

Combined Elevation of Temperature and CO₂ Levels Impacts the Production and Sugar Composition of Aphid Honeydew

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Abstract

Honeydew is the keystone of many interactions between aphids and their predators, parasitoids, or mutualistic partners. Despite the crucial importance of honeydew in the aphid-ant mutualism, very few studies have investigated the potential impact of climate change on its production and composition. Here, we quantified changes in the sugar compounds and in the amount of honeydew droplets released by *Aphis fabae* reared on *Vicia faba* plants, under elevated levels of temperature and/or CO₂. A combined elevation of these two abiotic factors increased honeydew production as well as the total amount in sugars, in particular the concentration of fructose and melezitose. Increased amount of sugars in phloem sap under elevated CO₂ conditions, along with a raise of aphid metabolism and sap ingestion to compensate for water loss under elevated temperatures might explain these observed changes increase in honeydew production and sugar content. A higher amount of excreted honeydew coupled with a higher concentration in melezitose and fructose are expected to enhance both the feeding behavior and the laying of a recruitment trail by ant foragers, thereby reinforcing the ant-homopteran mutualism under a scenario of elevated temperature and CO₂ levels. We discuss about the enhancing and counteracting effects of climate change on other biological agents (gut microorganisms, predators, parasitoids) that interact with aphids in a complex multitrophic system.

Introduction

Interactions between ants and aphids are among the most studied mutualistic relationships in terrestrial ecosystems. Aphids feed ants with their honeydew and, in return, they receive protection from their predators, as well as improved hygiene in their colony with reduced risks of fungal infection (El-Ziady and Kennedy 1956; Way 1963; Buckley 1987; Hölldobler and Wilson 1990; Yao et al. 2000). Aphid honeydew is a mixture of water-soluble carbohydrates, amino acids, proteins, minerals and vitamins originating from the plant phloem sucked and consumed by the homopterans (Way 1963; Woodring et al. 2004). Its role in the maintenance of the relationship between sap feeders and ants has long been recognized (El-Ziady and Kennedy 1956; Way 1963). Indeed, the quantity of aphid honeydew enhances ants' level of attendance (Breed et al. 1987, 1996; Mercier and Lenoir 1999; Völkl et al. 1999; Detrain et al. 1999; Maillieux et al. 2000; Maillieux et al. 2003).

The production of honeydew by an aphid colony depends on the size of the homopterans' population as well as their feeding metabolism, which influence the size and rate of renewal of honeydew droplets (Auclair et al. 1958). As for the composition of honeydew, it is highly variable, being dependent on the tended aphid species (Völkl et al. 1999), the aphid instars (Fischer et al. 2002), the aphid clone (Vantaux et al. 2011), and the host plants (Völkl et al. 1999; Fischer and Shingleton 2001; Fischer et al. 2005). Honeydew mainly contains monosaccharides (glucose and fructose), disaccharides (maltose, sucrose) and trisaccharides (melezitose, raffinose) (Auclair 1963; Hendrix et al. 1992; Völkl et al. 1999; Detrain et al. 2010), but is also made of small amounts of amino-acids, proteins and lipids (Völkl et al. 1999; Wäckers 2000; Wäckers 2001; Woodring et al. 2004). Not all sugars are equally important as enhancers of the ant-aphid relationship. Noticeably, melezitose and sucrose are the most attractive sugars for the

ants: both trigger a feeding response of the foragers as well as their laying of a trail pheromone towards aphid colonies (Boevé and Wackers 2003; Detrain et al. 2010; Detrain and Prieur 2014). Ant-tended aphids were found to produce a melezitose-enriched honeydew compared to unattended homopterans, thereby reinforcing their interactions with ants (Fischer and Shingleton 2001; Yao and Akimoto 2001). All these studies demonstrate that the mutualistic relationships between ants and aphids deeply rely on both the quality and quantity of honeydew excreted by the aphids. Consequently, in order to benefit from ant attendance, the aphid partners must be able to bear the costs of fulfilling the energetic needs of ant tenders (Stadler and Dixon 1998). Any environmental disruption that modifies the physiological and behavioral traits of aphids and hence their honeydew production may lead the ant colony to redirect its tending behavior to a more rewarding source of carbohydrates. As reported by Fischer and Shingleton (2001), mutualism can even switch to antagonism when the cost–benefit balance is altered, leading to ant predation on aphids, or to a cessation of their mutualistic interaction.

