EFFECTS OF RADIATION, DYE, DAY OF LARVAL HOPPING AND VIBRATION ON ECLOSION OF QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE)

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Summary

Marking pupae with fluorescent dye is a routine procedure of all releases of sterile Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) (Qfly) in Australia. Variable adult eclosion rates occur at field release sites possibly due to travel stress (vibration and temperature) over and above that due to other natural causes such as packaging, irradiation and treatment of pupae with fluorescent dyes. Results of six vibration treatments of up to 8 hours did not cause significant pupal mortality whereas the dying process increased mortality by 16% over the 22% losses due to mortality associated with packaging and irradiation. We conclude that there is an urgent need to re-assess current dye protocols used in the preparation of Qfly for sterile releases.

Keywords: adult eclosion, sterile insect technique

INTRODUCTION

Queensland fruit fly, *Bactrocera tryoni* (Froggatt), (Qfly) is one of the worst pests of horticultural crops in Australia (Sutherst *et al*. 2000). It is a critical economic and quarantine pest affecting the costs of horticultural production and influencing market access for Australian products (Horticultural Policy Council 1991).

Qfly is endemic to eastern Australia (Drew 1989), ranging from Cape York in Queensland to East Gippsland in Victoria. With the expansion of irrigated cropping, flies are now routinely caught beyond their natural range in western New South Wales (NSW) including Moree, Bourke, Narromine and Condobolin. Outbreaks periodically occur in south-western NSW (including the Fruit Fly Exclusion Zone (FFEZ)) and eastern Victoria, occasionally in metropolitan Adelaide in South Australia and rarely in Perth, Western Australia. The FFEZ is a quarantine area covering horticultural production areas along the Murray River and related rivers in NSW, South Australia and Victoria. Fruit flies are controlled inside the FFEZ and in the adjacent Risk Reduction Zone (RRZ) to optimise trade access to domestic and international markets.

Suppression of Qfly is currently achieved through a combination of cover and/or bait sprays supplemented by male annihilation techniques (in NSW, Victoria and Queensland) or with the Sterile Insect Technique (SIT) (in NSW, Victoria, South Australia and Western Australia (Jessup *et al.* 2007)). In NSW and Victoria the current use of SIT is limited to the RRZ.

Successful SIT requires large numbers of vigorous insects capable of locating and mating with their wild counterparts. In NSW Qfly SIT has experienced some issues associated with sub-optimal field eclosion of factory-reared pupae. Discrepancies in adult eclosion of sterile Qfly between the fruit fly production factory at Menangle in NSW and the rearing-out facility near to the release sites were reported with losses ranging from 14-100% for individual consignments (Dominiak *et al.* 2007, Worsley *et al.* 2008). Factors such as shock due to rough handling, jarring or vibration during transport were suggested as possible explanations (Calkins and Parker 2005, Dominiak *et al.* 2007, Campbell *et al*. 2008, Worsley *et al.* 2008). In this study we examined the effect of continuous vibration at a given temperature on eclosion success and sex-ratio of sterile Qfly used in SIT releases. We also aimed to quantify the effect of the current protocols for irradiation and application of fluorescent marker dye on eclosion.

MATERIALS AND METHODS

Effect of day of larval hopping and vibration

Qfly larvae were reared using a chaff-based diet held over vermiculite at the production facility operated by NSW Department of Primary Industries at the Elizabeth Macarthur Agricultural Institute (EMAI) at Menangle in south-western Sydney. When larvae are fully fed, they flick themselves ('hop') out of the larval medium and fall into a collection tray in the base of the rearing tower. 'Hopping' may continue for several days. Trays with larvae (and later pupae) from each 'hop day' are kept separately. Pupae for testing were randomly selected from large consignments normally irradiated by the Australian Nuclear Science and Technology Organisation (ANSTO) at Lucas Heights, Sydney. Pupae collected on the first and second 'hop days' from production batches on 28th November 2007, 23rd

January and 26th February 2008, were compared (Dominiak *et al*. 2002).

Effect of dye and irradiation

At EMAI, four polyethylene bags, each holding approximately 800 g of pupae from each batch date were packed into two standard boxes (2L capacity). The two boxes and their contents were maintained under the same conditions throughout, except when Box 1 underwent the actual irradiation process. Pupae in two of the four bags from the irradiated and non-irradiated boxes were dyed by dusting with a fluorescent marker (Swada, Fiesta® FEX Series, Astral Pink 1, DIC International (Australia) Pty. Ltd., Dandenong South, Victoria) at the rate of 10 g kg^{-1} of pupae. The dye was applied when packing pupae so that emerging flies would self-mark.

