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Elevated Carbon Dioxide Concentration Reduces Alarm Signaling in Aphids

Antoine Boullis[1] · Bérénice Fassotte[1] · Landry Sarles[1] · Georges Lognay[2] · Stéphanie Heuskin[2] · Maryse Vanderplanck[3] · Stefan Bartram[4] · Eric Haubruge[1] · Frédéric Francis[1] · François J. Verheggen[1]

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Abstract Insects often rely on olfaction to communicate with conspecifics. While the chemical language of insects has been deciphered in recent decades, few studies have assessed how changes in atmospheric greenhouse gas concentrations might impact pheromonal communication in insects. Here, we hypothesize that changes in the concentration of atmospheric carbon dioxide affect the whole dynamics of alarm signaling in aphids, including: (1) the production of the active compound (*E*)-β-farnesene (Eβf), (2) emission behavior when under attack, (3) perception by the olfactory apparatus, and (4) the escape response. We reared two strains of the pea aphid, *Acyrthosiphon pisum*, under ambient and elevated CO_2 concentrations over several generations. We found that an increase in CO_2 concentration reduced the production (i.e., individual content) and emission (released under predation events) of Eβf. While no difference in Eβf neuronal perception was observed, we found that an increase in CO_2 strongly reduced the escape behavior expressed by an aphid colony following exposure to natural doses of alarm pheromone. In conclusion, our results confirm that changes to greenhouse gases impact chemical communication in the pea aphid, and could potentially have a cascade effect on interactions with higher trophic levels.

Keywords Carbon dioxide · *Acyrthosiphon pisum* · Predator-prey interaction · (*E*)-β-farnesene · Signal dynamic

Introduction

Understanding and predicting how changes to the atmosphere impact biological interactions is a major challenge for all branches of ecology. For instance, plants respond to changes in atmospheric CO_2 concentration by altering C:N ratios and their production of secondary metabolites (e.g., Bidart-Bouzat and Imeh-Nathaniel 2008; DeLucia et al. 2012; Ode et al. 2014). Furthermore, modifications in atmospheric CO_2 concentrations impact the biology of herbivorous insect pests, at least indirectly through modifications of host plant physiology (Coviella and Trumble 1999; De Lucia et al. 2012; Zavala et al. 2013), as well as organisms from higher trophic levels (Boullis et al. 2015; Guerenstein and Hildebrand 2008).

Most insect species rely on olfactory cues released in their environment to exhibit appropriate behaviors, such as searching for food and sexual partners, escaping threats, or regulating population size (Hansson and Wicher 2016). Such odorant cues are fundamentally involved in the population dynamics of insects (Vet and Dicke 1992), especially in bottom-up (i.e., host plant–herbivorous insect) and top-down (i.e., herbivorous insect–natural enemies) trophic relationships (Verheggen et al. 2008). Thus, it is important to understand how elevated CO_2 concentrations (eCO_2) impact insect population dynamics through the modification of insect chemical communication within a framework of how agro-

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✉ François J. Verheggen
fverheggen@ulg.ac.be

[1] Functional and Evolutionary Entomology, Gembloux Agro-bio Tech, University of Liege (ULg), Passage des Déportés, 2, Gembloux, Belgium

[2] Analytical Chemistry, Gembloux Agro-bio Tech, University of Liege (ULg), Passage des Déportés, 2, Gembloux, Belgium

[3] Research Institute for Biosciences, University of Mons (UMONS), Place du Parc, 23 Mons, Belgium

[4] Max Planck Institute for Chemical Ecology, Ammerbach, Hans-Knöll-Straße 8, Jena, Germany

ecosystems are responding toward climate changes. In this context, more studies on multitrophic interactions are needed, not only focusing on plant-herbivore interactions, but also on extending the study to higher trophic levels, including the interactions between herbivores and their natural enemies to assess better the net effect of climate change on pest populations and their natural control. Unlike insect semiochemicals, the effects of eCO_2 on plant volatile organic compounds (VOCs) are well documented in both herbaceous and woody plants (Peñuelas and Staudt 2010). This focus on plants exists due to their apparently greater physiological modifications compared to insects. While the direct impact of eCO_2 on insects may be less disruptive than that to plants, alterations do occur (Boullis et al. 2016).

