

The influence of mixtures of parafformone lures on trapping of fruit fly in New South Wales, Australia

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Abstract

Tephritid fruit flies of economic importance are monitored using traps containing either cuelure (CL) or methyl eugenol (ME) as an attractant. There would be potential economic advantages if both lures could be combined in a single trap without compromising trapping efficiency. This study presents results from two trials testing combinations of cuelure (4.4 mL) and methyl eugenol (0.5 mL and 2.2 mL) in Lynfield traps near Griffith, NSW and in Sydney.

For the Griffith trial, the addition of 2.2 mL of methyl eugenol to the standard cuelure wick quadrupled the overall capture of sterile Queensland fruit fly (Qfly) although significant differences were detected in only one of four trials. Traps were placed between 5 and 55 m from the release point, and distance had no significant effect on the number of flies trapped. Time after trap deployment and all time interactions were significant. The proportion of sterile Qfly trapped within three weeks in the first three releases was >91% of total flies trapped in the CL–ME combinations while the CL only treatment recaptured <83% in the same period. Newman fruit fly were trapped with all treatments but not analysed.

In Sydney, the combined lure trapped fewer Qfly, although overall the treatments were not significantly different. There was a seasonal effect with cuelure alone attracting more flies than the combination lure in February and August and less in March and April. The combined lure lowered the capture of fruit fly attracted to methyl eugenol by 88%. Reasons for the discrepancies between the trials are discussed, as well as the

potential advantages for surveillance for Qfly and exotic fruit flies. Two additional species attracted to the lure combination are noted.

Keywords: Queensland fruit fly, cuelure, methyl eugenol, *Bactrocera*.

Introduction

There are about 4500 species of fruit flies world-wide. In the Pacific area alone, there are 350 species of which at least 25 species are regarded as being of major economic importance (Allwood 2000). The genus *Bactrocera* contains over 400 species, distributed primarily though the Asia-Pacific area including Australia (Drew 1974). Tephritidae fruit flies cause direct losses to many fresh fruit and some vegetable industries, resulting in adverse impacts on trade and potentially to the economies of many countries (Stephenson *et al.* 2003). With the increasing globalization of trade, fruit flies are a major quarantine concern triggering the implementation of regional surveillance programs (Stanaway *et al.* 2001, IAEA 2003, Reid and Malumphy 2009). Males of these species are generally regarded as being attracted to the parafformones cuelure or methyl eugenol (CL and ME respectively) but not both lures.

The National Exotic Fruit Fly monitoring program is deployed in most Australian ports as an early warning program for the entry of exotic fruit flies (Gillespie 2003). At each monitoring site for exotic *Bactrocera* species, separate monitoring traps are baited with either CL or ME (Drew 1974, Cunningham 1989, Gillespie 2003, IAEA 2003). The possible advantages of CL–ME mixtures in wicks has been reported in sub-tropical Australia (Hooper 1978) and in other countries with

different species (Umeya and Hirao 1975, Ito *et al.* 1976, Liu 1989, Vargas *et al.* 2000, Shelley *et al.* 2004). The economic benefit of combining lures in one trap would be considerable. In surveillance programs, each additional trap that requires inspection is a cost in staff and materials to service and administer. In a national stocktake, Oliver (2007) reported that \$128.7 million would be spent on fruit fly related activities in Australia from July 2003 to June 2008. Within this figure, \$34.3 million would be spent on surveillance. In neighbouring New Zealand that has no endemic fruit flies, approximately NZ\$1 million is spent annually on an early detection surveillance program for fruit flies (Stephenson *et al.* 2003). Any improvement in surveillance efficiency is likely to have significant financial benefits for Australia and New Zealand.

The objective of this study was to evaluate the efficacy of the addition of ME to standard CL wicks in attracting Qfly in the dry inland environment and the coastal environment in Sydney. Other species were trapped and are reported here but not analysed.

