First data on the repellent activity of essential oils of *Citrus limon* towards medfly (*Ceratitis capitata*)

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Abstract

The biological activity of peel essential oils of two Sicilian cultivars of C. limon*, 'Interdonato' and 'Lunario', was investigated by electrophysiological recordings (EAG) and field trials on* Ceratitis capitata *(Wiedemann) (Diptera Tephritidae). The EAG data showed a remarkable dose-response relationship and a low activation threshold dose (10^{-3}M) for both essential oil extracts. In field tests, essential oils showed a good repellent and antiovipositional activity on 'Navelina' oranges, and in particular the essential oils of 'Lunario' provided the same results of kaolin. Potential applications of essential oils from* C. limon *cultivars against* C. capitata *in organic farming are discussed.*

Keywords: *Mediterranean fruit fly, lemon peel, antiovipositional activity, EAG, orange orchards, Navelina, field trials.*

Introduction

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae), is one of the most injurious pests worldwide (McPheron and Steck, 1996) because of its direct economic impact and the strict quarantine restrictions imposed by many countries to avoid its entry (Follett and Neven, 2006). The species is spread in temperate regions and attacks a wide variety of hosts (Liquido et al., 1991). It has become a major pest of *Citrus* spp., causing extensive fruit losses worldwide and in particularly in Mediterranean countries, as well as in similar climatic regions (Staub et al., 2008; Aluja and Mangan, 2008); orange, clementines, mandarines, grape fruits, satsumas are attached by *C. capitata*, while lemons are almost totally resistant to it (Sproul, 1976; Spitler et al., 1984; Elekcioglu et al., 2008); just in some cases, overripe lemons can be attacked by the medfly (Porras et al., 2009). During the last decade, the development of crop protection methods had to take into account the need of preserving the precarious equilibrium among pests and their natural enemies, mainly affected by the application of chemical pesticides, as well as the need of using alternative control measures. For these reasons, several studies have been carried out to investigate the role of plant volatile compounds on medfly behaviour (Teranishi et al., 1987; McInnis et al., 1988; Warthen et al., 1989) with the aim of identifying biologically active compounds that could be used in sustainable control techniques (Lai et al., 2006; Peteu et al., 2010) and organic farming (Vincent et al., 2003). In organic farming the control of medfly is particularly difficult because of the lack of effective allowed insecticides, and it is the main limiting factor for the cultivation of some orchards. In the present paper, the repellence activity of peel essential oils of Sicilian cultivars of *Citrus limon* (L.) Burms.f. on medfly oviposition behaviour has been investigated by electrophysiological recordings (EAG) and field trials on orange orchards.

Material and methods

C. capitata adults for EAG tests were from artificially-reared colonies maintained, for several generations, in the insectary of the CIHEAM – IAMB Mediterranean Agronomic Institute of Bari (Italy).The electrophysiological recordings were carried out using *C. capitata* females, 8-10 days old, kept in Plexiglas cages (30x30x30 cm) at 22±3°C, 60±10% R.H., 12:12 L:D photoperiod and fed with a mixture of sucrose, casein and yeast (ratio 4:3:3) supplied on a wet cotton ball. The peel oils of the C. *limon* cvv. 'Lunario' and 'Interdonato' were obtained from fresh peels (all the fruits had been collected 24 hours before the extraction) by steam distillation in Clevenger-type apparatus: water ratio was 2. At the end of each distillation, which lasted about 4 hours, the oils were dissolved in 1 ml of n-pentane and were separated from the aqueous solution, dried by treating with anhydrous Na_2SO_4 (the solvent was evaporated by N_2). The oils were then transferred into dark glass flasks and kept at a temperature of 4°C. EAG responses were recorded as described in a previous paper (De Cristofaro et al., 2003). The essential oils were dissolved in spectrometric grade hexane at increasing concentrations (from 10^{-10}M to pure oil) and, from these solutions, odour cartridges were prepared for each stimulus, by absorbing 10 µl aliquots onto 1x2 cm pieces of filter paper, inserted into individual Pasteur pipettes. Once the insect was immobilized, the indifferent electrode, a glass micropipette (tip diameter: 2-4 µm), filled with Kaissling solution, was placed inside the insect abdomen. The recording electrode, a glass micropipette (tip diameter: 2-4 µm) containing Kaissling solution, was put in contact with the distal region of the terminal antennal segment. A constant flow (1.0 l/min) of charcoal-filtered and humidified compressed air was passed-over the antenna through a tube, connected

