Toxicity of citrus essential oils against Ceratitis capitata (Diptera: Tephritidae) larvae

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Keywords
Ceratitis capitata; citrus fruits; essential oils; hydrocarbon monoterpenes; limonene; oxygenated monoterpenes; pinene; toxicity.

Abstract
Citrus peel essential oils are considered to constitute the most important resistance factor of citrus fruits against fruit flies. Essential oils were obtained from three sweet orange varieties, one bitter orange and one lemon variety. Yield, chemical composition and toxicity against neonates of the Mediterranean fruit fly were determined. Based on chemical analysis, the toxicity of commercially purchased major and minor components (monoterpenes and sesquiterpenes) of essential oils was determined. In addition, fractions were prepared to evaluate the role of minor components in the toxicity of crude essential oils. Limonene was by far the most abundant ingredient (96.2–97.4%) in all sweet orange varieties and in bitter orange, while the concentration of limonene was much lower in lemon essential oils (74.3%). Orange and bitter orange essential oils were more toxic than lemon essential oils. The toxicity of orange and bitter orange essential oils was similar to that of their major component limonene. In tests of commercially purchased chemicals, the oxygenated components of essential oils were more toxic than hydrocarbons but their low concentration in citrus essential oils could not affect the toxic activity of essential oils. The presence of α-pinene and β-pinene seems to account for the lower toxicity of lemon essential oils in relation to other citrus essential oils. The importance of understanding the toxicity of essential oils in relation to their composition and their role regarding the resistance of citrus fruits to Ceratitis capitata infestation is discussed.

Introduction
Among the wide diversity of plant feeding insects, fruit feeding Diptera of the family Tephritidae (true fruit flies) comprise a very interesting group to address several issues of host plant–phytophagous insect interactions (White & Elson-Harris, 1992). Female fruit flies use a variety of chemical, visual and tactile stimuli to detect and accept a suitable fruit to lay their eggs (Papadopoulos et al., 2006a). Several activities of adult fruit flies such as sexual interactions take place on host fruits and they are stimulated by host plant properties. On the other hand, chemical and physical properties of the fruits may deter oviposition and cause egg and larva mortality conferring various degrees of resistance to respective fruit crops. Several fruit fly species of the genus Ceratitis, Anastrepha and Bactrocera constitute a major threat to citrus fruits (Robinson & Hooper, 1989a,b; White & Elson-Harris, 1992). Citrus species have developed several mechanisms to defeat infestations of fruit flies as well as infestations by other insect species. The chemical properties of fruits, especially the peel essential oils are considered to be the most important resistance mechanism of citrus fruits against fruit flies (Greany et al., 1983; Aluja et al., 2003;
towards breeding more resistant citrus varieties. Essential oils components might be of particular interest dose–response effects and the toxicity of each of the citrus fruits essential oils of several citrus fruits (species and varieties), including qualitative and quantitative aspects may include toxic effects of the peel essential oils (Back & Pemberton, 2006). Citrus fruit rind is the first barrier that eggs and pupae have to overcome. The flies used in the following trials were obtained from field-infested sweet oranges of the variety New Hall, collected in winter 2007 from the area of Arta. Fruits were brought to the laboratory and placed in plastic containers on a layer of dry sand. Ready to pupate, third instar larvae and pupae were collected daily. Upon emergence, adults were placed into wire-screened wooden holding cages (40 cm × 40 cm × 40 cm), and provided with standard adult food (a mixture of yeast hydrolysate and sugar, 1:4 weight, respectively). Approximately, 100 adults of both sexes (approximately 1:1 males and females) were placed in each cage. Females were allowed to oviposit into 5-cm prepunctured (30 holes of 0.5 mm in diameter), plastic, red, hollow domes that were fitted onto the lid of a Petri dish (Katsoyannos, 1989; Diamantidis et al., 2009). To stimulate oviposition, water was placed under each dome. Eggs were collected daily, placed on wet filter paper and maintained into plastic Petri dishes until hatch.

