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RELATIVE ATTRACTIVENESS OF ENRICHED GINGER ROOT OIL AND TRIMEDLURE TO MALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

This study describes field experiments that compare the relative attraction of male Mediterranean fruit flies (or medfly), *Ceratitis capitata* (Wiedemann), to trimedlure and ginger root oil, which contains the natural attractant α-copaene. The ginger root oil was embedded in a paste-like matrix, and the concentration of α-copaene was enhanced 20-fold above natural levels (hence the term "enriched" ginger root oil or EGRO). In tests conducted in a mixed fruit orchard in Waimanalo, Hawaii, 8 Jackson traps (4 baited with trimedlure, 4 baited with enriched ginger root oil) were placed in a circle (40 m radius) about a central point from which 500 males were released per replicate. Trap catches were scored 48 h after male release. In experiments using fresh (non-aged) lures, the amount of trimedlure used per trap was constant (1 ml), but the amount of EGRO-containing paste used in traps was 1, 10, or 20 drops. Significantly more males were captured in the trimedlure traps than the EGRO traps over all doses of EGRO. Similar experiments conducted in a small citrus grove yielded the same results. Additional experiments revealed that female medflies showed no attraction to either trimedlure- or EGRO-baited traps and that immature and mature males showed equal, short-range attraction to trimedlure and EGRO-baited traps.

RESUMEN

Este estudio describe los experimentos de campo para comparar la atracción relativa de los machos de la mosca mediterranea de la fruta, *Ceratitis capitata* (Wiedemann) al trimedlure y al aceite de la raíz de jengibre los cuales contienen un atrayente natural α-copaene. El aceite de la raíz de jengibre fué embutido en una pasta a manera de matríz y la concentración de α-copaene fué mejorada 20-veces por encima del nivel natural (de aqui el término "aceite de la raíz de jengibre "enriquecido" o EGRO por su sigla en ingles). En pruebas llevadas a cabo en huertos de frutas mezcladas en Waimanalo, Hawaii, 8 trampas de tipo Jackson (4 con el cebo de trimedlure, 4 con el aceite de la raíz de jengibre enriquecido) fueron colocadas en un circulo (40 m de radio) alrededor de un punto central de donde liberaron 500 machos por cada replicá. El contenido de las trampas fué contabilizado 48 h después de liberar los machos. En experimentos usando atrayentes frescos (no viejos), la cantidad de trimedlure usado por trampa fué constante (1 ml), pero la cantidad de pasta embutida con EGRO usada en trampas fué 1, 10, ó 20 gotas. Una cantidad significativamente mayor de machos fueron capturados en trampas de trimedlure que en las trampas de EGRO con las diferentes dosis de EGRO. Experimentos similares llevados a cabo en huertos pequeños de cítricos produjeron los mismos resultados. Experimentos adicionales revelaron que las hembras de la mosca mediterranea no mostraron ninguna atracción a ninguna de las trampas con cebo de trimedlure- o de EGRO y los machos maduros e inmaduros mostraron una atracción de corto alcance igual hacía las trampas con cebo de trimedlure como las de EGRO.

Females of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.) (medfly), may lay several hundred eggs over their lifetime and infest a wide variety of fruits and vegetables (Christenson & Foote 1960). Given this high intrinsic rate of population growth, early detection of incipient medfly outbreaks is critical for successful suppression or eradication. Following intensive screening of potential attractants in the 1950's (Beroza & Green 1963), trimedlure, *tert*-butyl 4 (and 5)-chloro-*trans*-2-methylcyclohexane-1-carboxlyate, emerged, and still prevails, as the standard attractant used to detect and monitor medfly populations (Beroza et al. 1961).