In the context of climate change, the expected rise in temperature and CO₂ levels is likely to act upon the biology of the aphids with potentially major consequences on their interaction with ants (Blanchard et al. 2019). Very few studies have investigated the impact of climate change on honeydew production and composition. A study conducted by Sun et al. (2009) on a non-myrmecophilous aphid species found that it produced a higher quantity of honeydew when being reared under elevated CO₂. By contrast, other studies on different non-myrmecophilous aphid species found that elevated CO₂ condition decreased the production of honeydew (Fu et al. 2010; Boullis et al. 2018) and increased its concentration in amino acids (Fu et al. 2010). As for the myrmecophilous aphid species, *Aphis craccivora*, colonies reared under elevated CO₂ concentration produced a three times higher amount of honeydew than under ambient levels of CO₂, thereby enabling a higher tending frequency and reinforcing aphid interactions with ants (Kremer et al. 2018). To date, no study has investigated how an increase of temperature, alone or in combination with an elevation of CO₂, can alter the production and the sugar content of the honeydew released by myrmecophilous aphids.

In this study, we investigate how an elevation of temperature and/or CO₂ levels based on the most accurate climate change scenarios (IPCC 2019) may influence the production and sugar content of the honeydew excreted by the myrmecophilous aphid *Aphis fabae*. We hypothesize that, following Kremer et al. (2018) findings, the honeydew production of *A. fabae* would increase under elevated CO₂ levels, coupled or not with an elevation of temperature. We also assume that the sugar content and concentration of the honeydew will be impacted by an elevation of temperature and/or CO₂, via changes in the phloem of host plant as well as in the metabolism of the sap-sucking aphids.

Methods And Materials

Tested conditions. Two abiotic factors were considered in this study: temperature and carbon dioxide concentration (CO₂). We used the meteorological data collected by the Royal Meteorological Institute of Belgium during the past seven years in Brussels to determine the levels of temperatures to be tested. At

the end of spring and in summer (May to September) and during daylight (8am to 22pm), the average temperature was close to 20°C. The report of the IPCC (Intergovernmental Panel on Climate Change) (IPCC 2019) foresees a raise of 2 to 4°C by the end of the 21th century. Thus, for the experimental conditions, we chose $20 \pm 0.5^\circ\text{C}$ as the current ambient temperature, and $23 \pm 0.5^\circ\text{C}$ as the predicted elevated temperature.

To determine the tested CO₂ concentrations, we used the CO₂ levels that were measured during the past seven years in Brussels by “Brussels Environment” public organization. The CO₂ levels were found to vary between 395 and 420 ppm. Besides, IPCC’s report stated a raise of about 400 ppm in the most foreseen climatic scenarios by the end of the century. We decided to choose 400 ± 50 ppm as the current ambient concentration and 800 ± 50 ppm as the foreseen elevated concentration for the tested experimental conditions.

Four combinations of these two abiotic factors were set up:

ambient CO₂ concentration (400 ppm) and ambient temperature (20°C) noted ambT°×ambCO₂; ambient CO₂ concentration (400 ppm) and elevated temperature (23°C) noted ambT°×elevCO₂; elevated CO₂ concentration (800 ppm) and elevated temperature (23°C) noted elevT°×ambCO₂; elevated CO₂ concentration (800 ppm) and ambient temperature (20°C) noted elevT°×elevCO₂.

Experimental set-up. Four closed climatic chambers, corresponding to each tested condition, were built with polycarbonate transparent panels (120×70×60cm). The ambient temperature inside each chamber was controlled using heat plates (HabiStat Reptile Radiator 75 Watts), coupled to a thermostat (HabiStat Digital Temperature Thermostat). An air pump (30 L/min air flow) renewed the ambient air inside the chamber, thereby helping to limit temperature fluctuations at around $\pm 0.5^\circ\text{C}$ during the whole duration of the experiments. Half of the chambers received the room air that was enriched with CO₂ by connecting a CO₂ gas bottle to the air pumps (>99% purity). The CO₂ concentration and temperature were monitored in each chamber using data loggers (MCH-383 SD, Lutron).

