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Methyl-isoeugenol, a significantly more attractive male lure for the methyl eugenol-responsive Pacific fruit fly, *Bactrocera xanthodes* (Diptera: Tephritidae)

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Abstract *Bactrocera xanthodes* (Broun) (Dacinae), the Pacific fruit fly, is a major pest that is widespread in the South Pacific. It infests the fruit of 34 hosts in 20 families and is a significant impediment to horticultural market access for South Pacific countries. It is weakly responsive to the male attractant methyl eugenol (ME) and consequently is difficult to detect and control. Recently, in Australia and Bangladesh, some weakly ME-responsive species were found to be more attracted to one or more of the eugenol analogues isoeugenol, methyl-isoeugenol and dihydroeugenol. We therefore considered it worthwhile to field test the relative attractiveness of these lures to *B. xanthodes*. Field trials were conducted in Tonga for 9 weeks between September and November 2017. The male attractants isoeugenol, methyl-isoeugenol, dihydroeugenol and a novel eugenol analogue, 1,2-dimethoxy-4-propylbenzene, were tested in comparison to ME, cue-lure and zingerone. More than three times more male *B. xanthodes* were caught in methyl-isoeugenol-baited traps (mean 33.0 ± 1.8 flies/trap/day(FTD)) than in ME traps (10.7 ± 1.0 FTD) with several individual weekly methyl-isoeugenol trap clearances catching over 600 flies. *Bactrocera xanthodes* was also caught in 1,2-dimethoxy-4-propylbenzene traps (mean 3.1 ± 0.5 FTD), but in numbers significantly less than in ME. This is the first record of this species responding to these novel male lures. The significantly greater response of *B. xanthodes* to methyl-isoeugenol would make it a considerably more effective attractant for use in surveillance and control programs.

Key words attractant, fruit flies, pest, phenylpropanoid, semiochemical.

INTRODUCTION

The male fruit fly lures methyl eugenol (ME) and cue-lure (CL) are highly attractive to many species of tropical Dacinae fruit fly, *viz*: *Bactrocera* Macquart, *Dacus* Fabricius and *Zeugodacus* Hendel (Drew 1989). These attractants are important for the management of pest species through male annihilation techniques and trapping to detect invasive pest populations. However, some species are only weakly responsive to these lures, making their detection and control difficult. *Bactrocera xanthodes* is one such species that is weakly attracted to ME (Allwood *et al*. 2002). It is a major pest in the South Pacific and inflicts heavy crop losses and causes trade restrictions (Drew and Romig 1997). Its weak lure response is an issue for its management in the Pacific and for neighbouring countries such as Australia and New Zealand which consider it a high priority biosecurity pest and need to monitor for incursions (e.g. DAWR 2016). Consequently, finding a more effective attractant for *B. xanthodes* is critical to its monitoring and control.

Bactrocera xanthodes is widespread in the South Pacific including Fiji, Samoa, American Samoa, Tonga, Wallis and Futuna and Niue (Leblanc *et al*. 2012). It invaded and established in the Cook Islands in the 1970s and outcompeted the local polyphagous pest *Bactrocera melanota* (Coquillett) (syn. '*B. melanotus*'). It also invaded French Polynesia in the 1990s outcompeting the native species *Bactrocera atra* (Malloch) (Duyck *et al*. 2004). *Bactrocera xanthodes* invaded Nauru but was eradicated in 1999 along with Oriental fruit fly *Bactrocera dorsalis* (Hendel) and melon fly *Zeugodacus cucurbitae* (Coquillett) (Allwood *et al*. 2002; Leblanc *et al*. 2012).

Bactrocera xanthodes has been recorded from 34 host fruit in 20 families. Its main hosts are breadfruit *Artocarpus altilis* (Parkinson) Fosberg, jackfruit *Artocarpus heterophyllus* Lam. (Moraceae), papaya *Carica papaya* L. (Caricaceae), avocado *Persea americana* Mill (Lauraceae), guava *Psidium guajava* L. (Myrtaceae), mango *Mangifera indica* L.(Anacardiaceae), pomelo *Citrus grandis* (L.) Osbeck (Rutaceae) and granadilla *Passiflora ligularis* Juss. (Passifloraceae) (Drew and Romig 1997; Heimoana *et al*. 1997; Purea *et al*. 1997; Vueti *et al*. 1997a, 1997b; LeBlanc *et al*. 2012, 2013). Breadfruit is an important host and its availability on most Pacific islands have made them suitable breeding grounds for *B. xanthodes* (Vueti *et al*. 1997a).

