Nat. Volatiles & Essent. Oils, 2017: 4(1): 1-7

NVEO Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Short range attraction of *Ceratitis capitata* (Diptera: Tephritidae) sterile males to six commercially available plant essential oils

Nancy D. Epsky[1]* and Jerome Niogret[2]

[1] Subtropical Horticulture Research Station, U.S. Department of Agriculture/Agricultural Research Service, 13601 Old Cutler Rd, Miami, FL 33158 USA

[2] Niogret Ecology Consulting LLC, 5961 SW 42nd Ter, Miami, FL 33155 USA

*Corresponding author. Email: nancy.epsky@ars.usda.gov

Abstract

Plant essential oils have a number of roles in insect pest management. For male *Ceratitis capitata*, this includes use of angelica seed oil as long range attractants and ginger root oil, which is exposure to sterile males to increase mating success. This is also called as aromatherapy in entomology? Neither of these plants are hosts for *C. capitata* and the chemical basis for these effects is unknown. Small cage bioassays were conducted to test short range attraction of sterile males to essential oils such as angelica oil, ginger root oil, Manuka oil, orange oil, cubeb oil, and tea tree oil. Previous research found all of these oils attracted males when undiluted oil (5 µL) was tested. Herein we compared attraction to undiluted and 100, 10, and 1 µg/µL diluted concentrations with *n*-hexane to determine if concentration affected short-range attraction, respectively. Undiluted angelica seed oil, cubeb oil and manuka oil attracted more males than dilutions of the same oils, however more males were attracted to orange oil and tea tree oil that was diluted to 100 µg/µL, and to ginger root oil that was diluted to 10 µg/µL. Overall, the highest attraction of sterile males (53%) was to tea tree oil (500 µg). Additional studies are needed to determine the chemicals responsible for this attraction, but bioassays of short range attraction to tea tree oil may be useful for quality control assessment of sterile males used in the sterile insect technique for pest control.

Keywords: Mediterranean fruit fly, attractant, tea tree oil, behaviour

Introduction

Plant essential oils have a number of roles in insect pest management and can be biologically active for insects as attractants, repellents and toxicants (Isman, 2000). Angelica seed oil, *Angelica archangelica* L., was found to be attractive to males of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), and traps baited with this oil were used for survey and detection for the eradication program in Florida in 1956 (Steiner et al., 1957). These traps were used so extensively both in Florida and in other areas vulnerable to *C. capitata* invasion during this time period that the 1956 production and older supplies of angelica seed oil were exhausted (Steiner et al., 1957). The chemical found to be responsible for attraction to this oil was α-copaene (Fornasiero et al., 1969), a chemical that is widely distributed in the plant kingdom. Tests of structural analogs of α-copaene found that those found in angelica seed oil were more attractive than those found in cubeb oil, *Piper cubeba* L. (Flath et al., 1994). Subsequent research to find a synthetic attractant equal in effectiveness to angelica seed oil resulted in the discovery of trimedlure ($C_{12}H_{21}ClO_2$), tert-butyl-4 (or 5)-chloro-2-methylcyclo-hexane-1-carboxylate (Beroza et al., 1961). This provided a cheap, abundant attractant for males, and Jackson traps baited with trimedlure are the standard male-targeted trapping system for *C. capitata* (IAEA, 2003).

Trimedlure is not of plant origin and there are no natural sources of trimedlure. The first demonstration of a benefit to male *C. capitata* was the finding that exposure to trimedlure volatiles increased mating success, although the effect was short lived (Shelly et al., 1996). This led to the discovery that exposure to ginger root oil, *Zingiber officinale* Roscoe, which contains α-copaene, also increased mating competitiveness of males and the effect was long lasting (Shelly, 2006). This approach is termed aromatherapy, since the males do not have to contact the oil to gain the benefit, and this approach has been used to increase effectiveness of sterile males released for control and eradication (Shelly, 2001; 2006).

Several essential oils have been found that are both long range attractants and that increase mating success. These include essential oils from both hosts (orange oil, *Citrus aurantium* L., Katsoyannos et al. 1997) and non-hosts (ginger root oil, manuka oil, *Leptospermum scoparium* Forst & Forst, and tea tree oil, *Melaleuca alternifolia* (Maiden and Betche) Cheel. (Shelly and Pahio 2002; Shelly et al. 2004; Shelly et al. 2008; Shelly & Epsky, 2015). Subsequent research found that all of these essential oils as well as cubeb oil are also short range attractants, but level of attraction was not correlated with concentration of α-copaene (Niogret et al., in press). Volatile chemicals from essential oils presented in too high of a concentration may cause males to arrest (Katsoyannos et al. 1997, Shelly 2001) and settle a distance away from the chemical source (Kennedy, 1978). Thus, concentration of the material tested is an important factor in evaluation of short range attraction.

