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Evidence of a female-produced sex pheromone in the European pear psylla, *Cacopsylla pyri*

Sonia GANASSI[1,2,5], Giacinto Salvatore GERMINARA[3], Sandra PATI[3], Stefano CIVOLANI[4], Stefano CASSANELLI[2], Maria Agnese SABATINI[1,2], Antonio DE CRISTOFARO[5]

[1]*Centro Interdipartimentale per il Miglioramento e la Valorizzazione delle Risorse Biologiche Agro-Alimentari (BIOGEST-SITEIA), Reggio Emilia, Italy*
[2]*Department of Life Sciences, University of Modena and Reggio Emilia, Italy*
[3]*Department of the Sciences of Agriculture, Food and Environment, University of Foggia, Italy*
[4]*Department of Life Sciences and Biotechnology, University of Ferrara, Italy*
[5]*Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy*

Abstract

Cacopsylla pyri (L.) (Hemiptera Psyllidae) is one of the most important pests of pear orchards in Europe that reduces the market value of pears. Summerform *C. pyri* males significantly preferred odours from living females or female cuticular extracts in the absence of visual stimuli in a Y-tube olfactometer. Conversely, males as well as females did not show any preference for odours from specimen of the same sex. Electroantennogram recordings showed that female cuticular extracts elicit dose-dependent responses in male antennae suggesting the presence of volatile compounds capable to stimulate the male peripheral olfactory system. Gas-chromatography coupled with mass spectrometry revealed marked quantitative differences between male and female cuticular extracts regarding 13-methylheptacosane, 11,13-dimethylheptacosane, 2-methylheptacosane, and 3-methylheptacosane. These compounds were found in larger amounts in female extracts which suggests their role in male attraction.

Key words: *Cacopsylla pyri*, sex pheromones, EAG, 13-methylheptacosane, 11,13-dimethylheptacosane, 2-methylheptacosane, 3-methylheptacosane.

Introduction

European pear psylla, *Cacopsylla pyri* (L.) (Hemiptera Psyllidae), is one of the most important pest of European pear, *Pyrus communis* L., in European pear growing areas. *C. pyri* damage includes necrosis of leaf and fruit tissues as well as the excretion of honeydew, which is quickly colonised by black sooty mould fungi, in turn reducing the market value of pears. In addition, *C. pyri* is the main vector of '*Candidatus* Phytoplasma pyri' responsible for pear decline disease that reduces tree vigour and sometimes causes tree death in Europe (Seemüller and Schneider, 2004). Adults of *C. pyri* are characterized by a marked seasonal dimorphism (Nguyen, 1972): a dark winterform appears at the beginning of September (Lyoussoufi *et al*., 1994; Civolani and Pasqualini, 2003) and overwinters individually or in small groups sheltered in cracks of the tree bark, at branch crossing or at the base of buds of the host plant, in a photoperiod-controlled reproductive diapause (Lyoussoufi *et al*., 1994). Oviposition in the field by post-diapause winterform begins in late winter as temperature increases (Nguyen, 1975). A small, lighter coloured adult summerform develops during the growing season. In Emilia-Romagna Region (Italy), the largest European pear growing area, *C. pyri* has five generations per year (Pollini, 2002). The control of *C. pyri* is currently based on a restricted number of insecticides, including highly efficient abamectin and spirotetramat (Civolani *et al*., 2015). Although such compounds are currently used to control this pest in late spring, according to regional integrated pest management (IPM) technical guidelines, *C. pyri* outbreaks are sometime observed in the following summer (Civolani *et al*., 2010). Observed outbreaks may be associated with the use of broad-spectrum insecticides which reduce natural enemies of *C. pyri*, particularly *Anthocoris nemoralis* (F.) (Hemiptera Anthocoridae), the main natural predator of pear psyllids (Nicoli *et al*., 1989; Shaltiel and Coll, 2004; Souliotis and Moschos, 2008). Inoculative releases of *A. nemoralis* adults may provide an alternative to chemical control of pear psyllids, but these efforts are often insufficient to keep the infestation at an economically acceptable level (Sigsgaard *et al*., 2006). Host plant resistance has been viewed for a long time as the best alternative and ecologically safe approach to insecticidal control of pear psyllids, but the identified resistant pear selections are not considered commercially acceptable (Pasqualini *et al*., 2006; Nin *et al*., 2012). For this reason, new alternative control methods of *C. pyri* are desirable. The identification of attractant semiochemicals could provide onset for new *C. pyri* control strategies within IPM context.

