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Comparative Trap Catches of Male *Bactrocera, Dacus,* and *Zeugodacus* Fruit Flies (Diptera: Tephritidae) With Four Floral Phenylbutanoid Lures (Anisyl Acetone, Cue-Lure, Raspberry Ketone, and Zingerone) in Queensland, Australia

Jane E. Royer,[1,3] Keng Hong Tan,[2] and David G. Mayer[1]

[1]Department of Agriculture and Fisheries, PO Box 267, Brisbane, Queensland 4000, Australia, [2]Mobula Research Sdn. Bhd., 20, Jalan Tan Jit Seng, 11200 Penang, Malaysia, and [3]Corresponding author, e-mail: jane.royer@daf.qld.gov.au

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Abstract

The male fruit fly attractants, cue-lure (CL) and raspberry ketone (RK), are important in pest management. These volatile phenylbutanoids occur in daciniphilous *Bulbophyllum* Thouar (Orchidaceae: Asparagales) orchids, along with zingerone (ZN) and anisyl acetone (AA). While these four compounds attract a similar range of species, their relative attractiveness to multiple species is unknown. We field tested these compounds in two fruit fly speciose locations in north Queensland, Australia (Lockhart and Cairns) for 8 wk. Of 16 species trapped in significant numbers, 14 were trapped with CL and RK, all in significantly greater numbers with CL traps than RK traps (at least in higher population locations). This included the pest species *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) (CL catches ca. 5× > RK), *Bactrocera neohumeralis* (Hardy) (Diptera: Tephritidae) and *Bactrocera bryoniae* (Tryon) (Diptera: Tephritidae) (CL catches ca. 3× > RK), and *Bactrocera frauenfeldi* (Schiner) (Diptera: Tephritidae) (in Cairns—CL catches ca. 1.6× > RK). Seven species were trapped with AA, and all were also caught in CL and RK traps in significantly greater numbers, with the exception of *B. frauenfeldi*. For this species, catches were not statistically different with CL, RK, and AA in Lockhart, and RK and AA in Cairns. Seven species were trapped with ZN, two at this lure only, and the remainder also with CL or RK but in significantly greater numbers. This is the first quantitative comparison of the relative attractiveness of CL, RK, AA, and ZN against multiple species, and supports the long-held but untested assumption that CL is broadly more attractive lure than RK.

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

Male fruit fly attractants, such as cue-lure (CL), raspberry ketone (RK), and methyl eugenol (ME), are important in the management of pest species in the tribe Dacini (Diptera: Tephritidae: Dacinae) through male annihilation and trapping to detect invasive species. CL and RK are phenylbutanoids, with a four-carbon primary substituent (a 'butanone' side chain), when compared with phenylpropanoids such as ME, which have a three-carbon primary substituent (an 'allyl' side chain). Dacini species are generally attracted to either phenylbutanoids or phenylpropanoids, but not both (Drew 1974, IAEA 2003, Tan et al. 2014, Royer 2015).

Phenylbutanoids and phenylpropanoids are volatile aromatic compounds that occur naturally in many plants, but most notably in fruit fly attracting ('daciniphilous') *Bulbophyllum* Thouars and *Dendrobium* Sw. (Orchidaceae) orchids (Nishida and Tan 2016). Male fruit flies are attracted to such wild sources and feed on them to produce pheromones, allomones against vertebrate predation, increase mating competitiveness or boost metabolism (Nishida et al. 1990a, b; Tan and Nishida 1996; Khoo and Tan 2000; Tan 2000; Wee et al. 2007; Shelly 2010; Kumaran et al. 2014b). In return, the orchid gains sexual benefits through cross pollination, by enticing the fruit fly pollinator to remove, transport, and deposit pollinia from another flower (Tan and Nishida 1998, Tan 2009, Tan and Tan 2018).

Daciniphilous orchids normally produce either phenylbutanoids (namely AA [anisyl acetone], CL, RK, ZN [zingerone]) or phenylpropanoids (such as ME, 2-allyl-4,5-dimethoxyphenol, *E*- and *Z*-methyl isoeugenol and *E*-coniferyl alcohol) (Nishida and Tan 2016). These compounds occur in flowers in relatively minute quantities which are still highly attractive to fruit flies (Tan and Nishida 2000, 2005, 2007; Nakahira et al. 2018; Katte et al. 2020).