Reported herein is research to 1) determine the effect of concentration of several plant essential oils on short range attraction of male *C. capitata* and 2) conduct comparative tests of those essential oils at concentrations that were found to elicit the highest level of attraction. Laboratory tests were conducted with sterile male *C. capitata* obtained from shipments sent to Florida as part of the preventative release program. Essential oils tested included angelica seed oil, ginger root oil, manuka oil, orange oil, cubeb oil and tea tree oil.

Materials and Methods

Insects

Sterile male *C. capitata* were obtained from the Programa Moscamed mass rearing facility (El Pino, Guatemala), where they were irradiated as pupae 2 d prior to emergence with 95 Gy of gamma radiation from a Co60 source. These are the temperature sensitive lethal strain flies (Franz 2005) that are used for the preventative release program (Dowell et al. 2000) in Florida. Thus only males were obtained, and only virgin males were used for testing. Irradiated pupae were shipped initially to the USDA-APHIS Medfly Project (Sarasota, FL, USA) and then to the USDA-ARS Subtropical Horticulture Research Station in Miami, FL. Holding conditions at Miami consisted of a 12/12h L/D photoperiod, 25 ± 2 °C, and 75 ± 5 % RH. Pupae were placed in collapsible small cages (20.3 cm^3) and there were ~100 pupae per cage. After eclosion, adult flies were provided with water (2 % agar blocks) and food (3:1 mixture of cane sugar and yeast hydrolysate). Flies used for all studies were 5 to 10 d-old, sexually mature virgin males.

Test substrates

Essential oils used in this study included angelica seed oil (SunRose Cosmetics, New York, NY); cubeb oil, ginger root oil and tea tree oil (Essential Oil India – SAT Group, Kannauj, India); manuka oil (Harmonic Skin Tones, Fallbrook, CA, USA); valencia orange oil (Florida Flavors, Lakeland, FL, USA). Serial ten-fold dilutions were made with *n*-hexane.

Laboratory bioassay

Small cage bioassays were used to quantify the short-range attraction of sterile males to test substrates (McInnis and Warthen 1988; Niogret et al. 2011; in press). All tests were no-choice tests that were conducted between 1 pm and 5 pm at room temperature. Dead flies and remaining pupae were removed prior to the start of a bioassay and the actual number of live flies per cage was determined at the end of each bioassay.

Each essential oil (5 µL) was added to the center of a filter paper disk (Whatman #1, 5.5 cm diam). The filter paper disk was air dried briefly to allow solvent from diluted substrates to evaporate when diluted substrates were tested. The filter paper was placed into the bottom of a plastic Petri dish (85 mm diam, 12 mm height), which was then placed in the center of the cage with flies. After 5 min, the lid was placed on the Petri dish to retain all responding flies, and the covered Petri dish was removed from the cage. Percentage of flies attracted, which was determined by dividing the number of flies in the closed Petri dish by the sum total of flies in the closed Petri dish and live flies remaining in the cage at the end of each bioassay, was used for analysis. Flies and Petri dishes were used only once and then were discarded. Cages were pressure washed with hot water between experiments to eliminate potential residual chemicals.

Two experiments using small cage bioassays were conducted. Both experiments were no choice tests, with one essential oil tested per cage and multiple cages per replicate. Experiment 1 determined if short range attraction to the essential oils could be improved by decreasing the concentration by *n*-hexane dilution. All essential oils were tested as undiluted material (1 mg/µL) and at concentrations of 100 µg/µL, 10 µg /µL, and 1 µg/µL. There were three replicates of each essential oil at each concentration, with replicates run on separate days. Since the tests of response to different concentrations of the essential oils were conducted using different batches of flies and attraction to essential oil-free controls were not included in Experiment 1, Experiment 2 compared among the concentrations that resulted in the numerically highest percentage attraction for each essential oil, which were designated the optimal concentrations. Two additional treatments were added as controls: 1) hexane only (5 µL added to filter paper) and 2) filter paper only to control for effects of the solvent. For experiment 2, there were four treatments tested per day, and tests were run for two days per week to complete tests of all eight treatments per replicate. Combinations tested per day were re-randomized for each replicate, and there were four replicates of these comparisons.