The boxes were prepared the day before irradiation (two to three days before adult eclosion), held over night at 18°C to induce a hypoxic atmosphere within the bags, and next day transported to and from ANSTO in an air-conditioned car. Box 1 received a sterilising dose of gamma irradiation (70-75 Gy) from a cobalt-60 source in the GATRI in-ground facility. Box 2 contained the non-irradiated pupae (Table 1). Changes in the hypoxic atmosphere within the sealed bags and the interaction with the larval 'hop day', dye and irradiation treatments were not determined.

Five replicates of approximately 100 pupae were taken from each batch prior to the commencement of the dying and packaging procedure. These samples served as control treatments and were subjected to the routine quality assessments (emergence parameters and sex-ratio) made at EMAI (Dominiak *et al.* 2002). These samples were compared with the untreated replicates subjected to packaging (including hypoxia) and transport to and from ANSTO.

Effect of vibration

On return to EMAI all pupae were held at 26±2°C and 60–65% relative humidity. Thirty individual samples of approximately 100 pupae were randomly drawn from each bag. Each sample was placed in a metal ring (35 mm inside diameter, 4 mm high) within a standard 90 mm petri-dish. Each ring was covered with a piece of medium density foam (60 x 60 x 10 mm) and the petri-dish lid replaced. Replicate dishes for each treatment were held together with two rubber bands to prevent physical movement of the rings or pupae.

The samples were vibrated in a Gallenkamp Orbital temperature controlled incubator (23±1°C) with the internal table rotating at 50 revolutions per minute. Replicates of each treatment combination were shaken for 0, 0.5, 1, 2, 4 or 8 h in an attempt to mimic the conditions of the post-irradiation transport to the release centres. On removal of the last samples from the shaker the foam insert and ring were removed from each petri-dish, the lid replaced and the flies left to emerge without disturbance. Emerged flies were denied access to food and water and died within the containers.

The petri-dishes were examined after seven days when the flies were dead. Individual flies were classified as: (i) fully-emerged with expanded wings; (ii) partially-emerged or deformed, when the end of the puparium was pushed off, or split, with the body remaining within, or attached to the puparium, or, the imago present but the wings not fully expanded; or, (iii) not-emerged and the anterior end of the puparium intact.

Table 1. Treatment of bags of pupae within the boxes transported to and from the ANSTO irradiation facility.

	Bag No.	Day of larval hopping*	Treatment	
			Irradiated	Dyed
Box 1	1	1	+	+
	2	1	+	-
	5	2	+	+
	6	2	+	-
Box 2	3	1	-	+
	4	1	-	-
	7	2	-	+
	8	2	-	-

* Pupae collected on the 1st or 2nd day of larval hopping. Each bag contained approximately 20000 pupae

Percentage emergence was assessed as the number of fully-emerged flies divided by the total number of pupae. The sex-ratio of fully-emerged flies apparently capable of flight was calculated as the number of males divided by the number of females.

Data analysis

The effects of dye, irradiation, day of larval hopping and vibration on emergence parameters (fully-emerged flies, partially-emerged pupae and deformed flies, and not-emerged pupae) was analysed by Generalised Linear Mixed Model with a binomial distribution and a logit link. The random terms fitted were batch, day of larval hopping and bag. The number of pupae per sample was used as the binomial totals. The data is presented as predicted percentages. The cumulative effect of sample preparation (packaging, transport to and from ANSTO but excluding the effect of dye and irradiation) on emergence parameters was assessed against the minimal disturbance control samples maintained at EMAI, using a General Linear Model with binomial distribution. Genstat 11 (Payne *et al*. 2008) was used for all analyses.

RESULTS

The day of larval hopping and interval of vibration had no significant effect on the emergence parameters (percentage fully-emerged flies, percentage of partially-emerged pupae and deformed flies, percentage of pupae not-emerged and the sex-ratio of emerged flies).

Effect of dye, irradiation and vibration on emergence parameters

The inclusion of fluorescent dye with pupae significantly influenced the percentage of fully-emerged flies under ideal laboratory conditions ($F_{1,19}$ 121.66, P = <0.001). Application of dye decreased successful emergence by an additional 18% (from 82 to 64%) over the background mortality expected during normal rearing. The effect of irradiation decreased the percentage of fully-emerged adults ($F_{1,19}$ 8.49, P = <0.009). There was a significant (P = <0.011) interaction between dye and time shaken. All other factors and interactions ('hop day', 'hop day' x time shaken, 'hop day' x radiation, time shaken x radiation, 'hop day' x dye, radiation x dye, 'hop day' x time shaken x radiation, 'hop day' x radiation x dye, time shaken x radiation x dye and 'hop day' x time shaken x radiation x dye) had no significant influence on fly emergence.

As with the fully-emerged pupae the proportion of partially-emerged/deformed flies present was significantly influenced by the presence of dye ($F_{1,19}$ = 231.26, P <0.001). As above, most other factors and interactions did not have significant effects on the emergence parameters. For the dyed pupae, the time of shaking caused a significant variation of partially-emerged/ deformed flies from 16.5 to 19.2% with no apparent trend. The presence of dye increased the incidence of partial emergence/deformity by 15%, from a background of 3% that occurred during normal rearing.