The phenylbutanoids, particularly AA, CL, RK, and ZN, have been found singly or in combination in several orchid species (Tan and Nishida 2000, 2005, 2007; Nishida and Tan 2016; Nakahira et al. 2018; Katte et al. 2020). They attract a similar range of fruit fly species when used separately in traps. CL attracts the same range of species as RK (Drew 1974, Drew and Hooper 1981), and ZN and AA attract several CL/RK-responsive species (Tan and Nishida 2000, Tan et al. 2014, Royer 2015). CL is more widely used in pest monitoring and management due to its reported greater attractiveness than RK, but this assumption appears to be based only on quantitative data for the melon fly *Zeugodacus cucurbitae* (Coquillett) (e.g., Beroza et al. 1960) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Monro and Richardson 1969). Comparative field testing of the relative attractiveness of these lures is needed to 1) confirm if CL is more attractive than RK across a range of species and 2) understand the differential field responses of Dacini fruit fly species to phenylbutanoid floral attractants for further enhancing the potential application of floral volatiles as fruit fly lures.

The purpose of this study was to assess the relative attractiveness of four floral phenylbutanoids, in relatively low quantities (closer to what is found in orchids [Tan and Nishida 2000, 2005, 2007; Nakahira et al. 2018; Katte et al. 2020], rather than higher quantities used in monitoring programs [FAO/IAEA 2018]) in Dacini-speciose regions of Australia. CL, RK, ZN, and AA were field tested in Lockhart River and Cairns in Queensland, Australia for 8 wk in the summers of 2018 and 2019.

Materials and Methods

Male Lures

Lures were made from one piece of 3.8 cm long dental wick (Livingstone Int., Rosebury, New South Wales) dosed at 0.5 ml (0.5 g for crystalline lures) lure to 0.08 ml malathion (Hy-Mal, 1150g/liter malathion). This rate was chosen to be closer to the ca. 10–100 µg of phenylbutaoids found in flowers (Tan and Nishida 2000, 2005, 2007; Nakahira et al. 2018; Katte et al. 2020), compared with the 2–3 ml used in trapping programs (FAO/IAEA 2018), while still maintaining attractiveness for the 8 wk trapping period (given CL's higher volatilization rate of 0.62 mg/day at 27°C, compared to RK's rate of 0.02 mg/day at 27°C [Metcalf and Metcalf 1992]). Male lures used were cue-lure (CL) (4-(*p*-acetoxyphenyl)-2-butanone, 99.4 % purity CAS 3572-06-3), raspberry ketone (RK) (≥98% purity CAS 5471-51-2), zingerone (ZN) (= vanillylacetone) (≥ 96% purity vanillylacetone, CAS 122-48-5), and anisyl acetone (AA) (≥ 98% purity CAS 104-20-1). The four different lures have different vapor pressures, but this has been found to be less important than chemical structure in fruit fly attraction (Park et al. 2016). Chemicals were obtained from Sigma Aldrich, Castle Hill, New South Wales, Australia. CL and AA were in liquid form and ZN and RK which were crystalline and mixed 3:1 with 100% ethanol to liquefy. Once liquefied, lures were applied with a graduated pipette to the dental wick. Lures were placed in Steiner traps (1 liter clear cylinder hung horizontally with 2.5-cm-wide ingress tubes on the flat sides) and hung in shady trees approximately 1.5 m from the ground.

Field Trials

Traps were placed at two locations in north Queensland, Australia: Lockhart River and Cairns. These sites were chosen to test the lures in areas with different fruit fly species assemblages (Drew 1989) and species populations (Royer 2015, unpublished data).

Lockhart River is on Cape York Peninsula (−12.79 143.30) at an altitude of 17 m, with mean annual rainfall of 2,047 mm and mean annual temperature 25.9°C (minimum 22.0°C, maximum 29.8°C) (BOM 2019a). Cairns is in north Queensland (−16.87 145.75) at an altitude 2 m, with a mean annual rainfall of 1,987 mm and mean annual temperature 25.0°C (minimum 20.8°C, maximum 29.1°C) (BOM 2109b). Traps were placed in rainforest or urban areas at locations that had previously trapped a variety of Dacini species (Royer 2015).