Materials and methods

Traps, lures and identification

Lynfield traps were used to monitor fruit flies in both locations. These traps consisted of a 1 L cylindrical clear plastic pot with a screw lid. The pots were 120 mm in diameter and 120 mm deep. There were four 2 mm drain holes in the bottom to prevent the accumulation of rainwater. Four equally-spaced 25 mm diameter holes were cut into the side of the trap. These holes allow the egress of pheromone and ingress of insects. Wicks are made using four dental cotton rolls (1 × 4 cm long) held together by metal clamp and suspended from the middle of the trap lid.

Four lure mixtures were evaluated. Treatment A consisted wicks baited with 5 mL of solution containing eight parts CL and one part Maldison (1150 gL⁻¹ active ingredient); this is the standard CL lure in New South Wales. Treatments B and C consisted of wicks baited with Treatment A to which was added either 0.5 or 2.2 mL of ME respectively. In Sydney, Treatment D was available with wicks baited with 2 mL of solution containing eight parts ME and one part Maldison (1150 gL⁻¹ active ingredient). Trapped flies were sent for identification to the Agricultural Scientific Collections Unit at the Orange Agricultural Institute, Orange, New South Wales (NSW). All detections and identifications were recorded on the state database and data retrieved later for analysis (Dominiak *et al.* 2007).

Griffith trials

A trial comparing Treatments A, B and C was established in an orchard near Griffith

in inland NSW. The three treatments were hung on adjacent trees with an average of 5.8 m between traps (range 3.1 to 9.3 m). The treatments were replicated at 10 different sites within the orchard. All traps were inspected 30 times from 19 March 2003 to 30 December 2003. Traps were not inspected in June or July (winter). Treatment D (ME only) was not used in the Griffith trial site since there are no naturally occurring ME-responsive species in that area.

Since wild Qfly are quickly eradicated at Griffith due to trade requirements, a test population of sterile Qfly from a mass rearing strain was released. Flies were mass reared, dyed, irradiated and transported to Griffith under standard protocols established for sterile releases (Dominiak *et al.* 2008). Sterile flies were released four times (5 March, 22 August, 1 November and 5 December 2003) in the orchard. A single release site was used and fruit flies were released using a pupal release technique similar to Dominiak *et al.* (2003a). No additional protein, sugar or water was provided for adults. The GPS coordinates of the release site and trap sites were taken using hand held equipment and the distance from the release point to each trap was calculated. The proportion of flies recaptured in the three weeks following release was calculated. *Dacus newmani* (Perkins) (Newman fly), an Australian native fruit fly came from the local environment. While the trappings are reported here, the results were not analysed as the species is of no economic importance.

Sydney trial

There is an extensive fruit fly trapping array in Sydney maintained as part of the National Exotic Fruit Fly Monitoring program to detect both CL- and ME-responsive species (Gillespie 2003). All flies came from the local environment. We used nine of these trapping sites in the present study. Each experimental site already had two Lynfield traps in separate trees (treatment A and D).

At the nine experimental sites, an additional Lynfield trap was deployed containing a mixture of CL and ME, corresponding to Treatment C above. Traps were inspected 22 times (fortnightly) from 12 January 2007 to 22 October 2007. New CL lures were deployed in January and September 2007 as part of the normal replacement procedure for the program.

Data analysis

In the Griffith experiment, the number of male sterile Qfly (Y) for each trap was fitted with a linear mixed model as follows: $\log_{10}(Y+1) = \text{fixed terms (treatment, release, time after release, distance and all interactions)} + \text{random terms (replicate and its interaction with cue lure and release)}$. All parameters were estimated

using Residual Maximum Likelihood (REML) estimation. All analyses were run on Genstat Windows Version 9 (VSN International Ltd 2006).

For the Sydney data, the number of male wild Qfly (Y) for each trap was fitted using a linear model: $\log_{10}(Y+1) = \text{fixed terms (treatment, fortnight and interactions)}$. Non-significant terms were dropped from the final model. All analyses were carried out in Genstat Versions 13 (VSN international Ltd 2010). Other species were not analysed due to the low numbers trapped.

