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Improved attractants for the melon fly, *Bactrocera cucurbitae*

J.E. Oliver[1*], V. Casaña-Giner[1], E.B. Jang[2], G.T. McQuate[2] & L. Carvalho[2]

[1]U.S. Department of Agriculture, Agricultural Research Service, Chemicals Affecting Insect Behavior Laboratory, Beltsville, MD 20705, U.S.A.

[2]U.S. Department of Agriculture, Agricultural Research Service, Pacific Basin Research Agricultural Center, Hilo, HI 96720, U.S.A.

Virtually all of the standard lures for the melon fly, *Bactrocera cucurbitae*, are based on raspberry ketone, and of these, cuelure (the acetate of raspberry ketone), has for many years been the most widely used. We have synthesized and evaluated additional potential melon fly attractants whose structures are also related to that of raspberry ketone, focusing in particular on analogs with enhanced volatility. We have found that raspberry ketone formate is a superior attractant, even to cuelure. Reasons for its superior performance are discussed.

INTRODUCTION

The history of melon fly attractants goes back at least to 1957 when Barthel *et. al.* (1957) reported in *Science* that anisylacetone attracted sexually mature males. The authors commented on its resemblance to methyl eugenol, which was already known to be an excellent attractant for the oriental fruit fly (Fig. 1). Anisylacetone was not an especially effective attractant, and a few years later Beroza *et al.* (1960) reported, also in *Science,* that 4-(*p*-acetoxyphenyl)-2-butanone (cuelure) was a much more potent attractant, and that it also attracted immature males. Cuelure has served as the standard melon fly lure since that time. It is interesting that both anisylacetone and cuelure were discovered through empirical screening procedures as opposed to any kind of rational extrapolation from known natural products.

In 1974, Drew (1974) cited work dating from 1959 wherein raspberry ketone (known as Willison's lure) had been developed as an attractant for male *Dacus tryon*. The history of this discovery is uncertain; presumably it was empirical in nature and it may not have been appreciated outside of Australia until considerably later. Doolittle *et al.* (1970) and Keiser *et al.* (1973) noted that as much as 50% raspberry ketone in cuelure did not decrease the efficacy of the latter as an attractant (raspberry ketone was regarded as a contaminant or degradation product of cuelure, not as a primary attractant).

In 1974 Drew (1974) published his pivotal observation that the Dacinae can be divided into two categories, one whose males are attracted to methyl eugenol, and one to whom raspberry ketone is attractive (he noted that there were some 92 species that responded to raspberry ketone, with about 40 responding to methyl eugenol). Both methyl eugenol and raspberry ketone are natural products, occurring in various plants, and Metcalf (1990) and Metcalf *et al.* (1979) discussed this subject in an evolutionary context. Various authors have shown that the male flies are able to sequester these compounds in their pheromone glands, where they increase competitiveness and mating success (a summary, with references, can be found in Shelly & Villalobos 1995; see also Shelly 2000). Thus, since about 1970, the thinking has gradually shifted from the early empirical approaches toward the evaluation of attractants within the contexts of fruit fly chemistry and behaviour.

In 1982, ethyl *p*-hydroxybenzoate was discovered as a component of the rectal gland secretion of the melon fly, although it was incorrectly identified at the time (Baker *et al.* 1982; Nishida *et al.* 1990; Perkins *et al.* 1990; Fletcher & Kitching 1995). Also about 1982, Flath & Ohinata (1982) identified 4-phenyl-2-butanone in flowers of the orchid *Dendrobium superbum,* which were attractive to melon flies. There is evidently a plant called fruit fly plant, which is attractive to several species of fruit flies, and from it has been identified benzyl acetate and 4-methoxybenzyl acetate (Lewis *et al.* 1988). Metcalf and coworkers (1986) synthesized and evaluated a series of substituted benzyl acetates, and found the most active to be 4-hydroxybenzyl acetate. The authors noted that this compound is isosteric to raspberry ketone (a CH_2 of the latter substituted by an oxygen). Recently, Tan & Nishida (2000) reported that flowers of the wild orchid *Bulbophyllum patens* attracted males of several fruit fly species, and identified zingerone as the principal attractive ingredient. In this case too, male flies seques-