Plant and insect rearing. Bean plants *Vicia faba* L. (variety “Major”, Grosse Ordinaire, Huy, Belgium) were sowed in individual plastic pots filled with soil. Eight plants were placed per climatic chamber and were left to grow for 16 days, before being used for the experiments. Black bean aphids, *Aphis fabae*, were reared under each tested climatic condition on *Vicia faba* L., grown in plastic containers filled with soil, for two months prior to experiments, to produce several generations. Because the composition of honeydew may vary between aphid clones (Vantaux et al. 2011), all the aphids used in the experiments were generated from the same aphid clone.

Quantification of honeydew. Before carrying out the experiments, standardized aluminum foil discs were cut out and weighed on a high-resolution scale (Mettler scale: Accuracy: 10^{-5}g). Similar-sized Whatman paper discs were cut out. Once the plants were 16- days old, each plant was infested with 70 adult aphids. An aluminum foil disc was placed around the plant at the basis of each stem, upon a Whatman

paper disc. Aluminum discs were left under the aphid-infested plant for three days during which they were progressively covered with the honeydew droplets released by the homopterans. After three days, the aluminum discs were withdrawn from the plant stem and left for four hours to dry at room temperature. After removing the dead aphids and molts, each disc was weighed on the scale at an accuracy level of 10^{-5} g. Among the eight plants followed per condition, two were not taken into account in the analyses because of the unexplained death of several adult aphids during the experiment. The discarded colonies showed a total fresh weight of aphids that was lighter at the end of the experiment than at its beginning. Thus, the two conditions corresponding to $\text{ambT}^\circ \times \text{ambCO}_2$ and to $\text{elevT}^\circ \times \text{ambCO}_2$ had seven replicates instead of eight.

Analysis of Honeydew composition. After having withdrawn the aluminum discs, within the next four hours, we placed the plants above plastic foils sterilized with alcohol. The dripping droplets were collected with capillary tubes (42 mm length; Minicaps® 2 μL ; Hirschmann® Laborgerate), directly on the plastic foils. We collected 1 μl of honeydew which was immediately mixed in 49 μl of miliQ water before sugar crystallization and stored in 200 μL glass inserts at -20°C until chemical analyses. On the eight replicates per condition, two were used prior to analyses for analytical method development. Six replicates were used in the following analyses for each condition. The collection of honeydew was carried out outside of the climatic chambers, under controlled laboratory conditions ($T: 19^\circ \pm 1^\circ \text{C}$; $\text{RH}: 60 \pm 10\%$; $[\text{CO}_2]: 450 \pm 50 \text{ ppm}$). Once the honeydew droplets were collected for further chemical analyses, all aphids were removed from each plant and their fresh weight was measured on the precision scale in order to consider possible changes in their population size throughout the experiment. Plant fresh weight was also measured to consider its possible effects on the production of honeydew by the aphids.

Each honeydew sample was then at first diluted to 1/50, i.e. 20 μl in 1000 μl of miliQ water. Each sample was put on a Carbowac™ PA-100 column (4 \times 250 mm) and then analyzed on a high-performance anion exchange chromatograph that was coupled with pulsed ED40 amperometric detection (Dionex, Sunnyvale, California). This setup is adequate for mono- and polysaccharide analyses (Yao and Akimoto 2001; Detrain et al. 2010). A control sample comprising a mix of 10 sugars of known concentration was injected prior to the analysis of the samples, and was used for sugar identification and quantification.

Statistical analyses. All analyses were carried out using R software (v. 3.5.0). Normality of data was assessed with Shapiro tests and homoscedasticity with Bartlett tests. The amount of collected honeydew was averaged per mg of aphids. The impact of climatic condition on honeydew production was analyzed by a Kruskal-Wallis test that was followed by Dunn post-hoc tests, with Bonferroni correction. The weight of aphids and plants were compared for the different tested conditions by using ANOVA tests that were followed, when significant, by Tukey post hoc tests. We used generalized linear models (GLMs) to analyze data that met the models' assumption and that showed no overdispersion based on model deviance/degrees of freedom values. The effects of aphids' weight and plants' weight on the total production of honeydew were assessed by using inverse gaussian GLMs with a $1/\mu^2$ link function. The total concentration of sugars in the collected honeydew as well as the concentration of each

identified sugar were compared across the four environmental conditions by using ANOVA tests that were followed by Tukey post hoc tests. All tests were two-tailed with a significance level of $\alpha = 0.05$.