In certain species cuticular hydrocarbons, molecules derived from fatty-acid compound that are produced on the adult cuticle shortly following eclosion (Blomquist, 2010), are known to be involved in chemical communication and a multitude of studies have shown that these components convey information about species and genus recognition (Howard and Blomquist, 2005; Saïd *et al*., 2005), sex (Syvertsen *et al*., 1995; Sullivan, 2002; Steiner *et al*., 2005; 2006; Sugeno *et al*., 2006; Geiselhardt *et al*., 2009; Ferveur and Cobb, 2010; Ginzel, 2010; Ruther *et al*., 2011; Kühbandner *et al*., 2012; Smith *et al*., 2012; Ingleby, 2015), and physiological state (Howard, 1993; Singer, 1998; Howard and Blomquist, 2005; Blomquist and Bagneres, 2010).

Previous studies on the role of chemical signals in mate location within the superfamily Psylloidea have shown male attraction to female odorants in two pear psylla species, namely *Cacopsylla bidens* (Sulc) (Soroker *et al*., 2004) and *Cacopsylla pyricola* (Foerster) (Horton and Landolt, 2007; Horton *et al*., 2007; 2008; Guédot *et al*., 2009a; 2011), in the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Wenninger *et al*., 2008), and in the potato psyllid, *Bactericera cockerelli* (Sulc) (Guédot *et al*., 2010). Some chemicals involved in sexual communication are known for the *C. pyricola* winterform and summerform morphotypes (Guédot *et al*., 2009b; Guédot *et al*., 2011) and for *C. bidens* (Soroker *et al*., 2004). Electroantennogram (EAG) recordings and behavioural tests showed that males of *C. bidens* gave the highest EAG responses and were attracted to volatiles from pears infested with females but not to males or uninfested pears (Soroker *et al*., 2004). A sex-attractant pheromone, 13-methylheptacosane, was identified from solvent extracts of winterform *C. pyricola* females, and was shown to attract winterform males in olfactometer and field tests (Guédot *et al*., 2009a; 2009b).

Here, the presence of a putative sex pheromone in *C. pyri* was investigated. The behavioural response of summerform males and females to conspecific of both sexes was first studied in Y-tube olfactometer bioassays. Then female cuticular extracts were assessed by electrophysiological and behavioural bioassays in order to characterize their bioactivity. Finally, male and female extracts were chemically analysed to point out possible differences between the chromatographic profiles.

Materials and methods

Insects

Adults summerform of *C. pyri* were collected in infested pear orchards near Ferrara (northern Italy) during May 2012 and mass-reared on two-year old potted pear plants (cv. Bartlett) placed in Plexiglas cages (42 cm length, 60 cm width, 37 cm depth) with two access openings closed by gauze (mesh 1 mm) to allow air exchanges for plant and insects care and maintained at 25 ± 2 °C under a L16:D8 photoperiod.

Adults were sexed and placed one per glass vial (20 ml) three hours before using in electrophysiological and behavioural bioassays or in groups of 12, 25, 50 specimens, per vial, for extracts preparation.

Cuticular extracts

Whole body solvent extracts were prepared as previously described (Guédot *et al*., 2011) with slight modifications. During May and July, between 12:00 and 16:00, 50 *C. pyri* females were immerged in 300 µl of *n*-pentane (0.166 insect equivalent/µl) in a 2 ml glass vial and agitated by hand for 10 min. For EAG tests, additional extracts were obtained by using 12 or 25 females in 300 µl of *n*-pentane (0.04 female equivalent/µl; 0.083 female equivalent/µl, respectively) or 50 females in 150 µl (0.333 female equivalent/µl). Each extract was then transferred to a clean glass vial and stored at −20 °C until needed for EAG, olfactometer and chemical analyses. Before using the vials were washed with soapy hot water, followed with a distilled water rinse, dry the water off with acetone, and dry in an oven at 300 °C for 4 hours.