Despite its wide use, however, trimedlure is not the most attractive compound known for male medflies. In the aforementioned screening efforts, botanically derived compounds were tested, and oil from the seeds of the angelica flower, *Angelica archangelica* L. was found to be highly attractive to *C. capitata* males. The active components were subsequently identified as α–copaene and α-ylangene, two structurally related tricyclic sesquiterpenes (Guitto et al. 1972). Field tests (Flath et al. 1994a,b) revealed that male medflies displayed greater attraction to α-copaene than α-ylangene and that α-copaene was actually more attractive than trimedlure (when presented in equal volumes). α-copaene was subsequently identified as a minor component in the essential oils of many host plants of *C. capitata*, including orange, *Cit-*

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rus sinensis L. MacLeod et al. 1988, Nishida et al. 2000), guava, *Psidium guajava* L. (MacLeod & De Troconis 1982, Oliveros-Belardo et al. 1986), and mango, *Mangifera indica* L. (Koulibaly et al. 1992).

Although attractive, α-copaene is not used in medfly detection programs, because 1) it occurs in very low concentrations in plants, making extraction impractical, and 2) it has a complex chemical structure, making synthesis laborious and expensive (Flath et al. 1994b). Ginger root, *Zingiber officinalis* Roscoe, oil is an inexpensive and readily available source of α-copaene, and a recently developed distillation procedure can greatly increase the concentration of this compound. α-copaene comprises 8% of this so-called enriched ginger root oil (hereafter EGRO) compared to only 0.4% in commercially available oil (Citrus and Allied Essences, Ltd., Lake Success, NY; F. Webster, personal communication).

The primary purpose of the present study was to compare trap captures of male medflies to trimedlure- and EGRO-baited Jackson traps in field tests. Similar tests were conducted in a mixed fruit orchard and a citrus grove to assess potential inter-habitat differences. At both sites, comparisons were made using different relative amounts of the attractants. As noted below, the amount of trimedlure used per trap was typical of that used in ongoing survey and detection programs. As such, the field experiments were designed to compare attractiveness, not between equal amounts of the active constituents of trimedlure and EGRO, but rather between presently used doses of trimedlure and a range of 'realistic' (in terms of practical application) doses of EGRO. In addition to field tests, we also examined potential age-dependent response of male medflies to trimedlure- and EGRO-baited traps using field-caged host trees.

Materials And Methods

Medflies

The flies used in this study were from a laboratory colony started with 300-400 adults reared from coffee, *Coffea arabica* L., berries collected near Haleiwa. The colony was 5-7 generations removed from the wild when used for the Waimanalo experiments and 11 generations removed when used for the Pearl City experiments (see below). Standard rearing procedures were employed (Tanaka et al. 1969), and prior to release adults were fed a sugar/protein (yeast hydrolysate) mixture (3:1 by volume). Adults were separated within 24 h of emergence, well before they attained sexual maturity at 6-8 d of age (T. E. Shelly, unpublished data). When used in the field experiments, males were 9-15 d old, and females were 9-14 d old.

Field Experiments – Waimanalo

Most of the field experiments were conducted at the University of Hawaii Agricultural Experiment Station in Waimanalo in a mixed fruit orchard that contained orange, guava, and mango trees. Jackson traps, containing a sticky insert, were placed singly in the canopies of 8 trees (2 m above ground) arranged in a circle (radius 40 m) about a central tree, which served as the release point. The Jackson traps contained trimedlure in 4 trees and EGRO in the remaining 4 trees (see below). The lures were applied to the traps at the laboratory (4 km from the study site), and the traps were then immediately transported to and placed in the test trees. At a given tree, the bait used was alternated between successive replicates, and for a given replicate, adjacent test trees contained different baits.

For the trimedlure-baited traps in all experiments, 1 ml of the lure was applied to a cotton wick (2.5 cm long), which was then placed in a perforated, plastic basket that was, in turn, suspended within the Jackson trap. This dose is ½ of that used in area-wide survey and detection programs (California Department of Food and Agriculture 1995). Trimedlure is composed primarily of eight isomers of which one (designated C) is the most attractive (McGovern et al. 1987), and 1 ml of trimedlure contains approximately 0.358 g of isomer C (trimedlure purity × concentration of the C isomer × weight of 1 ml trimedlure = 0.965 × 0.399 × 0.929g; J. Knapp, personal communication). For the EGRO-baited traps, the amount of lure used varied between experiments (see below) and was placed on a small piece of aluminum foil directly on the sticky insert within the Jackson trap. The oil was embedded within a paste-like matrix (Last Call™, IPM Technologies, Inc.), comprised primarily of tinuvin, that was applied with a calibrated pump (1 drop of paste = 50 µl or 0.05g). EGRO comprised 20% of the paste (by weight), α-copaene constituted 8% of the EGRO (by weight), and thus one drop of paste contained 0.0008 g of α-copaene (J. McLaughlin, personal communication). It should be noted that the distillation procedure used to increase the concentration of α-copaene increases the concentration of other sequiterpenes as well (e.g., α- and β-ylangene; F. Webster, personal communication) and that the combination of all sesquiterpenes is more attractive to male medflies than α-copaene alone (T. W. Phillips, personal communication).