Results

Griffith trials

The total number of flies recaptured in the Griffith trials is shown in Table 1. While trappings varied greatly between trials, there was no overall significant difference between treatments, but there were significant differences within releases ($P < 0.001$). For example, there were significant differences in capture rates between treatment A and C for the release on 11 November, but not for releases on 5 March, 22 August and 5 December. The distance parameter ($P = 0.60$) and all distance interactions were not significant. Trap catches decreased

as trapping time after release increased ($P < 0.001$). Regarding the proportion of sterile Qfly trapped within three weeks, the first three releases resulted in >91% of total flies trapped in the CL-ME combinations while the CL treatment recaptured <83% in the same period. The fourth release was monitored for only 20 days and was not included in these calculations. *D. newmani* was trapped in the August, November and December release periods in all three treatment lures (Table 1).

Sydney trial

The total number of each species trapped with each lure in the Sydney trial is shown in Table 1. In contrast to the Griffith trial, fewer CL-responsive flies were trapped in mixed lure traps (treatment C) than in CL traps (treatment A) ($P < 0.001$). The treatment by fortnight interaction was significant ($P < 0.001$), indicating different relative trapping rates through the year (see Figure 1). Treatment C trapped more Qfly than treatment A in the March-May period. During winter, both treatments trapped very small numbers of Qfly. In July-September, treatment A attracted more Qfly than treatment C, after which

Table 1. Numbers of each species trapped by each lure for the Griffith and Sydney trials.

Species	Types of lures			
	Treatment A CL (4.4 mL)	Treatment B CL (4.4 mL) + ME (0.5 mL)	Treatment C CL (4.4 mL) + ME (2.2 mL)	Treatment D ME (2.0 mL)
Griffith – CL responsive species				
5 March sterile Qfly release				
<i>B. tryoni</i> (sterile)	350	2042	261	*
<i>D. newmani</i>	0	0	0	*
22 August sterile Qfly release				
<i>B. tryoni</i> (sterile)	1023	2048	5283	*
<i>D. newmani</i>	528	118	260	*
1 November sterile Qfly release				
<i>B. tryoni</i> (sterile)	2223	326	9086	*
<i>D. newmani</i>	128	150	140	*
5 December sterile Qfly release				
<i>B. tryoni</i> (sterile)	1425	2300	2293	*
<i>D. newmani</i>	41	25	54	*
Sydney – CL responsive species				
<i>B. tryoni</i>	4848	*	2648	32
<i>D. aequalis</i> (Coquillett)	104	*	83	0
<i>D. absonifacies</i> (May)	74	*	45	2
Sydney – ME responsive species				
<i>B. cacuminata</i> (Hering)	0	*	277	2267
<i>B. endiandrae</i> (Perkins and May)	0	*	0	1

* = no data

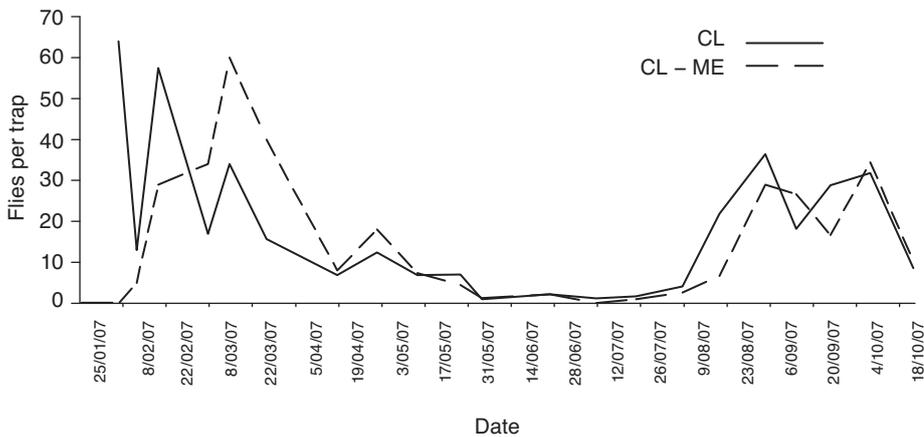


Figure 1. Trappings in Sydney of Qfly into cuelure and the cuelure-methyl eugenol combination traps.

no treatment was consistently more effective.