Olfactometer bioassay

A glass Y-tube olfactometer (each arm 23 cm long at 75° angle, stem 30 cm long, 3.0 cm i.d.) similar to that described in Germinara *et al.* (2011) was used to assess the attraction responses of *C. pyri* adults (of both sexes) to male and female odours and to female cuticular extracts. Each arm of the Y-tube was connected to a glass cylinder (9 cm long and 3.0 cm i.d.) as an odour source container. The device was put into an observation chamber (90 × 75 × 40 cm) and illuminated from above by two 36-W cool white fluorescent lamps providing uniform lighting (2500 lux) as measured by a photoradiometer (HD 9221 Delta OHM) at the cross section centre of the tube. A purified (activated charcoal) and humidified (bubble bottle) airflow, maintained at 12 cm/sec in each arm by a flowmeter was pumped through each arm. Olfactometer bioassays were carried out between 12:00 and 18:00. A first set of experiments evaluated the behavioural response of each sex to the odours of three live males or females vs clean air in order to recognize a possible sex specific attraction. In a second set of experiments, the response of males to 1 female equivalent extract (6 µl of 0.166 female equivalent unit extract) vs *n*-pentane (6 µl) was assessed in order to confirm the presence of attractive compounds. In this case, treatment and control stimuli were soaked onto two filter paper disks (0.5 cm^2) and suspended in the centre of the cross section of the odour chambers. One adult male or female was released at the open end of the stem and allowed to acclimate to the clean air flow for 15 min before exposure to the test stimuli. Each bioassay lasted 10 min. A choice was recorded when the insect exceeded the first 3 cm of an arm, marked by a horizontal line, for at least 10 sec. Insects that failed to make a choice within the first 10 min were discarded (Guedot *et al*., 2009b; 2011). Stimuli (control and treatment) were renewed for each insect tested. For each test stimulus at least 50 adults were used.

Before each bioassay, clean air was passed through the whole system for 15 min. After 5 psylla were assayed the olfactometer was rotated 180° in order to avoid positional bias. After 10 psylla were tested, glassware was rinsed with hexane and dried in an oven at 150 °C for at least 3 h. For each test stimulus a χ^2 test was used to determine significant differences between the number of psylla choosing the treatment and control. Statistical analyses were performed using SPSS 20.0 per Windows software (SPSS Inc., Chicago, IL, USA).

Electroantennography (EAG)

The EAG technique was similar to that used in previous studies (De Cristofaro *et al*., 2004; Germinara *et al*., 2012; Anfora *et al*., 2014). A male was dissected between the abdomen and the thorax and a glass micropipette (0.2-0.3 mm i.d.) filled with 10 mM NaCl solution,

acting as the neutral electrode, was inserted into the thorax. The last antennal segment was put in contact with the end of a similar pipette which provided the recording electrode. AgCl coated silver wires were used to maintain the electrical continuity between the antennal preparation and an AC/DC UN-6 amplifier in DC mode connected to a PC equipped with the EAG 2.0 program (Syntech Laboratories, Hilversum, The Netherlands). A stream of charcoal-filtered humidified air (500 ml/min) was directed constantly onto the antenna through a stainless steel delivery tube (1 cm i.d.) with the outlet positioned at approximately 1 cm from the antenna. Twenty five microliters of each female extract were absorbed onto a filter paper (Whatman No. 1) strip (1 cm × 2 cm) inserted in a Pasteur pipette (15 cm long) and used as an odour cartridge. Stimuli were allowed to evaporated for ca. 5 min before using. Over 1 sec, 2.5 cm^3 of vapour from an odour cartridge were blown by a disposable syringe into the air stream flowing over the antennal preparation. Stimuli were applied in ascending doses (1.04, 2.08, 4.16, 8.32 *C. pyri* female cuticular extract equivalent units).

Control (25 µl of *n*-pentane) and standard stimuli (25 µl of 1 M (Z)-3-hexen-1-ol *n*-pentane solution) were applied at the beginning and at the end of the experiment. Intervals between stimuli were 30 sec. EAG responses were recorded from 10 different male antennae.

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 *n*-pentane controls (Raguso and Light, 1998).

In dose-response curve, 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 than the previous dose (Germinara *et al*., 2009). Mean male EAG responses (mV) to four concentrations of the female cuticular extract were submitted to one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls (SNK) test ($P < 0.05$).

Gas-Chromatography coupled with Mass Spectrometry (GC-MS)

A 1 µL of extract (ca. 1 female equivalent) was analysed by a 6890N series gas chromatograph (Agilent Technologies) coupled with an Agilent 5973 mass selective detector (MSD) and equipped with a DB-WAX capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Inc., Folsom, USA). The carrier gas was helium at a flow rate of 1.0 mL/min. The injection was made in the splitless mode, the injector temperature was 250 °C. The column oven temperature was initially held at 40 °C for 3 min, then it was programmed to 220 °C at 3 °C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionization energy, 70eV) in a range of 30-500 amu at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading the mass spectrometer with solvent.

