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Attraction of Female Oriental Fruit Fly, Bactrocera dorsalis, to Terminalia catappa Fruit Extracts in Wind Tunnel and Olfactometer Tests【Research report】

以風洞及嗅覺檢定裝置測試欖仁果實萃取物對雌性東方果實蠅之誘引效果【研究報告】

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Abstract

Extracts of the tropical almond fruit, Terminalia catappa L., a preferred host of the oriental fruit fly, Bactrocera dorsalis (Hendel) (Tephritidae: Dacini), were tested for attractancy with both male and female flies in three separate bioassays. Multi-choice laboratory olfactometer tests showed female biased responses to an ethanol extract of T. catappa. These results were confirmed by laboratory wind-tunnel experiments, with mature mated females responding significantly more to the ethanol extract than males, immature, or virgin females. Outdoor olfactometer tests with the ethanol extract showed strong attraction over the control, but in contrast to the laboratory bioassays, there were no differences between the numbers of males and females caught. The male activity is presumably due to the presence of methyl eugenol, which was identified in the T. catappa extracts by GC/MS analysis. Surprisingly, when methyl eugenol alone was tested in the outdoor olfactometer, a little over a quarter of the flies caught were female. These results suggest that while the compound(s) responsible for the attraction of B. dorsalis to T. catappa are largely unknown, methyl eugenol may play an important role in the interaction of fly and host. Female attraction to methyl eugenol and the presence of this phenyl propanoid in T. catappa are discussed in relation to the 'ancestral host hypothesis'.

摘要

以三種不同試驗測試東方果實蠅寄主植物欖仁之果實萃取物對雌、雄蠅的誘引效果。室內多重選擇誘效測試顯示，雌蠅偏好欖仁果實乙醇萃取物。風洞試驗亦證實此項結果，且對成熟交尾過的雌蠅誘引效果明顯高於雄蠅、未成熟及未交尾雌蠅。於戶外誘效測試顯示欖仁果實乙醇萃取物處理組誘引東方果實蠅效果明顯優於對照組，但與室內試驗結果比較，捕獲的雌、雄蠅數目彼此間並無顯著差異。欖仁果實萃取物對雄蠅的誘引，可能是因為果實中含有甲基丁香油，此點已經由液相層析質譜儀分析獲得證實。令人意外的是，於戶外誘效試驗中，單獨使用甲基丁香油所捕獲的東方果實蠅中，約有四分之一是雌蠅。由上述試驗結果推測，雖然欖仁果實中對東方果實蠅誘引成分大部分仍未明瞭，甲基丁香油可能在果實蠅和寄主植物交互作用間扮演重要角色。甲基丁香油對雌蠅的誘引和欖仁果實中存在的Phenyl propanoid 與遠古寄主假說的關係於文中討論。

Key words: Female attractant, Tropical almond, methyl eugenol, semiochemicals, Tephritidae
關鍵詞: 雌蟲誘引物、欖仁、甲基丁香油、嗅覺化學傳訊物質、果實蠅科

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Attraction of Female Oriental Fruit Fly, *Bactrocera dorsalis*, to *Terminalia catappa* Fruit Extracts in Wind Tunnel and Olfactometer Tests

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ABSTRACT

Extracts of the tropical almond fruit, *Terminalia catappa* L., a preferred host of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Dacini), were tested for attractancy with both male and female flies in three separate bioassays. Multi-choice laboratory olfactometer tests showed female biased responses to an ethanol extract of *T. catappa*. These results were confirmed by laboratory wind-tunnel experiments, with mature mated females responding significantly more to the ethanol extract than males, immature, or virgin females. Outdoor olfactometer tests with the ethanol extract showed strong attraction over the control, but in contrast to the laboratory bioassays, there were no differences between the numbers of males and females caught. The male activity is presumably due to the presence of methyl eugenol, which was identified in the *T. catappa* extracts by GC/MS analysis. Surprisingly, when methyl eugenol alone was tested in the outdoor olfactometer, a little over a quarter of the flies caught were female. These results suggest that while the compound(s) responsible for the attraction of *B. dorsalis* to *T. catappa* are largely unknown, methyl eugenol may play an important role in the interaction of fly and host. Female attraction to methyl eugenol and the presence of this phenyl propanoid in *T. catappa* are discussed in relation to the 'ancestral host hypothesis'.