Statistical analysis

Data were analysed by analysis of variance (ANOVA, Proc GLM; SAS Institute, 2010) followed by LSD mean separation (P = 0.05) for significant ANOVAs. When necessary, data were transformed prior to analysis to satisfy conditions of equal variance (Box et al., 1978), non-transformed means ± standard deviations are presented. One way ANOVAs were used to test 1) effect of concentration on percentage of flies captured in Experiment 1, with separate analyses for each test substrate; 2) effect of test substrate on percentage captured in Experiment 2.

Results and Discussion

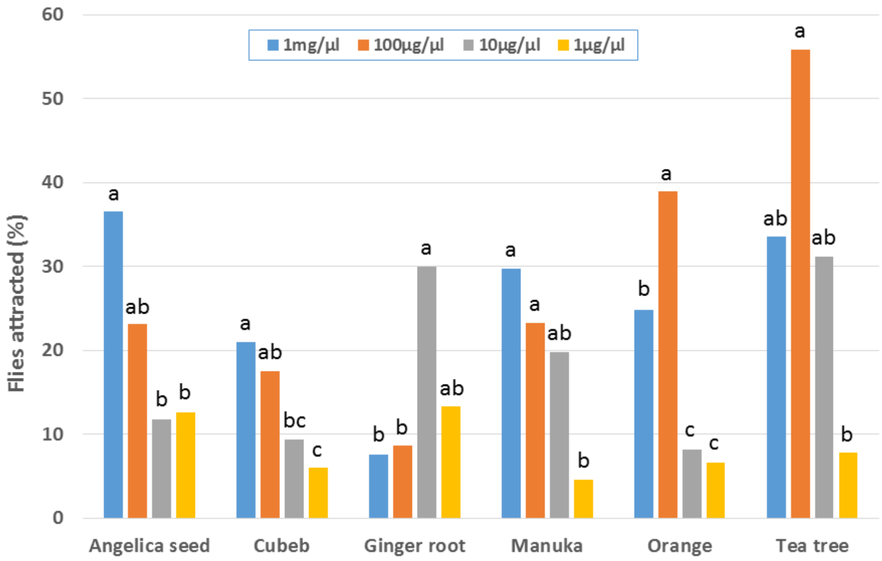
Concentration affected attraction of *C. capitata* males for all six essential oils (angelica seed oil $F_{3,8}$ = 16.14, P = 0.0009, cubeb oil $F_{3,8}$ = 11.04, P = 0.0032, ginger root oil $F_{3,8}$ = 3.49, P = 0.0499, manuka oil $F_{3,8}$ = 7.12, P = 0.012, orange oil $F_{3,8}$ = 70.31, P < 0.0001, tea tree oil $F_{3,8}$ = 6.3, P = 0.0168). The highest attraction of males was to undiluted (1 mg/µl) *Angelica* seed oil, cubeb oil and manuka oil so tests of lower concentrations did not increase response (Fig. 1). However, attraction to orange oil and tea tree oil increased when they were diluted to 100 µg/µL and attraction to ginger root oil increased when it was diluted to 10 µg/µl but then decreased as concentration decreased. Treatments then tested, in Experiment 2, with the same batch of flies so that percentage attraction could be compared directly at the optimal concentration for each oil as determined from Experiment 1. Attraction was affected by treatment ($F_{7,24}$ = 30.03, P < 0.0001). Attraction to tea tree oil (100 µg/µl) was greater than to any other treatment (Table 1). The next highest attraction was to undiluted orange oil and angelica seed oil, followed by ginger root oil (10 µg/µl) and undiluted manuka oil and cubeb oil. All oils, however, attracted more flies than either the hexane control or the untreated control.

All of the essential oils tested in this study attracted sterile male *C. capitata* that were at least within 10 cm, which is considered a short range response. This response occurred fairly quickly, that is, within 5 min of initial exposure. Attraction was affected by concentration of the essential oils, with higher attraction to some oils when they have been diluted. This indicates that the undiluted oils caused the flies to arrest outside of and not enter the Petri dish containing the oil. This was observed in field tests with traps baited with angelica seed oil (Steiner et al. 1957). The authors noted that flies entering newly baited traps stopped a distance from the bait but, after traps had been in the field for a while, flies entering the trap moved closer to the bait. Chemical analysis of these essential oils, using the same sources as those used in this study, found that manuka oil had the highest amount of α-copaene, the chemical hypothesized to be the primary kairomone responsible for attraction (Niogret et al., in press). As had been observed in earlier studies of short range attraction of sterile males to rasped wood, attraction was not correlated with amount of α-copaene (Niogret et al., 2011). Thus, additional research is needed to identify the chemicals responsible for the attraction.

