Attractiveness to *Anastrepha ludens* (Diptera: Tephritidae) of plant essential oils and a synthetic food-odour lure

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Abstract: Experiments were conducted in a citrus orchard to investigate the attractiveness of 26 plant essential oils individually and in combination with a synthetic food-odour lure to the Mexican fruit fly, *Anastrepha ludens* Loew. Anise, rose/grape seed, and tea tree oils were more attractive than unbaited traps but none approached the attractiveness of Advanced Pheromone Technologies’ AFF lure, a synthetic food-odour lure that emits several nitrogenous chemicals attractive to this fly. Traps baited with most of the oils were less attractive than unbaited traps. Rose/grape seed oil and pure-rose oil enhanced attractiveness of AFF lures to both males and females by about 68%. Grape seed oil did not enhance the attractiveness of AFF lures demonstrating that rose oil was the active component of the rose/grape seed oil. No other oil enhanced attractiveness of AFF lures and most decreased attraction to AFF lures. The possibility that highly attractive chemicals may be present in rose oil as minor components is discussed. Traps baited with the combination of clove bud oil and the AFF lure captured only 3% as many flies as traps baited only with the AFF lure indicating that clove bud oil is highly repellent to Mexican fruit flies.

Key words: AFF lure, attractant, Mexican fruit fly, rose oil, semiochemical

1 Introduction

Recently it was demonstrated that undiluted grapefruit peel oil (*Citrus paradisi*) was slightly attractive to Mexican fruit flies (*Anastrepha ludens* Loew) and enhanced the attractiveness of the AFF lure (see Materials and Methods), a synthetic food-odour lure for *Anastrepha* that emits several nitrogenous chemicals that are attractive to this fly (Robacker and Rios 2005). Increases in attractiveness when chemical blends that act on different appetitive drives were combined had been reported before in Tephritidae. For example, combination of pheromone with host odour increased attraction of papaya fruit fly (*Toxotrypana curvicauda*) (Landolt et al. 1992). Moreover, bacteria odour enhanced attraction of apple maggot fly (*Rhagoletis pomonella*) to host odour (MacCollom et al. 1994). However, in Mexican fruit fly, the enhanced attraction to the AFF lure by addition of grapefruit oil was unexpected. In earlier work with Mexican fruit fly, combinations of attractant blends that act on different appetitive drives had always resulted in a decrease in attraction. These included combinations of pheromone with fermented host fruit odour (Robacker and Garcia 1990) and a synthetic blend of host volatiles with a highly attractive mixture of ammonia, methylamine and putrescine (Robacker and Heath 1997). Similar decreases in attractiveness had also been observed with combinations of attractants in other Tephritidae (Haniotakis and Skyrianos 1981; Cornelius et al. 2000).

The unusual effect of grapefruit oil led Robacker and Rios (2005) to speculate that grapefruit oil may contain chemicals of a nature similar to the ‘parapheromones’ (Cunningham 1989) that are highly attractive to *Bactrocera* and *Ceratitis*. This seemed reasonable because two of the most attractive parapheromones had been discovered as constituents of essential oils. One of these, methyl eugenol, the powerful male attractant for numerous species of *Bactrocera* (Cunningham 1989), was discovered as a minor component of citronella oil (Howlett 1915). Likewise, α-copaene, a potent attractant for *Ceratitis capitata* (Cunningham 1989), was found as a component of angelica seed oil (Fornasiero et al. 1969; Giusotto et al. 1972). Although these chemicals are widely known as male attractants, both also attract sexually active females (Steiner et al. 1965; Nakagawa et al. 1970; Fitt 1981). The effect is not usually observed in field trapping probably because most females in nature are either immature or mated and thus not sexually active.

Contrary to the situations with the classical parapheromones discussed above, grapefruit oil attracted males and females of the Mexican fruit fly in equal proportions (Robacker and Rios 2005). In addition,
Attractants for Mexican fruit fly

2 Materials and Methods

2.1 Insects and test conditions

Mexican fruit flies were from a laboratory culture that originated from yellow chapote fruit, Casimiroa greggii (Rutaceae), a native citrus host of the fly, collected in Nuevo Leon, Mexico, in 2000. Laboratory conditions for holding flies were 22 ± 2°C, 50 ± 20% relative humidity, and 13 : 11 h (light : dark). Mixed-sex groups of 180–200 flies were kept in 473-ml cardboard cartons until released in the test orchard. Flies were irradiated, due to quarantine laws, with 70–92 Grays (Cobalt 60) 1–2 days before adult eclosion. Flies were fed sugar and water until they were released in test orchard. Flies were kept in 473-ml cardboard cartons until released in the test orchard. Flies were fed sugar and water until they were released in test plots 3–12 days after eclosion.