Results

Honeydew production. The weight of the aphid population at the end of the experiment (ANOVA, df = 3, F = 0.598, p-value = 0.622), as well as the fresh weight of the host plant (ANOVA, df = 3, F = 0.263, p-value = 0.852) did not differ across the four tested environmental conditions. As a corollary, the total amount of produced honeydew was not significantly influenced by the weight of aphid colonies (GLM, df = 3, t-value = 0.433, p-value = 0.667) nor by the plant fresh weight (GLM, df = 3, t-value = 0.168, p-value = 0.867). Noticeably, the average amount of produced honeydew was nearly twice as high under the condition combining an elevation of CO₂ levels and temperature (about 0.14 mg per mg of aphids) compared to the other conditions (about 0.07 mg per mg of aphids, Fig. 1). However, the impact of the environmental conditions on the total weight of honeydew released by the aphid colonies was not statistically significant (Kruskal-Wallis test, $\chi^2 = 6.95$, df = 3, p-value = 0.074; Fig. 1).

Honeydew sugar analyses. We found no significant impact of the environmental conditions on the total concentration of sugars detected in aphid honeydew (ANOVA, df = 3, F = 1.196, p-value = 0.338; Fig. 2). However, similarly to the higher amount of produced honeydew, the sugar content was slightly higher under conditions of elevated temperature and carbon dioxide.

Seven sugars were identified in *A. fabae* honeydew: melezitose, fructose, glucose, sucrose, raffinose, xylose, and trehalose (Fig. 3). Melezitose and fructose were the main sugars detected and quantified in *A. fabae* honeydew under the four experimental conditions. They showed the highest total concentrations, when pooling all the tested conditions (13.314 ± 1.210 g/L for melezitose and 9.499 ± 0.661 g/L for fructose). When averaging their relative amount in honeydew over all the replicates (n=24), regardless of the tested environmental condition, melezitose and fructose accounted respectively for 44% (SD $\pm 1\%$) and 31% (SD $\pm 1\%$) of the total amount of the analyzed honeydew sugars.

Glucose and sucrose were much less abundant (about 2.569 ± 0.238 g/L for glucose and 2.047 ± 0.211 for sucrose) and accounted for about 8% of the total sugar amount (0.082 ± 0.003 for glucose, 0.070 ± 0.008 for sucrose). Raffinose, xylose and trehalose were present at very low levels (respectively about 1.073 ± 0.080 g/L, 0.644 ± 0.018 g/L and 0.249 ± 0.017 g/L) and represented less than 5% of the total sugar weight (0.035 ± 0.002 for raffinose, 0.022 ± 0.001 for trehalose; 0.008 ± 0.001 for xylose).

When considering each sugar separately, we found a significant impact of the environmental condition on the concentration of fructose (ANOVA, df = 3, F = 4.149, p-value = 0.019; Fig. 3). Indeed, an elevation of both temperature and CO₂ levels led to fructose concentration that were higher than in the condition where only CO₂ level was elevated (Tukey post-hoc test, p-value = 0.012). As for the melezitose, its concentration was not impacted by the tested conditions (ANOVA, df = 3, F = 2.169, p-value = 0.124; Fig. 3). One can however see that its relative amount in honeydew was higher when both temperature and

CO₂ levels were elevated. For the other sugars that were less abundant in honeydew, we found no significant impact of the environmental conditions on the concentrations of sucrose (ANOVA, df = 3, F = 0.288, p-value = 0.814; Fig. 3), raffinose (ANOVA, df = 3, F = 1.681, p-value = 0.207; Fig. 3), xylose (ANOVA, df = 3, F = 0.911, p-value = 0.458; Fig. 3) and trehalose (ANOVA, df = 3, F = 1.034, p-value = 0.410; Fig. 3).

Discussion

For the first time, this study investigates the effect of temperature and carbon dioxide levels, alone and in combination, on the production of aphid honeydew and on its sugar content.