At each location, traps were placed at five sites. A randomized complete block design was used and each site was considered a block consisting of four traps baited with each of the lures. Traps within blocks were placed 30 m apart and blocks were a minimum of 300 m apart. At both sites traps were cleared weekly for eight clearances (Lockhart River from 13 December 2017 to 5 February 2018 and Cairns from 14 January 2019 to 11 March 2019). Traps were rotated each week to avoid any positional effect. Traps were hung approximately 1.5 m from the ground in shady trees that were host trees or near hosts such as *Terminalia catappa* L. (Combretaceae) and mango, *Mangifera indica* L. (Anarcardiaceae). Flies were examined under a stereomicroscope and identified by referencing Drew (1989).

Statistical Analysis

Analyses were conducted using generalized linear mixed models (GLMM) in GenStat (2016), adopting restricted maximum likelihood (REML). The Poisson distribution (standard or over-dispersed, as determined for each species) with the log link function was adopted for these analyses. Overall significance between the lures was determined using the Wald ratio (WR), which is the Wald statistic divided by the degrees of freedom. The fixed effects were lure, site, week of sampling (as trends—none, linear, or quadratic, depending on significance), and where appropriate the 'site.lure' and 'site.week' interactions. The random effects were the blocks and clearances, with the temporal correlations between successive clearances accounted for under an autoregressive (of order one) error structure. The variance components were restricted to not permit negative estimates. Cross-site analyses were preferred except where there was insufficient data at one site, in which case within-site analyses were quoted. The standard errors for each mean were formed on the link (natural logarithm) scale, and back-transformed to counts forming asymmetrical ranges. These were averaged to provide an approximate standard error for each mean. Significant differences between the treatment mean catch rates were determined by protected pairwise *t*-tests.

Results

Twenty species were trapped at both locations and all lures in numbers sufficient to analyze: 14 species in Cairns and 17 species in Lockhart River (Tables 1 and 2). Of these, four were trapped in insignificant numbers (*Bactrocera aeroginosa*, *Bactrocera manskii*, *Bactrocera rufofuscula*, and *Dacus bellulus*) and 16 were trapped in significant numbers in at least one location. The following results relate to the latter.

The 14 species trapped at both CL and RK were trapped in significantly greater numbers in CL traps than in RK traps in at least one site. For *B. frauenfeldi*, catches with CL were significantly greater than with RK in Cairns but not in Lockhart (a much lower

Table 1. Mean weekly trap catches of nonpest species with four phenylbutanoid male lures in Cairns (14 Jan. to 11 Mar. 2019) and Lockhart River (13 Dec. 2017 to 5 Feb. 2018)

	Cairns		SE	Lockhart		SE
Bactrocera abscondita (Drew & Hancock)	(WR[a] = 77.9; $P < 0.001$)					
AA	0.00	a	0.00	0.16	c	0.06
CL	0.37	a	0.23	27.99	a	9.74
RK	0.13	a	0.08	5.43	b	1.89
ZN	0.00	a	0.00	0.00	c	0.00
Bactrocera aeroginosa (Drew & Hancock)	(WR = 2.03; $P = 0.108$)					
AA	0.00	a	0.00	0.02	a	0.02
CL	2.00	a	0.33	0.54	a	0.49
RK	1.70	a	0.28	0.17	a	0.15
ZN	0.00	a	0.00	0.00	a	0.00
Bactrocera aglaiae (Hardy)	($t = 2.78$; $P < 0.001$)[b]					
AA	0.00	b	0.00	0.00	b	0.00
CL	0.00	b	0.00	0.00	b	0.00
RK	0.00	b	0.00	0.00	b	0.00
ZN	0.30	a	0.11	3.01	a	2.78
Bactrocera alyxiae (May)	(WR = 10.78; $P < 0.001$)					
AA	0.17	a	0.13	2.92	c	0.45
CL	4.75	a	3.66	115.93	a	17.94
RK	3.35	a	2.57	54.98	b	8.51
ZN	0.00	a	0.00	0.04	c	0.01
Bactrocera antigone (Drew & Hancock)	(WR = 40.26; $P < 0.001$)					
AA	NA			0.15	c	0.05
CL	NA			9.83	a	3.60
RK	NA			3.97	b	1.45
ZN	NA			0.00	d	0.00
Bactrocera breviaculeus (Hardy)	(WR = 22.45; $P < 0.001$)					
AA	0.00	b	0.00	0.00	b	0.00
CL	7.32	a	0.69	0.56	a	0.20
RK	2.58	b	0.24	0.16	b	0.06
ZN	0.00	b	0.00	0.00	b	0.00
Bactrocera erubescentis (Drew & Hancock)	(WR = 9.22; $P < 0.001$)					
AA	NA			0.06	b	0.05
CL	NA			17.98	a	15.68
RK	NA			2.79	b	2.43
ZN	NA			0.00	b	0.00
Bactrocera manskii (Perkins & May)	(WR = 1.82; $P = 0.141$)					
AA	NA*			0.00	a	0.00
CL	NA*			0.71	a	0.15
RK	NA*			0.35	a	0.07
ZN	NA*			0.00	a	0.00
Bactrocera peninsularis (Drew & Hancock)	(WR = 10.39; $P < 0.001$)					
AA	NA*			0.00	b	0.00
CL	NA*			1.20	a	0.27
RK	NA*			0.21	b	0.05
ZN	NA*			0.00	b	0.00
Bactrocera perkinsi (Drew & Hancock)	(WR = 17.39; $P < 0.001$)					
AA	NA			0.13	c	0.04
CL	NA			4.60	a	1.61
RK	NA			1.78	b	0.62
ZN	NA			0.00	c	0.00
Bactrocera quadrata (May)	(WR = 5.9; $P < 0.001$)					
AA	0.00	c	0.00	0.00	b	0.00
CL	1.38	a	0.24	0.47	a	0.21
RK	0.60	b	0.11	0.07	b	0.03
ZN	0.00	c	0.00	0.00	b	0.00
Bactrocera rufofuscula (Drew & Hancock)	(WR = 0.22; $P = 0.879$)					
AA	NA			0.00	a	0.00
CL	NA			0.10	a	0.05
RK	NA			0.05	a	0.03
ZN	NA			0.00	a	0.00