Key words: Female attractant, Tropical almond, methyl eugenol, semiochemicals, Tephritidae

Introduction

Fruit flies (Diptera: Tephritidae) in the subfamily Dacinae are a diverse, rapidly evolving group of more than 700 described species with a persistent effect on tropical agriculture (Metcalf, 1990). One dacine, the oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a serious pest throughout Southeast Asia and the

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Pacific Rim, where it is reported to attack over 173 different varieties of fruit and vegetables (Metcalf and Metcalf, 1992). The fly became established in Hawaii in 1946 (Steiner *et al*., 1965) and presents not only control problems within the state but quarantine issues which limit exports to the U.S. mainland. A key component in detection and control of this pest has been the use of semiochemical attractants (Jang and Light, 1996).

Tephritid attraction behavior is complex, with fly responses based on visual cues (Cornelius *et al*., 1999), the physiological state of the fly (Jang, 1995), and olfactory cues, including sex pheromones and various host and nonhost plant semiochemicals (Fletcher and Kitching, 1995). Dacine semiochemicals were first investigated by Howlett (1912), who observed that oil of citronella was attractive to males of *Dacus* (*Bactrocera*) spp. and subsequently identified methyl eugenol as the active attractant (Howlett, 1915). Methyl eugenol has subsequently demonstrated great usefulness in detection and/or control of the oriental fruit fly (Jang and Light, 1996), for example, its use in several successful eradication programs (Koyama *et al*., 1984; Nakamori *et al*., 1988), but its attractiveness is limited to males. Other food-type attractants, such as hydrolyzed protein products (e.g. NuLure) and synthetic chemical blends (e.g. BioLure), are moderately attractive to both males and females of many tephritid species (Jang and Light, 1996; Cornelius *et al*., 2000). The value of methyl eugenol as a male lure has encouraged efforts to develop female lures of comparable attractancy, with significant work focused on plant materials that are associated with flies in the environment.

While *B. dorsalis* is generally considered polyphagous, with wide host range, they nevertheless appear to display a strong preference for particular hosts (Drew, 1989; Clarke *et al*., 2001). One such plant is the tropical or Indian almond, *Terminalia catappa* L. Believed to have originated in Malaysia, this tree is generally confined to mesic and wet coastal habitats and is distributed throughout the Old World tropics and tropical America (Morton, 1985). Reaching heights of 15 to 25 m, *T. catappa* shows strong salt-, drought- and wind-tolerance and produces fruit (5-10 cm long) with a thin flesh surrounding a large fibrous nut. While the fleshy fruit is the target of larval infestation, *T. catappa* leaf extracts have also been shown to preferentially attract female oriental fruit flies (Chen and Dong, 2000). Clarke *et al*. (2001) found that *T. catappa* along with *Psidium guajava* L. constituted the major hosts for *B. dorsalis* in a survey of Thailand and Malaysia. In addition, *T. catappa* reared a particularly high number of larvae in proportion to the weight and number of fruit sampled, leading to the suggestion that it is a "primary native host" in the surveyed areas (Clarke *et al*., 2001). Similar disproportionately high numbers of larvae from *T. catappa* have been documented in Hawaii (Maehler, 1949), and these observations drew our attention to this host. Stands of tropical almond appear to be somewhat isolated from other known hosts of oriental fruit fly, but the fruit infestation levels suggest that this tree may contain a powerful attractant for female oriental fruit flies.