Solvent controls were analyzed to check for interferences. Hydrocarbons were tentatively identified by the comparison of their retention times vs C10, C20-C28 even straight-chain alkane standards and with the help of mass spectra interpretation, as no authentic standards were commercially available. Kovats method used linear *n*-alkanes as standards and was based on linearity between the carbon atoms number and the logarithm of specific retention time (Kovats, 1958). When temperature-programmed conditions instead of isothermal conditions are involved, as in this case, a generalization of the retention index system can be used, as proposed by Van den Dool and Kratz (1963). Briefly, the method includes linear temperature-programmed gas chromatography indices as follows: $I_x = 100[(t_x-t_n)/(t_{n+1}-t_n)+n]$, where I_x is the temperature-programmed retention index, t_n, t_{n+1} and t_x the retention time (in minute) of the two *n*-alkanes containing n and n + 1 carbons and of the compound of interest, respectively. As for mass spectra interpretation, methyl branched hydrocarbons gave enhanced diagnostic ions at branch points that allowed their tentative identification (Guédot *et al*., 2009b). Besides, comparison of MS fragmentation patterns with those included in the National Institute for Standards and Technology database (NIST 02, p > 80) were utilized to support tentative identification. Semi-quantitative analysis was carried out using the integrated peak area data from the GC-MS trace.

Results

Olfactometer bioassay

When males and females (n = 50 for each sex) were presented with odours from specimens of the same sex vs clean air the 68% and 56%, respectively, were responding. Among responding insects, the percentage of males (47.1%) and females (46,4%) choosing the arm containing respectively odours from males or females did not differ significantly from those of insects orienting to the empty arm (52.9% and 53.6% respectively) (male: $\chi^2 = 0.12$; df = 1; $P > 0.05$; female: $\chi^2 = 0.14$; df = 1; $P > 0.05$) (figure 1).

When exposed to females vs clean air, the 70% of males tested (n = 100) made a choice and the percentage of those (65.7%) choosing the arm with females was significantly higher than that of males entering the empty arm (34.3%) ($\chi^2 = 6.91$; df = 1; $P < 0.01$) (figure 1). When exposed to males vs clean air, the 60.0% of females tested (n = 60) made a choice but they did not show a significant preference for males (55.5%) compared with control air (44.5%) ($\chi^2 = 0.44$; df = 1; $P > 0.05$).

When presented with odours of the female cuticular extract vs control *n*-pentane, the 51.8% of males tested (n=108) made a choice and the percentage of males orienting to the extract (71.43%) was significantly higher than that of males entering the control arm (28.47%) ($\chi^2 = 10,286$; df = 1; $P < 0.01$) (figure 1).

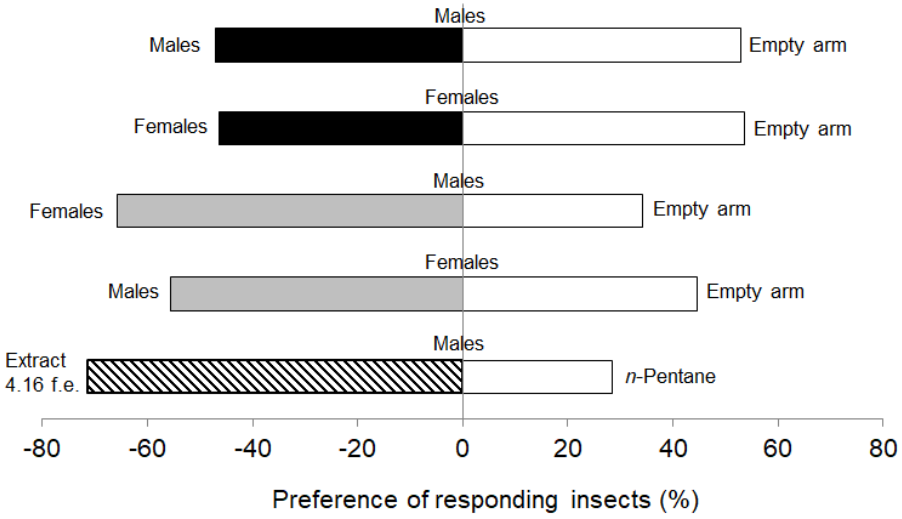

Figure 1. Preference of responding *C. pyri* (expressed as a percentage) to odours: preference of responding males and females to odours from specimens of the same sex vs clean air, respectively (black bars). Preference of responding males exposed to females vs clean air; and females exposed to males vs clean air; (gray bars). Preference of responding males to female cuticular extract (4.16 f.e.); (diagonal lines bar).