Aphids are primitive social insects that live in colonies and have limited mechanical defenses against natural enemies. However, aphids ubiquitously (i.e., in all development stages and in almost every aphid species) secrete liquid droplets from the two cornicles situated on the upper posterior surface of their abdomen in response to predation or other threats (Boullis and Verheggen 2016; Verheggen et al. 2010). For most species, these droplets contain chemical(s) acting as alarm pheromone for conspecifics (Kislow and Edwards 1972), with just one sesquiterpene hydrocarbon usually being active, namely (*E*)-β-farnesene (Eβf) (Francis et al. 2005). This chemical is generally sufficient to induce escape behavior in colony-mate individuals, and is of paramount importance in interactions between aphids and their natural enemies (Hatano et al. 2008; Vandermoten et al. 2012; Verheggen et al. 2007). The inclusive fitness theory (Hamilton 1964) suggests that alarm signals deployed during predation events have substantial inclusive fitness benefits for the aphid producing them, because the nearby individuals that benefit from the signal generally share the same genotype (Boullis and Verheggen 2016; Mondor and Roitberg 2004). Thus, elucidating how atmospheric gas concentrations impact alarm signaling by aphids should improve our understanding of how intraspecific and multitrophic interactions in insects are affected.

Some studies have addressed the question of how eCO_2 impacts aphid escape behavior, with their findings being generally consistent. Specifically, escape behavior is lessened under elevated CO_2 concentrations (Awmack et al. 1997a; Hentley et al. 2014; Mondor et al. 2004; Sun et al. 2010). Whatever the source of disturbance used in these experiments [crushed aphid (an unknown dose of alarm pheromone), known dose of synthetic Eβf, or natural enemy presence], these authors observed the same reduction in aphid escape behavior. However, the mechanisms have not been elucidated, and we do not know whether elevated CO_2 concentrations actually affect one or several of the different steps involved in the alarm signal (Fig. 1). In the present study, we used pea aphids, *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), reared under ambient or elevated CO_2 concentrations to test the hypothesis that changes in atmospheric composition disturb the dynamics of alarm signaling in aphids. Within this framework, we first studied the individual emitter by investigating (*i*) alarm pheromone (Eβf) production and (*ii*) the quantity of alarm pheromone released during an attack by a predator. Next, we studied the individual receiver and evaluated (*iii*) the olfactory perception of Eβf and (*iv*) the induced escape behavior. Because plant quality is likely to be impacted by changes in CO_2 concentration, the semiochemistry of aphids, as well as their behavior related to alarm signaling, may be altered. Finally, we discuss the potential impact of changes to greenhouse gases on herbivorous insect chemical communication and the potential cascade effect on higher trophic levels.

Methods and Materials

Conditioned Chambers Plants and insects were maintained in 12 chambers (60 × 50 × 50 cm) made of transparent sheets (PLEXIGLAS® GS, clear 0F00 GT, 8 mm thick; Evonik Industries, Essen, Germany), situated in a laboratory. In each chamber, a constant airflow (30 $l.min^{-1}$) was pushed through using an air pump (Koi flow 30; Superfish, Netherlands). Two different levels of CO_2 were used: half of the chambers received room air containing 450 ± 50 ppm CO_2 (termed aCO_2) and the other half received air enriched with CO_2 (termed eCO_2, corresponding to aCO_2 + 350 ppm), using a CO_2 gas tank (>99% purity; Airliquide, Paris, France). All chambers were maintained at 23 ± 1 °C and 60 ± 10% RH, with a 16:8 h light: dark photoperiod under cool white light-emitting diode (LED) lights (77 lmol/sqm/s). Carbon dioxide concentrations, temperature, and RH were continuously monitored in each chamber with MCH-383 SD data loggers (Lutron, Taipei, Taiwan).

Plants and Aphids Two genetically different populations (Y-R2 and L1–22) of *A. pisum* were maintained in the chambers, under either aCO_2 or eCO_2. These two aphid strains were kindly provided by the IGEPP Laboratory (University of Rennes 1, France). Aphids were reared on broad beans, *Vicia faba* L., which had been grown in 30 × 20 × 6 cm plastic pots containing a 1:1 mixture of perlite: vermiculite in other chambers under the same aCO_2 and eCO_2 conditions. Each week, aphids were transferred to young *V. faba* (one week after sowing) plants from the two CO_2 treatments to ensure their proper development. Under these conditions, aphids went through a minimum of 30 parthenogenetic generations before testing.