We found that a combined elevation of temperature and carbon dioxide concentration led to an increase in the amount of honeydew released by *A. fabae* aphids, in the total content of carbohydrates, as well as in the concentration of the main honeydew sugars, i.e. melezitose and fructose. Excepting for the fructose concentration, these impacts of abiotic conditions on aphid honeydew were however not statistically significant most probably due to the limited sample size.

Honeydew production

Phloem-feeding aphids can respond differently to changes in the sap of plants reared under conditions of elevated CO₂. In a non-myrmecophilous aphid species, *Acyrtosiphon pisum*, an elevation of CO₂ impacted negatively the production of honeydew (Boullis et al. 2018). Conversely, other studies showed that higher amounts of honeydew were produced by *Aphis craccivora* reared on lucerne (Kremer et al. 2018) and by *Aphis gossypii* reared on cotton (Sun et al. 2009), under higher CO₂ levels. These results, based on aphids and plants raised under daily temperatures of 26/18°C (Day-Night) (Kremer et al. 2018), and 28°C (Sun et al. 2009) are in accordance with our own findings, where an elevation of CO₂ at 23°C also enhanced the production of honeydew by *A. fabae* aphids. Thus, aphids seem to adapt their feeding behavior to the changing nutritional value of plants reared under elevated CO₂ conditions (Newman et al. 2003; Wang et al. 2004; Prichard et al. 2007; Sun and Ge 2011).

Noticeably, the enhancing effect of elevated CO₂ on honeydew production by *A. fabae* was not observed at 20°C and took place only at 23°C. This could be explained by some synergistic effects of elevated temperature and CO₂ levels on the ingestion of phloem sap by aphids. Indeed, higher temperatures induce higher rates of sap-feeding that go along with a generalized increase of aphid metabolism and that enable aphids to recover from water loss. On the other hand, elevated CO₂ conditions reduce the nitrogen to carbon ratio in the phloem sap (Awmack and Leather 2002; Stiling and Cornelissen 2007; Sudderth et al. 2005) what leads aphids to ingest more phloem sap in order to meet their needs in amino acids.

Besides, the release of honeydew droplets is obviously linked to the feeding efficiency of aphids. When aphids drill into plant tissues with their stylet, their capacity to find sieve tubes is linked to the sensing of sucrose and pH in the microenvironment around the stylet tip (Tjallingii 2006, Hewer et al. 2010). Further

studies should focus on how the feeding behavior of aphids also depends on temperature and/or CO₂-induced changes in the phloem sap of their host plant.

Sugar composition of honeydew

In this study, melezitose as well as fructose were the major sugars found in *A. fabae* honeydew, which together accounted for more than 70% of the total amount of identified sugars. *A. fabae* also contained smaller amounts of monosaccharides such as glucose, xylose and maltose, disaccharides such as sucrose and trehalose, and the raffinose trisaccharide. The honeydew analyzed in this study included several sugars that are attractive to the ants (Detrain et al. 2010) and for which temperature and/or CO₂-induced changes in concentration are expected to influence the interactions between ants and aphids. Previous studies showed that honeydew composition can be highly variable since it depends on the host plant of aphids (Hendrix et al. 1992; Völkl et al. 1999; Yao and Akimoto 2001; Pringle et al. 2014), on the aphid species (Wäckers 2000, Wäckers 2001; Fischer and Shingleton 2001; Hogervorst 2007; Woodring et al. 2004; Monticelli et al. 2020) and even on the aphid clone (Vantaux et al. 2011). Here, we found that the temperature and CO₂ levels can also influence the carbohydrate composition of aphid honeydew, albeit to different degrees depending on the sugar.

Several honeydew sugars, such as fructose, glucose, sucrose, or xylose are not synthesized by aphids, but come directly from the phloem sap of the plant (Hendrix et al. 1992). One can thus assume that the relative amount of these sugars in honeydew to be -at least partially- related to those in the phloem sap. However, in this study we found that the relative amount of these sugars in the honeydew of *A. fabae* aphids did not significantly change when we tested separately either the impact of an elevated temperature or the impact of a CO₂ enrichment.