*To whom correspondence should be addressed.
E-mail: oliverj@ba.ars.usda.gov

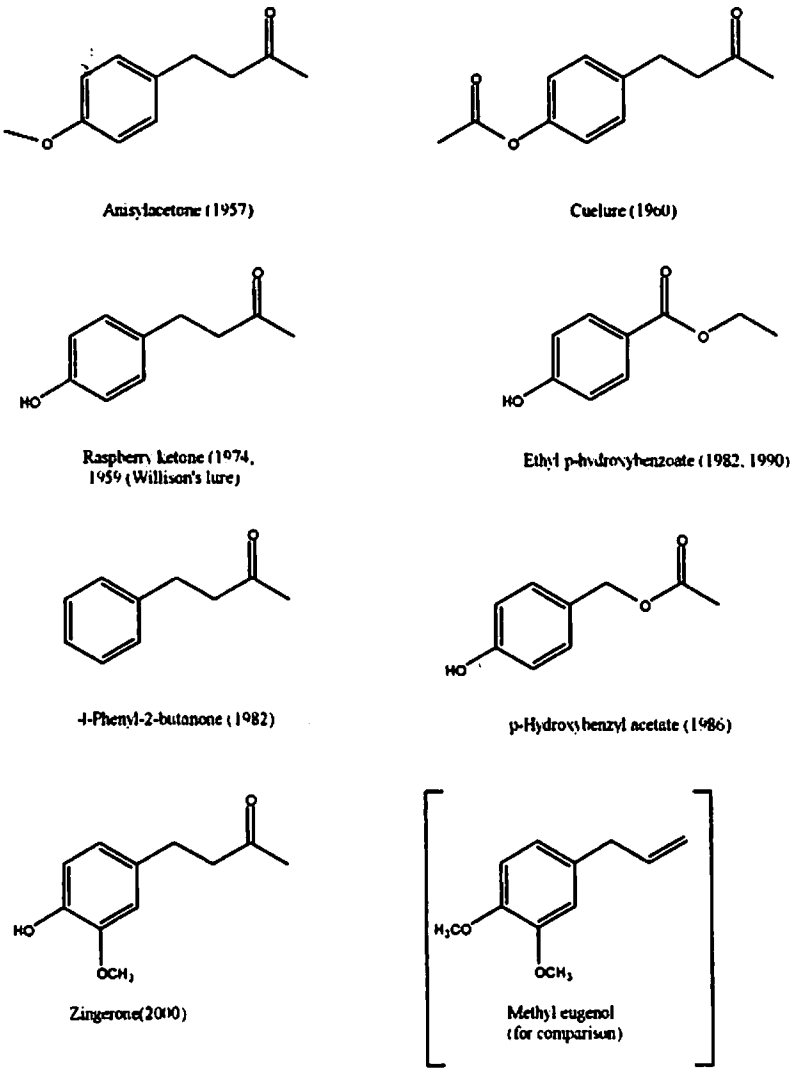


Fig. 1. Aromatic compounds attractive to *Bactrocera cucurbitae*.

tered zingerone in pheromone glands. In at least one species, zingerone is metabolized to the corresponding alcohol zingerol, which is used by the male to attract conspecific females. Zingerone is noteworthy in that it possesses structural features of both raspberry ketone and methyl eugenol, and is unique in its ability to attract both categories of flies, those normally responding to raspberry ketone as well as those attracted by methyl eugenol.

In spite of these advances, from a practical standpoint, cuelure has remained *the* melon fly attractant since its discovery. And cuelure is simply the acetate of raspberry ketone, a relationship that has escaped no-one since the central role of the latter became known. Esters of phenols are somewhat more easily hydrolysed than esters of aliphatic alcohols, and it has been speculated that facile hydrolysis of cuelure, e.g. in moist air, generates raspberry ketone which is the true attractant. This idea has been strongly emphasized by Metcalf & Metcalf (1992), but also, and earlier, by others (cited as a personal communication in Drew's paper (Drew 1974).

MATERIALS AND METHODS

Methods for syntheses of new compounds have been described elsewhere (Casaña-Giner *et al.* 2003a,b). Applications and a description of the olfactometer have also been described previously (Casaña-Giner *et al.* 2003b; Jang *et al.* 1997; Jang *et al.* 2001).