For the ME attracted species, the combined lure (treatment C) greatly reduced the numbers of flies trapped. Most notably, the number of *B. cacuminata* (Hering) attracted by the combined lure traps was only 12% of treatment D (ME-only).

Discussion

General

There is a general taxonomic concept that fruit flies are attracted to either CL or ME but not a combination of both lures (Drew 1974), even though both of these lures or their derivatives are plant extracts. More recent publications suggest that different mixture combinations or spatial proximity of different lures may affect trap catches. *B. cucurbitae* (Coquillett) is normally attracted to CL only. Shelley *et al.* (2004) found that the addition of ME placed in the same wick or within 3 m of CL resulted in an increase in the capture of *B. cucurbitae*. Vargas *et al.* (2000) found the response of *B. cucurbitae* to low levels of cross mixtures resulted in significant differences and that the season had a significant effect.

Liu (1989) found a mixture with 10% and 20% ME added to CL was more effective than CL alone at attracting *Dacus cucurbitae*. Hooper (1978) noted a Taiwanese report that reported *D. cucurbitae* trapping almost doubled as a result of adding ME to CL. Of the species normally attracted to CL, Hooper (1978) found that the addition of ME to the CL wick did not significantly decrease the capture of *Dacus tryoni*, *Dacus neohumeralis* (Hardy) or *Callandra aequalis* (Coquillett). However the capture rate was significantly improved when CL and ME lures were hung side by side.

Griffith trials

Our results have some similarities to those of Vargas *et al.* (2000) and Hooper (1978). Like Vargas *et al.*, we found that the

relative numbers of sterile Qfly trapped by the different lure mixtures varied greatly between the seasons. But like Hooper, we found no overall significant effect of the different treatment lures on numbers trapped. Our treatment B was similar to the 10% ME addition to CL tested by Liu (1989) who found a 10% ME mixture was more effective than CL alone. There are a number of possible confounding effects that could be affecting relative trapping rates. Firstly, there could be differences in fly physiology in different seasons affecting the reaction of the flies to lures. Secondly, environmental variation though the year (temperature and/or humidity) could affect the quantity or quality of the volatiles produced by the different mixtures. Differences in the availability of natural food sources could also vary seasonally, affecting fly responses. Thirdly, the responses of the mass reared strain may also be different to that of wild flies due to the genetic effects of adaptation to the mass rearing environment. Overall, the variability between the different trials at Griffith suggests that more trials will be required to identify factors affecting Qfly trapping rates.

Nevertheless for Qfly, treatment C did not result in a significant decrease in sterile Qfly numbers in three of the four evaluations. We infer that using this CL-ME mixture for Qfly is unlikely to have any detrimental impact on catches. However, for treatment B, there was a notable decrease in Qfly trapped in the third release, lending caution to the conclusion that ME has no detrimental impact on catches.

In our evaluations, a small number of traps caught most of the flies. This clumping effect was independent of distance (at distances up to 55 m) and was similar to the findings of Horwood and Keenan (1994) and Meats (2007). Meats (2007) reported that wild and sterile Qfly had clumped distributions, particularly at low densities.

We found that trappings did not vary significantly over short distances from the release point, i.e. within 55 m of the release point. Our results are consistent with Weldon and Meats (2007) who found no significant trend in the recapture rate with distance from release point up to 88 m. Fletcher (1974) however, proposed a rule that the number of the flies captured was proportional to the inverse distance from release point. Weldon and Meats (2007) suggested that Fletcher's rule probably became operational at some point after 100 m from the release point. Meats and Edgerton (2008) reconciled both short and longer distance trapping results by showing that a long-tailed (Cauchy) distribution provides an adequate dispersal model for all distances up to 1000 m.

Dacus newmani were trapped in the August, November and December periods but not in March. Our results are consistent with Gillespie (2003) who reported that this species has a major flight in spring and was captured in small numbers at other times of the year. Our report appears to be the first peer reviewed report of *D. newmani* being attracted to the CL-ME combination. The addition of ME to CL attracted very few non-target species. This would be a positive outcome if the lure combination was adopted as an enhanced male attractant. The trapping of large numbers of non-target species is an undesirable attribute of wet protein traps (Dominiak *et al.* 2003b, Dominiak 2006).