To ensure that bioassays were undertaken with standardized and naïve individuals (i.e., aphids of the same age that had never been exposed to their alarm signal), we prepared groups of 15 apterous reproductive adults from the four

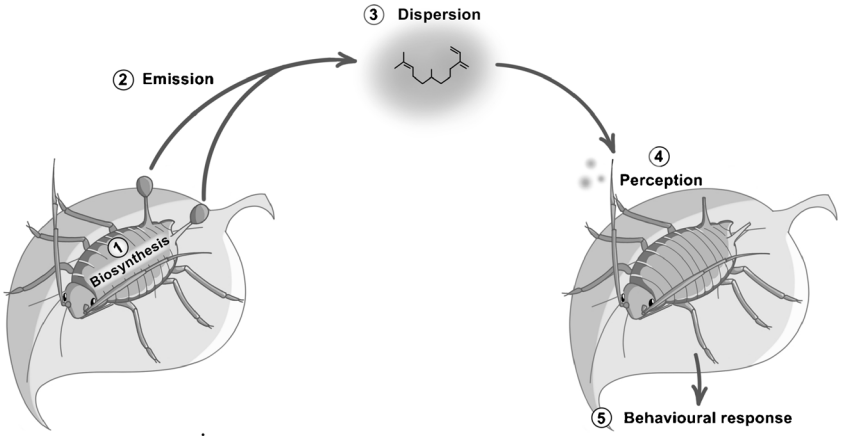

Fig. 1 Steps of aphid alarm signaling that are probably impacted by modifications to atmospheric CO_2 concentration (Graphic art by Carolina Levicek)

different modalities (i.e., two strains and two CO_2 concentrations) twice a week. These groups were then transferred to healthy plants from the respective CO_2 treatments. After 24 h, the adults were removed from the plants, and their offspring kept until they reached an age of 8 d (corresponding to the pre-reproductive developmental instar) for the experiments.

Chemicals Eβf (>98% purity) was used for the electrophysiological and behavioral assays, and chromatographic analyses, and was synthesized from farnesol (Tanaka et al. 1975). *N*-butylbenzene (>99% purity; Sigma-Aldrich, Saint-Louis, MO, USA) was used as an internal standard (IS) to quantify Eβf. *n*-Hexane (>97% purity; VWR International, Leuven, Belgium) and *n*-pentane (>95% purity, VWR International) were used for elution and dilution of Eβf solutions.

1st Experiment: Impact of eCO_2 on Aphid Eβf Content To assess how an increase in atmospheric CO_2 concentration impacted aphid Eβf content, a solvent extraction method was adapted from Fischer and Lognay (2012). Specifically, a single pre-reproductive aphid was gently removed from a plant (to avoid any disturbance and potential Eβf release) and placed in a threaded test tube containing 100 μl *n*-hexane dosed with *n*-butylbenzene (5 ng.μl^{-1}) as internal standard. A stir bar was placed inside the glass tube, which was sealed, and placed onto a working magnetic stir plate for 20 min to completely crush the aphid and to extract Eβf. Then, 50 μl of clean supernatant was recovered and transferred to a chromatography vial. Samples were maintained at −80 °C until analysis.

Eβf was quantified according to Fassotte et al. (2014) by gas chromatography, using a Trace™ GC Ultra (Thermo Scientific™, Interscience, Belgium) equipped with a flame ionization detector at 260 °C (GC-FID) and a splitless injector at 250 °C. In brief, 1 μl of sample was injected onto a non-polar capillary OPTIMA 5 column (30 m × 0.25 mm I.D., 0.50 μm thickness; Macherey Nagel, Düren, Germany) and the column oven programmed as follows: held at 40 °C for 2 min, then increased at 10 °C.min^{-1} to 300 °C, with a final hold of 5 min. Helium (constant flow of 1 ml.min^{-1}) was the carrier gas. Chromatograms were obtained and analyzed using ChromCard software (V. 2.7). For quantification, seven standard Eβf solutions diluted in *n*-hexane (0.2, 0.4, 0.5, 0.8, 1.0, 2.0, and 4.0 ng.μl^{-1}) were prepared with *n*-butylbenzene (5 ng.μl^{-1}) as an internal standard. Each solution was injected three times and a calibration curve established. The method of least squares fit analysis was used to calculate the calibration curve. Linearity was considered satisfactory when the correlation coefficient exceeded 0.996 (Heuskin et al. 2009). To confirm the accuracy of this calibration curve, a set of five solutions (0.2, 0.5, 1.0, 2.0, and 4.0 ng.μl^{-1}) was injected using the same method. The accuracy was considered to be satisfactory if values were between 90 and 110% of those calculated from the calibration curve (Heuskin et al. 2009). Eβf from aphids was identified based on comparison of retention time and mass spectrum (Fassotte et al. 2014) with those from synthetic Eβf.

