IMPACT OF FLUORESCENT MARKER DYES ON EMERGENCE OF STERILE QUEENSLAND FRUIT FLY, *BACTROCERA TRYONI* (FROGGATT) (DIPTERA: TEPHRITIDAE)

Bernie C. Dominiak,[1] Linda Schinagl[2] and Helen Nicol[1]

[1]NSW Agriculture, Locked Bag 21, Orange, NSW 2800
[2]NSW Agriculture, PMB 8, Camden, NSW 2570

Summary

In three trials, twelve fluorescent dye colours were applied to pupae of Queensland fruit fly (*Bactrocera tryoni* (Froggatt)) to evaluate the effect on emergence. Nova Red and orange colours appeared to have minimal adverse impact on emergence. Emergence percentages varied from 78% in the best treatments to 56% in the worst colour treatments. Recommendations are made for the use of different dye colours.

INTRODUCTION

The sterile insect technique (SIT) has been used for decades in many countries to control fruit flies. The effectiveness of SIT programs is evaluated by analysing the wild or sterile status of flies captured in monitoring traps. The first trials using SIT for suppression of Queensland fruit fly (Qfly) (*Bactrocera tryoni* (Froggatt)) were in Victoria, Australia, (Andrewartha *et al.* 1967) with further evaluations at Wodonga (MacFarlane and Betlinski 1988). SIT was used in Western Australia (Fisher 1992) to eradicate Qfly. More recently, Qfly suppression programs have occurred in New South Wales (James 1992; Horwood and Keenan 1994; Dominiak *et al.* 1998; Dominiak *et al.* 2000) and at Adelaide (Perepelicia and Bailey 1993; Perepelicia *et al.* 1994, Reynolds *et al.* 1995, Jackman *et al.* 1996; Perepelicia *et al.* 1997).

Sterile insect release programs rely on an efficient method of quickly identifying and separating sterile flies from wild flies caught in monitoring traps. Steiner (1965) reported using a method where pupae were coated in a dye which was retained in the ptilinum of the adult after emergence. This method used oil-soluble dyes and required crushing of the fly head for dye extraction which was time consuming and destructive. Holbrook *et al.* (1970) noted that the identification process was quicker when flies were marked with fluorescent dyes and determinations made by crushing the head while exposed under a UV lamp. Enkerlin *et al.* (1996) further shortened identification time by using fluorescent microscopy.

In early Australian SIT trials with Qfly, fluorescent dyes were used to mark released flies however the colour(s) used were not identified in some reports (MacFarlane *et al.* 1987; Perepelicia *et al.* 1993; Perepelicia *et al.* 1994). Yeates *et al.* (1992) used Arc Chrome A6 (orange) and Fire Orange A4 (pink); James (1992) used orange and red fluorescent dyes; Horwood and Keenan (1994) used Laser Red and Arc Chrome (orange); Reynolds *et al.* (1995) used red and orange (probably Nova Red and Arc Chrome); Jackman *et al.* (1996) and Dominiak *et al.* (1998) used Comet Blue, Nova Red and Arc Chrome; and Perepelicia *et al.* (1997) used Dayglo Blaze Orange.

While dyes are commonly used to mark sterile flies, there have been only some reports on ease of detection and few details on emergence. Holbrook *et al.* (1970) noted that Blaze Orange and Rocket Red were more easily discernible than Arc Yellow and Signal Green after one week exposure in the field. Jackman *et al.* (1996) noted that Comet Blue proved difficult to detect compared with Nova Red and Arc Chrome. In the suppression program at Wagga Wagga, New South Wales, Dominiak *et al.* (2000) noted that Comet Blue seemed more easily removed by fly preening than Nova Red or Arc Chrome. In the eradication program in Western Australia, Yeates *et al.* (1992) reported a recapture rate of 0.094% for flies marked with Fire Orange A4 compared with a recapture rate of 0.123% from flies marked with Arc Chrome A6.

Holbrook *et al.* (1970) noted that flies marked with Rocket Red powder exhibited an increased mating frequency compared with those marked with Arc Yellow, Signal Green or Blaze Orange however they made no comment about effects on emergence.

In recent Australian sterile release programs for suppression of wild flies, pupae and the resultant adult flies have been marked usually with orange or pink dye which are difficult to differentiate when found in a trap sample of flies marked with both dyes (Dominiak *et al.* 2000). During more recent trials and suppression programs, multiple dyes were needed to differentiate flies used in different release techniques at the same location. This paper describes the initial laboratory evaluation of the effect of twelve dyes on

emergence of Qfly and the identification of likely alternative colours that might be used with minimal impact on emergence.