Isolation of citrus essential oils

Samples of about 100 fruits of each variety were collected randomly from 20 to 40 trees. Before being used, fruits were thoroughly washed with tap water and dried at room temperature. The flavedo peel layer of subsamples of 30

The Mediterranean fruit fly (medfly), Ceratitis capitata (Wiedemann) (Diptera: Tephritidae) is one of the most notorious insect pests of citrus species causing extensive fruit losses worldwide (Eskafi, 1988; Katsoyannos et al., 1998; Mavrikakis et al., 2000). However, citrus fruits exhibit a number of resistance mechanisms that reduce survival, fecundity and longevity of the attacking Mediterranean fruit flies (Papachristos et al., 2008; Papachristos & Papadopoulos, 2009). Physicochemical characteristics of citrus peels such as the quality and quantity of essential oils of the flavedo layer (the outer part of citrus fruit rind, bearing oil glands and pigments), peel thickness, elasticity and mechanical resistance seem to constitute a strong barrier against attacks of C. capitata and that of other tephritid fruit flies (Back & Pemberton, 1915; Bodenheimer, 1951; Greany et al., 1983; Spitler et al., 1984; Birke et al., 2006; Papachristos et al., 2008).

Fruit fly females, such as medflies, force their ovipositor into citrus fruit peels, forming oviposition cavities into which eggs are deposited (Papaj et al., 1989; Birke et al., 2006). Citrus fruit rind is the first barrier that eggs and newly hatched larvae face. Larvae penetrating the citrus peel to the most nutritious fruit pulp have to overcome the toxic effects of the peel essential oils (Back & Pemberton, 1915; Papachristos et al., 2008). The toxic action of the citrus peel essential oils determines the resistance of citrus fruits to tephritids such as Anastrepha suspensa (Loew) (Greany et al., 1983; Styer & Greany, 1983). Citrus resistance to A. suspensa was positively correlated with the limonene to linalool ratio (Greany et al., 1983); however, in this study the role of other components of the citrus essential oils was not taken into account. Recently, citrus fruits essential oils have been recognised as the most critical mechanism conferring resistance to citrus species against C. capitata (Papachristos et al., 2008). However, the toxicity of citrus essential oils, and the role of each of their components, to larvae remains largely unknown. To our knowledge only Salvatore et al. (2004) have studied the toxicity of the lemon peel constituents (including essential oils) on C. capitata larvae. Studying the toxicity of the peel essential oils of several citrus fruits (species and varieties), including qualitative and quantitative aspects may facilitate the better understanding of the role of essential oils as a resistance mechanism against medfly and probably other fruit flies. On the other hand, determining dose–response effects and the toxicity of each of the citrus essential oils components might be of particular interest towards breeding more resistant citrus varieties.

The aim of the study was to identify the mechanisms of resistance of citrus fruits against C. capitata that are related to larvae toxicity. For this reason, the essential oils from fruits of various citrus species and varieties were isolated and their yield, chemical composition and toxicity against neonates of the Mediterranean fruit fly were determined. We also evaluated the toxicity of several major and minor components attempting to correlate their toxic action with that of the different citrus essential oils. Moreover, with the fractionation of essential oils and the evaluation of their toxicity we tried to clarify the role of minor components in the toxic activity of crude essential oils.

Materials and methods

Plant material

Fruits used in this study were collected from an unsprayed orchard in the area of Arta (Epirus, central western Greece) at the end of January 2007. The following four citrus varieties and species were tested: three sweet orange varieties (Citrus sinensis L.), (a) New Hall, (b) Merlin, (c) a local variety (Xino Artas, hereafter Artas), (d) a lemon variety (C. limon L., var Maglini) and (e) a local bitter orange (C. aurantium L.) variety.