2nd Experiment: Impact of eCO_2 on Eβf Emission under Predation A dynamic headspace sampling technique was used to quantify the amount of Eβf emitted by aphids under predation by a natural enemy. A single pre-reproductive aphid instar was placed in a 6 cm two-sided opened glass tube with an adult of the multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). The lady beetles never fed on aphids since their emergence, to avoid any risk of passive Eβf absorption, and were starved at least 48 h before the test to favor their foraging behavior. A charcoal-filtered air stream was pulled (100 ml.min^{-1}) into the glass tube with a GilAir™ Plus air sampling pump (Gilian®, West Caldwell, USA). A filter containing 15 mg of HayeSep Q® (80/100 mesh; Hayes Separation Inc., Houston, TX, USA) was placed between the pump and the glass tube to adsorb any released volatiles. The experiment was run for 90 min after the lady beetle attack. After 90 min, the filter was eluted with 50 μl of

n-hexane, containing *n*-butylbenzene (5 ng.µl^{-1}) for quantification. The eluate was kept in a glass chromatography vial and stored at −80 °C until analysis. Chromatographic conditions were identical to those in the 1st experiment. The reliability of our volatile quantification method was confirmed by testing Eβf recovery rate at three different doses (25, 50, and 100 ng). Five replicates were assessed for each cartridge and each quantity of Eβf. This recovery was considered satisfactory at 85–110% (Heuskin et al. 2012).

3rd Experiment: Impact of eCO_2 on Eβf Olfactory Perception To evaluate how eCO_2 impacts aphid olfactory perception toward alarm pheromone, we recorded the electrical depolarization produced by aphid antennae exposed to Eβf using an electroantennograph. After decapitating an aphid, the head was placed between two glass Ag-AgCl electrodes (Harvard Apparatus; 1.5 mm OD × 1.17 mm ID) that had been filled with saline solution (NaCl 7.5 g/l, $CaCl_2$ 0.21 g/l, KCl 0.35 g/l, $NaHCO_3$ 0.2 g/l) and that were in contact with metal wires. The ground glass electrode was placed in the posterior part of the aphid head, while the recording electrode was placed in contact with the excised tip of the antennae (both antennae in the same electrode). The recording electrode was linked to an amplifier (IDAC-4; Synthech®, Hilversum, Netherlands). A 6 cm^2 piece of filter paper (Whatman #1) was inserted into a glass Pasteur pipette, and 10 µl Eβf solution in *n*-hexane was applied. Four different solutions were tested on each individual in the following order: 0.5 µg (50 ng.µl^{-1}), 1 µg (100 ng.µl^{-1}), 5 µg (500 ng.µl^{-1}), and 10 µg (1 µg.µl^{-1}). After 45 s of solvent evaporation under a filtered air stream, each stimulus was presented to the antennae using an air puff (0.3 s) introduced into a constant airflow (1.5 ml.min^{-1}). In addition to the tested Eβf solutions, a negative control (10 µl *n*-hexane) was applied at the start and end of the test. One minute was left between two successive stimulations, allowing sufficient time for repolarization of the antennae. Electroantennograms (EAGs) were collected and analyzed using Autospike 3.0 (Syntech®). Six replicates were conducted for each modality (i.e., two aphid strains and two CO_2 concentrations).

4th Experiment: Impact of eCO_2 on Aphid Escape Behavior Here, we observed the proportion of aphids from a colony that established on a *V. faba* plant and initiated movement following exposure to Eβf. The colonies were obtained by isolating multiple groups of 15 individuals during 24 h. Then, all adults were removed and the offspring kept for 7 d until the test. The resulting colonies contained a maximum of 40 individuals.