EAG test

In the dose range tested, the mean EAG response of males varied from 0.128 mV to 0.585 mV (figure 2). The EAG response increased with the doses from 1 to 4 female equivalents and decreased from 4 to 8 female equivalents. The activation and the saturation thresholds were at 2 and 4 female equivalents, respectively (figure 2).

ANOVA revealed significant differences among the mean EAG responses of males to 1.04, 2.08, 4.16, 8.32 female equivalent doses ($F = 7.848$; $df = 3, 40$; $P < 0.01$). The mean EAG response to 4.16 female equivalent was significantly higher ($P < 0.001$) than those to the other doses (figure 2).

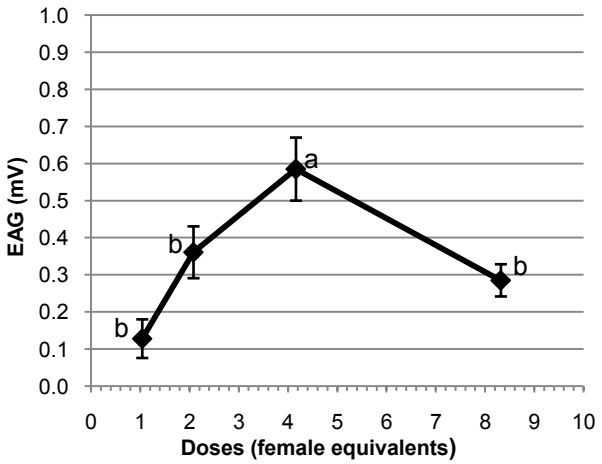

Figure 2. Mean EAG (mV) dose-responses curve of *C. pyri* males to female extracts in four ascending doses (1.04, 2.08, 4.16, 8.32 female equivalents). Vertical bars represent standard errors. Different letters show significant differences, Student-Newman-Keuls test ($P < 0.05$).

GC-MS

Chemical analysis of cuticular extracts from both male and females revealed the presence of a great number of hydrocarbons (figure 3). The cuticular hydrocarbons extracts included C10-C28 straight-chain alkanes, as compared with alkanes standard solution. Both chromatograms were quite similar with the exception of the region in the range of 72-82 min, illustrated in the inset of figure 3.

Peaks showing area at least three times as abundant in females compared to males were selected and listed in table 1, where information on retention times, tentative attribution, and MS ions, is reported. It is worth noting that compounds 1, 2, 3 and 4 were 11, 14, 3 and 14 times, respectively, more abundant in females than in males, and all eluted between hexacosane (retention time 70.32 min) and octacosane (retention time 81.36 min) standards. In accordance with Guédot *et al.* (2009b), LRI and interpretation of mass spectra were used to tentatively identify the target compounds. The molecular ion suggested the total number of carbons in the molecule, methyl-branched hydrocarbons gave enhanced diagnostic ions at branch points that allowed the positions of the methyl branches to be revealed, and the presence of methyl branches caused diagnostic shifts in retention times vs straight-chain standards. Compound 1 showing diagnostic ions at m/z 196 and 224 (Guédot *et al.*, 2009b) and showing a high similarity with that included in the MS library NIST 02 (p = 90) was attributed to 13-methylheptacosane. Peak 2 was characterized by diagnostic ions at m/z 168/239 and at 196/267 suggesting the presence of 11,13-dimethylheptacosane. Finally, compounds 3 and 4, with m/z 351 and 365 diagnostic ions, have been tentatively attributed to 2-methylheptacosane and 3-methylheptacosane, respectively (Guédot *et al.*, 2009b). The mass spectra of 11,13-dimethylheptacosane, 2-methylheptacosane and 3-methylheptacosane were not present in the NIST 02.

