Raspberry Ketone Formate as an Attractant for the Melon Fly (Diptera: Tephritidae)[1]

Víctor Casaña-Giner,[2] James E. Oliver,[2,3] Eric B. Jang,[4] Lori Carvalho,[4] Ashot Khrimian,[2] Albert B. DeMilo[2] and Grant T. McQuate[4]

USDA-ARS, Chemicals Affecting Insect Behavior Laboratory, B007, BARC-W, Beltsville, MD 20705 USA

J. Entomol. Sci. 38(1): 120-126 (January 2003)

Abstract Cuelure, the acetate of raspberry ketone, has been the standard melon fly (*Bactrocera cucurbitae*) attractant for at least 40 yrs. A closely related compound, raspberry ketone formate, is somewhat more volatile and has been found to be at least 1.7 times more attractive to both sterile and wild melon flies in field tests conducted in Hawaii, consistently outperforming cuelure for periods exceeding one month.

Key Words *Bactrocera cucurbitae,* melon fly, Tephritidae, lure, raspberry ketone, raspberry ketone formate, cuelure

The melon fly, *Bactrocera cucurbitae* (Coquillet) (Diptera: Tephritidae), is a polyphagous tephritid with more than 125 host plants (Metcalf 1990) that causes severe damage to a number of crops. In the Indo-Malayan region it is considered the most destructive pest of melons and related crops. It is found throughout most of southeastern Asia and in the Mariana Islands, and it greatly curtails the production of melons, cucumbers and tomatoes in Hawaii (Anonymous 1985).

Research focused on early detection of tephritid pests has been pursued in the United States for many years (McPhail 1939). Barthel et al. (1957) described anisylacetone [4-(4-methoxyphenyl)-2-butanone] as an effective lure for the male melon fly. Later, cuelure, the acetate of raspberry ketone (4-(4-acetoxyphenyl)-2-butanone), was developed (Beroza et al. 1960). In that work, cuelure, in a 61-d trial, attracted approximately 1.3, 2.11, 2.5, 4.5 and 12.8 times more male *B. cucurbitae* than raspberry ketone propionate, raspberry ketone itself, raspberry ketone butyrate, raspberry ketone isovalerate, and anisylacetone, respectively. Since then, cuelure has been employed worldwide for monitoring and control of the melon fly. Raspberry ketone, the precursor of cuelure, is a naturally-occurring compound (Bauer et al. 1955), and itself a melon fly attractant (Alexander et al. 1962). Raspberry ketone has relatively low volatility, and slow desorption from dispensers has been considered to be the primary limitation of its effectiveness as a bait. Although Metcalf (1990) reported that cuelure was substantially less attractive than raspberry ketone on filter paper, Alexander et al. (1962) described cuelure as 1.4 times more attractive for male melon flies

[1]Received 05 December 2001; accepted for publication 31 March 2002.

[2]USDA-ARS, Chemicals Affecting Insect Behavior Laboratory, BARC Bldg. 007, 10300 Baltimore Ave., Beltsville, MD 20705-2350.

[3]To whom correspondence should be addressed (email: oliverj@ba.ars.usda.gov).

[4]USDA-ARS, Pacific Basin Agricultural Research Center, P.O. Box 4459, Stainback Highway, Kulani Rd, Hilo, HI 96720.

than raspberry ketone. Although its boiling point is not particularly lower than that of raspberry ketone, cuelure is the superior bait for traps under field conditions, at least in part because of its higher release rate from the bait matrix (approximately 20× relative to raspberry ketone, as estimated by Metcalf and Metcalf (1992)).

During extensive searches for improved attractants, Metcalf and Metcalf (1992) reported that raspberry ketone formate was twice as attractive as cuelure in 2-d tests, but the work was evidently not actively pursued. At that time, Metcalf (1990) implied that the essence of the attraction of cuelure and other raspberry ketone esters resulted from their hydrolyses back to the inherently attractive raspberry ketone.