A charcoal-filtered air flow (50 ml.min^{-1}) was blown onto the colony, passing through a fine glass tube (4 mm I.D.) containing a 2 cm^2 piece of filter paper on which 4 µl of an Eβf solution diluted in *n*-pentane had been applied. Three different doses of Eβf were exposed to the different aphid colonies: 2 ng (0.5 ng.µl^{-1}), 20 ng (5 ng.µl^{-1}), and 200 ng (50 ng.µl^{-1}), mimicking natural doses of Eβf released by an individual under attack (Vosteen et al. 2016). After 30 s of solvent evaporation under a charcoal-filtered airstream, the Eβf-enriched airflow was directed into the center of the aphid colony, with the glass tube being placed 1 cm from the colony. The number of aphids initiating movement (i.e., running away or dropping off the plant) was quantified over 5 min. A control treatment (*n*-pentane) was also tested under the same conditions. Each colony was tested only once. Ten replicates were assessed for all treatments (i.e., for three Eβf doses, solvent alone, two CO_2 concentrations, two aphid strains).

Statistical Analyses Comparisons of Eβf content in aphids from different modalities were assessed using two-way analysis of variance (ANOVA), with the quantity of alarm pheromone recovered serving as the response variable, while both CO_2 concentration and aphid strain served as the explanatory variables. Before this parametric test, normality of the residuals and homoscedasticity were checked using Shapiro-Wilk and Bartlett tests, respectively ($P > 0.05$). The same statistical design was performed to compare Eβf emission by aphids that were preyed on; however, the data were square root-transformed as assumption violations occurred. The standard errors (S.E.) presented here were calculated using the mean square residual of these analyses.

Mean EAG performances were compared between aphids at different CO_2 concentrations and from different strains using a repeated-measures ANOVA, in which CO_2 concentration and aphid strain served as between-subject factors, while Eβf dose served as the within-subject factor. The EAG results were log-transformed to meet the assumptions (i.e., normality of the residuals and sphericity).

Finally, an analysis of covariance (ANCOVA) was used to test how aphid strain, Eβf dose, and CO_2 concentrations influenced the rate of aphid escape, using the size of the colonies as the covariate. ANCOVA assumptions, namely (i) residuals normality, (ii) homoscedasticity, (iii) independence of covariant and independent variables, (iv) homogeneity of regression slopes and (v) linear relationship between the dependent variables and the covariate, were checked prior to statistical analysis using orthogonal contrasts. We then performed a model simplification (deletion tests) to eliminate unnecessary parameters using a step function. As the Akaike information criterion (AIC) was better without the covariate, colony size was eliminated from the model and the ANCOVA replaced by a three-way ANOVA. Before this analysis, percentage data were arcsine-transformed to stabilize variance. Normality of the residuals and homoscedasticity were checked using Shapiro-Wilk and Bartlett tests, respectively ($P > 0.05$). All statistical tests were conducted using software R (V. 3.0.1 - R development core team 2013).

Results

Chemical Analyses To quantify Eβf, we developed a calibration curve using the ratio of peak areas (Eβf/Internal standard; y) and the quantity of Eβf present in the sample (x). A linear correlation was obtained (y = 0.9123× + 0.0017), with a correlation coefficient of r^2 = 0.998 (Supplementary, Fig. S1). Moreover, a non-significant difference was obtained for the accuracy of the measures against those from the calibration curve (i.e., mean of 103% of the value recovered), confirming very strong accuracy for this compound and this range of concentrations.

1st Experiment: Impact of eCO_2 on Aphid Eβf Content Eβf was found in all aphid strains at both CO_2 concentrations. Aphids from the pink strain Y-R2 contained 112.1 ± 4.5 ng (n = 24) and 96.9 ± 5.4 ng (n = 17) Eβf under aCO_2 and eCO_2 conditions, respectively. Aphids from the green strain L1–22 contained 68.7 ± 4.6 ng (n = 23) and 62.5 ± 4.6 ng (n = 23) Eβf under aCO_2 and eCO_2 concentrations, respectively. For both aphid strains, the Eβf content was lower ($F_{1,83}$ = 8.29, P = 0.005) in aphids reared under eCO_2 concentrations compared to those reared under aCO_2 concentrations. Also, individuals from the pink strain Y-R2 contained more ($F_{1,83}$ = 68.24, P < 0.001) Eβf compared to those from the green strain L1–22. No statistical interaction between the two qualitative variables was found ($F_{1,83}$ = 0.87, P = 0.35).