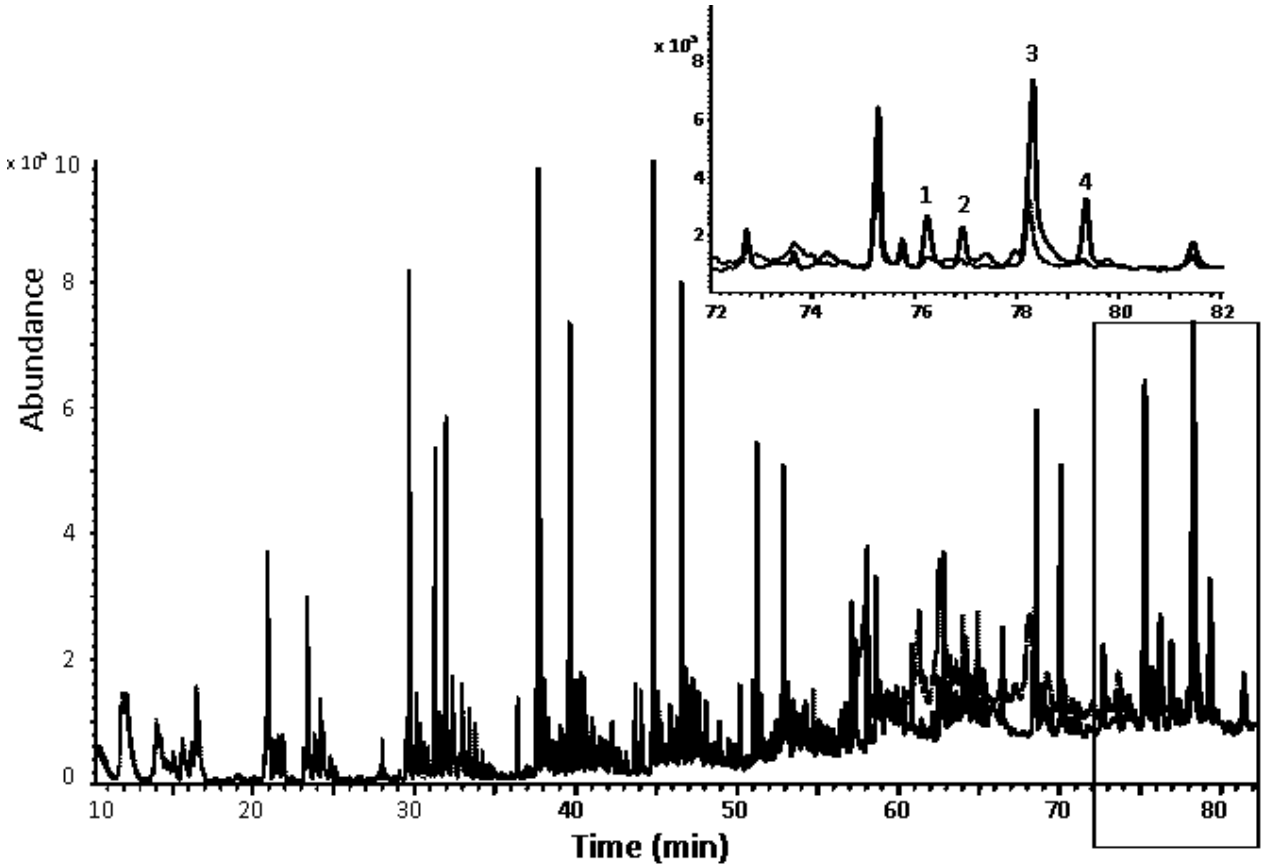

Figure 3. GC-MS analysis (overlapped) of cuticular extracts from both male (dotted line) and females (full line) *C. pyri*. The region of the GC-MS trace, in the range of 72-82 min, is magnified on the top right corner of the figure.

Table 1. GC-MS analyses of selected peaks found in *n*-pentane extracts of *C. pyri* female and male. Retention times, attribution, MS ions and $Area_f/Area_m$ ratio are reported.

peak	t_R/LRI	Compound	Diagnostic ions	$Area_f/Area_m$
1	76.2/2710	13-methylheptacosane	196; 224 (394, M^+)	11
2	76.9/2723	11,13-dimethylheptacosane	168/239; 196/267 (408, M^+)	14
3	78.3/2747	2-methylheptacosane	351, 379 (394, M^+)	3
4	79.3/2765	3-methylheptacosane	365, 379 (394, M^+)	14

t_R/LRI, retention time/ Linear Retention Index.
$Area_f/Area_m$, ratio of peak area found in female extract GC-MS trace to peak area found in male extract GC-MS trace.

Discussion

In behavioural bioassays, summerform *C. pyri* males were attracted by living summerform females and their cuticular extracts whereas females were not attracted by males or females. These observations provided evidences for the presence of a female-produced sex pheromone in *C. pyri*.

Similar behaviour responses have been also reported for other psyllid species including the two pear psyllids, *C. bidens* (Soroker *et al*., 2004) and *C. pyricola* (Horton and Landolt, 2007; Horton *et al*., 2007; 2008; Guédot *et al*., 2009a), the potato psyllid, *B. cockerelli* (Guédot *et al*., 2010) and the Asian citrus psyllid, *D. citri*.