We have reinvestigated raspberry ketone formate as a melon fly attractant, and here report that raspberry ketone formate is indeed superior to cuelure for field trapping of male melon flies over at least 4 wks, and that hydrolytic instability of raspberry ketone formate does not appear to be a limiting factor. Some preliminary experiments also suggest that alternative formulations may be used to suppress hydrolysis of raspberry ketone formate should it become a problem in subsequent trials.

Materials and Methods

Instrumentation. Capillary gas chromatography (GC) was performed on a Shimadzu GC-14A gas chromatograph equipped with a split injector fitted with a Hewlett Packard HP-1™ column (30-m × 0.25 mm ID, 25-μm-thick coating). The injector and flame ionization detector (FID) were held at 260°C and 250°C, respectively, with hydrogen (linear velocity 30 cm/sec) as carrier gas. GC-MS was performed using a HP5890 GC-mass spectrometer (Hewlett-Packard) at 70 eV, with a Hewlett-Packard HP-5™ column (0.25 μm film, 30-m × 0.25-mm ID). The oven temperature was programmed from 70°C (no initial hold) to 280°C at a rate of 5°C/min. Infrared spectra were obtained with a Perkin Elmer 882 infrared spectrophotometer. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Chemicals. Cuelure and raspberry ketone are commercially available and stock samples were used. The synthesis of formic acid 4-(3-oxobutyl)phenyl ester (raspberry ketone formate) was based on Stevens and VanEs (1964). A solution of formic acid (25.5 g, 0.55 mol), acetic anhydride (62.6 g, 0.61 mol), and imidazole (2.04 g, 0.03 mol) was stirred for 5 min under nitrogen at −10°C then raspberry ketone (54.6 g, 0.33 mol) was added slowly from a solids addition funnel. After 45 min the cooling bath was removed; when the mixture reached room temperature, a fresh solution of 23 g (0.50 mol) of formic acid and 20 g (0.25 mol) of acetic acid anhydride was added dropwise. After 6 h the mixture was added to approximately 200 mL each ice water and ether. The layers were separated and the aqueous phase was extracted (2 × 75 mL) with 1:2 ether-petroleum ether. The second extraction resulted in the separation of a third layer that was kept with the organic phases. The combined organic extracts were rinsed with cold water then with saturated sodium bicarbonate, dried over magnesium sulfate, concentrated on a rotary evaporator, and finally distilled to provide 59.4 g of raspberry ketone formate (93%), b.p. 157-163°C/0.25 Torr. GC (FID detector) indicated 97% raspberry ketone formate, 2.3% cuelure and 0.15% raspberry ketone. IR (cm^{-1}), 2940, 1770, 1750, 1725, 1520, 1210, 1180, 1120; MS, *m/z* (rel %) 192 (38, M^+), 164 (20), 149 (17), 121 (23), 108 (12), 109 (100), 94 (29), 77 (33), 65 (14), 63 (10), 51 (12), 40 (60). Because of the difficulty separating raspberry ketone

and raspberry ketone formate on a number of GC columns, we adopted the practice of treating mixtures that might contain raspberry ketone with trifluoroacetic anhydride. Raspberry ketone formate did not react, whereas raspberry ketone was converted to its trifluoroacetate, which elutes earlier than cuelure, raspberry ketone, and raspberry ketone formate from HP-1™ and HP-5™ columns.

Raspberry ketone formate hydrolysis experiment. In order to evaluate hydrolysis of raspberry ketone formate and cuelure under field conditions, cotton dental wicks were treated with 1 g of compound and then placed in delta traps. In the case of raspberry ketone formate, additional experiments employed a dispenser consisting of a cotton wick impregnated with 1 g of raspberry ketone formate wrapped with paper towel and secured with a rubber band, and then this wrapped cotton wick was placed inside a 7 × 5 mm paper towel sachet also containing 10 g of silica gel (blue indicating desiccant). Additional experiments were conducted by adding 3 mL of silicone oil to the cotton wick following application of raspberry ketone formate. The traps were hung at a height of 1.5 m in a wooded area of the Beltsville Area Research Center, MD, during an unusually rainy summer (2000). Each experiment was run in triplicate. Residues were measured after extraction of cotton wicks with dichloromethane and concentration. External standards were used for quantitation. Similar tests were subsequently performed during the summer of 2001 with wicks loaded with 0.1 and 0.01 g raspberry ketone formate, respectively, and unamended with desiccants or protecting agents.