However, we observed additive effects of elevated temperature and CO₂, which both tend to increase the total amount of honeydew sugars as well as the concentration of phloem-derivate sugars like fructose. According to the existing literature, an elevation of CO₂ levels was usually found to increase photosynthetic rates in plants, what can lead to the accumulation of carbohydrates in leaves (Moore et al. 1999; Ainsworth and Long 2005; Leakey et al. 2009), where an elevation of temperature commonly results in a decrease of carbohydrates' concentration in plants (Sami et al. 2016; Yelle et al. 1989; Olszyk et al. 2003; Farrar and William 1991). One would therefore predict that effects resulting from an increase of CO₂ (increased sugar content) would be levelled off by those expected from an increased temperature (decreased sugar content). This is not the case in the present study, in particular for the concentration of fructose that significantly increased under a combined elevation of temperature of CO₂ and temperature. This increase may result from a higher accumulation of this sugar in plant sap, under elevated CO₂ concentration but also from an enhanced metabolism of aphids at higher temperatures, which speeds up the enzymatic hydrolysis of sap sucrose into a fructose and a glucose unit. This confirms the need to consider all the processes involved in the production of honeydew, whose composition results not only from the characteristics of plant sap but also from the sugar metabolism of homopterans. Climate-

induced changes in the composition of the phloem sap do not imply similar effects on the honeydew released by sap-feeding homopterans. Differences across sugars in their rate of transports through the gut walls of the aphids, in their level of degradation by hydrolysis or conversely, in their need to be biosynthesized by the aphids themselves, have to be investigated in order to understand how climate change would alter the honeydew-mediated mutualism between aphids and ants.

Some honeydew sugars are directly synthesized by aphids. This is the case of melezitose, a trisaccharide which is synthesized in the aphid's gut by the coupling of two glucose units and one fructose unit (Bacon and Dickinson 1957; Ashford et al. 2000, Fisher and Shingleton 2001). We found that melezitose concentration increases under the combined elevation of temperature and CO₂ concentration. The biosynthesis of this trisaccharide was potentially facilitated by a higher sucrose content in the phloem sap under elevated CO₂ (Krumbein et al. 2010), which after being hydrolyzed in the gut of aphids, makes a glucose and a fructose unit available for the melezitose biosynthesis. Noticeably, since melezitose is made of two glucose units for only one fructose unit, the synthesis of this trisaccharide may leave fructose units in excess. This may explain why, in this study, the increase of fructose in honeydew went along with that of melezitose when *A. fabae* aphids were reared under a combined elevation of temperature and carbon dioxide.

Potential consequences for ant-aphid mutualism

The volume of honeydew excreted is a good indicator of phloem intake in sap-sucking insects, as well as a predictor of the potential strength of the ant–aphid mutualisms (Stadler and Dixon 1998).

Honeydew “richness” can be defined as the emission rate/volume of honeydew droplets coupled to their total concentration in sugars (Woodring et al. 2004). The total amount as well as the concentration of carbohydrates in honeydew is crucial for the initiation and maintenance of mutualistic relationships between ants and aphids (Beattie 1985; Blüthgen and Fiedler 2004; Buckley 1987; Engel et al. 2001; Stadler and Dixon 2005; Way 1963). In this study, the combined elevation of CO₂ and temperature had a positive impact on *A. fabae* honeydew richness as it tended to increase both the amount of released droplets and their total concentration in sugars. Ants increase their feeding behavior as well as the intensity of collective recruitment to food according to the energetic value of carbohydrates sources, i.e their volume and/or their sugar content (e.g. Detrain et al. 1999, Detrain et al. 2010, Dussutour and Simpson 2008, Mailleux et al. 2000, Mailleux et al. 2003, Völkl et al. 1999). Under a scenario of climate change, one may thus expect that a higher honeydew richness will reinforce the aphid-ant mutualism.