The objective of the present study was to characterize the attractiveness of *T. catappa* fruit extracts to oriental fruit fly and to establish if further chemical investigation is warranted. This was carried out in a number of bioassays arenas with attention given to female biased attraction. Preliminary GC/MS analysis of *T. catappa* extracts showed the presence of methyl eugenol, the strong male oriental fruit fly attractant, and the ecological ramifications of this discovery are discussed in light of the present understanding of dacine lure biology.

Materials and Methods

Insects

Adult flies used in this study were obtained as pupae from the mass-rearing unit at the USDA ARS Pacific Basin Agricultural Research Center, Mass-Rearing Facility in Honolulu, Hawaii. Larvae were reared on a standard wheat, sugar, yeast diet (Tanaka *et al*., 1969). Pupae were shipped by air to Hilo, HI, where they were placed into 30 cm × 30 cm × 30 cm cubical aluminum screen cages containing sugar, water, and hydrolyzed protein. Flies were held at 24ºC, 60-80% relative humidity, and 12L:12D photoperiod until use. Prior to testing, caged flies were placed in a 5ºC walk-in refrigerator to immobilize them before separated into groups of either 50 males or females. Flies were then placed in plastic containers with nylon mesh covers containing sugar, water, and hydrolyzed protein and held at room temperature (24ºC) for at least one hour before use. Mature flies used were approximately 9-11 days old when tested and presumed to be mated (ca. > 95% of females from mixed cages are mated by day 7) (Jang *et al*., 1997). For studies involving virgin females, flies were sexed within two days of adult emergence and held in groups of 50 as above. Immature flies were tested 3-4 days after adult emergence.

Instrumentation

GC/MS analysis was performed on an Agilent Technologies 6890N gas chromatograph interfaced with a Hewlett-Packard 5973 Mass Selective Detector equipped with an HP-5MS column (30 m × 0.25 mm ID 0.25 μm film thickness). The temperature program used was 45ºC to 240ºC at 10ºC/min with a 1 min start delay with the injector temperature set at 250ºC using helium as a carrier gas (1.1 ml/min.). GC analysis was performed on an Agilent Technologies 6890N gas chromatograph equipped with an HP-5 column (30 m × 0.32 mm ID 0.25 μm film thickness). The injector and flame ionization detector (FID) were held at 250ºC and 275ºC, respectively, with nitrogen as a carrier gas (8.4 ml/min.). Methyl eugenol from *T. catappa* was identified on the basis of mass spectra (NIST98 mass spectral database) and comparison of retention time with an authentic standard.

Plant Material and Extracts

Fallen fruits were collected from two locations, Onekahakaha Beach Park, Hilo, Hawaii and Anini Beach Park, Kauai Island, Hawaii, from October to December 2004. Fruits from Kauai were shipped by air to Hilo, and all fruit were extracted fresh or frozen at -70ºC until use. Newly ripened fruit were used for all extractions. Preliminary tests conducted with green unripe fruit showed only low level attractancy. Ripening fruit in the laboratory was also attempted, but once green fruits are removed/fall from the tree they do not continue to ripen. Extracts of *T. catappa* were made by soaking fruits for at least 24 hours in hexane, ethanol or water at a fruit/solvent ratio of 1 g/ml. For extracts used in the outdoor olfactometer the ethanol was removed by rotary-evaporation. Extracts were stored at -70ºC until used in bioassays.

For methyl eugenol quantitative analysis, the fleshy part of the fruit was cut away from the fibrous core, wrapped in cheesecloth, and pressed. Eicosane (100 ng) was added, as an internal standard, to a sample of the resulting juice (1 ml), which was then extracted twice with equal volumes of dichloromethane. Methyl eugenol content was quantified by GC-FID analysis utilizing a standard curve. *T. catappa* leaves were ground in 1:1 water/dichloromethane using an explosion proof blender. The resulting slurry was centrifuged, and the dichloromethane was removed and evaporated to dryness under

a nitrogen stream. Silica gel chromatography was performed with sequential elution using hexane, dichloromethane and methanol. Fractions were concentrated under a nitrogen stream before analysis. Methyl eugenol used in this study was obtained from Elan Chemical Co. Inc., Newark, NJ and distilled to provide a clear viscous liquid, purity 99.8% (GC).

