Impact of marking dye, transport and irradiation on eclosion of mass produced Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae)

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Abstract

In Sterile Insect Technique (SIT) programs, released flies are commonly marked with fluorescent dye prior to irradiation to assist subsequent identification. The impacts of dye, transport and irradiation on adult eclosion of Queensland fruit fly were investigated. Eclosion in the non-dyed, non-transported, non-irradiated pupae was 84.64%. Dyeing, transport and irradiation processes significantly reduced the adult eclosion rate to 72.88%. This reduction in adult eclosion was accompanied by a 10.2% increase in partially eclosed adults. In an additional treatment, the dye was removed from pupae following irradiation by rinsing with water. These pupae were allowed to eclose in a humid container, whereas the pupae from the other treatments were allowed to eclose in glass Petri dishes. The percentage of fully eclosed flies was 81.54% – a significant improvement compared with standard dyed transported irradiated pupae and very close to that found in untreated controls – and the percentage of partially eclosed flies was not significantly different from the control pupae. Both irradiation treatments had more uneclosed pupae compared to the control treatment although the three treatments differed by only 2.1% variation. Based on these results, it appears that dye and/or the eclosion environment have considerable impacts on the viability of Queensland fruit flies produced for SIT. There appears to be substantial room for improvement in either the marking system and/or the eclosion environment used prior to release.

Key words: Insect mass production, insect quality parameters, *Bactrocera*, sterile insect technique, insect marking.

Introduction

The Sterile Insect Technique (SIT) is a pesticide-free strategy originally developed by Knipling (1955) and used today to control many of the world's most damaging insect pests. In particular, SIT is used in many regions of the globe to combat fruit flies that threaten economic development and even subsistence in the case of some developing nations. The treated area is inundated with sterile male fruit flies, so that the vast majority of matings by the small numbers of wild female flies present yield no viable offspring. Reproductive failure results in greatly reduced pest numbers in the following generation.

To evaluate the success of SIT programs it is necessary to distinguish between wild and sterile flies captured in monitoring traps. The most common way to mark sterile flies for this purpose is to apply fluorescent dye dust to pupae prior to eclosion. This method was devised by Norris (1957), further developed by Steiner (1965), and is now recommended as the standard method of marking irradiated fruit flies (FAO/IAEA/USDA 1999, 2003, Enkerlin 2008). During eclosion the sterile flies accumulate the dye in their ptilinum, which can be seen with microscopic examination (Holbrook *et al.* 1970), allowing the positive identification of sterile flies recaptured in monitoring traps following release.

In Australia, SIT was first explored as a means to suppress or eradicate Queensland fruit fly (*Bactrocera tryoni* Froggatt, 'Qfly') in the early 1960s (Monro and Osborn 1967). SIT has been frequently used on an annual basis near or within the Fruit Fly Exclusion Zone (FFEZ) that has protected major fruit growing districts of New South Wales, Victoria and South Australia since 1996 (Dominiak *et al.* 1998, 2003a, Meats *et al.* 2003) and has been used at some time over the past two decades in all mainland Australian states except Queensland.

As in other fruit fly SIT programs; fluorescent dyes are used to mark sterile Qflies. However, several studies have reported that fluorescent dyes can reduce the quality of sterile Qflies by adversely affecting adult eclosion and recapture rates (Dominiak *et al.* 2000, 2003b, 2010, Weldon 2005, Campbell *et al.* 2009). Reduced adult eclosion rates obviously have very direct effects on numbers of sterile flies available for release. Further, reduced recapture rates in the field suggest reduced survivorship however this linkage needs to be verified. Both eclosion and recapture rates indicate that fluorescent dyes reduce SIT efficacy, and prompt investigation into the underlying causes. The purpose of this paper is to examine the impact of dye on the eclosion of adult Qflies.

Materials and methods

The experiment was conducted at the Elizabeth Macarthur Agricultural Institute (EMAI) Qfly mass-production facility. Conditions under which the Qflies are reared at this facility have been reported previously (Dominiak *et al.* 2008). Data were collected using 13 weekly production batches generated between 18 February 2003 and 13 May 2003. For each of the 13 weekly batches, samples of pupae at 80% maturity (i.e. eight days old, or two days prior to adult eclosion) were set aside for treatment with dye, transport and irradiation or as controls. For the control group, 100 pupae were placed into each of three covered glass Petri dishes to evaluate eclosion. Pupae in the control group were not subjected to transport, dye or irradiation.

Pupae for the two irradiated treatments were dyed at EMAI, at the rate of 4 g of fluorescent dye (Day Glo® series – Astral Pink) per 400 g (approximately 1 L) of pupae. They were then transported (about 2 h each way) by air-conditioned vehicle for irradiation at the Australian Nuclear Science and Technology Organisation (ANSTO), Lucas Heights, by a Co_{60} GATRI in-ground gamma irradiator. Pupae were irradiated at a nominal dosage of 73 ± 2 Gy in anoxia and were then returned to EMAI. 100 dyed transported irradiated pupae were placed in five covered glass Petri dishes, identical to the control group. To investigate the combined effects of dye removal and humidity, some pupae were rinsed in a small amount of water to remove the dye and were then dried on paper towels. Five samples of 100 pupae were placed on top of a 1 cm deep bed of damp vermiculite (four parts vermiculite to one part water) in a 9 cm deep plastic container. The plastic container had a mesh lid to prevent the escape of emerged flies.

Percentage adult eclosion from treated and control pupae was monitored in the facility's quality control laboratory which was maintained at 25°C (± 1°C) and relative humidity of 65% (± 5%) with

artificial lighting (12 h light, 10 h dark with 1 h brightening to simulate dawn and 1 h dimming to simulate dusk). The rooms also have a skylight (Deece *et al.* 2000). No food and water was provided for the flies. Counts of fully eclosed, partial eclosed and uneclosed flies were collected. The average percentages of fully eclosed, partially eclosed and uneclosed flies were calculated for each treatment and analysed using ANOVA (Payne *et al.* 2007).