Not only the amount but also the nature of honeydew sugars matters for triggering ant attendance. On the one hand, some sugars including maltose, xylose or melibiose elicit only a weak feeding response from ant foragers (Boevé and Wäckers 2003, Detrain et al. 2010). In contrast, melezitose, sucrose, fructose and glucose are regularly consumed by the majority of ants, even though species-specific sugar preferences may occur (Blüthgen and Fiedler 2004, Völkl et al. 1999). In a climate change perspective, based on our chemical analyses, we expect an enhanced feeding of ants due the increased amount of

fructose and melezitose in *A. fabae* honeydew. As for sucrose, which makes up 80% of the organic components of the phloem sap in most C₃ plants (including bean species), it is an effective phagostimulant for herbivorous and sap-feeding insects (Moon 1967; Hawker 1985, Weibull 1990). However, unlike a previous study (Detrain et al. 2010), we found a surprisingly little amount of this disaccharide in *A. fabae* honeydew in each environmental condition, what makes sucrose playing a marginal role in the ant-aphid interaction.

Finally, a special attention should be paid to melezitose sugar, which was the main sugar produced in *A. fabae* honeydew and whose concentration increased under a combined elevation of temperature and carbon dioxide. Melezitose is known to attract aphid-tending ants such as *Lasius niger* (Fischer et al. 2005; Völkl, et al. 1999; Woodring et al. 2004), whose foragers prefer to exploit aphid clones that secrete a melezitose-rich honeydew (Kiss 1981; Völkl et al. 1999; Fischer et al. 2001; Vantaux et al. 2011). In addition to stimulating ants' feeding, melezitose triggers the laying of a recruitment trail by ant foragers (Detrain et al. 2010; Detrain and Prieur 2014), thereby playing a key-role in the initiation and the maintenance of high flows of ants travelling towards the aphid colonies. When considering all these effects of melezitose on ant behavior, one may assume that the increased production of this trisaccharide by the *A. fabae* aphids would lead to the reinforcement of aphid attendance and honeydew exploitation by ants under conditions of climate change.

The benefits acquired by aphids from these enhanced interactions could however be mitigated in the long term since the increased production of a sugar-enriched honeydew may incur higher physiological costs and may impair homopterans' development (Stadler and Dixon 2005). Beside the ant mutualists, one should consider how honeydew changes can impact the survival and reproduction of aphid predators and parasitoids (Wäckers 2000). For instance, some species (e.g. *Cotesia glomerata* L., a parasitoid wasp) achieve the longest lifespan when feeding on sucrose, fructose and glucose and the shortest longevity in the presence of melezitose (Wäckers 2001). Attention should also be paid to the honeydew volatiles that enable aphid predators such as hoverflies to find suitable oviposition sites (Leroy et al. 2011) or mutualists such as ants to find aphid partners at a distance (Fisher et al. 2015). Finally, the bacterial community in the aphid gut (Fischer et al. 2015) can shape the profile of honeydew volatiles and potentially alters the predator/mutualist ratio of aphids. Some bacteria also enhance the resistance of aphids to parasitoids and to thermal stress (Renoz et al. 2019). Future research should investigate how climate change could indirectly alter aphid fitness by modifying their bacterial community and whether bacteria conferring thermal resistance could be naturally selected by making aphids more tolerant to heat waves.

To conclude, aphids and their ant partners take part to a multitrophic network involving amplifying processes, negative feed-backs as well as cascading effects. Like any other complex systems, it is difficult to predict how slight changes in local interactions between agents would influence the global functioning and the stability of the whole multitrophic system. Based on our results, one may assume that, in a scenario of global warming, the changes induced in *A. fabae* honeydew will enhance their mutualism with ant partners. In addition to testing the generic value of this prediction for other plant-

aphid-ant combinations, much work is however needed to see whether the predicted impact of global warming on aphid-ant relationship could be further enhanced or counteracted by changes in plant phloem sap, aphid metabolism, parasitoid/predator behavior as well as in the bacteria community of sap-feeding insects.

Declarations

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All authors contributed to the study conception and design. Material preparation and data collection were performed by Solène Blanchard. Analyses were performed by Solène Blanchard and Isabelle Van de Vreken. The first draft of the manuscript was written by Solène Blanchard and Claire Detrain and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

The authors have no competing interests to declare that are relevant to the content of this article.