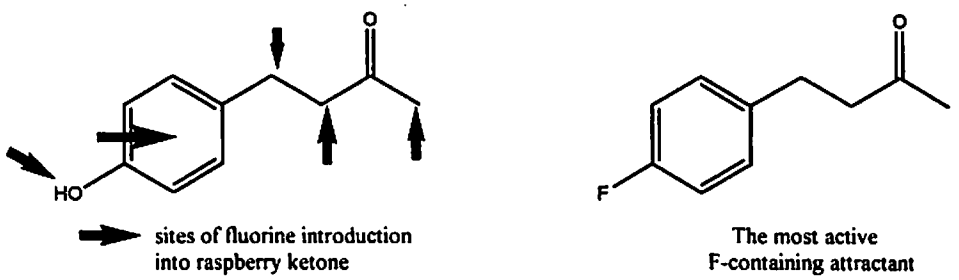


Fig. 2. Fluorinated analogs of raspberry ketone.

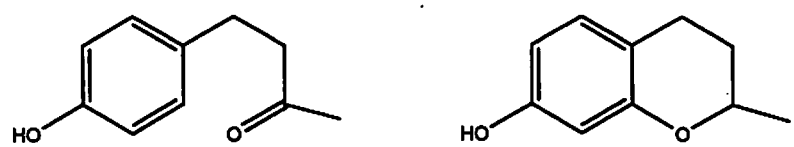
Most field data were obtained with released flies as previously described by Casaña-Giner *et al.* (2003b), and studies with wild melon flies were conducted in Kapoho on the island of Hawaii. Traps were placed 10–15 m apart in a citrus orchard, and serviced weekly.

RESULTS AND DISCUSSION

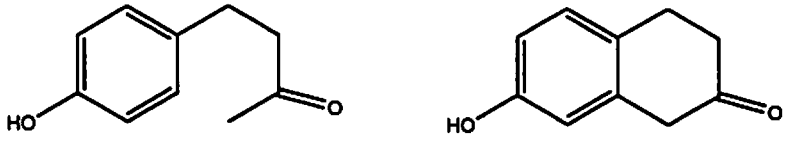
Conventional wisdom states that raspberry ketone is intrinsically highly attractive, but, from a practical standpoint, is simply too non-volatile to serve as an effective bait in traps (the real issue is release from a formulating matrix, a phenomenon influenced by several factors of which volatility is only one). Although itself possessing limited volatility, cuelure enters the air more rapidly than raspberry ketone so that it performs much better under field conditions [Metcalf & Metcalf (1992) reported that cuelure was released ~20 times faster than raspberry ketone from filter papers treated with 0.1 µg]. However, in spite of its advantages, it has been noted that cuelure is a less effective attractant for melon flies than methyl eugenol is for oriental fruit flies (Shelly & Villalobos 1995), and various groups have cited a need for a better melon fly attractant, citing the limited volatility of cuelure as its major shortcoming. It was in this context that we were asked to try to develop a more active, particularly a more volatile, melon fly attractant. For the reasons outlined, we chose to not deviate drastically from the general raspberry ketone framework, but rather to attempt relatively subtle modifications that might lead to enhanced release from formulation matrices. Accordingly, additional analogs have been synthesized and evaluated. One approach was one already tried by Metcalf *et al.* (1986), and involved fluorine-substituted analogs. Fluorine can mimic, at least in some cases, the effect of a hydroxyl group (Welch 1991), and with three non-bonded electron pairs, can also participate in hydrogen bonding. Fluorine atoms are only slightly larger than hydrogen atoms (Dixon & Smart 1991), and multiply fluorinated compounds are sometimes more volatile than their hydrogen-containing counterparts. Figure 2 demonstrates some of the sites of fluorine substitution of analogs we prepared. Discussion of these analogs warrants little time because the approach was simply unsuccessful. The analog with the phenolic OH replaced by fluorine was somewhat attractive, and a few others demonstrated marginal attractiveness, but none were nearly as attractive as raspberry ketone or cuelure (Casaña-Giner *et al.* 2003a).

A few non-fluorinated analogs were also synthesized and evaluated. Neither of the bicyclic analogs (Fig. 3) was attractive. These were not expected to be particularly more volatile than raspberry ketone, but rather were synthesized to see if they might shed light on a raspberry ketone receptor. If the receptor were to accommodate the molecule in a folded configuration (as illustrated in Fig. 3), one or the other of these compounds might fit that receptor quite neatly. Their inactivity seems to suggest that raspberry ketone fits into the receptor with the chain extended away from the ring.