Insects. Laboratory irradiated melon fly pupae were obtained from the USDA-ARS-PBARC rearing facility in Honolulu, HI. One hundred mL of irradiated pupae (approximately 3300 insects) were held in screened action packers (modified RubberMaid® container for temporary storage and transporting insects as described in Jang et al. [2001]). Flies were given sugar, water and hydrolyzed yeast protein and held at 26°C under a 12:12 L/D photoperiod. Sterile (irradiated) melon flies (150 Gy two days preemergence) were released in an open field during tests no. 1 and 3.

Attractiveness comparison of raspberry ketone, raspberry ketone formate and cuelure in field tests. Field test no. 1 was conducted in a macadamia nut orchard near Hilo, HI. Attractants tested were cuelure, raspberry ketone and raspberry ketone formate. Twenty mg of each compound was added to a 1.2 cm dental wick as a solution in 100 µL acetone, then the wick was inserted into a delta trap with a 2 g Revenge® pest strip (Roxide International, NY). Traps were hung within the perimeter canopy of every other tree and every row, i.e., defining rectangles of approximately 20 × 15 m. Flies were released about 50 m upwind of the nearest row of traps, as evenly as possible along all of the columns. One release was made for each test and replication for the 3-wk period. There were 6 replicates placed in a randomized complete block design. Melon flies from four action packers (~3300 insects/action packer) were released (one action packer released per row). Tests were performed at 20 to 30° and 60 to 90% relative humidity. Traps were left in the field for 24 h after which traps removed and male and female trap captures were recorded.

Field test no. 2 compared only raspberry ketone formate and cuelure in Molokai using Moroccan traps (Epsky et al. 1999) with wild melon flies. Twenty mg of each treatment was presented on a 1.2-cm cotton wick and placed in a Moroccan trap with a 2 g Revenge® pest strip. Traps were placed on the borders of various melon fly host crops. Trap captures were recorded 24 h after emplacement. Field test no. 3 was conducted in the same field and under the same conditions as test no. 1. The initial load was 20 mg. Every 7 d, 8 action packers containing irradiated melon flies were

released and catches were recorded 24 h after the release. Catches from the same cotton wicks were monitored through 4 wks. Only male melon fly catches are reported due to the negligible number of females caught.

Statistical analysis. Statistical analyses were performed with SAS software (SAS Institute 1989). Residuals were examined graphically for homogeneity and normality. The distribution of groups of attractants within the same category—similar attraction—was done with transformed data, arcsine [sqrt {x}], by means of the Tukey's Studentized Range (HSD) (SAS Institute 1989).

Results and Discussion

Although Metcalf and Metcalf (1992) reported experiments with raspberry ketone formate, to the best of our knowledge the synthesis of raspberry ketone formate has not been published. We obtained a high yield of nearly pure raspberry ketone formate based on a procedure of Stevens and VanEs (1964). We noted that raspberry ketone formate and raspberry ketone are quite difficult to separate by gas chromatography on any of several columns, and that derivatization was required to detect raspberry ketone that might otherwise be present as an unnoticed impurity.