Table 1. Continued

	Cairns		SE	Lockhart		SE
Dacus bellulus Drew & Hancock	(WR = 1.89; *P* = 0.129)					
AA	0.00	a	0.00	NA		
CL	0.23	a	0.16	NA		
RK	0.03	a	0.02	NA		
ZN	0.00	a	0.00	NA		
Zeugodacus fallacis (Drew)	(WR = 6.24; *P* < 0.001)					
AA	0.06	b	0.04	0.02	c	0.02
CL	0.35	a	0.24	0.81	a	0.57
RK	0.00	c	0.00	0.28	b	0.20
ZN	0.00	c	0.00	0.00	d	0.00
Zeugodacus strigifinis (Walker)	(WR = 10.6; *P* < 0.001)					
AA	0.03	c	0.01	0.00	c	0.00
CL	5.04	a	1.05	8.54	a	1.58
RK	3.28	a	0.68	2.74	b	0.51
ZN	0.12	b	0.03	0.00	c	0.00

NA = not trapped, generally outside known range (Hancock et al. 2000); NA* = trapped in insufficient numbers to analyse; *n* = 40 (each lure at each location); within locations, means within species with different letters are significantly different.

[a]WR = Wald Ratio as defined in Materials and Methods—Statistical analysis.

[b]Analyzed ZN data only, and *t*-test compares ZN capture rate to zero which occurred for all other lures.

Table 2. Total trap catches of species caught in low numbers (insufficient to statistically analyze) at four phenylbutanoid lures in Cairns (14 Jan. to 11 Mar. 2019) and Lockhart River (13 Dec. 2017 to 5 Feb. 2018)

	Lure			
Species	AA	CL	RK	ZN
Bactrocera aurea (May)				5 (Lockhart)
Bactrocera bryoniae (Tryon)		1 (Lockhart)		
Bactrocera frauenfeldi (Schiner) **female**[a]	1- Cairns		4 (Cairns)	
Bactrocera manskii (Perkins & May)		2 (Cairns)	1 (Cairns)	
Bactrocera peninsularis (Drew & Hancock)		3 (Cairns)		
Bactrocera silvicola (May)		1 (Cairns)		
Bactrocera speewahensis Fay & Hancock				1 (Cairns)
Bactrocera tryoni (Froggatt)		3 (Lockhart)		
Dacus axanus (Hering)		2 (Cairns) 2 (Lockhart)	1 (Cairns)	
Dacus hardyi Drew		1 (Cairns)		
Dacus sp. nr. *pusillus*				7 (Lockhart)
Zeugodacus choristus (May)		1 (Cairns)		

[a]Only species with females captured.

catching site) (Fig. 2), and for *Zeugodacus strigifinis* catches with CL were significantly greater than with RK in Lockhart but not Cairns (Table 1). No species were trapped in significantly greater numbers in RK than CL. For several of the CL/RK species, including the pest species *B. bryoniae*, *B. frauenfeldi*, *B. neohumeralis*, and *B. tryoni*, catches with CL were 2–5 times greater than with RK (Figs. 1, 2, 4, and 5).