Five hundred males were released per replicate for all experiments. As wild flies were rare at the study site, released flies were not marked, and we assumed that all captured flies were from the releases. Successive replicates were run a minimum of 2 d apart to allow previously released flies time to disperse from the test area. Ten replicates were performed per experiment. Flies were re-

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leased between 1000-1200 hours by placing 4 buckets (volume 5 liters and each containing 125 flies) on the ground beneath the release tree and gently removing the screen cover from the bucket. The buckets were not tapped or shaken, and the flies exited the bucket on their own volition. Trap catches were counted 2 d later. Field work at Waimanalo was conducted during September-December, 2000, and daily minimum and maximum temperatures ranged from 17-21° C and 24-28°C, respectively.

The number of males captured at trimedlure-baited traps was compared with traps baited with 1 (50 µl), 10 (0.5 µl), or 20 (1 ml) drops of the paste containing EGRO. When multiple drops of EGRO-bearing paste were used, the individual drops were placed close together but were not overlapping. To investigate temporal decline in lure attractiveness, male catches were compared between trimedlure-baited traps and traps baited with 1 drop of the EGRO-containing paste using baits that had been aged 5 d prior to use (lures were placed in Jackson traps and placed outdoors in a covered area several km from the study site). In a final experiment, the female attraction to trimedlure-baited traps and traps baited with 1 drop of the paste containing EGRO was compared following the procedures outlined above for males.

Following the trimedlure-EGRO comparisons, we ran 2 additional experiments (using the protocol described above) that examined the potential attractiveness and repellency, respectively, of the paste in which the EGRO was delivered. In the first case, we compared male captures in 4 traps baited with 1 drop of the EGRO-containing paste versus traps baited with the one drop of paste to which no EGRO was added. In the second, we placed 0.5 ml of ginger root oil (Citrus and Allied Essences, Ltd., Lake Success, NY) on wicks for all 8 traps. In 4 of the traps, we also placed 10 drops of paste (lacking EGRO) on a piece of aluminum foil fastened to the sticky insert, while in the remaining 4 traps, we placed the foil only and no paste. Six replicates were run of each of these 2 experiments.

Field Experiments—Pearl City

To examine potential habitat differences in lure attractiveness, a second set of field experiments was conducted in a small citrus grove (1 ha) at the University of Hawaii's Urban Garden Center in Pearl City. Fieldwork at this site was conducted during June-July, 2001, and daily minimum and maximum temperatures ranged from 22-26°C and 30-33°C, respectively. The protocol used was identical to that described above, except that 1) trimedlure plugs containing 2 g of trimedlure (Farma Tech International, Fresno, CA), were used in place of liquid trimedlure and 2) only 4 traps, 2 baited with trimedlure and 2 baited with EGRO, were used per replicate. Trimedlure –baited traps were compared only with traps baited with 1 or 10 drops of the EGRO-containing paste, and no experiments were run involving aged baits or females. Eight replicates were performed per experiment.