Short-term and long-term field results showed that raspberry ketone formate was superior to cuelure as an attractant for both sterile and wild male melon flies. In test no. 1, raspberry ketone formate caught twice as many sterile male melon flies as cuelure in 24 h; ($\bar{X} \pm SE$), raspberry ketone formate = 261 ± 44a; cuelure = 130 ± 17b; raspberry ketone = 95b; different letters show statistical difference in the Tukey's Studentized Range, n = 18, $P < 0.05$. In test no. 2 with wild flies, raspberry ketone formate also caught more flies than cuelure, ($\bar{X} \pm SE$), raspberry ketone formate = 177 ± 42a; cuelure = 130 ± 33b; control (acetone) = 0.00 ± 0.00c; different letters show statistical difference in the Least Significant Difference (LSD) test, n = 17, $\alpha = 0.075$, df = 48.

In test no. 3 (Fig. 1), a longer term experiment, raspberry ketone formate captured 1.7 times more melon flies than cuelure over 28 d. The difference in attractiveness between the two baits was greater in the first 2 wks than in the last 2 wks, but the traps baited with raspberry ketone formate caught more flies than those baited with cuelure during all time intervals.

These results are consistent with those of Metcalf and Metcalf (1992) who observed that raspberry ketone formate was twice as attractive as cuelure in a limited field test. The latter work followed laboratory assays from which they had also concluded that raspberry ketone formate was a superior attractant to either raspberry ketone or cuelure. It is uncertain why the development of raspberry ketone formate as an attractant was not pursued more aggressively at that time. A likely explanation lies in their reservations about presumed hydrolytic instability of raspberry ketone formate. The formate ester of raspberry ketone formate is certain to be more easily hydrolyzed than the acetate of cuelure, and Metcalf (1990) stated that cuelure itself was so sensitive to hydrolysis that it was virtually impossible to measure its intrinsic attractiveness. It was implied (Metcalf and Metcalf 1992) that the attractiveness of cuelure and other esters of raspberry ketone was a result of facile hydrolysis of the esters back to raspberry ketone.

More recently, however, the alleged hydrolytic instability of cuelure has been questioned. Cheng et al. (1996) found only 12 to 20% hydrolysis of cuelure over a 2-mo period under field conditions. Similarly, in outdoor tests, with wicks impregnated with

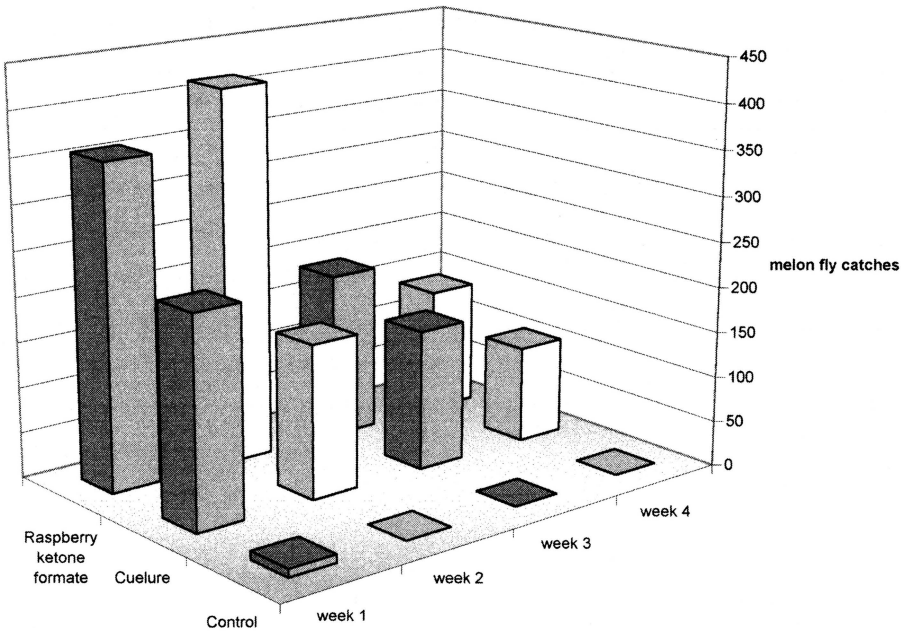

Fig. 1. Comparison of male melon fly catches in traps baited with raspberry ketone formate and cuelure in a 4-wk field test, Hilo, HI. Jackson trap, sticky insert, 5-mm cotton wick, 2 g pest strip (Revenge®). Irradiated melon flies. Releases consisted of 8 packers x 1750 insects/packer/test. Doses of attractant were 20 mg. Catches of males were recorded 24 h after release. Means were determined from 12 replicates.