2.2 Traps and lures

Yellow-bottom multilure traps (Better World Manufacturing, Inc., Miami, FL, USA) were used in all experiments. Multilure traps are plastic McPhail-like traps with a clear, colourless top and an opaque, coloured bottom that serves as a liquid reservoir for drowning captured flies. The synthetic food-odour lure used in this work was the AFF lure (Advanced Pheromone Technologies, Inc., Marylhurst, OR, USA). This lure emits ammonia, methyamine, putrescine, and 1-pyrroline (Robacker and Czokajlo 2005).

Essential oils used in experiments were obtained from two sources. Oils from New Foods (Bloomingdale, IL, USA) were: anise oil (Pimpinella anisum) from seeds; basil oil (Ocimum basilicum) from leaves; chamomile oil (Ormenis multicadis) from flowers; eucalyptus oil (Eucalyptus globulus) (unspecified plant part); ginger oil (Zingiber officinale) (unspecified); lavender oil (Lavandula spp.) (unspecified); patchouli oil (Pogostemon cablin) (unspecified); and rose absolute oil, a 5% mixture of rose oil (Rosa centifolia) from flowers in a base of grape seed oil (Vitis vinifera) from seeds. Note that the term ‘absolute’ in rose absolute oil was the label designation although the bottle contained a mixture of two oils and hereafter will be called ‘rose/grape seed’ oil. All oils from New Foods were listed as 100% natural. Oils from aromatherapywebsite.com were: balsam peru oil (Myroxylon pereirae) (unspecified plant part); bay oil (Pimentum racemosum) (unspecified); cedarwood oil (Juniperus virginiana) from wood; citronella oil (Cymbopogon nardus) from foliage; clove bud oil (Eugenia caryophyllata) from flower buds; coriander oil (Coriandrum sativum) from seeds; fennel (sweet) oil (Foeniculum vulgare) (unspecified); geranium oil (Pelargonium graveolens) from flowers and leaves; grape seed oil (Vitis vinifera) from seeds; lemongrass oil (Cymbopogon citratus) from foliage; nutmeg oil (Myristica fragans) (unspecified); orange bitter oil (Citrus bigaradia) from peels; peppermint oil (Mentha piperita) (unspecified); rose moroc absolute oil (Rosa centifolia) from flowers (hereafter called ‘pure-rose’ oil); rosewood oil (Aniba rosaedora) from wood; spearmint oil (Mentha spicata) from leaves; tea tree oil (Melaleuca alternifolia) from leaves; and ylang ylang oil (Cananga odorata) from flowers. All oils from aromatherapywebsite.com were listed as pure undiluted.

2.3 Experiments

Experiment 1 tested eight oils: anise, basil, chamomile, eucalyptus, ginger, lavender, patchouli, and rose/grape seed. Eighteen treatments were evaluated: each oil alone, the AFF lure alone, each oil in combination with the AFF lure, and no lure. All traps contained 300 ml of water with 0.01% Triton® (Rohm and Haas Co., Philadelphia, PA, USA) as a wetting agent to drown captured flies. Oil lures were 4 ml glass vials each containing 1 ml of undiluted oil with a cotton wick. Oil vials were taped inside the top of the traps. For AFF lures, the two plastic bags containing the attractants were removed from the factory supplied mesh bag, carefully folded, and put into the lure baskets in the trap tops. The experiment was conducted in a mixed citrus orchard near the laboratory in Weslaco, Texas. The orchard contained several varieties of oranges, lemons and tangerines. One row of Valencia sweet oranges (Citrus sinensis) and one row of Valley lemons (Citrus limon) were used for tests. Each row contained 20 traps placed in consecutive trees comprising one trap for each oil, one trap for each oil in combination with the AFF lure, two traps with AFF lures only, and two traps with no lures. Treatments were placed in random order with restrictions that traps containing the same oil, traps with AFF lures only, and traps with no lures were separated by at least three trees. Traps were hung one to a tree, north of centre, at 1–2 m height. Each week approximately 2000 flies were distributed uniformly onto rows of trees adjacent to the test rows. Traps were serviced weekly. Flies were removed and counted, water/Triton was replaced, and traps were moved sequentially along the length of the 20-tree blocks with the treatment on the last tree moved to the first tree of the row. Vials with oils were replaced every 2 weeks. AFF lures were not replaced during the experiment. The duration of the experiment was 10 weeks from February to April 2005.