More encouraging results were realized with silyl ether derivatives of raspberry ketone (Fig. 4). In the olfactometer, raspberry ketone trimethylsilyl ether was 71% as attractive as raspberry ketone and 67% as attractive as cuelure. More importantly, the attractiveness of the trimethylsilyl ether in a field test was comparable to that of cuelure [no statistical difference in the LSD test ($\alpha = 0.05$)], and more interesting, it caught significantly more flies than did raspberry ketone. These latter results illustrate that completely parallel results between olfactometer and field results are not always obtained – possibly because the olfactometer experiments are exclusively short range in nature. In this experiment, cuelure and raspberry ketone were comparably attractive in the olfactometer, both attracting more flies than the trimethylsilyl ether, but in the field test, the trimethylsilyl

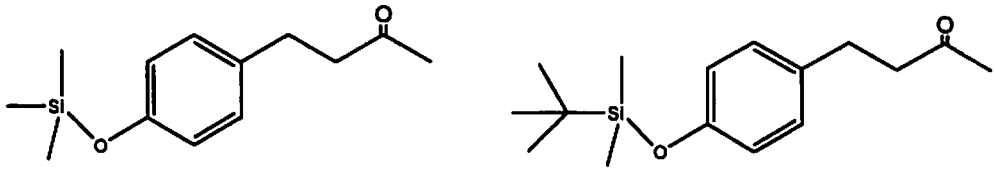


Raspberry ketone



Raspberry ketone

Fig. 3. Bicyclic analogs of raspberry ketone.



Raspberry ketoneTMS ether

Raspberry ketoneTBDMS ether

Fig. 4. Silyl ethers of raspberry ketone.

ether and cuelure were comparably attractive, both catching at least twice the number of flies as raspberry ketone. Interestingly, the related t-butyldimethylsilyl ether was also measurably attractive. This compound is significantly less volatile than the trimethylsilyl ether, and the bulky functional group would decidedly change the shape and space requirements of that end of the molecule – i.e. its ability to fit a receptor.

We decided to re-examine another compound that had briefly been investigated earlier, specifically raspberry ketone formate (Fig. 5). In 1982, Metcalf & Metcalf reported that it was superior to either raspberry ketone or cuelure in attractancy, catching twice as many flies in a limited field test, and it is uncertain why the development of RKF as an attractant was not pursued more energetically at that time. Somewhat later, in our own laboratory, DeMilo and Khrimian prepared a sample of the formate and also found it to be highly attractive to male melon flies (unpubl.).

More recently, we resynthesized the material and initiated experiments in which both sterile and wild male melon flies were consistently attracted to raspberry ketone formate in short-term (24-h) field tests (Casaña-Giner *et al.* 2003b). In the first test, the formate caught twice as many sterile male melon flies as cuelure in 24 h (261 ± 44 by the formate, 130 ± 17 by cuelure, 95 for raspberry ketone). In the second test, this time with wild flies, the formate again caught more flies than cuelure (177 ± 42 to 130 ± 32). In a longer term experiment, raspberry ketone formate captured 1.7 times more melon flies than cuelure over 28 days. The difference in attractiveness between the two baits was greater in the first two weeks than in the last two weeks, but the traps baited with the formate consistently caught more than those baited with cuelure through the course of the experiment. In an ongoing experiment on the Hawaiian island of Pohoiki, where several baits and formulations are being evaluated against wild melon flies, a formulation of raspberry ketone formate has consistently caught more flies than a similar formulation of cuelure over at least 14 weeks (Fig. 6).

As mentioned, earlier investigators did not follow up the encouraging results with raspberry ketone formate. A likely explanation is their apprehension about presumed hydrolytic stability.