Insects

The flies used in the following trials were obtained from field-infested sweet oranges of the variety New Hall, collected in winter 2007 from the area of Arta. Fruits were brought to the laboratory and placed in plastic containers on a layer of dry sand. Ready to pupate, third instar larvae and pupae were collected daily. Upon emergence, adults were placed into wire-screened wooden holding cages (40 cm × 40 cm × 40 cm), and provided with standard adult food (a mixture of yeast hydrolysate and sugar, 1:4 weight, respectively). Approximately, 100 adults of both sexes (approximately 1:1 males and females) were placed in each cage. Females were allowed to oviposit into 5-cm prepunctured (30 holes of 0.5 mm in diameter), plastic, red, hollow domes that were fitted onto the lid of a Petri dish (Katsoyannos, 1989; Diamantidis et al., 2009). To stimulate oviposition, water was placed under each dome. Eggs were collected daily, placed on wet filter paper and maintained into plastic Petri dishes until hatch.

Isolation of citrus essential oils

Samples of about 100 fruits of each variety were collected randomly from 20 to 40 trees. Before being used, fruits were thoroughly washed with tap water and dried at room temperature. The flavedo peel layer of subsamples of 30
fruits was separated from peels with a chirurgical lancet (approximately 200–300 g of plant material). Essential oils were obtained by subjecting plant materials to hydrodistillation using a Clevenger apparatus (Winzer, Wertheim, Germany), for 3 h at 100°C, then dried over anhydrous sodium sulphate and stored in a freezer at −10°C until use. The procedure was repeated three times for each of the plant materials obtained from the above subsamples in order to estimate oil yield (given as an average of three values). Following hydrodistillation and yield estimation, essential oil yields of the three subsamples were combined into one sample for each citrus species, which was used for chemical analysis and bioassays.

Essential oils analysis
The essential oils were analysed using a Hewlett Packard II 5890 gas chromatography (GC) system, equipped with a Flame Ionization Detector (FID) detector and HP-5 MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220°C and 290°C, respectively. GC oven temperature was programmed from 60°C to 240°C at a rate of 3°C min⁻¹ and held isothermally for 10 min. Helium was the carrier gas at a flow rate of 1 mL min⁻¹. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 μL were injected manually in the splitless mode. Quantitative data were obtained electronically from FID area without the use of correction factors. Qualitative analysis of the essential oils was performed using the same conditions as GC, and the Hewlett Packard II 5890 gas chromatograph equipped with Hewlett Packard II 5972 mass selective detector in the electron impact mode (70 eV). Almost all ingredients of the essential oils were identified by comparing GC relative retention times and mass spectra with those of pure standards. Tentative identification of the few remaining components was based on the comparison of their mass spectra and elution order with those obtained from the NIST 98 and Wiley 275 library data of the Gas Chromatography-Mass Spectrometry (GC-MS) system and literature data (Adams, 2001).

Essential oils fractionation
A gram of the isolated crude essential oils derived from the sweet orange variety New Hall, bitter orange and lemon were submitted to chromatography on a silica gel column and eluted with a mixture of pentane/diethyl ether as a binary mixture with increasing polarity (pentane/diethyl ether: 100/0, 97/3, 95/5, 93/7, 90/10, 80/20). The resulting subfractions of each chromatographic procedure were monitored by Thin Layer Chromatography (TLC) with pentane/diethyl ether (9.7/0.3 v/v) as the developing reagent. Authentic compounds eluted on same TLC plates in order to specify the desired subfractions. Visualisation was conducted under ultraviolet (UV) lamp and by spraying later with a mixture of 1% vanillin and 5% sulfuric acid solution (in ethanol), heating at 120°C and/or phosphomolybdic acid solution (phosphomolybdic acid 7.5% w/v in ethanol) and charring on a hot plate. The chosen subfractions were combined and elution solvents were removed by a rotary flash evaporator. The distillation was terminated when the volume of solvents was reduced to approximately 3 mL and completed by flushing through nitrogen. Thus, the procedure resulted in one fraction from sweet orange New Hall (F₀₀₀), one fraction from bitter orange (F₉₀₀) and two fractions (F₁₁₀ and F₁₂) from lemon essential oil. The chemical composition of the isolated fractions was determined according to GC-MS analytical conditions above.