Experiment 2 tested eight more oils: citronella, clove bud, fennel, geranium, lemongrass, orange bitter, peppermint and ylang ylang. It was conducted for 8 weeks from May to July 2005. Experiment 3 tested another eight oils: balsam peru, bay, cedarwood, coriander, nutmeg, rosewood, spearmint and tea tree. It was conducted for 10 weeks from September to November 2005. Procedures for these two experiments were identical to those of Experiment 1. Experiment 4 was conducted to reassess the effects of rose/grape seed oil, lemongrass oil and orange bitter oil, on attractiveness of AFF lures observed in the first two experiments. Procedures were the same as for Experiment 1 with the following differences. Four treatments were evaluated: each oil tested in combination with the AFF lure;
and the AFF lure alone. Within each row, two linear blocks of eight trees each were chosen with two buffer trees between blocks. One each of the four treatments was tested in each block with one buffer tree between each treatment. Positions of treatments within each block were randomized for the first week then traps were moved sequentially within each block during weekly servicing. The experiment was conducted for 8 weeks from December 2005 to January 2006.

Experiment 5 was conducted to determine whether the effect of rose/grape seed oil on attractiveness of the AFF lure was due to the rose or grape seed component of the oil mixture. Four treatments were evaluated: the AFF lure combinations with rose/grape seed oil, pure-rose oil and grape seed oil, and the AFF lure alone. Procedures were similar to those of Experiment 4 except that four linear blocks of four consecutive trees each were chosen within each row with one buffer tree between blocks. The experiment was conducted for 4 weeks during February 2006.

2.4 Statistical analyses

All experiments were analysed as randomized complete blocks. Replications over time were treated like replications over space (blocks of trees) for the purpose of statistical analyses. Data sets from the first three experiments were divided into one subset containing the no-lure trap and each of the oils by themselves, and another subset containing the AFF lure and the combinations of the oils with the AFF lure. Data sets were subjected to analysis of variance using Superanova (Abacus Concepts 1989). Means separations were done by Fisher’s protected least significant difference method (LSD) (Snedecor and Cochran 1967).

3 Results

3.1 Experiment 1: Anise, basil, chamomile, eucalyptus, ginger, lavender, patchouli and rose/grape seed oils

Traps baited with anise and rose/grape seed oils were significantly more attractive than no-lure traps to both males (F = 4.9; d.f. = 8,173; P < 0.0001) and females (F = 4.1; d.f. = 8,173; P < 0.001) (fig. 1a).

The AFF lure combination with rose/grape seed oil was the only combination more attractive than the AFF lure by itself to both males (F = 10.1; d.f. = 8,173; P < 0.0001) and females (F = 13.1; d.f. = 8,173; P < 0.0001) (fig. 1b). All other AFF lure/oil combinations were significantly less attractive than the AFF lure by itself.

3.2 Experiment 2: Citronella, clove bud, fennel, geranium, lemongrass, orange bitter, peppermint, and ylang ylang oils

None of the traps baited only with oils were more attractive than no-lure traps and most were significantly less attractive than no-lure traps to both males (F = 2.5; d.f. = 8,143; P < 0.05) and females (F = 5.2; d.f. = 8,143; P < 0.0001) (fig. 2a).

Traps with only clove bud oil caught no flies. No combinations were significantly more attractive than traps with only AFF lures and all but lemongrass and orange bitter combinations were significantly less attractive than the AFF lure by itself to both males (F = 7.0; d.f. = 8,143; P < 0.0001) and females.

Fig. 1. Numbers of Mexican fruit flies captured in traps baited with plant essential oils in a citrus orchard. (a) Oils only. (b) Oils in combination with AFF lures. + indicates a mean is significantly greater (P < 0.05) and − indicates significantly lower (P < 0.05) than means for traps with no-lures (a) or with only AFF lures (b) by Fisher’s protected LSD.
The combination of the AFF lure and clove bud oil caught fewer than half as many flies as the no-lure trap (not significant by ANOVA of full data set).