12 g of cuelure, 99.3% of the cuelure reportedly remained in the wick after 12 mo (Keiser et al. 1974). We also have found hydrolysis of cuelure under ambient conditions to be relatively slow (about 4% in 10 d in warm, moist air), and Liquido (pers. commun.) found that vigorous agitation of an ether solution of cuelure with water for 10 min caused no breakdown of cuelure to raspberry ketone. Part of the Metcalf and Metcalf (1992) conclusion was based on higher observed attractiveness of raspberry ketone esters applied to moist filter paper compared to the attractiveness of the same esters applied to dry filter paper. Their interpretation was that hydrolysis was releasing raspberry ketone which was the true attractant. We suggest that the difference may have resulted from more rapid volatilization of the esters from the moist paper, either because of lowered surface activity of the moist paper, or because of co-evaporation of the esters with water from the surface. We have found (unpubl. data) that the presence of either water or organic solvents facilitates evaporation of raspberry ketone when air is passed through a vessel containing that attractant.

We were gratified to find that raspberry ketone formate was in fact adequately stable under warm, moist field conditions as typically encountered during trapping of melon flies (15 to 30% hydrolysis over 50 d [Table 1]). Heavy loading of the cotton wicks employed as dispensers may be a key, inasmuch as more rapid hydrolysis was

Table 1. Mean (±SE) percentage of hydrolysis of raspberry ketone formate under field conditions*

Day	Unamended	With silicone oil	With silica gel	Refrigerated and sealed
1	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
7	3.4 ± 0.13	5.4 ± 2.20	1.1 ± 0.75	0.5 ± 0.05
14	16.8 ± 0.92	11.8 ± 5.91	8.2 ± 3.58	0.6 ± 0.03
21	14.5 ± 1.64	13.9 ± 6.83	10.9 ± 4.47	0.8 ± 0.02
28	21.7 ± 3.97	14.5 ± 7.66	9.9 ± 6.30	0.7 ± 0.12
35	19.8 ± 4.81	10.8 ± 6.31	13.8 ± 5.05	1.1 ± 0.84
42	22.9 ± 6.75	17.6 ± 8.40	16.7 ± 4.80	1.5 ± 0.32
50	25.9 ± 8.77	19.3 ± 6.89	15.2 ± 7.31	1.4 ± 0.69

* Conducted in Beltsville, MD, July-August 2000. Cotton wicks (5 mm x 40 mm) were loaded with 1 g of raspberry ketone formate with or without additive (see Materials and Methods) on d 0 and not refilled. Measured hydrolysis is corrected for 3.7% raspberry ketone initially present in the sample.

observed on wicks loaded with 0.1 g raspberry ketone formate, and still more rapid hydrolysis occurred with loadings of 0.01 g (Oliver and Dua, unpubl. data). At the lower loading levels, the cotton wicks expanded and became visibly moist upon exposure to humid air, and it is likely that this hydrated matrix contributed to the more extensive hydrolysis of raspberry ketone formate. Because high loadings (at least 1 g) seem to be almost always used with these baits, the hydrolysis threat does not appear to impose a serious limitation. If lower loading levels should be desirable, alternative release matrices should be investigated; it is likely that a less hygroscopic substance than cotton might prove beneficial. Some preliminary experiments have indicated that it may be possible to reduce raspberry ketone formate hydrolysis by the incorporation of desiccants or other protecting agents into dispensers. We tried only one desiccant (silica gel) and one moisture protection agent (silicone oil), and found that both seemed to have potential for reducing hydrolysis (Table 1, experiments conducted at the 1 g loading level). The utility of such agents is as yet uncertain because neither release rates nor trap catches have yet been measured from the amended baits. In any event, because the hydrolysis product, raspberry ketone, is itself a melon fly attractant, and because 50 d is not unsatisfactory for trap longevity, we consider that hydrolytic stability is unlikely to be a serious limitation to the use of raspberry ketone formate.