Chemicals
Based on chemical analyses of the essential oils, several monoterpenes and sesquiterpenes were chosen for evaluating their toxic action against larvae. The selection of the chemicals for the bioassays below was based on their proportion in crude essential oils. To represent all the chemical groups of the identified components in the essential oils [oxygenated (alcohols, aldehydes, monoterpenes esters), non-oxygenated (monoterpenes and sesquiterpenes)], we obtained and used the following compounds from commercial sources: (-)-limonene (97%), S-(−)-limonene (97%), terpinen-4-ol (96%) and α-terpineol (96%), α-(−)-pinene (99%), myrcene (90%) and citral (62% nerol and 33% geranial) obtained from Sigma–Aldrich (Steinheim, Germany) and α-(+)-pinene (99%), β-(−)-pinene (99%), β-(+)-pinene (99%), γ-terpinene (97%), linalool (97%), linalyl acetate (>95%), geranyl acetate (>99%), neryl acetate (>99%), (+)-valencene (>70%) and trans-(-)-caryophyllene (>98.5%) obtained from Fluka Chemie (Steinheim, Germany).

Bioassays
A proportion of the specific citrus essential oil, and respective compound or a fraction was applied on larval diet as it is described below. Larval diet was prepared by mixing solid larval food (Boller, 1985) with water in a proportion of 1:2 (food:water). One gram of this food was placed into cylindrical glass vessels (5 mL volume: 1.8 cm diameter and 2 cm height). The appropriate amount of the essential oils was diluted in 200 μL acetone and was applied into the larval food within the glass vessel. They

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were then left for 10 min at room temperature in order for acetone to evaporate. Twenty newly hatched larvae were placed on a small filter paper and then on the larval food. Each glass vessel was covered with a metal tape. Five holes (1 mm diameter) were perforated in each glass vessel cover in order to ensure good aeration and humidity equilibrium. Larval mortality was determined by opening the glass vessels 2 days later and counting both live and dead larvae. Four to six doses were tested for each essential oil (based on preliminary test) with four replicates for each dose. As a result of differential toxicity, doses of the compounds tested varied from 0.8 to 60 μL per g of food. Two controls were included in our experiments, consisting of either food treated with 200 μL acetone or untreated. However, because there were no differences in larval mortality between acetone treated and untreated food the data were combined and considered as a single common control. Fractions and pure monoterpenes and sesquiterpenes as well as an artificial mixture (prepared with the addition of α-pinene (10 μL) and β-pinene (69 μL) in the essential oil of the variety New Hall (921 μL) were tested using the same experimental techniques. The artificial mixture was prepared in order to resemble lemon essential oils regarding α-pinene and β-pinene components. All bioassays were conducted at 25 ± 1°C, 70 ± 5% relative humidity (RH) and a photoperiod of 16:8 (L:D) h.

Statistical analysis

Differences among citrus varieties in essential oil yields were determined by one way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test for means separation (Sokal & Rohlf, 1995). Data obtained from each dose–response bioassay were subjected to probit analysis and LC50, LC90 values and 95% fiducial limits were estimated. The comparisons among LC50 or LC90 values were based on the overlap of fiducial limits (Finney, 1971). All analyses were conducted using the statistical package SPSS 14.0 (SPSS Inc., Chicago, IL, USA 2004). Abbot’s formula (Abbott, 1925) was used to correct control mortality.

Results

Oil yields and chemical composition

Yields of essential oils were significantly affected by the citrus variety (F = 127.732; d.f. = 4, 10; P < 0.001). Average oil yield ± SE (μL per g fresh weight of the flavedo tissue) was the highest in Artas (14.6 ± 0.3) and lemon (14.5 ± 0.4), intermediate in Merlin (10.7 ± 0.2) and New Hall (10.6 ± 0.2) and the lowest in bitter orange (7.2 ± 0.2).