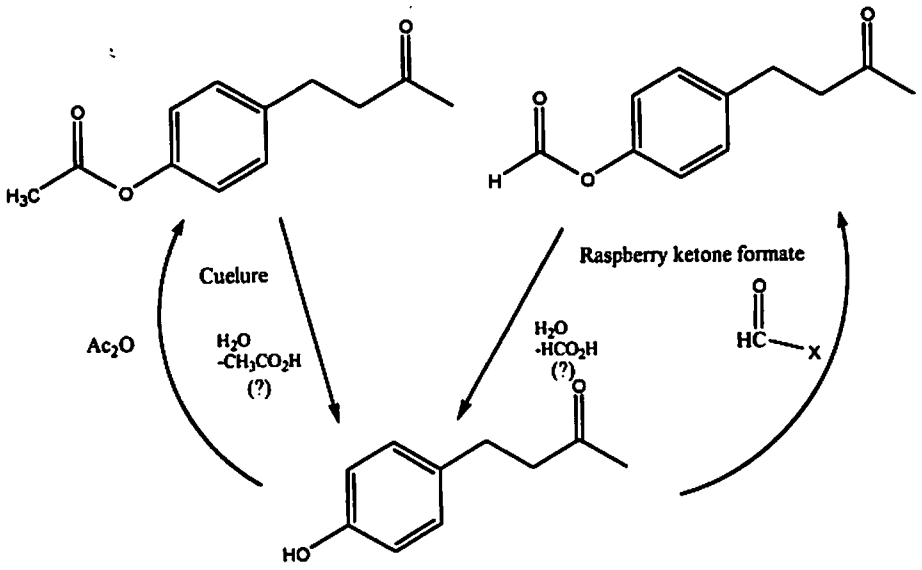


Fig. 5. Cuelure and raspberry ketone formate.

Metcalf (1990) stated that cuelure is so sensitive to hydrolysis that it is virtually impossible to measure its intrinsic attractiveness, and strongly suggested (Metcalf & Metcalf 1992) that the attractancy of cuelure and other esters of raspberry ketone is a result of facile hydrolysis of the esters back to raspberry ketone. Formates are generally more easily hydrolysed than acetates, so one would expect raspberry ketone formate to be more rapidly hydrolysed than cuelure (Fig. 5 illustrates the conversion of raspberry ketone to cuelure and the formate, and also hydrolyses of the latter back to raspberry ketone).

However, the hydrolytic instability of cuelure been questioned by some, including ourselves (Casaña-Giner *et al.* 2003b). Cheng *et al.* (1996)

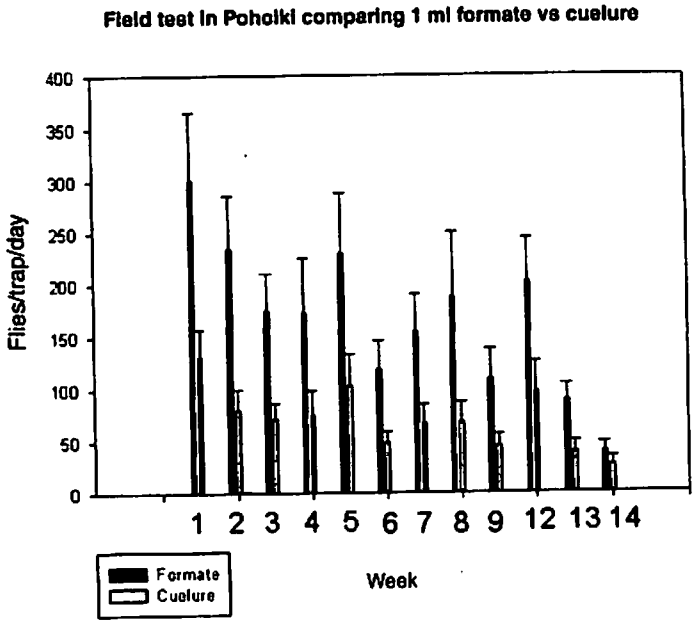


Fig. 6. Melon fly catches by cuelure and raspberry ketone formate.