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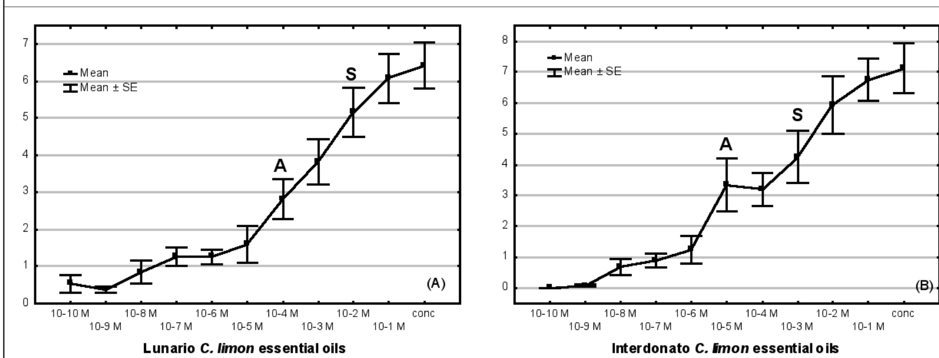
with the primed cartridge and positioned ca. 1 cm from the antenna. When activated, the system diverted the purified air through the stimulus cartridge (inserted in the tube), where evaporating volatiles were carried onto the antenna. Stimulation lasted 1.0 s and it was followed by an interval of ca. 1 minute of clean air. For each stimulus, EAG responses were recorded from 10 females flies. Control stimulus (10 µl of the hexane solvent) was interspersed at the beginning and at the end of each experiment. EAG responses were evaluated by measuring the amplitude of negative deflection (mV) elicited by a given stimulus and then by subtracting the amplitude of the response to the hexane control. The amplitude (mV) of the EAG response to each test stimulus was adjusted to compensate for solvent and/or mechanosensory artefacts by subtracting the mean EAG response of the two nearest hexane controls (Raguso and Light, 1998). To compensate for the decrease of the antennal responsiveness during the experiment, the resulting EAG amplitude was corrected according to the EAG response to the standard stimulus (Den Otter et al., 1991). In dose-response curves, the activation threshold was considered to be the lowest dose at which the lower limit of the standard error of the mean response was greater than the upper limit of the standard error for the lowest dilution tested (Sant'ana and Dickens, 1998). Saturation level was taken as the lowest dose at which the mean response was equal to or less that the previous dose (Germinara et al., 2009). One-way analysis of variance (ANOVA) followed by Tukey-HSD test (critical values corresponding to P = 0.05) was used to classify the mean EAG responses to different concentration of an essential oil. Field trials were carried out in October 2011 in an organic orange orchard (Cooperativa Arcobaleno) in Villafranca Sicula (Agrigento Province, Sicily). The trial orchard cultivated with *Citrus sinensis* (L.) Osbeck, cv. 'Navelina', consisted of 28 rows with 8 trees per row. C. capitata males were monitored by eight pheromone traps (Traptest® Isagro), baited with trimedlure and were checked every 10 days from September until November. To evaluate the efficacy of the different treatments, plots were arranged in one area of the experimental orchard, involving 5x3 trees for each plot, separated from the other ones by a row of untreated trees. The following treatments were compared: untreated control; kaolin (95% kaolin formulated product: Surround® WP, Engelhard Corp. Iselin NJ, USA) was used at a rate of 5 kg in 100 L of water (Lo Verde et al., 2011); bentonite (formulated product DalCin Formula 7/3-2010) was used at the same rate of kaolin; 'Lunario' essential oil formulation (from *C. limon* cv. 'Lunario'); 'Interdonato' essential oil formulation (from *C. limon* cv. 'Interdonato'). Essential oils treatment mixture consists of 0.3 % of pure essential oil and 0.3 % of Tween-80® in 1 L of water; it was prepared the day before the treatment and preserved at 4°C (Blum and Roitberg, 1999; Moretti et al., 2002). Kaolin and bentonite treatments were performed with an atomizer, spraying all the canopy of the tree, while essential oil treatments were performed on each orange with a small hand sprayer. The first treatment was carried out on 11 October at the beginning of the veraison, when fruits become susceptible to *C. capitata* attack; the second treatment was performed on 27 October. One-way analysis of variance (ANOVA) followed by Tukey-HSD test (critical values corresponding to P = 0.05) was used to evaluate the percentage of visible punctures for each data and ANOVA repeated measures followed by Tukey-HSD test (critical values corresponding to P = 0.05) was applied to compare the trend of visible punctures recorded in the two sampling dates.