Laboratory and Outdoor Olfactometer Bioassays

Attractancy tests with *T. catappa* extracts were carried out in three different behavioral arenas. The first two arenas tested attraction in more controlled, smaller scale, short duration assays, while the third was more natural, larger and encompassed a greater portion of the day. The first bioassay, a laboratory multiple-trap rotating cage olfactometer (0.9 m × 0.9 m × 0.9 m) described by Jang and Nishijima (1990), was used to compare different extracts in competitive tests. Three glass McPhail traps containing 5 ml of the respective solvent extracts in 10 ml of water along with a negative control (15 ml water) were hung on opposite arms of the rotating hub. Two drops of the IGEPAL nonionic surfactant were added to each trap. The test duration was 1 hr at 26-28ºC under fluorescent lights (ca. 2000 lux) with the hub revolving at a rate of 2 r.p.m. Fifty mature flies of each sex were released into the cage and allowed to choose freely among the different extracts. Tests were run between 13:00 and 16:00 hr, a time when females are known to be responsive to plant attractants (Jang *et al*., 1997). At the end of each run, entrapped flies were removed and counted.

The second arena was a glass/metal laboratory flight tunnel consisted of a 0.9 m × 0.9 m × 2.8 m rectangular glass arena equipped with inlet and exit fans, which produced a laminar flow of air (Jang *et al*., 1997). The ethanol extract and the negative control in the two-choice flight-tunnel tests were placed in small glass McPhail traps as previously described. For each assay, 50 males and 50 females or 50 virgin females only were released from the downwind end of the flight tunnel and allowed to respond freely for 2 hr. Groups of immature males and female were tested in the same way. At the end of each test, trapped flies were counted, remaining flies removed, and the flight tunnel cleaned. All assays were run between 13:00 and 16:00 hr at a temperature of 26-28ºC, under fluorescent lights (2000 lux) (Jang *et al*., 1994).

An outdoor multiple rotating trap olfactometer was used to evaluate attractancy on a larger scale, over a longer period of time and under less controlled, more natural conditions. The olfactometer consisted of a 3 m × 3 m × 2.5 m rectangular wood-framed screen cage (Jang *et al*., 1997). A rotating hub with arms approximately 62 cm long was hung from the center of the cage approximately 1.8 m from the floor. The outer arms of the motorized hub unit revolved at a rate of 1.5 r.p.m. Treatments of the ethanol extract (15 ml extract/15 ml water) or methyl eugenol (500 μg/30 ml water) vs. a negative control (30 ml water) were carried out using small invaginated glass McPhail traps with 2 drops of the nonionic surfactant IGEPAL added to each trap. Approximately 500 flies of each sex were placed in the outdoor cage. The duration of the tests was approximately 19 hours (initiated 13:00; terminate 8:00 following day). Tests were conducted under ambient outdoor conditions (usually 23-28ºC), and only natural (indirect) light was used. At the end of the test, flies were removed from the traps and counted.

Data Analysis

Results of the two-choice flight tunnel tests were compared using *t*-tests (Proc TTEST) (SAS, 2000). Multiple-choice test results were analyzed using ANOVA, and

Table 1. Laboratory rotation olfactometer bioassay. Mean number (± SE) of flies caught per trap by solvent extracts of *T. catappa* fruit [1)]

Treatment	N	EtOH (mean ± SE)	Hexane (mean ± SE)	H_20 (mean ± SE)	Control (mean ± SE)
Mature mated					
Female	4	17.2 ± 2.0 a	3.4 ± 0.9 b	2.0 ± 0.9 b	0.0 ± 0.0 b
Male	4	4.2 ± 0.7 b	2.7 ± 1.2 b	3.2 ± 1.4 b	0.0 ± 0.0 b
Total		21.4	6.1	5.2	0.0

[1)] Means followed by different letters within treatment are significantly different at $p < 0.05$; Tukey's HSD (SAS, 2000).