Eleven species were caught in AA traps (but only seven in significant numbers), all of which were also caught in CL and/or RK traps, but in significantly greater numbers (Table 1; Figs. 1, 4, and 5), with the exception of *B. frauenfeldi*. For *B. frauenfeldi* in Cairns, catches in AA traps and RK traps were not significantly different (although catches were greater in AA traps), and in Lockhart, catches in AA, RK, and CL traps were not significantly different (Fig. 2).

Seven species were caught in ZN traps. Two species were caught in ZN traps only (*Bactrocera aglaiae* and *Bactrocera jarvisi*) and the remaining five were also caught at CL and RK traps, but in significantly greater numbers (Table 1; Fig. 3).

Total captures of the remaining species caught in numbers insufficient to analyze are shown in Table 2. This includes the only captures of females at the male lures (*B. frauenfeldi*).

Discussion

Fourteen species were captured in significantly greater numbers at CL than RK, in at least one location, and no species was caught in greater numbers in RK than CL (Table 1; Figs. 1, 2, 4, and 5). Of the pest species, catches of 1) *B. tryoni* with CL were ca. 5× greater than with RK, 2) *B. neohumeralis* and *B. bryoniae* with CL were ca. 3× greater than with RK, and 3) *B. frauenfeldi* with CL in Cairns (the location with greater catches) were ca. 1.6× greater than with RK (Table 1). This is the first record of CL's greater attractiveness than RK across a wide range of Dacini fruit fly species.

CL has previously been reported to trap twice as many melon fly (*Z. cucurbitae*) than RK (Beroza et al. 1960) and 1.5× times as many *B. tryoni* than RK (Monro and Richardson 1969). This is

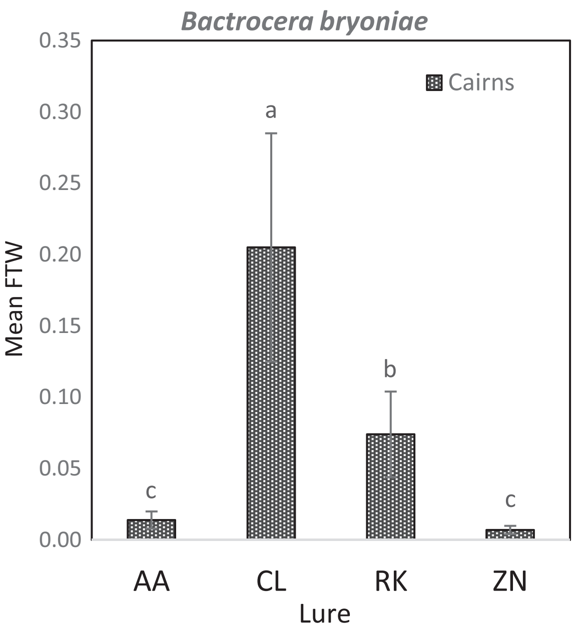

Fig. 1. Mean weekly trap catches of *Bactrocera bryoniae* with four phenylbutanoid male lures in Cairns (14 January to 11 March 2019) (insufficient trapped in Lockhart to analyze) (WR = 8.66; $P < 0.001$). WR = Wald Ratio as defined in Materials and Methods—Statistical analysis. $n = 40$ (each lure at each location). Within locations, means within species with different letters are significantly different.

