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Impact of the Mediterranean fruit fly (Medfly) *Ceratitis capitata* on different peach cultivars: The possible role of peach volatile compounds

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ABSTRACT

The relationship between susceptibility of different peach cultivars (*cvs*) to the Mediterranean fruit fly (medfly), *Ceratitis capitata*, and the volatile composition of ripe fruit of each *cv* has been investigated, since understanding the fruit-insect interaction mechanism is crucial for developing control strategies for such a pest. Volatile compounds were analyzed by SPME–GC–MS in three *cvs* highly susceptible to medfly attack (Fair Time, Flaminia, Sicilia Piatta), and in two less susceptible *cvs* (Percoca Romagnola 7 and Doctor Davis). Among the volatile compounds detected, 88 could be identified. The main differences found in the volatile composition of the *cvs*, concerned the relative abundance of esters. The least susceptible *cvs*, above all Percoca Romagnola 7, contained the higher amounts of hexenyl, hexyl, 3-methylbutyl, butyl and 2-methylpropyl esters; among these, some C_6 derivatives detected, such as (*Z*)-3-hexenyl acetate, are known to act as priming agents, enhancing plant defence response to insects. Instead, a lower relative content of methyl esters, such as methyl hexanoate and methyl octanoate, known to act as medfly pheromone and attractant respectively, was found in the least susceptible *cvs*.

1. Introduction

Ceratitis capitata Wiedemann (*C. capitata*) is a fly which, in the larval stage, feeds on a wide variety of fruits and can affect about 250 different species (Tremblay, 1994). *C. capitata* has the ability to adapt to a wide variety of environmental conditions, which causes concern in zones not yet colonized and thus potentially at risk (Fimiani, 1989). Currently, medfly control is done, almost exclusively, with chemicals that are harmful for human health and the environment (Bolognesi & Merlo, 2011). In organic fruit production, the problem is even more serious, since European law regarding organic farming prohibits the use of synthetic substances. For this reason, growers tend to limit the problem by avoiding medium-late varieties *cvs*, preferring early season *cvs*, which mature before fly damage occurs. Given the seriousness of this problem, ninety peach *cvs* were evaluated for their susceptibility to medfly, during the 2006–2009 season, in a research program carried out at the Fruit Tree Research Center near Rome (Italy). From the results obtained in field and laboratory tests, a few *cvs* were identified as being less susceptible to fly damage. Several authors have demonstrated how fruit of different *cvs* have a different profile of volatile compounds and how the release of these compounds increases or decreases during the ripening process (Horvat et al., 1990; Wang et al., 2009). For example, it has been found that some substances, such as lactones and open chain esters are found in higher concentrations during ripening (Hernandez, Vargas-Arispuro, Adelantado, & Primo-Yufera, 1999; Visai & Vanoli, 1997). Repeated field observations confirm that there is a clear preference of the medfly for nearly ripe fruit. This has been confirmed by Hernandez et al. (1999) in electrophysiological studies, where the greatest electroantennography (EAG) response occurred for nearly ripe fruit. This leads to the hypothesis that, though *C. capitata* is quite adaptable and able to feed on many kinds of fruit, the preference for some *cvs* over others could be due to different profiles of volatile compounds in their fruit. In this paper, the results for two less susceptible and three more susceptible peach *cvs* will be shown, and the possible relationship between the susceptibility to medfly and profile of volatile compounds, with potential effects on *C. capitata*'s behaviour, will be explored.

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2. Materials and methods

2.1. Peach cvs

Within the experimental orchards of the Agricultural Research Council, Fruit Tree Research Center (CRA-FRU, Rome), the national germplasm of fruit species has about 700 different peach *cvs*, cling peaches and nectarines. From 2006 to 2009, 90 *cvs* of peach were evaluated for their susceptibility to *C. capitata* using field and laboratory tests. Among these, five late-maturing *cvs* (two *cvs* of cling peaches, Percoca Romagnola 7 (PR7) and Doctor Davis, and three *cvs* of peaches, Sicilia Piatta, Flaminia and Fair Time), which showed a clear and differentiated response to the fly in the previous screening trials, were chosen for further investigation and for the analysis of the volatile compounds in their fruit skin. Sicilia Piatta is harvested in late August, Fair Time and Doctor Davis in early September, followed 2 weeks later by Flaminia, and finally PR7 is harvested in late September.

2.2. Insect bioassays

In May and June, before medflies are present, approximately 30 fruit per *cvs* were enclosed in biodegradable paper bags to prevent insect damage. At ripening, the bagged fruit were used for laboratory tests, while the remaining fruits were sectioned to evaluate the percentage of natural *C. capitata* infestation in the field. Trees (two per *cvs*) were located in an area with high and homogeneous natural infestation and the records covered at least two years of observations. The laboratory experiments were performed in plexiglas cages with five couples of sexually mature adults of *C. capitata*, previously reared in the laboratory. Two fruit samples were placed in a cage containing food and water and each cage was considered a replicate. The peaches were exposed to the medflies for 24 h, then they were placed in appropriate containers and stored at room temperature for between 6 and 8 days, to allow larval development. Fruit were then sectioned and the larvae inside each peach were counted. Each fruit was assigned an infestation index of 1–4, according to the following scale: 1 = absence of larvae; 2 = 1–9 larvae; 3 = 10–29 larvae; 4 = 30 or more larvae.