3.3 Experiment 3: Balsam peru, bay, cedarwood, coriander, nutmeg, rosewood, spearmint, and tea tree oils

Traps baited only with tea tree oil were more attractive to females than no-lure traps \( (F = 5.5; \text{d.f.} = 8.182; P < 0.0001) \) (fig. 3a). Traps baited with the other oils were significantly less attractive than no-lure traps to females and most of these were also significantly less attractive than no-lure traps to males \( (F = 3.2; \text{d.f.} = 8.182; P < 0.01) \) (fig. 3a). No combinations were significantly more attractive than traps with only AFF lures and all but cedarwood, spearmint and tea tree oil combinations were significantly less attractive than the AFF lure by itself to both males \( (F = 5.6; \text{d.f.} = 8.182; P < 0.0001) \) and females \( (F = 5.1; \text{d.f.} = 8.182; P < 0.0001) \) (fig. 3b).

3.4 Experiment 4: Reassessment of lemongrass, orange bitter and rose/grape seed oils

Traps baited with AFF lures and rose/grape seed oil were more attractive than traps baited only with AFF lures to both males \( (F = 6.2; \text{d.f.} = 3.109; P < 0.001) \) and females \( (F = 6.7; \text{d.f.} = 3.109; P < 0.001) \) (fig. 4).

Combinations with the other two oils did not differ in attractiveness from AFF lures alone.

3.5 Experiment 5: Determination of active component of rose/grape seed oil

Traps baited with combinations of AFF lures with rose/grape seed oil or pure-rose oil were more attractive than traps baited with AFF lures alone or in combination with grape seed oil to males \( (F = 5.7; \text{d.f.} = 3.120; P < 0.01) \) and females \( (F = 4.1; \text{d.f.} = 3.120; P < 0.01) \) (fig. 5). Traps with rose/grape seed oil and pure-rose oil were equally attractive. Furthermore, traps with AFF lures alone or in combination with grape seed oil were equally attractive.

4 Discussion

Certain procedures used in Experiments 1–3 may have affected the validity of the results. These were: (1) treatments (except no-lure and AFF control traps) were tested in only two traps; (2) treatments were not randomly represented throughout the orchard due to incomplete rotation of 20 traps through trees in each block; (3) weekly fly captures from the same trap were not independent because AFF lures were not changed for the duration of each experiment and oil lures were changed only biweekly; and (4) Fisher’s LSD means separation test tends to generate false significant...
differences. The effect of testing most treatments in only two traps each was overcome by moving the traps around the orchard and recording data from them each week during the experiments. Although each treatment did not occupy every position in each block, it is unlikely this factor had much effect on the results. The reasons are that tree size, fruit load on trees and fly distribution were relatively uniform throughout the orchard. Lack of independence of weekly data from the same traps would create a bias if variability was different using a different trap and lure(s) each week vs. using the same trap and lure(s). This is unlikely because both the AFF lures and the oil lures were made to a high degree of standardization so there should be little difference between using the same ones

Fig. 4. Numbers of Mexican fruit flies captured in traps baited with combinations of plant essential oils and AFF lures in a citrus orchard. ‘+’ indicates the mean is significantly greater ($P < 0.05$) than the mean for traps with only AFF lures by Fisher’s protected LSD.

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or different ones each week. Finally, Fisher’s LSD probably did create false significant differences. However, it was also important to minimize false nonsignificant differences so that possible differences could be identified. The problem of false significant differences was alleviated by conducting Experiment 4 to retest the most important findings of Experiments 1–3.

Several of the oils were attractive to Mexican fruit flies, but they were much less attractive than AFF lures. Most of the oils were repellent as indicated by lower fly captures in traps with oils compared with traps without lures. Repellency would be less likely at lower concentrations and some of the oils might be attractive at lower concentrations. However, because the objective was to search for oils that contained attractive components, possibly minor components, undiluted oils were tested. As discussed in the Introduction, citronella and angelica seed oils, from which the powerful parapheromones methyl eugenol and a-copaene were found as minor components, were attractive to Bactrocera and Ceratitis in undiluted form (Nakagawa et al. 1970; Cunningham 1989).