Table 2. Oriental fruit flies attracted to ethanol extract of *T. catappa* fruit in wind tunnel bioassay [1)]

Treatment	N	Ethanol extract (mean ± SEM)	Control (mean ± SEM)
Mature mated			
Female	4	16.6 ± 2.5 a	1.2 ± 0.6 b
Male	4	1.8 ± 0.9 b	0 ± 0 b
Total		18.4	1.2
Immature			
Female	4	3 ± 0.9 a	0.3 ± 0.3 a
Male	4	0.5 ± 0.3 a	0.3 ± 0.3 a
Total		3.5	0.6
Mature virgin			
Female	4	0.8 ± .5 a	0.3 ± 0.3 a

[1)] Means followed by different letters within treatment are significantly different at $p < 0.05$; Tukey's HSD (SAS, 2000).

means compared using the Tukey's HSD studentized range test (Proc GLM) (SAS, 2000). All analyses of significance were made at the $p < 0.05$ level of significance or lower.

Results

Multi-choice laboratory olfactometer tests of *T. catappa* fruit extracts showed significant attractiveness to both sexes ($p < 0.05$) with all solvents tested, when compared to the water control (Table 1). The ethanol extract of *T. catappa* was significantly more attractive to females when compared to all other treatments ($p < 0.05$), but male catches were not significantly different than male or female catches with water or hexane extracts.

Two choice wind tunnel bioassays with mature, mated flies showed significant differences ($p < 0.05$) in catch numbers between female flies caught with the ethanol extract and all other counts within the treatment (Table 2). There were no significant differences between the ethanol extract and control in the immature and mature virgin female tests.

The outdoor olfactometer tests showed significantly different ($p < 0.01$) catches with both males and females caught in treatments compared to controls (Table 3). No differences were seen between female and male catches with the ethanol extract, but significantly more males were caught

Table 3. Oriental fruit flies attraction in outdoor olfactometer bioassay [1)]

Treatment	N	Treatment (mean ± SEM)	Control (mean ± SEM)
Ethanol extract			
Female	11	137.4 ± 12.6 a	0.1 ± 0.1 b
Male	11	108.6 ± 13.4 a	0.0 ± 0.0 b
Total		246.0	0.1
Methyl Eugenol			
Female	11	44.3 ± 6.8 b	0.0 ± 0.0 c
Male	11	120.2 ± 12.3 a	0.3 ± 0.1 c
Total		164.5	0.3

[1)] Means followed by different letters within treatment are significantly different at $p < 0.05$; Tukey's HSD (SAS, 2000).

by methyl eugenol.

Initial GC-MS analysis revealed the presence of methyl eugenol in the ethanol extracts from *T. catappa*. Subsequent GC-FID quantification with eicosane as an internal standard showed 1.3 ± 0.3 μg/ml (N = 4) of methyl eugenol in pressed juice. Methyl eugenol was also identified by GC-MS and GC-FID in *T. catappa* leaves but was not reliably quantified due to its low abundance.

Discussion

Laboratory olfactometer and wind tunnel bioassay studies demonstrate the attractiveness of the *T. catappa* fruit ethanol extract to mature mated *B. dorsalis* females. These results are similar to previous reports of female biased attraction of oriental fruit fly to volatiles from leaves of several non-host plants (Jang *et al.*, 1997; Chen and Dong, 2000). The laboratory olfactometer tests suggest that the female attractive compound(s) are of a moderate polarity as shown by the activity of the ethanol extract over the other extracts. The unresponsiveness of virgin and immature females to the ethanol extract is consistent with a 'behavioral switch', where females only respond strongly to host odors after they are mated and prepared to lay eggs in fruit (Jang, 1995).