The chemical composition of the isolated essential oils is given in Table 1. In accordance with previous research (Shaw, 1979), limonene was by far the most abundant ingredient, ranging from 96.2% to 97.4% in all sweet orange varieties and in bitter orange. All other chemicals were detected in amounts ranging from 0.1% to 1.2%. Concentration of limonene was much lower in lemon essential oils (74.3%), while that of the remaining chemicals ranged from 0.1% to 7.0%.

The chemical composition of fractions is also listed in Table 1. The fractionation of the essential oils was prepared in order to evaluate the role of oxygenated components in crude essential oils, and moreover to understand the reason of the lower toxicity of lemon essential oils (see below). Thus, in all fractions we made an effort to remove all oxygenated components. Moreover in lemon, we attempted to prepare two fractions, one with similar amounts of pinenes (less toxic group) and a second with lower amounts of pinenes compared to crude essential oils. All fractions consisted of hydrocarbon monoterpenes, with the addition of small quantities (lower than 0.7%) of hydrocarbon sesquiterpenes in all except those obtained from bitter orange that contained only hydrocarbon monoterpenes. New Hall and bitter orange fractions consisted almost entirely of limonene (≥99%). In lemon fraction 1, limonene was the most abundant component, followed by β-pinene, γ-terpinene and α-pinene (Table 1). Lemon fraction 2 was richer in limonene (90.0%) than lemon fraction 1 and contained much lower quantity of pinenes than lemon fraction 1.

Toxicity against larvae

Sweet oranges and bitter orange essential oils exhibited similar toxicity against C. capitata larvae, which was significantly higher than that of the lemon essential oil (Fig. 1).

The fractions from orange and bitter orange exhibited a similar activity to that of crude sweet orange and bitter orange essential oils, suggesting that oxygenated compounds (removed through fractionation) did not affect the toxicity of citrus essential oils. Lemon fraction 1 was of the same toxicity as the crude lemon essential oil. However, fraction 2 (containing higher limonene quantities) from lemon was more toxic than lemon essential oils and fraction 1.

Because the presence of α-pinene and β-pinene in lemon essential oil was considered to account for the lower toxicity of lemon essential oils in relation to that of the sweet oranges and bitter orange, we prepared an artificial mixture from essential oils of New Hall with the addition of α-pinene and β-pinene in order to confirm the role of pinenes in the toxicity of citrus essential oils. The toxicity of the mixture was lower than that of New...
### Table 1: Chemical composition (%) of essential oils and their fractions derived from the fruit peel flavedo layer of three sweet orange varieties, bitter orange and lemon

<table>
<thead>
<tr>
<th>Compound</th>
<th>Merlin Oil</th>
<th>Artas Oil</th>
<th>New Hall Oil</th>
<th>Fraction</th>
<th>Bitter Orange Oil</th>
<th>Fraction</th>
<th>Lemon Oil</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
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<tbody>
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<td>α-Thujene</td>
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<td>0.2</td>
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<tr>
<td>α-Pinene</td>
<td>0.4</td>
<td>0.3</td>
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<td>–</td>
<td>0.4</td>
<td>0.1</td>
<td>1.4</td>
<td>1.8</td>
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<td>–</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
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<tr>
<td>β-Pinene</td>
<td>–</td>
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<td>–</td>
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<td>7.0</td>
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<td>α-Terpine</td>
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<td>–</td>
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<td>96.2</td>
<td>98.8</td>
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<td>76.8</td>
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<tr>
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<td>–</td>
<td>6.4</td>
<td>8.5</td>
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<td>–</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>β-Bisabolene</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nootkatone</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
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**Monoterpenes**

- Hydrocarbon: 97.9, 97.6, 98.2, 99.8, 98.6, 100.0, 91.7, 99.2, 99.3
- Oxygenated: 0.7, 1.5, 0.7, –, 0.9, –, 6.4, –, –

**Sesquiterpenes**

- Hydrocarbon: 0.2, 0.4, 0.2, 0.2, –, –, 1.2, 0.7, 0.6
- Others: 0.1, 0.2, 0.1, –, –, –, –, –, –