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In host fruit surveys in American Samoa, *B. xanthodes* was found infesting approximately 50% of all breadfruit, avocado, papaya and soursop. In Wallis and Futuna and Fiji, it was recorded infesting approximately 40% and 15% of breadfruit, respectively (Leblanc *et al.* 2013). In Tonga, it was found to infest over 20% of papaya and breadfruit, and it is considered one of the most damaging and aggressive pest species there (Heimoana *et al.* 1997; Leblanc *et al.* 2013). In the Cook Islands, agricultural production is one of the major avenues of earning foreign exchange, but commercial crops such as papaya, oranges, mangoes, grapefruit and guava are target hosts of *B. xanthodes* (Leweniqila *et al.* 1997). As exporting countries, the Cook Islands, Fiji, Samoa and Tonga are required to treat produce for *B. xanthodes* and provide background information on this pest to importing countries, such as New Zealand and Australia, for import risk analyses before produce can be accepted for trade (Cowley *et al.* 1991; Leweniqila *et al.* 1997; DAWR 2018; MPI 2018). Consequently, accurate distribution data gained from male lure trapping is important.

In Fiji, Tonga and the Cook Islands, *B. xanthodes* is widely found throughout the year in orchards and villages where fruit trees such as breadfruit are abundant (Leweniqila *et al.* 1997; Purea *et al.* 1997). In Tonga, populations peak in the summer months of December and January, which coincides with the fruiting of breadfruit (Leweniqila *et al.* 1997). In the Cook Islands populations peak from August to October (Purea *et al.* 1997), and in Fiji populations peak November to June which coincides with fruiting of both breadfruit and its native hosts, *Ochrosia oppisitifolia* (Lam.) K. Schum (Apocynaceae) and *Barringtonia edulis* Seem. (Lecythidaceae) (Vueti *et al.* 1997b). The mean life-cycle parameters for *B. xanthodes* at 26^{o} C are: complete lifecycle (egg to egg) 35 days, egg hatch 44 h, larval development 8 days, pupal development 13 days and adult maturation to egg laying 12 days. In common with other Dacini species, *B. xanthodes* mates at dusk (Clare 1997).

Bactrocera xanthodes has been recorded as weakly responsive to ME, which was thought to be a contributing factor to it being difficult to eradicate on Nauru (Allwood *et al.* 2002). In recent field studies in Australia and Bangladesh, several species that were weakly ME-responsive were trapped in greater numbers at one or more of the eugenol analogues: methyl-isoeugenol, isoeugenol or dihydroeugenol (Royer 2015; Royer *et al.* 2018b). For example, in Bangladesh, the weakly-ME-responsive cucurbit flower pest *Zeugodacus diversus* (Coquillett) was trapped in methyl-isoeugenol baited traps in numbers 50 times greater than in ME traps (Royer *et al.* 2018b). We therefore considered it worthwhile to field test these eugenol analogues against *B. xanthodes*. The purpose of this study was to determine if isoeugenol, methyl-isoeugenol or dihydroeugenol would be more attractive to *B. xanthodes* than ME. Other lures included for comparison were 1,2-dimethoxy-4-propylbenzene ((1,2D4) a eugenol analogue previously found to be an effective attractant for *B. dorsalis* (DeMilo *et al.* 1994)), cue-lure (CL) and zingerone, a newer male lure (Tan and Nishida 2000) that has attracted species that are non-responsive or weakly responsive to CL or ME (Fay 2012; Royer 2015).