Results

The EAG data showed a high sensitivity (about -6.5/-7.0 mV) of the medfly antennae to the essential oils of *C. limon* cultivars and a clear dose-response relationship. Stimulation with various concentrations of essential oil in hexane elicited a typical sigmoid-shaped dose-dependent response (Fig. 1). The activation doses were 10^{-4} M for 'Lunario' and 10^{-5} M for 'Interdonato'; the saturation doses were 10-2 M for 'Lunario' and 10^{-3} M for 'Interdonato'.

Figure 1 - *Mean (±SE) responses of* C. capitata *antennae males after stimulation (n=10) to increasing doses of 'Lunario' (A) and 'Interdonato' (B)* C. limon *essential oils. A= activation threshold dose; S= saturation threshold dose.*



One-way ANOVA on each essential oil data indicated significant differences among the EAG responses to different concentrations (Tab.1). Field trials have been conducted in an organic orange orchard (*C. sinensis* cultivar 'Navelina'). During field observation (September 30-November 27), the number of males captured was an average of 13 males/trap, showing a low risk of infestation. Two sprays for each treatment were carried out (only one for kaolin) on 11 October and 27 October and two sampling procedures on 3 November and 18 November (Fig. 2). The visual analysis of fruits was carried out on 600 oranges for each sampling procedure.

Table 1 - *Mean (±SE) responses (mV) of* C. capitata *antennae males (n=10) stimulated by increasing doses of* C. limon *'Lunario' and 'Interdonato' essential oils in hexane.*

	Absolute mean EAG response in mV (±SE)[a]			
Dose	*Lunario*		*Interdonato*	
10^{-10}M	0.5 ± 0.1	a	0.0 ± 0.0	a
10^{-9}M	0.4 ± 0.1	a	0.07 ± 0.04	a
10^{-8}M	0.9 ± 0.4	ab	0.7 ± 0.3	ab
10^{-7}M	1.3 ± 0.4	ab	0.9 ± 0.2	ab
10^{-6}M	1.3 ± 0.1	ab	1.2 ± 0.5	ab
10^{-5}M	1.6 ± 0.4	abc	3.2 ± 0.9	bc
10^{-4}M	2.8 ± 0.8	bc	3.4 ± 0.5	bc
10^{-3}M	3.8 ± 0.8	cd	4.2 ± 0.8	cd
10^{-2}M	5.2 ± 0.8	de	5.9 ± 0.9	cd
10^{-1}M	6.1± 0.7	e	6.8± 0.6	d
Conc.	6.2 ± 0.9	e	7.1 ± 0.8	d

[a] Values followed by different letters are significantly different at P = 0.05 (Tukey-HSD test).