METHODS

The TriState Fruit Fly Production Facility is located at Camden in New South Wales. It produces up to 16 million sterile Qflies per week for research and suppression programs in New South Wales, South Australia and Victoria. The facility has laboratories to study quality aspects of production and these facilities were used to study the impact of dyes on emergence. All treatments were held in the quality control room at the Camden production facility (Terras *et al.* 1997) with the environmental conditions maintained at 26°C and 55–60% relative humidity.

Three trials were conducted using different dye colours in different trials. All vermiculite was removed from pupae prior to dying. Three hundred pupae were dyed with different dye colours and separated into three, one hundred pupae repeat samples and placed in petri dishes. Pupae were dyed by mixing pupae gently with dye at the rate of 9 g per 800 g (about 80,000 pupae or two litres) of pupae. Each trial also had three repeat samples of one hundred undyed pupae. Pupae were placed in petri dishes without covering and adults allowed to emerge.

All dyes were obtained from Fernz Specialty Chemicals, Villawood, in New South Wales. Based on data sheets provided by this company, there were no differences in specific gravity, average particle size, purity or other formulation details between Series A and Series E dyes except that Series E had a reported improved colour strength for colouring paper products.

Each trial was assessed for empty pupal cases, partly emerged adults and unemerged pupae at the end of 14 days. The experimental design was completely randomised within each trial. Percentage emerged were analysed by ANOVA using Genstat 5 (Genstat 5 Committee 1993). Residual plots were examined and found to be close to normal; no transformation of data was done.

RESULTS AND DISCUSSION

Percentage emergences of adult flies, after dye treatment, are shown in Table 1. The three trials supported the use of red and orange colours but variability between trials and the fact that not all colours were used in each trial made it difficult to draw finite conclusions. In Trial 1 and Trial 3, emergences of undyed pupae were similar to the factory control emergences and these trials used pupae with a weight of 8.8 mg. Trial 2 used larger pupae (10.0 mg) and there was a larger variation between the nil treatment pupae and the factory control.

In Trial 1, all colours had a significantly lower emergence than the control. The probability of the F test was not significant for Trial 3. In Trial 2, Flame Orange, Arc Chrome, Solar Yellow and Fire Orange did not differ significantly from the control; Strong Pink, Blaze, Strong Magenta and Comet Blue had significantly lower emergence than the control and the first four mentioned colours; Strong Magenta and Comet Blue had the lowest emergence and were significantly different to other colours.

In terms of emergence, Nova Red would appear to be the most favourable dye to use. Similarly Orange (Arc Chrome and Flame Orange) appear to be almost as good. However these dyes are sometimes difficult to separate in the field due to the closeness of colour to Nova Red (Dominiak *et al.* 2000). Based on trial emergences, Green (Stella Green) and Yellow (Lunar Yellow and Solar Yellow) appear to be the next dyes of preference; Magenta colours gave variable results.

Steiner (1965) reported that the Calco blue dye was removed from the flies or faded within two weeks. Jackman *et al.* (1996) noted that Comet Blue proved difficult to detect within several weeks as did Dominiak *et al.* (2000). Comet Blue appears to be the least favourable because of the detrimental effect on emergence and the problems of detection in the field.

Table 1. Percentage emergence of adult Qflies from pupal cases using different dye colours. For each trial, figures followed by the same letter are not significantly different.

Fluorescent dye colour used	Trial 1	Trial 2	Trial 3
Nil	88.0 a	73.7 a	80.7
Nova Red 2—Series A	71.7 b		77.7
Stella Green 8—Series A	69.0 b		73.7
Flame Orange 4—Series A	78.0 b	70.6 a	72.7
Lunar Yellow 27—Series E	69.7 b		71.7
Magenta 10—Series A	71.0 b		71.3
Arc Chrome 6—Series A		72.3 a	
Solar Yellow 7—Series A		69.3 a	
Fire Orange 4—Series A		66.7 a	
Strong Pink 1/3—Series A		62.6 b	
Blaze 5—Series A		61.3 b	
Strong Magenta 21–Series E		58.7 bc	
Comet Blue 60—Series A		55.6 bc	
Mean of the entire test	74.6	65.7	74.6
Probability of F test	0.007	0.016	0.329 NS
Batch from factory	D3	D5	D6
Average adult emergence %	82.0	74.6	80.7
Pupal weight—Day 6 first hopping	8.8 mg	10.0 mg	8.8 mg

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