2nd Experiment: Impact of eCO_2 on Eβf Emission under Predation The average elution recovery was considered satisfactory, with values ranging from 89.6–109.1% depending on the three Eβf quantities, with relative standard deviations of repeatability (RSDs) ranging between 1.6–7.7%.

Eβf was the only volatile compound found in all samples. Eβf emissions differed between the two aphid strains ($F_{1,130}$ = 14.81, P < 0.001) and between CO_2 concentrations ($F_{1,130}$ = 7.97, P = 0.006). Under ladybeetle predation, aphids from the pink strain Y-R2 emitted 45.6 ± 4.3 ng (n = 38) and 29.3 ± 4.2 ng (n = 40) Eβf under aCO_2 and eCO_2 concentrations, respectively. Aphids from the green strain L1–22 emitted 21.9 ± 4.9 ng (n = 28) and 14.5 ± 4.9 ng (n = 28) Eβf under aCO_2 and eCO_2 conditions, respectively. No statistical interaction between the two qualitative variables was found ($F_{1,130}$ = 0.32, P = 0.58).

3rd Experiment: Impact of eCO_2 on Eβf Olfactory Perception All tested antennae produced electrical depolarization in response to Eβf and exhibited a dose-response relationship ($F_{3,51}$ = 83.03, P < 0.001). However, both aphid strains exhibited similar dose-response relationships ($F_{1,17}$ = 0.05, P = 0.83). Aphids from the same strain showed similar dose-response relationships for the two CO_2 concentrations ($F_{1,17}$ = 3.63, P = 0.07; Fig. 2). No significant interaction between CO_2 concentration, aphid strain, and/or Eβf dose was found ([CO_2]: aphid strain, $F_{3,51}$ = 1.45, P = 0.24; [CO_2]: Eβf dose, $F_{3,51}$ = 2.17, P = 0.10; aphid strain: Eβf dose $F_{3,51}$ = 1.49, P = 0.23; [CO_2]: aphid strain: Eβf dose, $F_{3,51}$ = 0.35, P = 0.79).

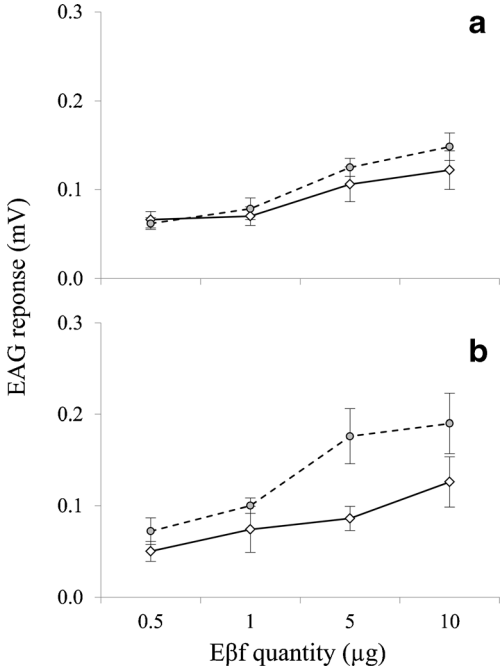

Fig. 2 Mean (± S.E.) electroantennogram (EAG) responses of pea aphids toward different doses of aphid alarm pheromone (Eβf) after being reared under ambient CO_2 (*solid lines*) and elevated CO_2 (*dotted lines*) conditions. **a** Aphids from the pink strain Y-R2. **b** Aphids from the green strain L1–22

4th Experiment: Impact of eCO_2 on Aphid Escape Behavior No modification to aphid behavior was recorded following exposure to solvent (Fig. 3). In contrast, as Eβf dose increased, the proportion of aphids exhibiting escape behavior also increased ($F_{2,114}$ = 70.23, P < 0.001). Three-way ANOVA indicated that aphid escape behavior was not similarly affected by CO_2 concentration in both aphid strains, due to a significant interaction between both variables ($F_{1,114}$ = 12.68, P < 0.001). Under Eβf exposure, aphids from the pink strain Y-R2 behaved similarly regardless of whether they originated from aCO_2 or eCO_2 (Tukey's HSD; P = 0.73). In comparison, the escape behavior exhibited by green strain L1–22 aphids was lower when they originated from eCO_2 compared to aCO_2 conditions (Tukey's HSD; P < 0.001).