MATERIALS AND METHODS

Male lures

Lures were made from two pieces of 2.5 cm long dental wick (Livingstone Int., Rosebury, New South Wales) wired together and dosed at 3 mL lure to 0.5 mL malathion (Hymal 1150 g/L maldison). This dosing rate was chosen as an earlier study in Queensland showed only a 34% loss of ME lure from wicks (2.9 to 1.9 g) over an 8 week period (Queensland Government unpublished data 1998). The Australian Pesticides and Veterinary Medicines Authority (permit 13785 for trapping fruit flies) also recommends a similar dosing rate for CL and ME wicks of 3.5 mL lure to 0.5 mL malathion (1150 g/L), with lures changes at 8 to 12 week intervals. Male lures used were methyl eugenol ((ME) 4-allyl-1,2-dimethoxybenzene, CAS 95-15-2), isoeugenol (2-methoxy-4-propenylphenol, CAS 97-54-1), methyl-isoeugenol (1,2-dimethoxy-4-propenyl-benzene, CAS 93-16-3), dihydroeugenol (2-methoxy-4-propylphenol, CAS 2785-87-7), cue-lure ((CL) 4-(p-acetoxyphenyl)-2-butanone, CAS 3572-06-3) and zingerone (vanillylacetone, CAS 122-48-5) 1,2D4 (1,2-dimethoxy-4-propylbenzene, CAS 5888-52-8). Lures were obtained from Sigma Aldrich, Castle Hill, New South Wales, Australia. Lures were in liquid form except for zingerone, which was crystalline and mixed 2:1 with 100% ethanol to liquefy. Once liquefied, lures were applied with a graduated pipette to the dental wick. Lures were placed in Probodelt® cone traps (Amposta, Spain). These have a yellow funnel shaped base and clear top with four ingress tubes 2.5 cm wide and 6 cm from the top and a flap at the base of the trap for removing flies. Trap dimensions are 15 cm wide at the top, 3 cm wide at the base and 19 cm long. Traps were hung approximately 1.5 m from the ground in shady trees that were fruiting or near fruiting trees (see below). Traps were placed a minimum of 10 m apart (as 3 m separation between CL and ME traps has been shown not to cause an interference effect (Shelly *et al.* 2004)). Lures were not replaced during the trial.

Traps were placed at three locations in the Kingdom of Tonga on the island of Tongatapu: Popua village in a residential site with many breadfruit trees (−21.14537 −175.17071 elevation 6 m), Vaini Agricultural Research Station in a stand of breadfruit amongst mixed agricultural land (−21.20627 −175.19837, elevation 29 m) and ‘Utulau village in a row of breadfruit surrounded by mixed use agricultural land (−21.18066 −175.26975 elevation 21 m) (mean annual rainfall 1700 mm, mean annual temperature 24^{o}C (mean minimum 20^{o}C, mean maximum 27^{o}C)). A randomised complete block design was used, and each site was considered a block consisting of the seven lures. Traps were maintained in the same position as similar environments were used for all traps and sufficient replication was used to smooth out any minor location effects. Traps were set up on 12 September and cleared weekly for nine clearances until the 15 November 2017. Flies were examined under a stereomicroscope and identified by referencing Drew (1989).

Statistical analysis

Each trap catch at each location was considered as an independent experimental unit. Flies/trap were converted to flies/trap/day (FTD) by dividing by the respective days in each clearance. These values were analysed using a generalised linear model (McCullagh and Nelder 1989) with the Poisson distribution and log link, using GenStat (2017). In this model, the standard errors are proportional to the fitted means. An over-dispersed Poisson model was adopted for the species which displayed this feature. The effects of 'location' and 'clearance date' were fitted first to account for patterns in abundance, followed by 'lure'. Interactions were tested but proved to be non-significant so were omitted from the final model. Adjusted means were estimated, and significant differences between the mean catch rates for the lures were obtained using protected pairwise *t*-tests.

RESULTS

Mean captures of *B. xanthodes* in methyl-isoeugenol baited traps were more than three times greater than captures in traps baited with ME. *Bactrocera xanthodes* was also captured in lower numbers in 1,2D4 traps (Fig. 1), but in numbers that were significantly greater than catch at all other lures (12 flies trapped in isoeugenol traps across seven individual trap clearances,