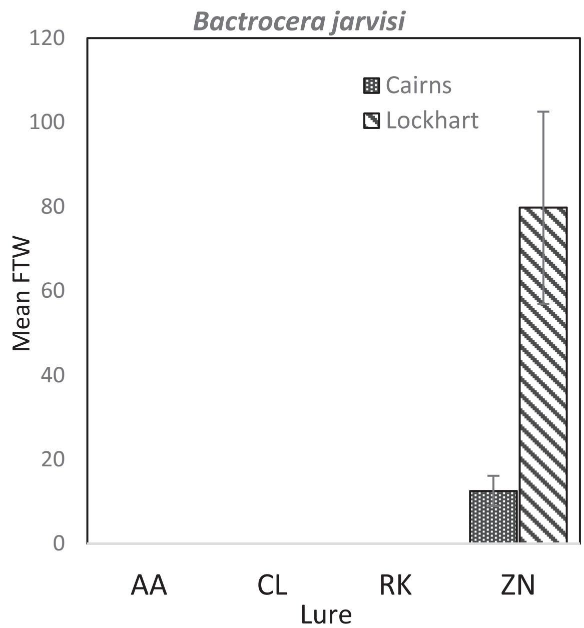

Fig. 3. Mean weekly trap catches of *Bactrocera jarvisi* with four phenylbutanoid male lures in Cairns (14 January to 11 March 2019) and Lockhart River (13 December 2017 to 5 February 2018) ($t = 3.50$; $P < 0.001$). Analyzed ZN data only, and *t*-test compares ZN capture rate to zero which occurred for all other lures. $n = 40$ (each lure at each location). Within locations, means within species with different letters are significantly different.

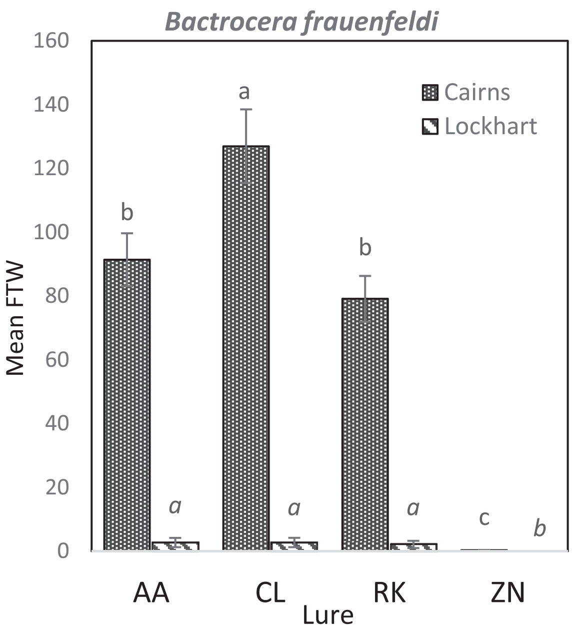

Fig. 2. Mean weekly trap catches of *Bactrocera frauenfeldi* with four phenylbutanoid male lures in Cairns (14 January to 11 March 2019) and Lockhart River (13 December 2017 to 5 February 2018) (WR = 10.43; $P < 0.001$). WR = Wald Ratio as defined in Materials and Methods—Statistical analysis. $n = 40$ (each lure at each location). Within locations, means within species with different letters are significantly different.

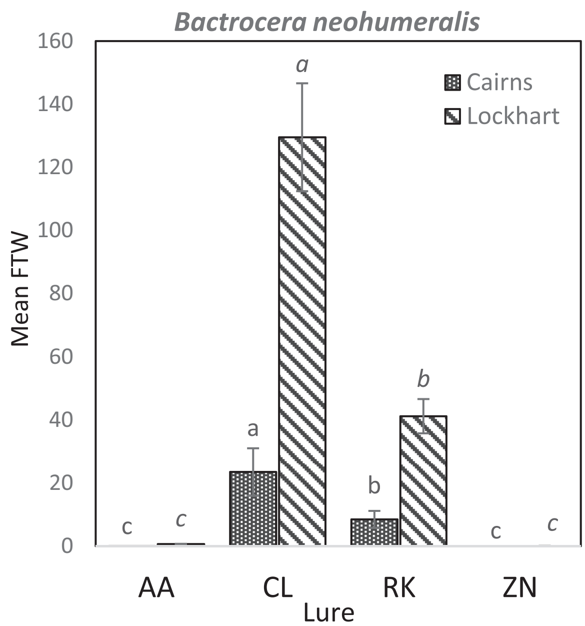

Fig. 4. Mean weekly trap catches of *Bactrocera neohumeralis* with four phenylbutanoid male lures in Cairns (14 January to 11 March 2019) and Lockhart River (13 December 2017 to 5 February 2018) (WR = 21.92; $P < 0.001$). WR = Wald Ratio as defined in Materials and Methods—Statistical analysis. $n = 40$ (each lure at each location). Within locations, means within species with different letters are significantly different.

possibly due to CL's higher vapor pressure (ca. five times that of RK) and release rate (Metcalf 1990), but could also be attributable to the different chemical structures affecting ligand–receptor binding. It was previously assumed that CL hydrolyzes to RK, which is the true attractant (Drew 1974, Metcalf 1990). However, a recent study found that hydrolysis of CL is negligible and it stays intact in the atmosphere in the time-frame of the compound acting as a fruit fly lure (Park et al. 2016).