In contrast to the laboratory bioassays, the attraction of both females and males in the outdoor olfactometer was unexpected. However, preliminary GC/MS analysis identified methyl eugenol in *T. catappa* fruit extracts which presumably accounts for the observed male activity. The fact that males only responded to *T. catappa* fruit extracts in this bioassay is perplexing but may be due to changes in behavior reflecting the larger scale, longer time period and less artificial conditions of this bioassay. A similar attraction of both male and female oriental fruit flies was reported in field cage tests with asarylaldehyde, a compound found in Indian calamus root oil (Jacobson *et al.*, 1976).

Tests with methyl eugenol against a negative control were conducted to establish its role, if any, in attraction of females to *T. catappa* fruit extracts. These showed a considerably higher female:male ratio in *T. catappa* extracts than with methyl eugenol only. However, direct comparisons are difficult as the methyl eugenol levels used were subsequently found to be higher than those found in *T. catappa* extracts and both treatments were not run head-to-head. Allowing for this, the comparison of methyl eugenol and *T. catappa* extracts catches suggests that, while methyl eugenol

may be responsible for some female attraction, and is most likely responsible for the male attraction, females are largely attracted to an as yet unidentified compound(s). Further study to characterize additional attractants present in *T. catappa* seems warranted.

The number of females caught with what is generally considered to be a male lure was itself a somewhat unexpected result. While the attraction of male dacine flies to methyl eugenol is well known, the relationship with females is much less clear. Female attraction to methyl eugenol was first noted in field studies by Steiner *et al.* (1965) with similar results also reported more recently (Verghese, 1998). More extensive investigation by Fitt (1981a) and Raghu and Clarke (2003b) have found that responsiveness is limited mostly to virgin females. Methyl eugenol is also reported to stimulate oviposition in females oriental fruit flies (Metcalf *et al.*, 1983), and it simulates strong electrophysiological responses (Lee *et al.*, 1997). The relatively high female:male ratio observed in the outdoor olfactometer are similar to those reported by Verghese (1998), suggesting these female catches are not simply an artifact of the bioassay. A majority of the females responding to methyl eugenol in this study would be expected to be mated (ca. > 95% of females mated by day 7) (Jang *et al.*, 1997), which puts these results somewhat at odds with previous work (Fitt, 1981a; Raghu and Clarke, 2003b). Additionally, females caught in methyl eugenol baited traps may be in part responding to pheromone released into the trap by dying males complicating the results.

The presence of methyl eugenol in the tropical almond may have implications for our understanding of the ecological relevance/evolutionary history of this semiochemical. While methyl eugenol has remarkable attractancy for many *Bactrocera* spp., its biological role is not altogether clear with two principal hypotheses proposed to explain dacine responses to this naturally occurring phenylpropanoid. These hypotheses are often viewed as alternative to each other, but they are not necessarily mutually exclusive (Raghu, 2004). Most recent research has pointed to the importance of methyl eugenol as a pheromone precursor with proximate benefits and behaviors seen in males that acquire the compound (Fitt, 1981a, b; Shelly and Dewire, 1994; Tan and Nishida, 1998). In *B. dorsalis*, methyl eugenol is oxidized to 2-allyl-4,5-dimethoxyphenol and coniferyl alcohol, which are released during courtship (Nishida *et al.*, 1988). While a competitive advantage is well established for *B. dorsalis,* males of a related species, *B. cacuminata,* failed to show significant benefits after feeding on methyl eugenol (Raghu and Clarke, 2003a).