Discussion

The present study evaluated how an increase in atmospheric carbon dioxide concentration affects insect chemical communication, including the whole dynamic of pheromonal

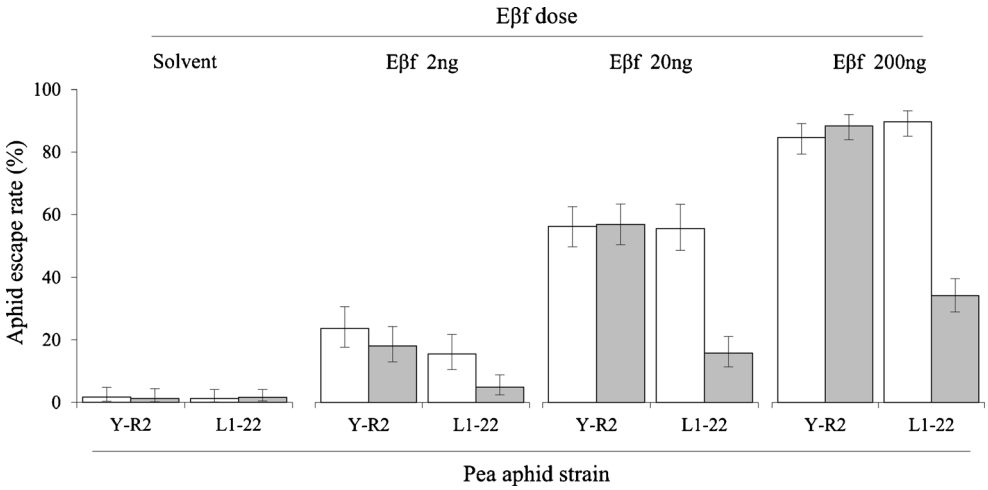

Fig. 3 Mean (± C.I.) escape rate of pea aphids within a colony reared under ambient CO_2 (*white bars*) or elevated CO_2 (*grey bars*) conditions toward different doses of aphid alarm pheromone (Eβf)

signaling. We found that increased CO_2 concentration reduced the production and emission of Eβf, aphid alarm pheromone. While no difference in Eβf neuronal perception by aphids was observed, we found that an increase in CO_2 strongly reduced the escape behavior expressed by an aphid colony following exposure to natural doses of alarm pheromone. To our knowledge, this study is the first to take all steps that a chemical signal must pass through into account, from the emitter to the receiver.

An aphid under attack by a predator releases Eβf from cornicle secretions (Joachim et al. 2013). Therefore, Eβf is probably produced before disturbance and is stored in the aphid body until release. Here, we observed that pea aphids reared under elevated CO_2 concentrations produced 10–15% less Eβf compared to aphids reared under normal CO_2 concentrations. We hypothesize that this reduction in Eβf body content is related to a decline in the biosynthesis of this molecule. The biosynthetic pathway in aphids for producing this sesquiterpene has been poorly elucidated to date (Vandermoten et al. 2012). In contrast, the biosynthesis of Eβf in plants is well known and involves the enzymes Eβf synthase and farnesyl diphosphate synthase that transform farnesyl pyrophosphate to Eβf (Sallaud et al. 2009). We speculate that the observed drop in Eβf production under eCO_2 occurs during the development of aphids, and results from a modification in the host plant, either by a decline in biosynthetic enzymes or to the reduced availability of a precursor (affected by cascade effects caused by changes in host plant composition). This hypothesis is consistent with studies on terpene synthases in plants, in which enzymatic activity is altered by eCO_2 concentration (Misra and Chen 2015). Alternatively, elevated CO_2 concentrations might also affect aphid fitness through life history traits, thereby indirectly impacting pheromone production. Within the context of climate change it is worth noting that while our study was limited to addressing whether CO_2 concentration might impact pheromone production, accompanying increases in average temperature might also modify pheromone biosynthetic pathways by affecting enzymatic activity, as observed in other insects (Boullis et al. 2016; Sentis et al. 2015).