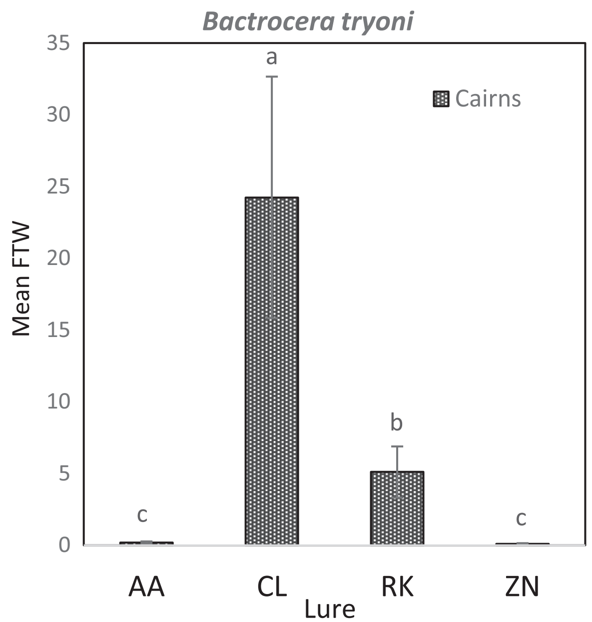

Fig. 5. Mean weekly trap catches of *Bactrocera tryoni* with four phenylbutanoid male lures in Cairns (14 January to 11 March 2019) (insufficient trapped in Lockhart to analyze) (WR = 45.99; $P < 0.001$). WR = Wald Ratio as defined in Materials and Methods—Statistical analysis. $n = 40$ (each lure at each location). Within locations, means within species with different letters are significantly different.

Furthermore, ingested CL results in a sex pheromone that is more attractive to female *Z. cucurbitae* (Khoo and Tan 2000) and *B. tryoni* (Kumaran et al. 2014a). However, ingested CL has never been detected in the male rectal (pheromone) gland of these species or *Zeugodacus tau* (Walker), although RK has (Nishida et al. 1990a, b; Tan and Nishida 1995; Nakahira et al. 2018). This suggests that CL is spontaneously hydrolyzed in the fly's crop to RK; and this biochemical process warrants further research to confirm the site of biotransformation.

CL was considered a synthetic lure only (Metcalf 1990), until recently, with it being discovered in two daciniphilous flowers—*Bulbophyllum hortorum* (Tan et al. 2014, Nishida and Tan 2016, Katte et al. 2020) and *Passsiflora maliformis* L. (Park et al. 2018). These findings occurred more than 50 yr after CL was first discovered as a synthetic lure for melon fly, through mass screening of chemical compounds similar to AA (the first male melon fly lure [Beroza et al. 1960]). Similarly, RK ('Willison's lure') was discovered in Australia in 1959 through chemical synthesis as an attractant for *B. tryoni* (Drew 1974), and independently discovered in Hawaii at a similar time, through the aforementioned mass chemical screening (Beroza et al. 1960). Decades later, Nishida et al. (1993) found RK in flowers of an orchid species, *Dendrobium superbum* (syn. of *Dendrobium anosmum*), which was first observed to be attractive to male melon flies (Ichinohe et al. 1983). RK is now known from six daciniphilous orchid species (Tan and Nishida 2005, Nishida and Tan 2016).

AA trapped 11 CL-responsive species, which were also caught at CL and RK in significantly greater numbers, except for *B. frauenfeldi*. Catches of *B. frauenfeldi* at AA and RK in Cairns, and AA, RK, and CL in Lockhart were not significantly different from each other (Fig. 2). This is the only known occurrence of AA being highly attractive to a species, apart from a preliminary field test using sticky traps in Papua New Guinea that showed AA captured more *Bactrocera atramentata* (Hering), *B. bryoniae* and *B. frauenfeldi* male flies than RK and ZN lures (Tan et al. 2014). The response of *B. frauenfeldi* to AA (both under laboratory and field conditions) will be covered in more detail elsewhere (Wee et al. 2019, unpublished data).