Only rose/grape seed oil and pure-rose oil enhanced the attractiveness of the AFF lure. The enhancement occurred for both male and female flies and averaged 68% over the three experiments testing these two oils (figs 1, 4 and 5). Grape seed oil had no effect on attractiveness of the AFF lure indicating that rose oil was the attractive component of the rose/grape seed oil.

Traps containing anise oil and AFF lures were less attractive than those with AFF lures alone (fig. 1b) despite the significant attractiveness of anise oil when it was tested by itself (fig. 1a). Effects of this type have been reported before for Mexican fruit flies as discussed in the Introduction. However, in this case the effect may be nothing more than a random result caused by the low number of replications of treatments.

What is the physiological basis for attraction of Mexican fruit flies to rose oil? It is not likely that attraction to rose oil is a host response because rose is not a host of the Mexican fruit fly (Norrbom 2003). It also seems unlikely the attraction was a general response to plant odours because attraction to other oils would have elicited similar responses. In previous work using Mexican fruit flies, grapefruit oil also was slightly attractive by itself and increased the attractiveness of AFF lures (by 25%) (Robacker and Rios 2005). Assertions were made that attraction to undiluted grapefruit oil was not a host response because attraction to the oil did not increase after flies had oviposition experience with grapefruit (Robacker and Rios 2005), although their attraction to grapefruit peel extract did increase after experience with grapefruit (Robacker and Fraser 2005). Thus grapefruit peel extract, tested in undiluted form, apparently did elicit a host response.

Similarities in responses to undiluted rose and grapefruit oils suggest similar reasons for the attraction responses. The most plausible explanation is that specific chemicals in the oils, perhaps minor components, elicited attraction. Evidence that minor components may be the attractive principals of grapefruit oil is that the oil was attractive in pure form and 10% dilutions but not at lower concentrations (Robacker and Rios 2005). If this hypothesis is correct, then flies were not attracted to the essence of grapefruit or rose but only to some of the components of the oils. Fractionation of these oils is needed to determine which chemicals are responsible for the attractiveness.

These cases are reminiscent of the Bactrocera and Ceratitis parapheromones except that rose and grapefruit oils are equally attractive to males and females. Although parapheromones were not understood less than 20 years ago, the biological significance of attraction to these chemicals by male Bactrocera has now been worked out in several cases. It has been demonstrated that parapheromones such as methyl eugenol can be precursors of both male-produced pheromones to bring the sexes together (Tan and Nishida 1996, 1998; Lee and Tan 1998, 2004, 2005) and allomones to deter predation (Nishida and Fukami 1990; Tan and Nishida 1998), or can act directly as attractants for females (Nishida et al. 1997, 2000, 2004). Whereas the reason for the unique attraction of males of Bactrocera to parapheromones is now understood, the physiological basis of attraction of male and female Mexican fruit flies to essential oils is not yet known. Perhaps males may acquire certain compounds from grapefruit and rose oils that are used as precursors of pheromones, but the possible role of these chemicals in attraction of females is without precedence. This phenomenon certainly warrants further investigation.

Many of the oils were repellent and clove bud oil was especially repellent. Traps with AFF lures and clove bud oil caught only 3% as many flies (97% reduction) as traps baited only with AFF lures (fig. 2). Repellent effects caused by adding plant volatiles to an attractive mixture of nitrogenous chemicals were discussed in the Introduction (Robacker and Heath 1997). Reduction in captures was only about 30% with the plant volatiles. The repellent effects of clove bud oil vs. the plant volatiles probably differ at the physiological level because the plant volatiles were attractive by themselves but repellent when combined with nitrogenous attractive chemicals (Robacker and Heath 1997), whereas clove bud oil was repellent by itself and in combination with nitrogenous attractants (fig. 2).

The repellent effects of the clove bud oil and many of the other oils tested here probably have a physiological basis similar to oviposition deterrent effects of orange peal oil observed with Mediterranean fruit flies (Levinson et al. 2003). Lethality to eggs and larvae of oil glands in citrus peels probably is the underlying basis for the oviposition deterrence by adult female flies (Back and Pemberton 1918; Greany et al. 1983, 1985). The possibility that many of these oils may deter oviposition by Mexican fruit flies is a topic for future investigation.

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