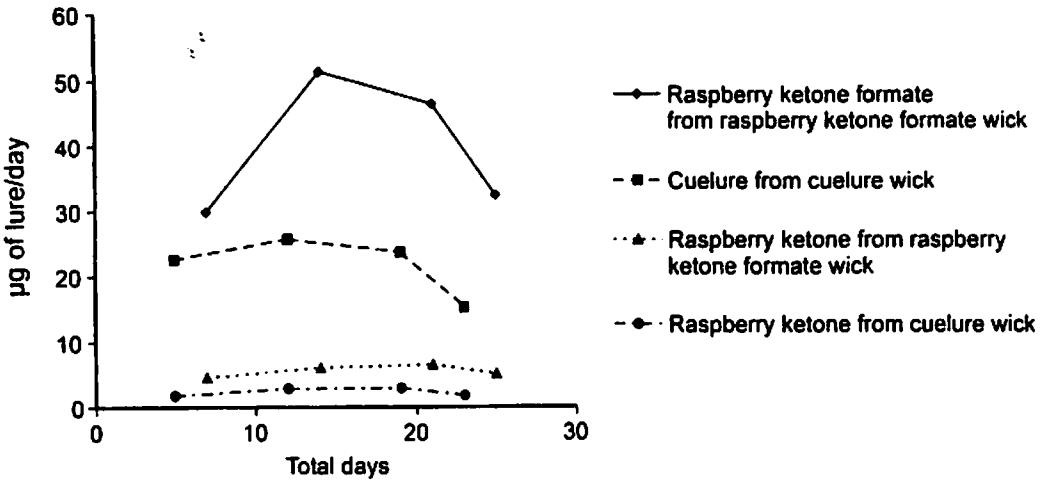


Fig. 7. Lures trapped from air on Super Q. The lower lines represent raspberry ketone trapped although only cuelure or raspberry ketone formate had been applied to the cotton wicks.

reported that only 12–20% hydrolysis of cuelure occurred over a two-month period under field conditions. We similarly found hydrolysis of cuelure under ambient conditions to be relatively slow (about 4% in 10 days in warm, moist air). Liquido (pers. comm.) found that vigorous agitation of an ether solution of cuelure with water for 10 minutes caused no breakdown of cuelure to raspberry ketone. Part of the Metcalf & Metcalf (1992) conclusion was based on higher observed attractancy of raspberry ketone esters applied to moist filter paper compared to the attractancy of the same esters applied to dry filter paper. Their interpretation was that hydrolysis was releasing raspberry ketone which was the true attractant. We suggested (Casaña-Giner *et al.* 2003b) that other contributing factors might not have been considered: more rapid volatilization of the esters from the moist paper because the moisture lowered the surface activity of the paper, for example, or because of co-evaporation of the esters with water. We have found, for example (unpubl.) that the presence of either water or organic solvents facilitates evaporation of raspberry ketone when air is passed through a vessel containing that attractant. The issue is not yet resolved; Metcalf & Metcalf measured insect response, at short range but through air, whereas the other conclusions were based on residue analyses of cuelure recovered from baits or other matrices.

We were gratified to find that raspberry ketone formate was in fact reasonably stable under warm, moist field conditions such as typically encountered during trapping of melon flies (15–30% hydrolysis over 50 days). Heavy loading of the cotton wicks employed as dispensers may be a factor, inasmuch as more rapid hydrolysis was observed on wicks loaded with 0.1 g of the formate, and still more rapid hydrolysis occurred with loadings of 0.01 g. 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. Since high loadings (at least 1 g) seem to be typically used with these baits, the hydrolysis threat does not appear to impose a serious limitation, particularly since the hydrolysis product is itself a melon fly attractant. If lower loading levels should be desirable, alternative release matrices should be investigated; it is possible that a less hygroscopic substance than cotton may prove beneficial, and evaluations of alternative formulations are currently underway.

Questions about the exact nature of the attractant are still being addressed: are the formate and acetate esters inherently attractive, or does hydrolytic conversion to raspberry ketone, perhaps in the vapor phase after evaporation from the release matrix, account for the observed attractancy (as invoked by Metcalf)? By forming large aggregations in the liquid phase, raspberry ketone esters may quite effectively shield the bulk of the molecules from water, whereas single, or smaller clusters of, molecules at the very surface of a matrix or in the vapor phase may be more vulnerable. Ongoing experiments seem to point toward a disproportionately large amount of raspberry ketone in trapped volatiles produced by either raspberry ketone formate or cuelure

(i.e. relatively more raspberry ketone is found in *volatilized* attractant than in residual attractant) (Fig. 7). Rapid ester hydrolysis on an insect's antennal surface is also worth considering. Esterases, hydrolases, etc., are widely distributed in nature and could be invoked as instruments whereby the esters (cuelure and raspberry ketone formate) could be converted to, and perceived as, raspberry ketone by olfactory receptors. However, recall that the two silyl ethers of raspberry ketone (Fig. 4) were also moderately good attractants. Because of the lack of naturally occurring organosilicon compounds, specific enzymes facilitating their hydrolysis would be quite unexpected.

In summary, raspberry ketone formate appears to be significantly superior to cuelure as a melon fly attractant, and adequately stable for practical applications. Further evaluations are underway, as are experiments to better understand the exact nature of the attractant and attraction process.

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