percentage of deformed adults at Camden and Yanco for different days of larval hopping.

Site	Variables	Day of larval hopping					
		1	2	3	4	5	6
Camden	emergence	0.050	0.058	0.169	0.551**	0.180	0.666**
	non-emerged	-0.004	-0.033	-0.155	-0.547**	-0.520**	-0.578**
	deformed	-0.329**	-0.140	-0.128	-0.028	-0.361**	-0.805**
Yanco	emergence	0.305*	0.110	0.078	0.481**	0.461**	0.327**
	non-emerged	-0.234	-0.105	-0.107	-0.451**	-0.455**	-0.484**
	deformed	-0.254*	-0.111	-0.062	-0.208	-0.035	0.402**
	stuck	-0.329**	-0.031	0.115	-0.150	-0.327**	-0.672**
	failed	-0.156	-0.105	-0.137	-0.448**	-0.407**	-0.372**

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

Table 3. Correlation between percentage emergence and non-emergence of pupae, and percentage deformed adults between Yanco and Camden for different days of larval hopping.

Variables	Day of larval hopping					
	1	2	3	4	5	6
emergence	0.386**	0.564**	0.584**	0.802**	0.125	0.534**
non-emerged	0.618**	0.712**	0.696**	0.204	0.920**	0.904**
deformed	0.162	0.280*	0.170	0.204	0.147	-0.460**

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

indicate that there was a significant difference in emergence rates at Camden and Yanco. This indicates that in terms of fly emergence, pupal weight was equally important at both sites. It appears stresses during transport, or as a consequence of the transport conditions has caused the large decline in fly emergence. If these could be reduced emergence at Yanco should approach that achieved at Camden.

Trends for percentage 'deformed adults' at Camden and Yanco were less clear in relation to the hopping day, however most trends were negative. This suggests that that bigger pupae result in fewer deformed adults. The percentage deformed adults (Tables 2 and 3) appeared to be poorly correlated with any of the measured parameters. The relationship of percentage deformed adults at Camden and Yanco for each hopping day was unclear and we feel that there is no benefit to be derived from research aimed at reducing the percentage of deformed adults due to transport.

The percentages of 'emerged' and 'non-emerged' pupae at Camden was mostly positively and highly significantly linked to the same parameters at Yanco (Table 3). Improvements in emergence parameters at Camden are likely to flow on to the Yanco release facility.

The comparison in our results for emergence and non-emerged pupae, and to a lesser extent the deformed adult categories, demonstrated a highly significant relationship between most of the quality parameters measured at Camden and Yanco. Our results also demonstrate that the last three days of larval hopping do exert a positive influence on the emergence rates at Yanco and contributed to the success of field releases, if pupal weight remains high. Production of larger pupae at Camden, resulting in higher emergence, and fewer 'non-emerged' or incompletely emerged pupae at Yanco would have a significant beneficial effect on the success of the release program. However our results indicate there was a marked decline in quality parameters, principally emergence, as a result of the transport of pupae from Camden to Yanco. The factors causing this decline need further investigation.

These emergence parameters are just the start of the process to maximise the number of competitive adults released into the field. The number of pupae held for fly emergence in the release containers, the growing conditions within the rearing room and the nutritional and hydration status of the emerged flies all contribute in varying degrees to the number of viable, competitive flies released into the field. The quality of pupae produced at Camden influences the Yanco operations and must be maximised to benefit the overall release program.

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