Field-Cage Experiments – Waimanalo

Four experiments were conducted during June - September, 2001, at Waimanalo using field-cages (height 2.5 m, diameter: 3.0 m) that contained rooted guava trees. In the first, we investigated age-dependent attraction of males to trimedlure by comparing trap catches of immature (1 d old) and mature (9-13 d old) males. Groups of 100 immature and 100 mature males were released between 0900-1100 hours in a field-cage containing 1 baited (treated) and 1 unbaited (control) trap, and trap catches were recorded 24 h later. For the purpose of identification, males were marked by age group by cooling them for several minutes and then placing a small dot of enamel paint on the thorax. Immature males were marked in the late afternoon of the day of adult emergence to allow hardening of the exoskeleton; mature males were marked 1-2 d before testing. Traps were rectangular pieces of white cardboard (9 by 16 cm) coated on both sides with Tanglefoot™ and suspended in the canopy (1.5 m above ground) with wire hooks. A wick containing 1 ml of trimedlure was placed in the center of one side of the treated trap, and a cotton wick (without trimedlure) was applied to the control trap. The trimedlure used had a deep red color, and consequently we added a small amount of diluted red food coloring (McCormick & Co., Inc.) to the wick on the control trap to equalize visual stimuli. The same trap sites were used over all replicates, but the positions of baited and control traps were alternated between successive replicates. The second experiment examined age-dependent response of males to EGRO following the same procedures described above. In this case, 1 drop of EGRO-containing paste was placed on a small piece of aluminum foil, which was then placed in the center of one side of the trap. For the control trap, aluminum foil was placed on the trap, but no paste was applied. In the third and fourth experiments, we investigated close-range attraction of females to trimedlure and EGRO, respectively. The same protocol was followed except that only 1 group of 100 females (9-13 d old) was released per replicate, and their numbers on baited and control traps were compared. Seven replicates were conducted for each of the 4 field-cage experiments.

Statistical Analyses

Pairwise comparisons of trap catches were made using the Mann-Whitney test (test statistic

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T), a nonparametric equivalent of the Students t-test. Multiple comparisons were made using the Kruskal-Wallis (test statistic H), and if significant variability was detected, the multiple comparison Tukey test (test statistic q) was used to assess pairwise differences. Nonparametric tests were employed to avoid assumptions of normality and equal variance for the sampled populations. Analyses were conducted using SigmaStat Statistical Software (Version 2.0).

RESULTS

Field Experiments – Waimanalo

On average, the trimedlure-baited traps captured more males than the EGRO-baited traps in all experiments (Table 1A). With fresh (non-aged) baits, the trimedlure-baited traps captured approximately 1.6-2.6 times more males, on average, than did the EGRO-baited traps. When aged baits were used, the trimedlure-baited traps caught 19 times as many males as the EGRO traps (experiment 4). The number of males captured in trimedlure-baited traps did not differ significantly across the 4 experiments ($H = 2.7$, $df = 3$, $P > 0.05$), indicating that the 5-d aging period did not reduce trimedlure's attractiveness. In contrast, male numbers in EGRO-baited traps varied significantly among experiments ($H = 23.4$, $df = 3$, $P < 0.001$), with the male catch for aged EGRO-baited traps being significantly lower than those recorded for any of the freshly-baited traps ($P < 0.001$ in all cases). Among traps having fresh EGRO-containing paste, the number of males captured varied independently of the amount of paste used ($H = 3.5$, $df = 2$, $P > 0.05$).

Neither trimedlure nor GRO was attractive to females. Not a single female was caught in any trap over 10 replicates.

The paste used to deliver EGRO was not attractive or repellant to male medflies. Similar to experiment 1, traps baited with 1 drop of EGRO-containing paste caught an average of 11.1 (range: 5-18) males per replicate. However, traps baited with paste that lacked EGRO captured no males at all over 6 replicates. In testing for potential repellency of the paste, we found no significant difference in captured males between traps with paste ($\bar{x} = 11.3$; range: 8-18) versus traps without paste ($\bar{x} = 10.7$; range: 7-14; $T = 40.0$, $P > 0.05$) adjacent to the wick containing ginger root oil.

Field Experiments - Pearl City

Results obtained at Pearl City were similar to those reported above for Waimanalo (Table 1B). The trimedlure-baited traps, on average, captured significantly more males than did the EGRO-baited traps. In relative terms, the difference between the baits was even more pronounced

TABLE 1. NUMBERS OF MALES CAPTURED IN TRIMEDLURE- OR EGRO-BAITED JACKSON TRAPS AT WAIMANALO AND PEARL CITY STUDY SITES[1].