Although rapid hydrolysis in moist air may not be the major factor in the attractiveness of esters of raspberry ketone, rapid hydrolysis on an antennal surface is a possibility that is worthy of consideration but has not yet been addressed experimentally.

In summary, we have found raspberry ketone formate to outperform the standard bait cuelure as an attractant for male melon flies. Its hydrolytic stability under field conditions is better than previous workers had predicted, and it can be efficiently prepared and handled. Although more extensive field tests are needed to confirm its superior attractiveness in a wider variety of locations and climates, we suggest that

raspberry ketone formate be considered as a replacement for cuelure for many applications.

References Cited

Alexander, B. H., M. Beroza, T. A. Oda, L. F. Steiner, D. H. Miyashita and W. C. Mitchel. 1962. The development of male melon fly attractants. J. Agric. Food. Chem. 10: 270-276.

Anonymous. 1985. California adds another fruit fly to invader list. Citrograph August: 223-224.

Barthel, W. F., N. Green, I. Keiser and L. F. Steiner. 1957. Anisylacetone, synthetic attractant for male melon fly. Science 126: 654.

Bauer, L., A. J. Birch and A. J. Ryan. 1955. Studies in relation to biosynthesis. VI. Rheosmin. Austral. J. Chem. 8: 534-538.

Beroza, M., B. H. Alexander, L. F. Steiner, W. C. Mitchel and D. H. Miyashita. 1960. New synthetic lures for male melon fly. Science, 131: 1044-1045.

Cheng, E. Y., T. Y. Ku, C. H. Kao and Y.-B. Huang. 1996. Instability of the currently used poisons baits for the oriental fruit fly and melon fly—the study on decomposition of naled and cuelure. Zhonghua Nongye Yanjiu 45: 422-435; Chem Abstr (1997) 127: 105561c.

Epsky N. D., J. Hendrichs, B. I. Katsoyannos, L. A. Vasquez, J. P. Ros, A. Zumreoglu, R. Pereira, A. Bakri, S. I. Seewooruthun and R. R. Heath. 1999. Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. J. Econom. Entomol. 92: 156-164.

Jang E. B., A. S. Raw and L. A. Carvalho. 2001. Field attraction of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) to synthetic stereoselective enantiomers of the ceralure B1 isomer. J. Chem. Ecol. 27: 235-242.

Keiser, I., R. M. Kobayashi, D. H. Miyashita, E. J. Harris and D. L. Chambers. 1974. Comparative volatilization of three lures for male tephritids in Hawaii: methyl eugenol (oriental fruit fly), cue-lure (melon fly), and trimedlure (Mediterranean fruit fly). Environ. Letters 7: 47-52.

McPhail, M. 1939. Protein lures for fruit flies. J. Econ. Entomol. 32: 558-761.

Metcalf, R. L. 1990. Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 83: 1017-1030.

Metcalf, R. L. and E. R. Metcalf. 1992. Fruit flies of the family Tephritidae, Pp. 139-142. *In* T. A. Miller and H. F. van Emden [Eds.], Plant kairomones in insect ecology and control. Chapman and Hall, New York and London.

SAS Institute. 1989. SAS/STAT user's guide, version 6.0, 4th ed. SAS Institute, Cary, NC.

Stevens, W. and A. VanEs. 1964. Mixed carboxylic acid anhydrides. II. Esterification of alcohols with the formic acid/acetic anhydride reaction mixture. Rec. Trav. Chim. Pays-Bas 83: 1287-1293.