Results

The three treatments differed significantly in the percentage of pupae that fully eclosed as adult flies ($F_{2,24} = 55.22$, $P < 0.001$, Table 1). Pupae that had not been dyed, transported or irradiated had the highest percentage of fully eclosed flies (84.64%) whereas pupae that had been dyed, transported and irradiated had the lowest percentage of fully eclosed flies (72.88%). Washing the dye off pupae following irradiation and subsequently rearing the flies in a humid environment resulted in an intermediate rate of full eclosion (81.54%).

The three treatments differed significantly in the percentage of partially eclosed flies ($F_{2,24} = 77.20$, $P < 0.01$); dyed transported irradiated pupae had a significantly higher percentage of partially eclosed flies than undyed unirradiated pupae or pupae that had been washed to remove dye and eclosed in humid conditions (Table 1).

The three treatments differed significantly in the percentage of uneclosed flies ($F_{2,24} = 5.03$, $P = 0.015$); fewer untreated control pupae failed to eclose than was the case for either dyed irradiated pupae or pupae that had been washed to remove dye and eclosed in humid conditions (Table 1).

Discussion

The percentage of full eclosion was highest in the untreated controls. Dyeing, transport and irradiation caused a significant 11.76% decline in eclosion and this is consistent with findings of other studies (Dominiak *et al.* 2007a, Campbell *et al.* 2009). Washing the dye off after irradiation and transport, and maintaining humidity during eclosion resulted in a significant increase in eclosion, to within 3% of the untreated controls (Table 1). The percentage of partial eclosion increased significantly for pupae that had been dyed, transported and irradiated. Washing these pupae and then maintaining them in humid conditions lowered the incidence of partial eclosion to levels similar to those found in untreated controls. Both groups of pupae that had experienced dyeing, transport and irradiation had a significantly increased percentage of uneclosed pupae, and there was a general trend towards increasing percentages of uneclosed pupae with each handling.

Washing dyed and irradiated pupae followed by eclosion in humid containers resulted in improved eclosion and decreased partial eclosion compared with the standard dyeing, transport and irradiation process followed by eclosion in glass Petri dishes. These differences may be attributed to a number of factors, including the dye powder, the washing treatment or the environment in which the pupae were allowed to eclose. First, as a very direct effect, the dye powder itself may physically hinder eclosion. Effects of dye might also be indirect, resulting from damage to the pupae during their ongoing development. For example, Weldon (2005) speculated that the dye powder could contribute to dehydration of the pupae, resulting in a decrease in pupal weight, which has been correlated with many quality parameters including eclosion (Dominiak *et al.* 2002, 2007b, 2008). Washing the dye from pupae after irradiation may allow a small amount of water to be absorbed by the pupae, despite towel drying afterwards. Similarly, eclosion in a humid container (provided by damp vermiculite) could also have helped to maintain pupal hydration and weight, compared with eclosion in the standard Petri dish.

Hydration of pupae following irradiation, during transport and at eclosion may be an important factor to consider when trying to improve eclosion rates. Some steps have been made in addressing this issue as moist vermiculite is recommended as a covering for pupal release containers (Dominiak *et al.* 2003a, Reynolds *et al.* 2010).

The percentage of uneclosed pupae was significantly higher in treatments that experienced dyeing, transport and irradiation. However, the range across treatments was only 2.13% and hence it seems that dyeing, transport and irradiation did not have a considerable impact on this parameter. There does seem to be a trend towards increasing percent uneclosed pupae with additional handling that warrants further investigation. Previous studies have found a 16.7% decline in eclosion in pupae after transport to release areas (Dominiak *et al.* 2007a). Whether this was caused by travel stress or handling stress is not clear. Campbell *et al.* (2009) found that dye had a stronger effect than irradiation on eclosion rates but that vibration stress (mimicking travel stress) did not adversely affect eclosion. The issue of handling stress needs to be evaluated further in Qflies.

The dyeing process is the only international recognized method of marking irradiated pupae (FAO/IAEA/USDA 1999, 2003). Washing the dye off after irradiation is obviously not an option for SIT, but the results of this study do point to some practical actions that can be taken within SIT operations. We suggest that the continued hydration of pupae may be important and that the deleterious effects of the dye may be minimized by maintaining humidity around the irradiated pupae or by maintaining higher pupal weight. Reynolds *et al.* (2010) used moist vermiculite in field pupal releases and found higher relative humidities resulted in generally higher eclosion. The international standard for the amount of dye per kg of pupae has decreased to 1.5 g L^{-1} (FAO/IAEA/USDA 1999, 2003). Dominiak *et al.* (2010) reported increased eclosion rates with decreasing concentration of dye. Recent research into optimizing irradiation to minimize adverse effects (Collins *et al.* 2008, 2009) and improving release methodology (Campbell *et al.* 2008) are steps forward. Further research is needed to explore potential adverse effects of the current dyeing procedure, or to develop a replacement marking method, that will improve Qfly SIT.

Table 1. Percentage of fully eclosed, partially eclosed and uneclosed pupae subjected to the control: dyed transported and irradiated; and dyed, transported, irradiated and washed, and reared in humid environment.

Treatment	Fully eclosed	Partially eclosed	Uneclosed
Control	84.64 a	3.31 a	12.05 a
Dyed, transported and irradiated pupae	72.88 c	13.57 b	13.72 b
Dyed, transported, irradiated and washed pupae reared in a humid environment	81.54 b	4.28 a	14.18 b

Figures in the same column followed by the same letter are not significantly different ($P = 0.05$)

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