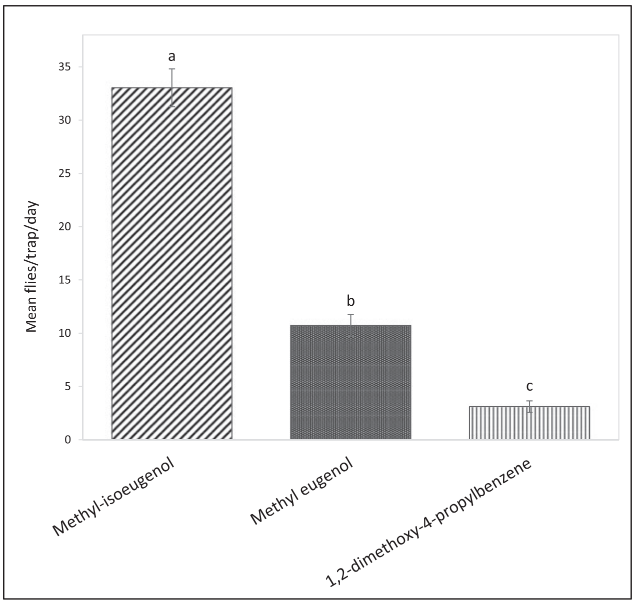

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Fig. 1. Mean daily trap catches (±S.E.) of *B. xanthodes* males at traps baited with three male lures in Tonga 12 September 2017 to 14 November 2017 (deviance ratio = 192.6, $df = 6124$, $P < 0.001$) (low catches at isoeugenol, dihydroeugenol and cue-lure and nil catch at zingerone are not shown – they were significantly lower than these three but not different from each other).
Means with different letters are significantly different.

single flies captured once each in CL and dihydroeugenol and nil flies in zingerone). This is the first record of *B. xanthodes* responding to methyl-isoeugenol or 1,2D4.

Methyl-isoeugenol consistently trapped more *B. xanthodes* than ME over the trapping period (Fig. 2). *Bactrocera xanthodes* numbers peaked in early October with one individual methyl-isoeugenol trap clearance containing 913 *B. xanthodes*. When the methyl-isoeugenol trap at 'Utulau village was being set, about six *B. xanthodes* were seen in the trap in the first half hour with another four on the outside.

The only other species trapped were the pest species *Bactrocera distincta* (Malloch), *Bactrocera facialis* (Coquillett) and *Bactrocera kirki* (Froggatt). All were caught at CL traps only (except for two *B. facialis* at zingerone) and were therefore not analysed further. Means for these species at CL were: *B. facialis* 38.7 FTD, *B. kirki* 7.6 FTD and *B. distincta* 1.3 FTD.

DISCUSSION

Over three times more *B. xanthodes* were captured in methyl-isoeugenol baited traps (33.0 ± 1.8 FTD) than in traps baited with its known lure, ME (10.7 ± 1.0 FTD) (Fig. 1). This is the first record of this species responding to methyl-isoeugenol. Several individual weekly trap clearances of methyl-isoeugenol traps contained 600 to 900+ *B. xanthodes*. A higher mean number of *B. xanthodes* were trapped at methyl-isoeugenol than ME at every clearance over the 9 week trapping period (Fig. 2).

Other fruit fly species have also shown a stronger attraction to methyl-isoeugenol than ME. Captures of *Zeugodacus diversus* (Coquillett), a weakly ME-responsive cucurbit flower pest in Asia (Drew and Romig 2013), were 50 times greater in methyl-isoeugenol baited traps than in ME traps (Royer *et al.* 2018b). In Australia, the weakly ME-responsive non-pest species *Bactrocera barringtoniae* (Tryon), *Bactrocera murrayi*

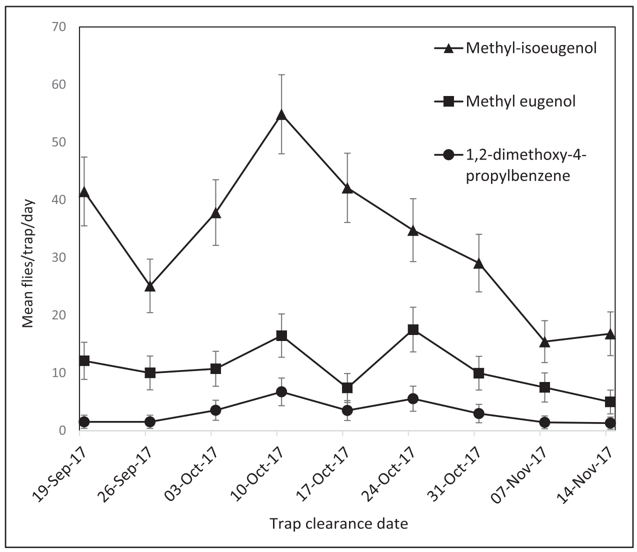

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Fig. 2. Mean *B. xanthodes* male catches at traps baited with three male lures in Tonga (traps set 12 September 2017 and cleared weekly til 14 November 2017).