The percentage of pupae failing to emerge and the sex-ratio of fully-emerged flies were not significantly influenced by any of the treatments ($P \leq 0.05$).

Effect of transport to and from ANSTO on emergence parameters

The cumulative effects of packaging and vehicle transport to and from the irradiation site at ANSTO significantly reduced successful fly emergence ($\chi^2_{1,50} = 18.3$, $P \leq 0.001$) from 85.4% under laboratory conditions to 81.0% .

The percentage of partially-emerged pupae and deformed flies was significantly influenced by the combined cumulative effects of packaging and transport to and from the irradiation site ($\chi^2_{1,50} = 38.0$, $P \leq 0.001$). The cumulative effect of sample preparation and transport increased the percentage of partially-emerged pupae and deformed flies by 2.6% over the 1.2% expected as a result of undisturbed adult eclosion under ideal laboratory conditions.

The proportion of males was significantly reduced ($\chi^2_{1,44} = 9.22$, P =0.002) from 0.455 to 0.410 by the combined effects of packaging and transport.

DISCUSSION

Vibration of pupae in the transport phase after irradiation to release sites should not influence subsequent fly emergence. This is contrary to the belief that transport stress (a combination of vibration and temperature) is the major factor in reducing field emergence of Qfly following sterilisation (Dominiak *et al.* 2007, Campbell *et al.* 2008, Worsley *et al*. 2008). It should be noted that our tests were conducted at only one temperature (23±1 °C) and given the earlier reports (Dominiak *et al.* 2007, Campbell *et al.* 2008, Worsley *et al*. 2008), the effect of vibration at different temperatures is worthy of further investigation.

No significant effect due to the irradiation procedure was detected. This eliminates this variable as a possible cause of the observed decline in field emergence and is largely consistent with the conclusions of Collins *et al.* (2008) who found few changes in insect quality parameters following pupal irradiation. However, the combined effects of packaging (including the induction of hypoxic conditions) and transport to ANSTO contributed significantly to pupal mortality, causing a 10% loss above that which could be expected as the normal

background in the laboratory (12% mortality).

The application of 10 g kg^{-1} dye to Qfly pupae and the transport to and from ANSTO was associated with a 16% increase in mortality above the mortality of the untreated controls. It is anticipated that under the current protocols field emergence at release facilities following transport and associated stresses would seldom exceed 80% based on our results. This confirms evidence by previous reports regarding the adverse effects of dye on the emergence of pupae (Dominiak *et al.* 2000, 2003, Weldon 2005). Under the Australian procedure the amount of fluorescent dye applied to pupae is significantly greater than that applied to mark Medfly pupae in the United States of America (1.5 g of Day-Glo® $L^{-1}$of pupae (Nestel *et al.* 2007).

The need to discriminate between wild-type and released sterile flies caught in the field is contentious. The current system of incorporating fluorescent dye into the ptilinum of emerging flies is antiquated when compared with other available technologies such as visible genetic or trace element markers (Hagler and Jackson 2001, Alphey 2007). Moreover, the current identification process is labour intensive. The dye is often not visible to the naked eye and thus requires the use of ultra-violet or blue light and may require the crushing of individual fly head capsules. Incorporating genetic markers or fluorescent proteins into the larval medium, provided they were not deleterious to pupal survival or adult fitness, could reduce handling associated with preparation of consignments for radiation and improve the identification of mixed trappings of wild and sterile flies. DNA profiling techniques, suitable for routine screening of large numbers of insects, are currently not available in Australia to separate wild from sterile Qfly (Gilchrist *et al.* 2006).

Markers used in a SIT program should ideally have no impact on insect behaviour, growth and reproduction, be non-toxic to the insect and environment, be durable, easily applied, readily visible and inexpensive (Hagler and Jackson 2001). Based on these criteria the application of Fiesta, Astral Pink 1 pigment is inappropriate because of its adverse impact on adult emergence.

Based on our results the factors influencing pupal mortality occur at the factory prior to irradiation and transport to the release sites. The current pupal packing and pre-irradiation protocols should be revised. In particular the application rate of dye needs to be reconsidered. An alternate method of marking, or a compromise between the amount of dye applied and confidence in identification of sterile flies, is required if SIT is to be used to its full potential in Australia. However, any change to current production protocols can only be justified if significant improvements occur in the quality of the released flies.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Laura Jiang, Selliah Sundaralingam and the fruit fly factory staff at EMAI for their assistance during this project. This project was funded by the NSW Department of Primary Industries and the manuscript improved by comments made by Drs. Jenny Ekman and Idris Barchia.

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