**Total**

- 98.9, 99.7, 99.2, 100.0, 99.5, 100.0, 99.3, 99.9, 99.9

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*Compounds listed in order of elution from an HP-5 MS column.*

*Tentative identification based on data obtained from NIST 98 and Wiley 275 library of the GC-MS system and literature data.*

*Comparison with pure standards.*

*Compounds applied for bioassays.*

*Trace (< 0.06%).*
Essential oils toxicity against *Ceratitis capitata* larvae

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**Figure 1** Toxicity of citrus essential oils and their fractions against *Ceratitis capitata* larvae. The number of larvae tested is given in parenthesis. $F_{\text{NH}}$, fraction from sweet orange New Hall essential oil; $F_{\text{BO}}$, fraction from bitter orange essential oil and $F_{\text{L1}}$ and $F_{\text{L2}}$, fractions from lemon essential oil.

Hall essential oils and similar to that of lemon essential oils (Fig. 1).

In all essential oils, doses lower than 0.8 µL g$^{-1}$ food were not active against larvae (mortality $\leq$ 5%). Doses higher than 13 µL g$^{-1}$ food for sweet orange and bitter orange essential oils and 16.5 µL g$^{-1}$ food for lemon essential oils killed the highest proportion of larvae ($\geq$ 99%) (Fig. 2).

The most active hydrocarbons were $R$-(+) and $S$-(−) limonene and $\gamma$-terpinene ($LC_{50}$ 6.2–7 µL g$^{-1}$ food), followed by myrcene ($LC_{50}$ 9.6 µL g$^{-1}$ food) (Fig. 3). Pinenes were the least active components with $LC_{50}$ values significantly higher than the other monoterpenes and sesquiterpenes. Oxygenated monoterpenes (citral, linalool, $\alpha$-terpinol, terpinen-4-ol, linalyl acetate, germacrene and neryl acetate) exhibited similar activity; 1.5–1.9 times more active than the most active hydrocarbon (Fig. 3). The toxicity of hydrocarbon sesquiterpenes ($LC_{50}$ values 8.3 and 10.4 µL g$^{-1}$ food for caryophyllene and valencene, respectively) was slightly lower than that of the hydrocarbon monoterpenes (Fig. 3).

**Discussion**

Our results show that lemon essential oil was approximately 1.4 times less toxic against *C. capitata* larvae compared to sweet orange varieties and bitter orange essential oils. Based on their toxic activity against *C. capitata* larvae, citrus essential oils constituents are classified into three groups. The most active were the oxygenated monoterpenes followed by hydrocarbon monoterpenes (with the exception of $\alpha$-pinene and $\beta$-pinene) and sesquiterpenes. Both $\alpha$-pinene and $\beta$-pinene were the least toxic components of the citrus essential oils with $LC_{50}$ values four to six times higher than that of limonene. The higher toxicity of some oxygenated monoterpenes,
such as citral, compared with that of the hydrocarbon monoterpenes, such as limonene and $\alpha$-pinene, have also been reported earlier for larvae of A. suspensa that also attack citrus fruits (Styer & Greany, 1983).

There were some small differences in the composition of sweet oranges and bitter orange essential oils in minor components (e.g. three times more linalool in the essential oil of Artas compared with New Hall, Merlin and bitter orange) that did not affect their toxicity against medfly larvae. Toxicity of the sweet oranges and bitter orange essential oils was similar with that of their predominant component limonene ($\geq 96\%$ of the crude essential oils). The oxygenated monoterpenes were significantly more toxic than crude essential oils, limonene and other hydrocarbons of citrus essential oils. However, because of their low concentration in crude essential oils ($\leq 1.5\%$) oxygenated monoterpenes do not increase the toxicity of citrus essential oils against medfly larvae. On the other hand, the toxicity of lemon essential oil was lower than that of limonene ($74\%$ of the crude lemon essential oil). The higher proportion of $\alpha$-pinene and $\beta$-pinene (8.4% of the crude lemon essential oil) that were practically inactive in relation to other monoterpenes and sesquiterpenes may account for the above reduced toxicity of the lemon essential oil. Therefore, the proportion of each hydrocarbon monoterpenes in the total hydrocarbons content of the essential oils significantly affects the toxicity of the citrus essential oils. Similar with the sweet oranges and bitter orange essential oils, the oxygenated monoterpenes did not affect the toxicity of the lemon essential oil. Results from the bioassays using fractions of the citrus essential

