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The responsiveness of *Bactrocera jarvisi* (Diptera: Tephritidae) to two naturally occurring phenylbutaonids, zingerone and raspberry ketone

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ABSTRACT

The males of different species of *Bactrocera* and *Zeugodacus* fruit flies are commonly attracted to plant-derived phenylpropanoids (e.g. methyl eugenol (ME)) or phenylbutanoids (e.g. raspberry ketone (RK)) but almost never to both. However, one particular plant-derived phenylbutanoid, zingerone (ZN), which possesses an intermediate chemical structure between ME and RK, weakly attracts both ME- and RK-responding fruit fly species. *Bactrocera jarvisi*, an Australian fruit fly species, is weakly attracted to cue lure (an analogue of RK) but strongly attracted to ZN. Here, we investigated the minimum olfactory threshold and optimum sensitivity of *B. jarvisi* males to ZN and RK as a function of dose, time and sexual maturation. Our results show that *B. jarvisi* males had a marked preferential response to ZN, with a much lower olfactory threshold and faster response time to ZN than RK. Probit analysis demonstrated that ZN was at least > 1600× more potent than RK as a male attractant to *B. jarvisi*. Although fruit fly male attraction to the phytochemicals is generally associated with sexual maturity, in *B. jarvisi* immature males were also attracted to ZN. Our results suggest that *B. jarvisi* males have a fine-tuned olfactory response to ZN, which appears to play a central role in the chemical ecology of this species.

1. Introduction

The males of Dacini fruit flies (Diptera: Tephritidae, members of the genera *Bactrocera* Macquart and *Zeugodacus* Hendel predominantly) exhibit strong, positive chemotaxis to a small group of plant-derived secondary metabolites (referred to in the wider fruit fly literature, and hereafter, as 'male lures' or just 'lures', because of their history in applied entomology) (Raghu, 2004; Tan and Nishida, 2012). The response of flies to these phytochemicals is so strong that when mixed with a toxicant in a lure-and-kill pest management approach, and used in combination other control tactics such as protein bait spray, they can drive local populations to extinction (Cunningham and Steiner, 1972; Cantrell et al., 2002).

As late as the 1980s why the flies responded to the lures was still unknown (Cunningham, 1989), but it is now widely recognised that the lures are associated with the mating systems of the flies (see reviews by Shelly, 2010; Tan and Nishida, 2012). Lure-fed males gain a competitive mating advantage over unfed males through incorporation of phytochemical-derived compounds into their pheromones which are subsequently more attractive to females (Shelly and Dewire, 1994; Tan and Nishida, 1998; Wee et al., 2007, 2018a). Males may also show increased activity after lure feeding (Kumaran et al., 2014a); while mating with a lure-fed male (in some cases) may also have direct benefits (higher fecundity) (Kumaran et al., 2013) and cause complex indirect effects to females (Kumaran et al., in press). However, as research continues, it is also becoming clear that the lure effect varies between fruit fly species (see e.g. Kumaran et al., 2014a; Shelly, 2017) and within species based on lure (Kumaran et al., 2014b).

Only a few years ago the association between lures and fly response was considered straightforward. Males of a species were thought to only ever respond to one of two lure types, those lures being methyl eugenol (ME) and cue lure (CL) (or raspberry ketone [RK], the hydrolysed form of CL) (Fig. 1), or the species was 'non-lure responsive' (Drew, 1974; Drew and Romig, 2013). Species may have been 'weakly' or 'strongly' attracted to a given lure (Drew, 1989), but nevertheless it was still one or the other. However, increasingly, it is clear that this simple dichotomy is inadequate to capture the complexity of the Dacini lure response, with new attractive chemicals being found (Royer, 2015; Siderhurst et al., 2016; Wee et al., 2018b), and previously 'non-lure responsive flies' being attracted to novel chemicals (Royer et al., 2018).

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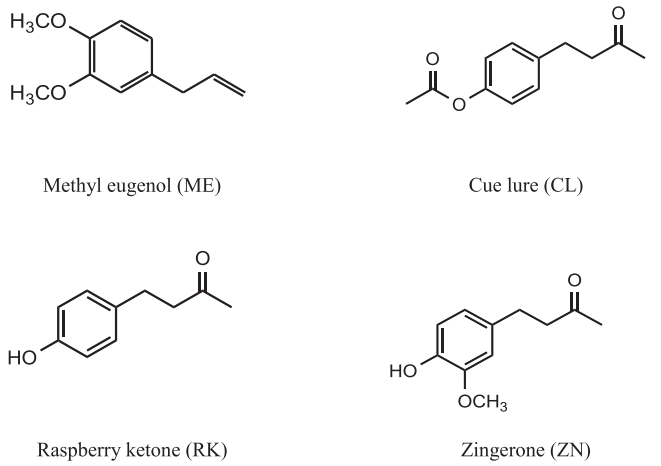

Fig. 1. Chemical structures of methyl eugenol (ME; 3,4-dimethoxy-allylbenzene), cue lure (CL; 4-(p-acetoxyphenyl)-2-butanone), raspberry ketone (RK; 4-(4-hydroxyphenyl)butan-2-one) and zingerone (ZN; 4-(4-hydroxy-3-methoxyphenyl)-2-butanone).

This new line of research can be largely attributed to the discovery that certain orchid species, e.g. *Bulbophyllum patens* and *Bu. baileyi,* attract both ME and CL responsive species (Tan and Nishida, 2000) and that the attractive chemical is the phenylbutanoid zingerone (ZN) (the essence of ginger) (Fig. 1) (Tan and Nishida, 2007). ZN, as a phenylbutanoid, possesses an intermediate chemical structure between ME (a phenylpropanoid) and RK (a phenylbutanoid) explaining, from a chemical structure perspective, its attractiveness to both ME- and RK/CL responsive groups of fruit flies (Tan and Nishida, 2000).

