Effect of gamma–irradiation, antibiotic treatment, and probiotic enriched diets on cuticular hydrocarbon profiles of mass-reared Ceratitis capitata males

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INTRODUCTION

The medfly (*Ceratitis capitata*) is one of the major agricultural pests controlled through sterile insect technique (SIT) programs (Dyck *et al.*, 2006). However, mass-rearing, domestication and gamma irradiation of medfly used in SIT can negatively impact fly quality and performance (Collins *et al.*, 2008; Kumano *et al.*, 2008). Symbiotic bacteria supplied as probiotics to mass-reared fruit flies may help to overcome some of these issues (Ben Ami *et al.*, 2010).

Until recently, the majority of studies investigating medfly gut communities have focused on several aspects related to the rearing efficiency and biological quality of the medfly such as host fitness and sexual competitiveness (Ben Ami *et al.*, 2009; Hamden *et al.*, 2013, Augustinos et *al.*, 2015). While, their impact on chemical communication such as cuticular hydrocarbon (CHCs) remains unknown. In addition, recent studies suggest a microbial involvement in the production of certain signaling compounds, such as CHCs that work as pheromones; for example, in *Drosophila melanogaster*, gut microbes affect mate choice and flies are attracted to mate with conspecifics that have a similar gut microbiota (Sharon *et al.*, 2010).

The characterization of CHCs profiles could be useful for the selection of volatile compounds to be further investigated in biological assays to improve knowledge of the key CHCs involved in medfly behavior. Hence, the aim of this study was to investigate factors affecting male CHCs profiles of *C. capitata* to increase the efficacy of SIT.

METHODS

We firstly, studied the CHCs profiles of mature males from laboratory and wild *C. capitata* populations using gas chromatography–mass spectrometry (GC-MS).

Secondary, we investigated whether a disruption of the microbiota through antibiotic treatment or irradiation affects CHCs profiles in the male of *C. capitata*. Three independent experiments that differentially knock down the multiple bacterial symbionts of medflies were conducted by subjecting medflies to antibiotic treatments, gamma-irradiation or probiotic enriched diets and analyzing their CHCs profiles in comparison to untreated controls by GC-MS.

1-Samples preparations:

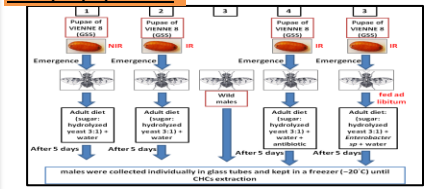
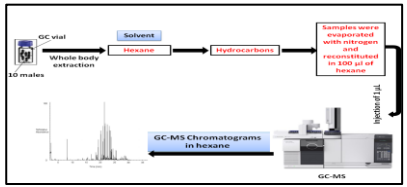


Figure 1: Scheme of preparation of males flies for cuticular extraction

2-Cuticular hydrocarbon extraction:

*** The cuticular hydrocarbon are extracted by hexane.**

*** Sample is injected into the GC-MS and a chromatogram obtained.**



3- Statistical Analysis:

The peaks were identified by their mass spectra in comparison to previously published analyses of *C. capitata* cuticular hydrocarbon profiles. Peak areas were automatically integrated using the MS Workstation Software. Finally, the success of this integration was controlled manually for every peak. The abundance of each peak was calculated relative to the internal standard. Results from the three comparative analyses were separately subjected to statistical analysis. Principal components analyses (PCA) were used to visualize overall treatment effects on hydrocarbon profile, and treatment effects on the compounds that represented the top two principal components were tested with one-, two-, and three-way analyses of variance (ANOVA) models in R.

RESULTS

Our findings show that the cuticular hydrocarbon profiles of males *C. capitata* adults are complex, with multiple variations attributable to population, and treatments. Adults of the wild population produced greater amounts of cuticular hydrocarbons than those of the laboratory strain, suggesting that CHC content may be useful as a biomarker to differentiate between wild and laboratory populations.

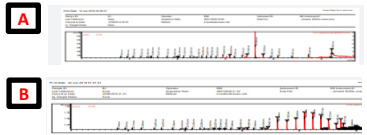
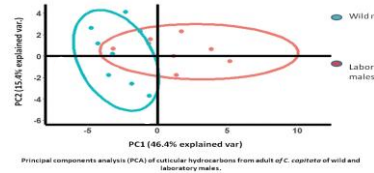


Figure 2 : Cuticular hydrocarbon profiles of (A) wild males and (B) laboratory males of *C. capitata*

Most notably, several qualitative and quantitative differences were observed between wild and laboratory males in minor or trace components. Our data also suggest that the CHCs composition may be influenced by food, since wild and laboratory males were differed in the diets. All disruption of the microbiota through antibiotic treatment or irradiation affects CHCs profiles suggesting a possible implication of microbiota disturbance on mate choice decisions. Also probiotic enriched diets affect their CHCs profiles in comparison to untreated controls.



Principal components analysis (PCA) of cuticular hydrocarbons from adult of *C. capitata* of wild and laboratory males.

CONCLUSION

There is a growing body of evidence showing that artificial fruit fly rearing changes reproductive behavior as compared to wild population. Our observation that the CHCs profiles of laboratory and wild males differ both qualitatively and quantitatively provides another aspect to the general consensus. Among other factors, larval food quality may be responsible for the observed differences. Besides food, also, rearing conditions might affect adult reproduction success.

Different selection pressures in the wild and in the laboratory may result in complex behavioral and physiological differences. All disruption of the microbiota through antibiotic treatment, irradiation or probiotic enriched diets, affects CHCs profiles suggesting a possible implication of microbiota disturbance on mate choice decisions. Understanding factors associated with variation in hydrocarbon profiles is important for identifying potential vulnerabilities relating to pest ecology and life histories and for developing tools for pest monitoring and management strategies.

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SUMMARY

Cuticular hydrocarbons (CHCs) are the main components of the epicuticular wax layer that in many insects functions as a barrier against desiccation. CHCs also play many other roles, including serving as sex pheromones, kairomones, primer pheromones, and colony-, caste-, species- and sex-recognition signals. In insects, CHC profiles can vary depending upon age, species, sex, and strain. Understanding factors associated with variation in hydrocarbon profiles is important for identifying potential vulnerabilities relating to pest ecology and life histories and for developing tools for pest monitoring and management strategies. In this study, we assessed potential sources of variation in CHC profiles in the medfly (*Ceratitis capitata*), an economically important pest through the word. Using coupled gas chromatography-mass spectrometry, we characterized and compared CHC profiles between adults of wild and laboratory males of *C. capitata*. We further investigated whether a disruption of the microbiota through antibiotic treatment or irradiation affects CHCs profiles in the male of *C. capitata*. Hydrocarbon profiles comprised different CHCs in detectable quantities and included straight-chain alkanes and a variety of mono-, di-, and tri-methylalkanes.