We found that eCO_2 did not affect the ability of aphids to perceive their alarm signal in either aphid strain. Olfactory perception of Eβf in *A. pisum* is mediated by a specific odorant binding protein (*Apis*OBP3; Qiao et al. 2009) that allows Eβf to reach specific olfactory receptors (OR) that are responsible for signal activation (Vogt 2005). After the chemical signal is transduced to an electrical signal and transmitted to the antennal lobes, odorant-degrading enzymes (ODEs) rapidly degrade the active molecules. This action deactivates the transduced signal and vacates the associated receptors (Leal 2013). Because there is no modification in Eβf perception capacity related to CO_2 concentration, we hypothesized that *Apis*OBP3 mechanisms are not directly affected by this change in atmospheric composition.

One of the tested strains of the pea aphid (i.e., Green strain L1–22) reared under eCO_2 conditions exhibited reduced escape behavior. This change in behavior was recorded at all doses of Eβf. Several previous studies demonstrated a relationship between CO_2 concentration, host plant quality, and feeding behavior of herbivorous insects, with insects increasing feeding behavior to compensate for the lack of nutrients available from the host plant (Bezemer and Jones 1998). Under eCO_2, phloem composition is generally altered (Wang and Nobel 1995). As aphids are extremely sensitive to changes in sap composition, they are able to adjust their feeding behavior in response to changes in CO_2 concentration (Hughes and Bazzaz 2001; Sun et al. 2016). The metabolic cost associated with a drop in the quality of nutrients probably favors aphids leaving their feeding site (Hentley et al. 2014). However, this particular assumption requires testing. The other strain (i.e., Pink strain Y-R2) was unaffected by changes in CO_2 concentration. This difference in response may be due to differences between the genotypes, resulting in different consequences to intraspecific interactions. Mondor et al. (2005)

reported that eCO_2 could impact two distinct genotypes of the pea aphid differently, consistent with our results. Specifically, no change in population dynamics was observed in their pink *A. pisum* genotype under eCO_2, whereas there was a change in the green *A. pisum* genotype. Furthermore, Mondor et al. (2005) found that the pink strain had a more conserved phenotypic plasticity (i.e., similar winged induction between aCO_2 and eCO_2 groups) compared to the green strain. During rearing in the current study, wing induction did not seem to be affected by changes in CO_2 concentration (i.e., no visual differences were detected between aCO_2 and eCO_2 populations) in the pink strain Y-R2. In contrast, wing induction was visibly inhibited (i.e., lower proportion of winged morphs under eCO_2) in the green strain L1–22. The expression of wing induction in the pea aphid is strongly related to alarm signaling (Kunert et al. 2005), with alate (winged) aphids being more able to disperse and respond to alarm pheromone compared to wingless individuals. Thus, we hypothesize that a reduction in escape response to Eβf observed in our green strain L1–22 grown under eCO_2 might be related to changes in phenotypic frequency.

Because chemical communication is a centerpiece in the stability of insect interactions, it is important to study how climate change impacts insects so as to predict modifications that might arise in ecosystem dynamics. The current study showed that alarm signaling in aphids was impacted by elevated CO_2 concentrations. These results support those of previous studies (Awamack et al. 1997a; Hentley et al. 2014; Mondor et al. 2004; Sun et al. 2010). Thus, predator-prey interactions based on chemical communication might be impacted by rising CO_2 concentrations over the next 100 years, as part of climate change. However, other interactions might also be impacted including, for example, phytovirus transmissions via insect vectors. Indeed, phytoviruses are transmitted to neighboring plants via aphid dispersal, which is associated with aphid alarm communication (Lin et al. 2016). Reduced aphid dispersal might also affect the extent to which plants are damaged, as well as the efficiency of their natural enemies. Such changes could have a cascade effect, disturbing ecological interactions across an entire multitrophic system. By impacting different genotypes in different ways, natural selection within aphid species might be affected by rapid climate change. This study suggests that changes to CO_2 concentration impact intraspecific chemical communication in insects, and could potentially have cascade effects on interactions with higher trophic levels.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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