Bactrocera jarvisi (Tryon) (Diptera: Tephritidae) (a.k.a. Jarvis's fruit fly) is an endemic Australian fruit fly distributed across northern Australian and down the Australian east coast to the southern end of the subtropics (Drew, 1989). While recorded in the older literature as either non-lure responsive (Drew et al., 1978) or weakly responsive to CL (Drew, 1989), Fay (2012) reported the species was strongly attracted to ZN, a result confirmed by Royer (2015). As a species which sits between ME and CL/RK responsive species, understanding the biology and physiology of the species' lure response is critical to helping develop a more informed knowledge of the larger lure-response pattern across the Dacini. *Bactrocera jarvisi* is only one of numerous Dacini fruit flies across Asia and the Pacific that are now known to respond to ZN (Tan and Nishida, 2000, 2007; Fay, 2012; Royer, 2015; Royer et al., 2018), so detailed research on a predominantly ZN responsive species fills a key gap in fruit fly male lure research.

In this paper, we investigate the lure selectivity and sensitivity of *B. jarvisi* males towards the two naturally-occurring phenylbutanoids, RK and ZN, in association with male sexual maturation. Probit analysis (Finney, 1971) is used to determine the minimal response threshold and optimum sensitivity of the fly to each of the lures. In addition, we investigate the temporal aspect of phytochemical lure perception, i.e. total time taken to elicit a positive response to lure, to gain more insight into *B. jarvisi*'s fruit fly-lure interactions. This is the first paper of an intended series, which aims to build a comprehensive understanding the chemical ecology of this ZN responsive fruit fly.

2. Materials and methods

2.1. Insects

Bactrocera jarvisi of 6–8th generation (reared from field-infested mangoes) were obtained as pupae from the [Queensland] Department of Agriculture and Fisheries, Brisbane, Queensland. Emerged flies were provided with protein hydrolysate and sugar mixture (ratio 3:1) and water *ad libitum*. The rearing room was illuminated with fluorescent lighting between 07:00 and 16:00 hr daily in addition to natural sunlight. Except for flies used for the sexual maturity experiment, which were sex-segregated within a few hours of emergence, all flies were sexed within two days of emergence and maintained separately in screen cages (40 × 40 cm) at 27 °C and 70% RH. Sexually mature virgin flies (14–21 day-old) were used for all studies unless otherwise stated.

2.2. Chemicals

Based on the results of a preliminary test, zingerone (ZN; 4-(4-hydroxy-3-methoxyphenyl)-2-butanone; CAS 122-48-5) and raspberry ketone (RK; 4-(4-hydroxyphenyl)butan-2-one; CAS 5471-51-2) (both > 96% purity, Sigma-Aldrich) were diluted serially in absolute ethanol (≥99.8% purity; Sigma-Aldrich) into the desired concentrations (ZN: 10, 50, 100, 400, 800 ng/10 µl; RK: 25, 50, 100, 300, 500, 1000 µg/10 µl) for Probit bioassays. For age-dependent lure response bioassays, 500 µg/10 µl of RK or ZN was used.

2.3. Sexual maturation

One-hundred each of first-day emerged *B. jarvisi* males and females, after full-body colouration had developed, were placed in a medium size screen cage (40 × 40 × 40 cm) with food and water *ad libitum*. Daily observations for mating pairs were conducted after scotophase (between 20:30–21:30 hr) under dimmed red light conditions until the experimental flies were 50 days old. As soon as a pair settled in mating, the pair *in copula* was carefully coaxed into a specimen vial and removed from the cage. The experiment was replicated four times. At the end of the 50-day observation period, a graph of cumulative mating percent was plotted as a function of age after adult emergence. An explicit assumption was made that mating is directly correlated with sexual maturation attainment and that the sexual maturation development rate was the same in both sexes, as has been done elsewhere (Ooi and Wee, 2016; Wee et al., 2018a,b). This approach may slightly overestimate the time required for flies to reach sexual maturity (i.e. if flies are sexually mature for one or more days before mating), but it cannot underestimate the time taken to reach sexual maturity as sexually immature *Bactrocera* do not mate.

2.4. Olfactory threshold determination via Probit analysis

The response of male *B. jarvisi* to either ZN or RK was evaluated based on a precise evaluation method via the complete sequential total male lure reflex, which involves a sequential behavioural attraction, arrestment and feeding (hereafter referred to as complete sequential reflex) (Metcalf et al., 1979). A positive response was scored when an individual male, activated by the presence of the test chemical, approached by an oriented zig-zag flight, landed and fed on the lure source within a 10-minute bioassay duration. This zig-zag flight was readily observed in the observation arenas.

Lure response bioassays were conducted in the morning between 09:00–11:30 hr in a laboratory which was illuminated with fluorescent lighting in addition to natural sunlight received through glass doors and windows. Room temperature and relative humidity were maintained between 22 and 25 °C and 65–70%, respectively. During each trial a group of 20 male flies, housed in a screen cage (20 × 20 × 30 cm), was assayed for their response to increasing quantities of either ZN or RK which was tested on different days. For each bioassay, 10 µl of lure solution was dispensed onto a 3.5 cm diam. filter paper that was placed in a 3.5 cm diam. disposable Petri dish using a pre-calibrated glass pipette (Drummond®, USA).

A male fruit fly's sensitivity to a chemical attractant can be measured both as a function of dose (dose-response via Probit analysis) as well as a function of time, i.e. time taken to complete the sequential response reflex (hereafter known as response time). We further differentiated the response time into two categories, i.e. the minimum time