Experiment	Baits aged?	Amount of EGRO-containing paste	Males/trap		
			Trimedlure	GRO	T
A. Waimanalo					
1	no	50 µl	21.7A,a (11-27)	8.3B,b (3-17)	152.0***
2	no	0.5 ml	18.5A,a (11-26)	11.2B,b (4-18)	143.0**
3	no	1.0 ml	18.4A,a (10-26)	11.2B,b (4-16)	141.0*
4	yes	50 µl	19.2A,a (6-30)	1.8B,c (0-4)	155.0***
B. Pearl City					
1	no	50 µl	24.1A,a (11-37)	6.6B,b (4-17)	98.0***
2	no	0.5 ml	19.4A,a (6-27)	7.7B,b (2-9)	92.0**

[1]In all experiments, 1 ml of trimedlure was used in the trimedlure-baited traps. Values represent mean numbers of males captured per replicate; ranges are given in parentheses. Ten and 8 replicates were conducted for experiments conducted at Waimanalo and Pearl City, respectively. T values were computed for Mann-Whitney tests comparing male captures for the 2 attractants within a given experiment (row). For a given location, values in the same row followed by the same uppercase letter were not significantly different, and values in the same column followed by the same lowercase letter were not significantly different following the Kruskal-Wallis or Tukey test ($P = 0.05$). Significance levels: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

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at Pearl City, where the average trap catches were 3-4 times higher for trimedlure-baited traps than EGRO-baited traps. Additionally, no difference in male captures was detected between traps having 1 or 10 drops of the EGRO-bearing paste over the two experiments ($T = 75.0$, $P > 0.05$). Male captures were also similar for the trimedlure-baited traps across the 2 experiments ($T = 78.0$, $P > 0.05$).

Inter-habitat comparisons revealed that, for trimedlure-baited traps, the number of males captured per trap did not differ significantly between Waimanalo and Pearl City ($T = 431.5$, $n_1 = 30$, $n_2 = 16$, $P > 0.05$). For the EGRO-baited traps, however, trap catches were significantly greater at Waimanalo ($x = 10$ males/trap) than at Pearl City ($x = 6.5$ males/trap; $T = 245.5$, $n_1 = 30$, $n_2 = 16$, $P < 0.05$).

Field-Cage Experiments – Waimanalo

No difference was detected in the number of immature and mature males captured on treated versus control traps for the experiments involving trimedlure or EGRO (Table 2). Data pooled for immature and mature males revealed that, on average, treated traps captured approximately 13 times as many males as control traps in a given replicate for both trimedlure (80/6) and EGRO (66/5). Consistent with the field experiments, the average number of males captured per replicate (age groups combined) was higher for trimedlure- than EGRO-baited, although this difference was not statistically significant ($T = 64.0$, $P > 0.05$). In the third and fourth experiments, females showed no short-range attraction to trimedlure or EGRO. On average, the trimedlure-baited trap captured 2.7 (range: 0-6) females per replicate compared to 2.4 (range: 1-5) for the control trap ($T = 54.5$, $P > 0.05$). Similarly, the EGRO-baited trap captured 3.7 females per replicate compared to 2.7 females for the control trap ($T = 63.5$, $P > 0.05$).

Discussion

Based on field trapping tests conducted in 2 habitats, trimedlure-baited traps were more attractive to male medflies than EGRO-baited traps. This difference was evident over a wide range of EGRO dosage: at Waimanalo, traps baited with 1 ml of (non-aged) trimedlure caught 1.6 -2.6 more males than traps baited with 1-20 drops of EGRO-containing paste. Likewise, at Pearl City traps baited with trimedlure plugs captured 4.0 and 2.6 times as many males as traps baited with 1 and 10 drops of EGRO-containing paste, respectively. At both sites, the relative consistency in trap catches across a wide range of EGRO doses was unexpected. Among EGRO-baited traps, the number of males captured per trap did not vary significantly among traps with 1-20 drops of paste at Waimanalo or between traps with 1 or 10 drops of paste at Pearl City.