Longevity of sterile flies in the field is a significant issue impacting on the frequency of release. Some species survive less than a week and require weekly releases (Hernandez *et al.* 2007). The March and November releases for CL attracted 82.5% and 71.3% respectively (within three weeks) of the total treatment catch. This is consistent with Dominiak and Webster (1998) who reported 85.7% recaptured after three weeks. The CL-ME combinations seem to attract more flies within the 21 day period compared with CL alone in the March and November releases. Given the perception that the ME plume travels a longer distance than the CL plume, we suggest that the addition of ME might attract more flies from longer distances more quickly compared with CL alone. This could be an advantage for the trapping out technique to quickly deplete a population, prior to a sterile release deployment. This chemical combination could also be useful in the male annihilation technique. Vargas *et al.* (2000) found the combination lure lasted well in fibre-board discs in the field.

Sydney trial

The Sydney trial contrasted with the Griffith trial. In Sydney, the mixed lure traps caught only half of the number of Qfly which were trapped in CL traps. We can

only speculate the reasons for these differences. The environmental conditions in the drier inland may create a different result compared with the moister environment of the Sydney basin (our results) or the Queensland coast (Hooper 1978, Dominiak *et al.* 2006). Alternatively the difference between the trials may be due to strain differences: sterile flies were used in the Griffith trial and the wild flies were trapped in the Sydney trial. Weldon and Meats (2010) reported no significant differences in the capture of sterile and wild flies in Sydney, but that result may be relevant to the harsher inland environment. Our Sydney trial and that of Hooper (1978) were conducted in humid coastal environment. Hooper used lower amounts of lure (1.5 mL of CL and ME) than the present trials.

The range of species trapped in this trial was consistent with those reported for Sydney by Osborne *et al.* (1997) and Gillespie (2003). This trial showed that treatment C attracted CL responsive species (Qfly, *D. aequalis* and *D. absonifacies*) but only attracted 10% of the ME responsive *B. cacuminata* compared with ME alone. Hooper (1978) found that captures of *B. cacuminata* were reduced by the CL–ME mixture in comparison to ME alone. Shelly *et al.* (2004) also found the same asymmetry between CL and ME responsive species. They speculated that this may indicate that ME response evolved later in Dacinae than CL response. Since *B. cacuminata* is not of economic importance this reduction should not influence the use of combined traps for surveillance. However, since some economically important exotic *Bactrocera* species are ME-responsive, this aspect requires further investigation. As in the Griffith trial, the CL–ME mixture attracted very few non-target species.

Variation between trials

Overall, our results show that relative effectiveness of different lures was dependent on season and location. Fitt (1983) found the response of male *Dacus opiliae* (Drew and Hardy) to methyl eugenol traps varied with seasonal patterns of humidity associated with 'wet' and 'dry' seasons. Recent research has shown that the attractiveness of CL can be improved by the addition of other compounds. Apart from ME as discussed earlier, Khoo and Tan (2000) reported that zingerzone added to CL had potential to improve the monitoring of *B. cucurbitae*. More research is required before the CL–ME mixture can be recommended as a replacement for the standard CL monitoring lure for Qfly or Newman fly. In the Australian context, our results are consistent with Hooper (1978) indicating that *B. tryoni* and *D. aequalis* were attracted to the CL–ME combination. This paper appears to be the first to report that *D. newmani* and *D. absonifacies* are

attracted to the CL–ME combination. Any improvement in surveillance efficiency is likely to have significant financial benefits for all countries monitoring fruit flies.

Additionally, the CL–ME lure combination could also be useful in the male annihilation technique in drier inland areas (Dominiak *et al.* 2009) and is worthy of additional research. Vargas *et al.* (2000) found the combination lure lasted well in fibreboard discs in the field. Our results indicate that, on occasion, large numbers of CL-responsive flies are attracted to mixed lure traps. However, that response was highly variable and we know little about the factors leading to the highly clumped distribution of Qfly in that region. The CL–ME combination in monitoring and male annihilation is worthy of further research.

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