AA was only recently identified as a natural component in daciniphilous orchids (Nishida and Tan 2016, Katte et al. 2020). Historically, AA was the first male attractant for melon fly, identified through mass screening of synthesized compounds (Barthel et al. 1957). AA was subsequently used in fruit fly trapping in Australia from 1959 to 1962 and attracted 11 species (Queensland Government, unpublished data). It largely fell into disuse following the discovery of CL as a much stronger attractant (Beroza et al. 1960). It was more recently revisited in field studies, and found to weakly attract several CL-responsive species in Australia (Fay 2010, Royer 2015) and New Caledonia (Royer et al. 2019), in addition to the aforementioned species that were strongly attracted in Papua New Guinea (Tan et al. 2014).

ZN trapped seven species, two of which were only trapped at this lure (*B. aglaiae* and the pest *B. jarvisi*). The remaining five species were also trapped at CL and RK, in significantly greater numbers. ZN also trapped three other species, but in numbers insufficient to analyze (*B. aurea*, *B. speewahensis*, and *Dacus* sp. nr. *pusillus*; Table 2). All the species responding to ZN have previously been reported as attracted to this lure (Fay 2012, Royer 2015).

ZN is unique among the four phenylbutanoids in that it attracts ME- and CL- responsive species, at least in south-east Asia (Tan and Nishida 2000, 2007). In Australia and the Pacific it has only attracted CL/RK-responsive species (in addition to ZN-responsive species) (Royer 2015; Royer et al. 2018, 2019). ZN is the only daciniphilous phenylbutanoid first identified from a natural source. It was isolated from the daciniphilous orchid *Bulbophyllum patens* King in Malaysia (Tan and Nishida 2000) and is now known to exist in flowers of eight orchid species (Nishida and Tan 2016).

The overlapping species catches by traps baited with CL, RK, AA, and ZN somewhat parallels their occurrence as floral volatiles in *Bulbophyllum* orchids. ZN is known as the only daciniphilous volatile in *Bu. baileyi* F. Muel. (Tan and Nishida 2007), a var. of *Bu. ecornutum* J.J. Sm. subsp. *verrucatum* J.J. Verm., P. O'Byrne & A.L. Lamb (Tan et al. 2019, unpublished data), *Bu. macranthum* Lind. var. ex. Malaysia and Thailand (except var. ex Philippines; Nakahira et al. 2018), and *Bu. patens* (Tan and Nishida 2000). Likewise, several fruit fly species in Oceania and south-east Asia only respond to ZN (Fay 2012; Royer 2015; Leblanc et al. 2018a, b; Royer et al. 2018, 2019). ZN, AA and RK occur together in *Bu. hortorum* J.J. Verm., P. O'Byrne & A.L. Lamb, and *Bu. macranthoides* Kraenzl. subsp. *tollenoniferum* J.J. Sm. (Katte et al. 2020). Likewise, several fruit fly species respond to two or more of these lures (Tan and Nishida 2000, 2007; Royer 2015; Royer et al. 2019).

Interestingly, these floral attractants are always accompanied by minute quantities of their respective alcohol analogues (Katte et al. 2020). Although these alcohol analogues have either very weak or no attractiveness to melon fly males (Tan and Nishida 2000), their consistent presence in the orchid flowers and in the rectal glands of male flies that have consumed the respective floral phenylbutanoid attractants (Tan and Nishida 2000, Nishida and Tan 2016) certainly warrants further investigations to ascertain their specific roles, if any, in the co-evolutionary process between daciniphilous orchids and Dacini fruit flies. The only females trapped in this study were *B. frauenfeldi* females—four specimens trapped at RK and one at AA in Cairns. Females are not usually attracted to male lures, but are reported to occasionally respond when male populations are low (Fitt 1981). However, this response occurred in Cairns where

B. frauenfeldi populations were high. Any ecological reason for this, and for females only being trapped with RK and AA and not CL, would be speculative. In summary, this study demonstrates for the first time that CL is significantly more attractive than RK to a range of fruit fly species. It is unclear whether this is due to the greater volatility of CL, or the different chemical structures affecting ligand–receptor binding, or a combination of both factors. This study also shows that 1) AA weakly attracts a range of CL-responsive species, but strongly attracts *B. frauenfeldi*, 2) ZN weakly attracts a range of CL-responsive species, as well as species that respond to ZN only (as found elsewhere), and 3) lure combinations in fruit fly-attracting orchids reflect several fruit fly species' range of lure attractions, which suggests that assessing volatiles in orchids may be used to predict lures for Dacini fruit fly species.

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