Figure 2 - *Trend of visible punctures on fruits damaged by* C. capitata *on different dates as treated with essential oils 'Interdonato', essential oils 'Lunario', Kaolin and Bentonite treatments.*

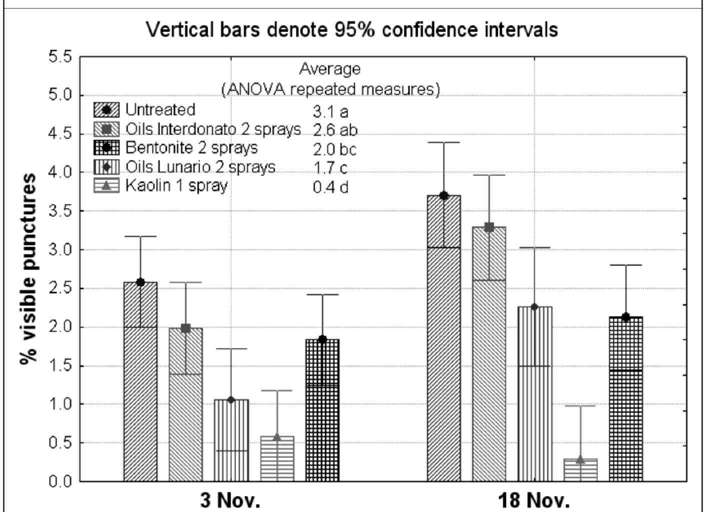
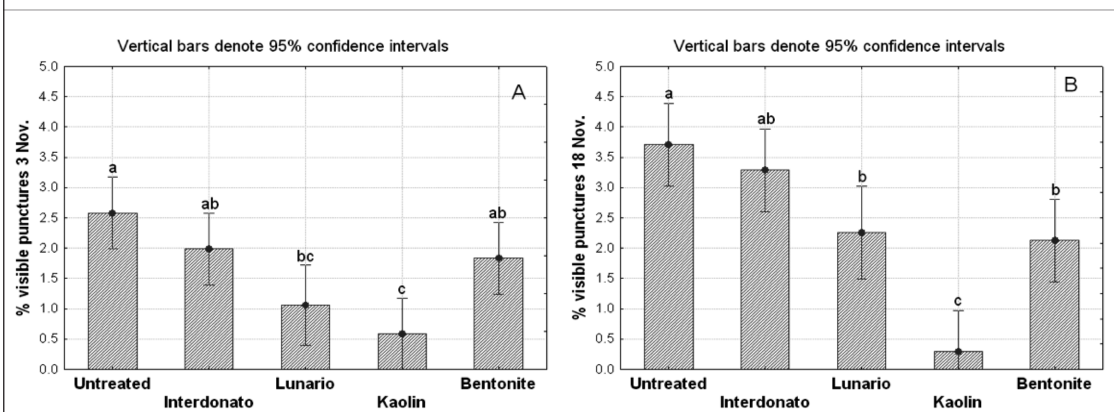


Figure 3 - *Percentage of visible punctures on fruits damage ed by* C. capitata *on 3 (A) and 18 (B) November treated with 'Interdonato' essential oils, 'Lunario' essential oils, kaolin and bentonite treatments.*



Discussion

The present study provided remarkable results on the potential use of *C.limon*; essential oils in the control of *C. capitata*. EAG analysis aimed at assessing the electrophysiological response of medfly, showed that the insect is sensitive to essential oils at low concentrations and activation doses are 10^{-4} M for 'Lunario' and 10^{-5} M for 'Interdonato'. In field trials, these oils applied on oranges exhibited a good repellent activity (Fig. 2). In the first sampling (Fig. 3A), 'Lunario' essential oils have given a result statistically different from the untreated, but equal to the kaolin, here utilized as an effective product (Lo Verde et al. 2011). The second sampling (Fig. 3B), carried out in the middle of harvest season, also has provided interesting results, because the percentage of visible punctures recorded in kaolin and bentonite remained basically unchanged, while the value recorded for essential oils of 'Lunario' has confirmed that one of bentonite, but statistically different from 'Interdonato' and untreated.

The repellent activity exerted by 'Lunario' *C. limon* essential oils provides an opportunity to deepen this study and enhance the used formulation.

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