2.3. Volatile compounds analysis

The analysis of volatile compounds was performed on whole fruit, since the goal was to examine the role of the skin volatile compounds on insect-fruit interaction as related to *cv* susceptibility. Indeed, the whole fruit have a different pattern of volatiles than the sliced and homogenized fruits (Takeoka, Flath, Guntert, & Jennings, 1988). The volatile profile was determined by Solid Phase Micro Extraction (SPME)–GC–MS. Peach samples were collected at the proper degree of ripeness, stored for a maximum of 2 days at 4 °C, and conditioned at 25 °C for 1 h before analysis. One whole fruit was analyzed in each analysis. For each *cv*, two fruits were analyzed, in order to keep down the storage period, thus avoiding possible alterations of the volatile profile due to the formation of bruises, moulds, etc. Only for PR7, was it possible to analyze a higher number of samples (nine), since its ripening was slower and fruit could be collected on different days. Sample fruits to be analyzed were chosen of approximately the same volume. Volatile compounds were extracted by SPME using a polydimethylsiloxane–polydivinylbenzene fiber (PDMS–DVB, 65 μm coating, Supelco) (Wang et al., 2009), exposed overnight (15 h) at 25 °C to the headspace of a sealed flask containing the whole peach and then analyzed by gas-chromatography (Agilent Technologies 6890N) equipped with a single quadrupole mass spectrometer detector (Agilent Technologies 5973). The same procedure was followed for blank samples. The capillary chromatographic column was a 30 m × 0.25 mm × 0.25 μm film thickness HP-5 ms (Agilent Technologies). Injector temperature was 260 °C, splitless time was 5 min. Oven temperature was held at 35 °C for 5 min, then raised to 220 °C at 10 °C min^{-1} and held for 15 min. Finally, temperature was raised to 300 °C at 25 °C min^{-1} and held for 5 min. The initial carrier gas (helium) flow rate was 2.0 ml min^{-1}. Straight chain alkanes C_6–C_{19} were used to calculate retention indices. Pure reference standards of farnesene, γ-octalactone, γ-decalactone, δ-deca-2,4-dienolactone, δ-decalactone, ethyl acetate, benzaldehyde, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 3-methylbutyl acetate, methyl octanoate, methyl nonanoate, methyl decanoate, limonene, (*Z*)-3-hexenol, hexanol and alkanes C_6–C_{19} were purchased from Sigma–Aldrich. The esters (*Z*)-3-hexenyl acetate, butanoate, 3-methylbutanoate, hexanoate, octanoate and hexyl 3-methylbutanoate, hexanoate, octanoate have been synthesized by reacting an excess of hexanol (1.5 ml) and (*Z*)-3-hexenol (1.5 ml) with acetic (0.12 mmol), butanoic (0.25 mmol), 3-methylbutanoic (0.5 mmol), hexanoic (0.75 mmol) and octanoic acid (1 mmol) in presence of 95–98% sulfuric acid (50 μl). The reaction mixture was stirred magnetically at room temperature for 3 days in order to allow the formation of the expected products in sufficient yield, even though a few hours were sufficient to detect the products. The reaction was monitored by GC–MS. To quench the reaction, brine was added (2 ml), after diluting the mixture with dichloromethane (10 ml); the organic layer was separated and then washed twice with saturated aqueous sodium hydrogen carbonate (2 ml). One product could be detected for each alcohol and acid pair. No isomerization or formation of other by-products was observed. The identities of the synthesized products have been confirmed by mass spectrometry and by comparison of their retention indices and mass spectra with literature data. Thus, the volatile compounds detected in the fruits, have been identified by comparison of their retention indices and their mass spectra with those of authentic standards (purchased or synthesized), with reference spectra from the US National Institute of Standards and Technology (NIST) and with retention indices from literature (NIST, 2008), as specified in Table 2.

2.4. Statistical analysis

One-way ANOVA test was performed to analyze the percentage of damaged fruits present in the field. For the inference tests relative to the individual volatile compounds and for data from the infestation index, due to the non-homogeneity of variance, the non-parametric Kruskal–Wallis test was used. The volatile compounds detected in the blank samples (as benzaldehyde, nonanal and decanal) and compounds considered exogenous to the fruit, probably deriving from a post-harvest contamination (phthalates, aromatic hydrocarbons, diisopropylnaphthalene, 2-ethylhexanol) were not considered in the statistical analysis.

3. Results

3.1. Insect bioassays

For the results obtained in field and laboratory tests, a high level of correlation was found: $r^2 = 0.864$ ($p = 0.022$) for linear and $r^2 = 0.983$ ($p = 0.001$) for logarithmic relation. The *cv* PR7 was not chosen by female medflies for egg deposition, either in the field or in the laboratory, when confined in the cages with the flies. In fact, after the time of incubation, the fruits were completely healthy and no larvae were found. Doctor Davis, though slightly damaged by flies in the laboratory (infestation index 1.7), was almost as unattractive to flies as PR7 in the field (Table 1). Instead,