The highest attraction was obtained when flies were exposed to 500 µg of tea tree oil. Studies conducted in Hawaii found that traps baited with tea tree oil captured about 50% as many fertile males as traps baited with trimedlure in field cage tests, but only 8% as many wild males in field tests (Shelly & Epsky, 2015). Although tea tree oil is not promising as a long range attractant for use in traps, the results of our study indicate that bioassay of short range response may provide another measure of quality control for assessing fitness of sterile males used for laboratory experiments or field release. Sterile males are subjected to a number of stress factors including irradiation, packaging and shipping that may cause a decrease in fitness that may be difficult to assess. Standard protocols are in place to assess quality, such as percentage emergence and flight ability, but addition of a measure of short range attraction using a 5 min bioassay may provide additional useful information on new shipments of flies.

Identification of the chemicals responsible for short range attraction may lead to development of new or improved measures of detection or control of *C. capitata*. Methyl eugenol is a kairomone that is both a long range attractant and a feeding cue for males of the Oriental fruit fly, *Bactrocera dorsalis* Hendel, and has been used successfully for *B. dorsalis* control and eradication. Methyl eugenol can be combined with pesticide to kill attracted flies (Steiner and Lee 1955). This approach is called male annihilation (Cunningham 1989) and is the basis for the male annihilation technique (MAT). Trimedlure is not a feeding cue for male *C. capitata* and, because the attracted flies do not contact or feed on trimedlure, it is rarely used for MAT. Research on biologically active chemicals from tea tree oil may provide the identification of *C. capitata* kairomones that could be used for development of MAT for this pest.

Figure 1. Average percentage sterile male *C. capitata* attracted in small cage bioassays of short range attraction. Essential oils were presented as 5 µL samples of undiluted oil (1 mg/µl) and at concentrations of 100 µg/µl, 10 µg/µl, and 1 µg/µl material diluted with *n*-hexane.



Means within a test substrate headed by the same letter are not significantly different (LSD mean separation test, $P = 0.05$), n = 4 per concentration and essential oil; ~80-120 flies per bioassay)

Table 1. Percentage (mean ± std dev) of sterile male *C. capitata* attracted in no choice, small cage bioassays. Essential oils were tested at concentrations that were found to elicit the highest level of response in comparisons among several concentrations.

Essential oil (5 µL)	Concentration	Flies attracted (%)
Tea tree oil	100 µg/µl	52.7 ± 6.8a
Orange oil	100 µg/µl	37.8 ± 12.7b
Angelica seed oil	1 mg/µl	29.5 ± 6.9b
Ginger root oil	10 µg/µl	16.2 ± 5.4c
Manuka oil	1 mg/µl	12.9 ± 6.9c
Cubeb oil	1 mg/µl	11.7 ± 5.0c
Hexane only	-	3.2 ± 2.4d
Filter paper only	-	3.0 ± 0.4d

Means followed by the same letter are not significantly different (LSD mean separation test [$P = 0.05$] on square root [$x + 0.5$]-transformed data, untransformed means presented).

ACKNOWLEDGMENT

The authors thank Teri Allen, Elena Schnell, and Micah Gill (USDA/ARS, Miami, FL, USA) for technical assistance, and Todd Shelly (USDA/APHIS, Waimanalo, HI, USA), Peter Landolt (USDA/ARS, Wapato, WA, USA) and Nurhayat Tabanca (USDA/ARS, Miami, FL, USA) for comments on an earlier version of this manuscript.

REFERENCES

Beroza, M., Green, N., Gertler, S. I. (1961). New attractants for the Mediterranean fruit fly. *Journal of Agricultural and Food Chemistry*, 9(5), 361-365.

Box, G. E. P., Hunter, W. G., Hunter, J. S. (1978). Statistics for experimenters. An ıntroduction to design, data analysis, and model building. J. Wiley & Sons, New York, NY.

Cunningham, R. T. (1989). Male annihilation. *In* A.S. Robinson & G. Hooper [Eds.], World crop pests 3A fruit flies. Their biology, natural enemies and control, (pp 345-351). Elsevier, Amsterdam.