The difference in trap capture between the 2 attractants was even more dramatic when aged baits were compared. For trimedlure, the number of males trapped using aged baits did not differ significantly from that trapped with fresh baits. This result is consistent with previous studies (McGovern et al. 1966; Nakagawa et al. 1981; Rice et al. 1984) showing trimedlure maintains attractiveness for 1-6 weeks depending on local weather conditions. In contrast, traps with EGRO-bearing paste aged 5 days caught only about 10% as many

Table 2. Numbers of 1-d old (immature) and 9-13 d-old (mature) males captured in control and treated (a-trimedlure; b-egro) panel traps in field-cages at Waimanalo[1].

	Male Age		
Trap type	1 day	9-13 days	T
A. Trimedlure			
Control	2.6A	3.0A	54.5
		(0-5)	(0-8)
Treated	42.8A	37.0A	59.5
	(29-54)	(21-58)	
B. EGRO			
Control	2.2A	3.1A	54.5
			(0-7)
Treated	32.5A	34.6A	
	(24-42)	(27-42)	

[1]Values represent mean numbers of males captured per replicate; ranges are given in parentheses. Seven replicates were performed for all field-cage experiments. T values were computed for Mann-Whitney tests comparing captures for the 2 age groups within a given experiment (row). Values in the same row followed by the same uppercase letter were not significantly different ($P = 0.05$).

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males as traps baited with fresh paste. Thus, data from both fresh and aged baits indicate that trimedlure is superior to EGRO as a detection and monitoring tool in medfly control programs.

Although only 2 sites were involved, the field experiments indicate, preliminarily at least, that the attractancy of EGRO-baited traps may vary more among different habitats than that of trimedlure-baited traps. Trap catches were similar between the 2 sites for trimedlure-baited traps, whereas a significantly higher number of males was captured at Waimanalo than Pearl City for the EGRO-baited traps. While this difference could reflect the small sample of sites included, it may also indicate differences in the 'aromatic' environment at the 2 sites that differentially affected (interfered with) the effectiveness of the 2 baits. For example, volatiles emanating from the citrus trees at Pearl City may have more closely resembled (or mimicked) those given off from EGRO-baited traps than trimedlure-baited traps. If so, the citrus trees might have effectively "swamped" or outcompeted the olfactory stimuli of EGRO-baited traps and thus effectively lessened the attractiveness of this bait to male medflies.

Females were not attracted to either bait in the field trials or the field-cage tests. The lack of female response has been documented previously for trimedlure (Delrio & Zumreoglu 1983, Howse & Knapp 1996) and α-copaene (Nishida et al. 2000). As with pure α-copaene, the non-attraction of females to EGRO was unexpected, because α-copaene, which is present in many medfly hosts (see aforementioned references), has been considered a rendezvous stimulus that brings the sexes together for mating (Nishida et al. 2001). Although not attractive to females by itself, α-copaene may still affect the mating system of the medfly by enhancing female response to the male pheromone. Dickens et al. (1990) reported that green leaf volatiles boost female response to male medfly pheromone, and it seems likely that particular plant compounds, such as α-copaene, may act in a similar way (see examples in Landolt & Phillips 1997).

Although differing in their overall attractiveness, trimedlure and EGRO were both attractive to immature and mature males. Initial experiments (Shelly 2001) showed that mature male medflies exposed to the aroma of ginger root oil had a mating advantage over non-exposed males in tests conducted 2 days after the exposure. Follow-up tests (Shelly 2001) further revealed that 1-day old males exposed to ginger root oil had a mating advantage over non-exposed males in tests conducted 8-10 days after exposure. Given these findings, it was not surprising that male attraction to EGRO was age independent: attraction to an α-copaene source appears to confer an immediate benefit to mature males and a delayed benefit to immature males. This explanation, however, does not appear applicable for trimedlure. Although exposing mature males to trimedlure boosts their mating success, the effect is short-lived, and mating enhancement was evident only within 24 h of exposure (Shelly et al. 1996). Although we have not exposed 1-day old males to trimedlure, it appears unlikely that such early exposure would affect mating performance a week following exposure. Consequently, the attraction of immature males to trimedlure cannot apparently be explained in the context of sexual selection.

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