The attraction of *C. pyricola* summerform males to females was further confirmed while summerform females did not respond to odours produced by live summerform males, their solvent extracts and odours from live females regardless of their mating status (Guédot *et al*., 2011). In field studies, *C. pyricola* females showed no preferences among traps baited with live females, live males, and unbaited traps (Brown *et al*., 2009). In *D. citri*, males were attracted to female cuticular extracts (Mann *et al*., 2013) but females were not attracted to male extracts (Wenninger *et al*., 2008). Moreover, GC-MS analyses revealed that dodecanoic and tetradecanoic acids were present in female *D. citri* cuticular extracts in higher amounts compared with males (Mann *et al*., 2013).

In this study, female cuticular extracts elicited dose-dependent EAG responses in *C. pyri* males and attracted them in a highly significant manner in behavioural bioassays, thus suggesting the presence of volatile compounds responsible for male attraction.

Subsequent GC-MS analyses pointed out marked differences between *C. pyri* female and male extracts regarding likely the presence of 13-methylheptacosane, 11,13-dimethylheptacosane, 2-methylheptacosane, and 3-methylheptacosane which were found in larger amounts in female extracts. Tentative assignments of structure were carried out as no commercially available standard was found. Structure confirmation should be performed in further studies with authentic synthesized standards. Methyl-branched hydrocarbons have been identified as cuticular components in many species of insects (Blomquist *et al*., 1987; Lockey, 1988; Nelson, 1993; Nelson and Blomquist, 1995; Blomquist, 2010; Yocum *et al*., 2011). 2-methylheptacosane, 3-methylheptacosane and 13-methylheptacosane were also detected in the cuticular extracts of both sexes of *C. pyricola* but only the latter is produced by females in a significantly larger amount (Guédot *et al*. 2009a; 2009b). This compound was proposed as the sex-attractant pheromone of *C. pyricola* females since it was as attractive to males as the whole female extracts. 13-methylheptacosane was detected also in Western flower thrips *Frankliniella occidentalis* (Pergande), where it is more than two times lower in adults in comparison to larvae (Gołębiowskia *et al*., 2007). Moreover, differences regarding 13-methylheptacosane between fertile queens and workers of the ant *Camponotus floridanus* (Buckley) were found to be involved in regulating worker reproduction (Endler *et al*., 2004).

In *C. pyri*, the additional compounds tentatively identified as 11,13-dimethylheptacosane, 2-methylheptacosane and 3-methylheptacosane, which were detected in significantly higher quantities in female extracts, probably play a role in the reproductive isolation of this species. Tentative structure assignments need further confirmation with authentic synthesized standards. Detailed behavioural and electrophysiological investigations carried out utilizing the above mentioned compounds, tested individually and in blends, are needed to clarify their role in *C. pyri* mating behaviour. 2-methylheptacosane, and 3-methylheptacosane are sex pheromone components common to many insect species (El-Sayed, 2014). Kühbandner *et al*. (2012) showed that 3-methylheptacosane is a key component of the females contact sex pheromone in the parasitic wasp *Lariophagus distinguendus* (Forster); however, it triggers courtship behaviour only if an olfactory background of other cuticular lipids is present. 11,13-dimethylheptacosane was reported as a sex pheromone component of *Cataglyphis* species (Hymenoptera Formicidae) (Dahbi *et al*., 1996).

Different studies were focused on sound production and the interactions between acoustic and chemical signals in the courtship behaviour of psyllids (Lubanga *et al*., 2014). Mechanoreception generally appeared prominent and may play a significant role in much of pear psylla behaviour, including mating, and a high sensitivity of *C. bidens* male and female antennae to mechanical stimuli was reported (Soroker *et al*., 2004).

Studies carried out by Wenninger *et al*. (2009) showed that *D. citri* produces substrate-borne vibrational signals for mate finding. In this species, indeed, not only host location but also mate finding are probably mediated by several modalities, including an integration of olfactory, visual, and vibrational cues (Mann *et al*., 2013). Recently, Eben *et al*. (2014) found evidence of acoustic signals emitted by male and female *C. pyri* during mate search and pre-copulatory behaviour. The low volatility of candidates *C. pyri* sex pheromone components identified in this study suggests a short-range attractant function (Bradbury and Vehrencamp, 1998; Singer, 1998; Ferveur, 2005; Griffith and Ejima, 2009; Martin and Drijfhout, 2009) and a possible need of a combination of different sensory modalities (Griffith and Ejima, 2009; Lubanga *et al*., 2014). Detailed studies of the chemical ecology of the species in the Psyllidae are needed further to understand their mating behaviour (Lubanga *et al*., 2014). Moreover, the knowledge of a possible interaction of chemical and acoustical signals in mating behaviour of *C. pyri* (Eben *et al*., 2014) could be essential for the design of behaviour-modifying biological control methods like mating disruption and push and pull strategies.