Figure 3 Toxicity of the citrus essential oils monoterpenes and sesquiterpenes against Ceratitis capitata larvae (insert graph shows the LC$_{50}$ and LC$_{90}$ values for $\alpha$-pinene and $\beta$-pinene). The number of larvae tested is given in parenthesis.
oils that were free from most of the minor components, and especially the most toxic of them (oxygenated monoterpenes), suggest no synergistic effects between the constituents of the citrus essential oils.

In a recent study, Papachristos et al. (2008) found that newly hatched medfly larvae cannot survive in the flavedo layer of the sweet orange varieties New Hall, Artas and Merlin and lemon. However, larvae survive in the flavedo area of bitter oranges. Concentration of the essential oils in the flavedo area of the different citrus varieties and their differential toxicity, as both determined in our study, help in explaining the results of Papachristos and coworkers. Concentration of essential oils in all sweet orange varieties and lemon tested is higher than that required to kill 99% of the larvae. Although the toxicity of bitter orange essential oil is similar with that of the sweet orange varieties, the concentration in the flavedo layer of the bitter orange fruit is much lower than that required to kill 99% of medfly larvae. It is noteworthy to report here that the concentration of essential oils in the flavedo layer of lemon and Artas is double that of bitter oranges.

Our results show that citrus essential oils confer resistance to citrus fruits against C. capitata (see also Papachristos et al., 2008) as well as other tephritid fruit flies (Greeny et al., 1983; Aluja et al., 2003; Birke et al., 2006). They also seem to play a critical role in the citrus resistance against a wide range of insects other than tephritids as well as plant pathogens. Habib et al. (1972) found a negative correlation between the concentration of essential oils in leaves and fruits of citrus and their susceptibility to red scale Aonidiella aurantii (Maskell). Moreover, Salama & Saleh (1984) found that citrus species with higher hydrocarbon content are more susceptible to red scale infestation compared to those with higher aliphatic terpenes. Caccioni et al. (1995 and 1998) have reported the inhibitory effects of citrus essential oils and their components against the fungal pathogens Penicillium digitatum (Pers.) Sacc. and Penicillium italicum Whem. Kuete et al. (2006) detected a positive correlation between the activity of citrus essential oils against the fungus Phacorumularia angolensis (T. Carvalho & O. Mendes) P.M. Kirk (causing the citrus leaf and fruit spot diseases) and the resistance of the corresponding species to disease.

Citrus essential oils apart from the toxic effects against C. capitata larvae that we report here are involved in several other complicated interactions with the Mediterranean fruit fly. The presence of essential oils in citrus peels seems to reduce female oviposition (Levinson et al., 2003; Papachristos & Papadopoulos, 2009). However, more work is needed to clarify the role of citrus essential oils on the oviposition behaviour of the Mediterranean fruit fly. On the other hand, sexually mature male medflies are strongly attracted to citrus fruit essential oils (Katsyoyannos et al., 1997; Nishida et al., 2000), and those males exposed to citrus essential oils acquire a significant mating advantage over unexposed males (Papadopoulos et al., 2001; Papadopoulos et al., 2006b). Besides the wealth of studies on effects of citrus species on medfly biology and behaviour, there are several issues that need to be studied further.

Based on the toxicity of citrus essential oils and the role of their components against medfly larvae, it seems that the selection of varieties with large amounts of essential oils or varieties with high proportion of oxygenated monoterpenes might be of vital importance in the development of resistant varieties against the Mediterranean fruit fly.

References