(Perkins) and *Bactrocera yorkensis* Drew & Hancock were captured in significantly greater numbers in methyl-isoeugenol baited traps than ME traps (Royer 2015). However, the preference for methyl-isoeugenol or ME is species-specific. For example, the ME-responsive Oriental fruit fly, *Bactrocera dorsalis* (Hendel), exhibited a weaker response to methyl-isoeugenol than ME in Hawaii and Papua New Guinea (Steiner 1952; Royer *et al.* 2018a) as did the banana fruit fly, *Bactrocera musae* (Tryon), in Australia and Papua New Guinea (Royer 2015; Royer *et al.* 2018a).

Bactrocera xanthodes was trapped in lower but significant numbers at 1,2D4 (1,2-dimethoxy-4-propylbenzene) (Figs 1 and 2). This compound has a similar chemical structure to ME but with a saturated three carbon primary sidechain. It had previously been found to be a reasonable substitute for ME for *B. dorsalis*, with an equivalent low limit of response, initial attraction and persistence (Mitchell *et al.* 1985), and a statistically similar level of attraction as ME in 7d field tests (DeMilo *et al.* 1994). 1,2D4 was also weakly attractive in field tests to three Australian ME-responsive species (*Bactrocera cacuminata* (Hering), *Bactrocera endiandrae* (Perkins & May) and *B. musae*) (J. E. Royer, 2014, unpublished data).

Bactrocera xanthodes was trapped in low numbers in isoeugenol traps (12 flies across seven weekly trap catches), but this was not significant. Isoeugenol was found to be a more effective attractant (along with methyl-isoeugenol) for the very weakly-ME-responsive Australian species *Bactrocera bidentata* (May), for two CL-responsive pest species, the New Caledonian *Bactrocera curvipennis* (Froggatt) (Royer *et al.* 2019) and the Australian *Bactrocera kraussi* (Hardy), as well as the 'non-responsive' Australian species *Bactocera halfordiae* (Tryon) (Royer 2015).

The only other species trapped were the pest species *Bactrocera distincta* (Malloch), *Bactrocera facialis* (Coquillett) and *Bactrocera kirki* (Froggatt) which were captured in CL traps only, except for two *B. facialis* at zingerone. Elsewhere, zingerone has attracted weakly-CL responsive species (e.g. *Bactrocera jarvisi* (Tryon) in Australia (Fay 2012)) and 'non-responsive' species (e.g. *Bactrocera aglaiae* (Hardy) in Australia and Papua New Guinea (Fay 2012; Royer 2015) and *Bactrocera fulvifacies* (Perkins) in New Caledonia (Royer *et al.* 2019).

Male fruit fly attractants, ME, CL, raspberry ketone and zingerone, occur in many plants that are commonly not hosts of Dacini (Metcalf and Metcalf 1992; Tan and Nishida 2000; Tan and Nishida 2012; Nishida and Tan 2014, 2016; Park *et al.* 2018). Fruit flies seek and feed on plant sources of lures in the wild (Tan 2008) and biotransform or sequester them to later release (with or without endogenously synthesised compounds) as sex pheromones to attract females (Nishida *et al.* 1990; Tan and Nishida 1996; Wee *et al.* 2007; Shelly 2010; Nishida and Tan 2016). Methyl-isoeugenol is also known from fruit fly-attracting plants. It was identified, along with ME and isoeugenol, as a fruit fly-attractive component of citronella oil (from lemongrass *Cymbopogon nardus* (L.) Rendle (Poaceae)) (Howlett 1915). Methyl-isoeugenol occurs in basil *Ocimum tenuiflrom* L. (Lamiaceae), along with ME and isoeugenol, (Vasudevan *et al.* 1999), and the plant is known to attract fruit flies when damaged releasing phenylpropanoids (Tan and Nishida 2012). The fruit fly orchid *Bulbophyllum cheiri* releases methyl-isoeugenol as well as ME (Nishida *et al.* 2004). Of the 450+ plants known to contain ME, over 30 also contain methyl-isoeugenol (Tan and Nishida 2012). While several studies have looked at the role of ME, CL or raspberry ketone in pheromone synthesis, the role of newer eugenol analogues, such as methyl-isoeugenol, is yet to be studied.

Bactrocera xanthodes is a polyphagous pest of commercial fruit in the South Pacific. It is weakly responsive to ME, and consequently, there has been a need for a more effective attractant for it. This study found methyl-isoeugenol to be over three times more attractive to *B. xanthodes* than ME and to consistently trap more flies than ME over the 9 week trapping period. Methyl-isoeugenol would therefore make a significantly improved lure for the monitoring and control of this species.

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