The second hypothesis primarily addresses the evolutionary relationship between dacine flies and their ancestral host(s) (Drew, 1989; Metcalf, 1990; Metcalf and Metcalf, 1992). Dacines are thought to have originated in the Indian block of Gondwana during the Cretaceous and Tertiary periods, where they were saprophagous, feeding on rotting fruit (Rohdendorf, 1974; Munro, 1984; Drew and Hancock, 2000). Para-coumaric acid from these rotting fruits is thought to have been a kairomone for ancestral dacines and also a precursor of both methyl eugenol and raspberry ketone (Metcalf, 1990). Host plant semiochemicals are particularly important in facilitating host plant location, which is the focal point for the ecology of the fly (Drew, 1989). Therefore Metcalf speculated that the ecological role of methyl eugenol was as a mating rendezvous cue at the host plant (Metcalf, 1990). This 'ancestral host hypothesis' is supported by the observation that mature virgin female *Bactrocera* spp. are attracted to methyl eugenol (Fitt, 1981a; Raghu and Clarke, 2003b).

Metcalf's hypothesis has been discounted

(Fitt, 1981a) by the observation that, while methyl eugenol has been isolated from a number of plants (Metcalf, 1990; White, 2000; Raghu, 2004), few of these plants are encountered by Dacinae flies. Countering this, Raghu and Clarke (2003b) showed that *B. cacuminata* (Hering) partition their behavior between spatially separate resources so that a mating cue could be emitted from a non-host plant. However, many *Bactrocera,* including *B. dorsalis*, are more closely associated with their hosts (Drew and Hancock, 2000) and hosts releasing methyl eugenol would therefore be expected under this hypothesis. In agreement with this, six *Bactrocera* host fruits are now known to contain methyl eugenol (Gaydou *et al.*, 1986; Ryman, 1991; Vernin *et al.*, 1998; Pino *et al.*, 2002; Sajjadi and Mehregan, 2003), including the widely attacked common guava, *P. guajava,* (Paniandy *et al.*, 2000), and Strawberry guava, *P. cattleianum* Sabine (Vernin *et al.*, 1998). Given the evidence that *T. catappa* is an important native host for oriental fruit fly, the discovery of methyl eugenol in this plant adds additional weight to the ancestral host hypothesis.

Arguments for and against both hypotheses can be made but perhaps the most interesting questions arise when the hypotheses are viewed as complimentary. For instance, if methyl eugenol as a mating cue is ancestral it would be possible that is was the preadaptation for the development of methyl eugenol as a pheromone precursor. These questions highlight the underlying complexity of the interactions involving methyl eugenol and dacine fruit flies and point to a need to better understand what role methyl eugenol plays in female behavior.

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以風洞及嗅覺檢定裝置測試欖仁果實萃取物對雌性東方果實蠅之誘引效果

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摘　要

以三種不同試驗測試東方果實蠅寄主植物欖仁之果實萃取物對雌、雄蠅的誘引效果。室內多重選擇誘效測試顯示，雌蠅偏好欖仁果實乙醇萃取物。風洞試驗亦證實此項結果，且對成熟交尾過的雌蠅誘引效果明顯高於雄蠅、未成熟及未交尾雌蠅。於户外誘效測試顯示欖仁果實乙醇萃取物處理組誘引東方果實蠅效果明顯優於對照組，但與室內試驗結果比較，捕獲的雌、雄蠅數目彼此間並無顯著差異。欖仁果實萃取物對雄蠅的誘引，可能是因爲果實中含有甲基丁香油，此點已經由液相層析質譜儀分析獲得證實。令人意外的是，於户外誘效試驗中，單獨使用甲基丁香油所捕獲的東方果實蠅中，約有四分之一是雌蠅。由上述試驗結果推測，雖然欖仁果實中對東方果實蠅誘引成分大部分仍未明瞭，甲基丁香油可能在果實蠅和寄主植物交互作用間扮演重要角色。甲基丁香油對雌蠅的誘引和欖仁果實中存在的 Phenyl propanoid 與遠古寄主假說的關係於文中討論。

關鍵詞：雌蟲誘引物、欖仁、甲基丁香油、嗅覺化學傳訊物質、果實蠅科。