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Authors' addresses: Sonia GANASSI (corresponding author, sonia.ganassi@unimol.it), Antonio DE CRISTOFARO, Department of Agricultural, Environmental and Food Sciences, University of Molise, Via De Sanctis, 86100 Campobasso, Italy; Giacinto Salvatore GERMINARA, Sandra PATI, Department of the Sciences of Agriculture, Food and Environment, University of Foggia, Foggia, Italy; Stefano CIVOLANI, Department of Life Sciences and Biotechnology, University of Ferrara, Italy; Maria Agnese SABATINI, Stefano CASSANELLI, Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.

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corrigenda

Evidence of a female-produced sex pheromone in the European pear psylla, *Cacopsylla pyri*

Sonia GANASSI, Giacinto Salvatore GERMINARA, Sandra PATI, Stefano CIVOLANI, Stefano CASSANELLI, Maria Agnese SABATINI, Antonio DE CRISTOFARO

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Change "11,13-dimethylheptacosane" to "11,15-dimethylheptacosane" along all the text.

Page 61, add figure 4, the experimental mass spectrum relevant to the peak 2 and the fragmentation scheme for the compound 11,15-dimethylheptacosane, similar to the one reported by Kenig *et al*. (1995).

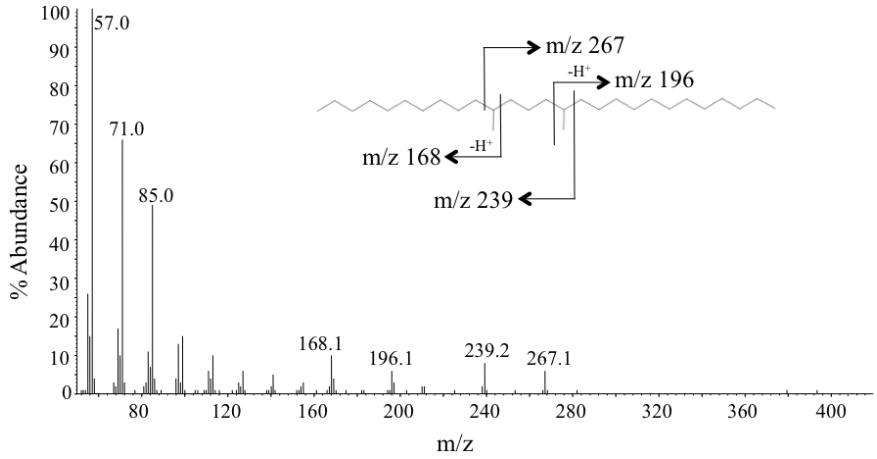

Figure 4. Mass spectrum, subtracted for the background, relevant to the peak 2. In the inset, fragmentation scheme of the compound 11,15-dimethylheptacosane.

Page 61, table 1, add this information in the footnotes "Molecular ions in the brackets were not visible in the spectrum, but could be inferred by the diagnostic ions".

Page 62, delete "11,13-dimethylheptacosane was reported as a sex pheromone component of *Cataglyphis* species (Hymenoptera Formicidae) (Dahbi *et al*., 1996)."

Page 63, References, delete "DAHBI A., LENOIR A., TINAUT A., TAGHIZADEH T., FRANCKE W., HEFETZ A., 1996.- Chemistry of the postpharyngeal gland secretion and its implication for the phylogeny of Iberian *Cataglyphis* species (Hymenoptera: Formicidae).- *Chemoecology*, 7: 163-171."

Page 63, References, add "KENIG F., SINNINGHE DAMSTÉ J. S., KOCK-VAN DALEN A. C., RIJPSTRA W. I. C., HUC A. Y., DE LEEUW J. W., 1995.- Occurrence and origin of mono-, di-, and trimethylalkanes in modern and Holocene cyanobacterial mats from Abu Dhabi, United Arab Emirates.- *Geochimica et Cosmochimica Acta*, 59: 2999-3015."

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