Dowell, R.V., Siddiqui, I. A., Meyer, F., Spaugy, E. L. (2000). Mediterranean fruit fly preventative release programme in southern California. In K. H. Tan (Ed.), *Area-wide control of fruit flies and other insect pests* (pp. 369-375). Penerbit Universiti Sains Malaysia Pulau Pinang, Malaysia.

Flath, R. A., Cunningham, R. T., Mon, T. R., John, J. O. (1994). Male lures for Mediterranean fruitfly (*Ceratitis capitata* Wied.): structural analogs of α-copaene. *Journal of Chemical Ecology*, 20(10), 2595-2609.

Fornasiero, U., Guitto, A., Caporale, G., Baccichetti, R., Musajo, L. (1969). Identification of the attractant of Ceratitis capitata males contained in the Angelica archangelica seed oil. *Gazzetta Chimica Italiana* 99(7), 700-710.

Franz, G. (2005). Genetic sexing strains amenable to large scale rearing as required for the sterile insect technique. In V. A. Dyck, J. Hendrichs, and A. S. Robinson [Eds.], The sterile insect technique: Principles and practice in area-wide integrated pest management (pp. 427-452). Springer, Dordrecht, The Netherlands.

(IAEA) International Atomic Energy Agency. (2003). Trapping guidelines for area-wide fruit fly programmes. International Atomic Energy Agency, Vienna, Austria.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection,* 19(8-10), 603-608.

Katsoyannos, B. I., Kouloussis, N. A, Papadopoulos, N. T. (1997). Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions. *Entomologia Experimentalis et Applicata*, 82(2), 181-188.

Kennedy, J. S. (1978). The concepts of olfactory ‘‘arrestment’’ and ‘‘attraction’’. *Physiological Entomology,* 3, 91-98.

McInnis, D. O., and Warthen Jr., J. D. (1988). Mediterranean fruit fly (Diptera: Tephritidae): Laboratory bioassay for attraction of males to leaf or stem substances from *Ficus* and *Litchi. Journal of Economic Entomology* 81(6), 1637-1640.

Niogret, J., Montgomery, W. S., Kendra, P. E. Heath, R. R., Epsky, N. D. (2011). Attraction and electroantennogram responses of male Mediterranean fruit fly (Diptera: Tephritidae) to volatile chemicals from *Persea*, *Litchi*, and *Ficus* wood*. Journal of Chemical Ecology,* 37(5), 483–491.

Niogret, J., Gill, M. A., Espinoza, H. R., Kendra, P. E., Epsky, N. D. (in press). Attraction and electroantennogram responses of male Mediterranean fruit fly (Diptera: Tephritidae) to six plant essential oils. *Journal of Entomology and Zoology Studies.*

Shelly, T. E. (2001). Exposure to α-copaene and α-copaene-containing oils enhances mating success of male Mediterranean fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 94(3), 497-502.

Shelly, T. E. (2006). Aromatherapy and medfly SIT. In R. L. Sugayama, R. A. Zucchi, S. M. Ovruski, and J. Sivinski (Eds.), *Fruit flies of economic importance: From basic to applied knowledge* (pp. 59-69). Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2005, Salvador, Brazil.

Shelly, T. E., Epsky, N. D. (2015). Exposure of tea tree oil enhances the mating success of male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist,* 98(4), 1127-1133.

Shelly, T. E., Pahio, E. (2002). Relative attractiveness of enriched ginger root oil and trimedlure to male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist* 85(4), 545-551.

Shelly, T. E., Whittier, T. S., Villalobos, E. M. (1996). Trimedlure affects mating success and mate attraction in male Mediterranean fruit flies. *Entomologia Experimentalis et Applicata*, 78(2), 181-185.

Shelly, T., Dang, C., Kennelly, S. (2004). Exposure to orange (*Citrus sinensis* L.) trees, fruit, and oil enhances mating success of male Mediterranean fruit flies (*Ceratitis capitata* [Wiedemann]). *Journal of Insect Behavior,* 17(3), 303-315.

Shelly, T. E., Cowan, A. N. Edu, J., Pahio, E. (2008). Mating success of male Mediterranean fruit flies following exposure to two sources of α-copaene, manuka oil and mango. *Florida Entomologist*, 91(1), 9-15.

Steiner, L. F., Lee, R. K. S. (1955). Large-area tests of a male-annihilation method for oriental fruit fly control. *Journal of Economic Entomology*, 48(3), 311-317.

Steiner, L.F., Miyashita, D. H., Christenson, L. D. (1957). Angelica oils as Mediterranean fruit fly lures. *Journal of Economic Entomology*, 50(4), 505.