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Figures

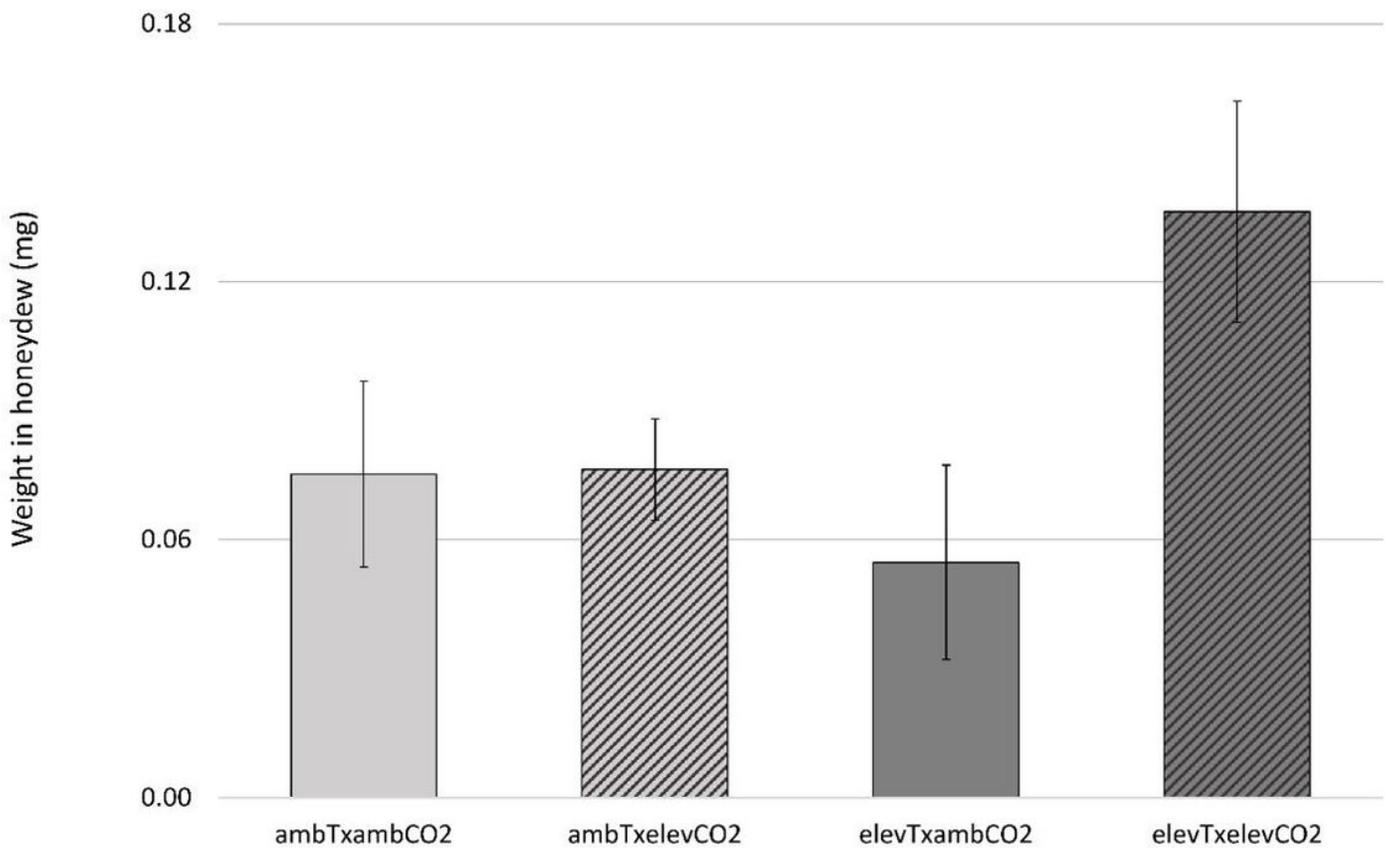


Figure 1

Impact of four environmental conditions on the production of honeydew, in mg per mg of aphids, by *A. fabae* colonies. Means and standard errors (n=7 replicates for ambT°×ambCO2 and elevT°×ambCO2 and n=8 replicates for ambT°×elevCO2 and elevT°×elevCO2) represent the dry weight of honeydew produced over four days by an aphid colony and are expressed in milligrams per mg of aphids in order compare honeydew production across colonies. “elev.” stands for elevated and “amb.” for ambient. A Kruskal-Wallis test was performed with non-significance at the level $\alpha=0.05$

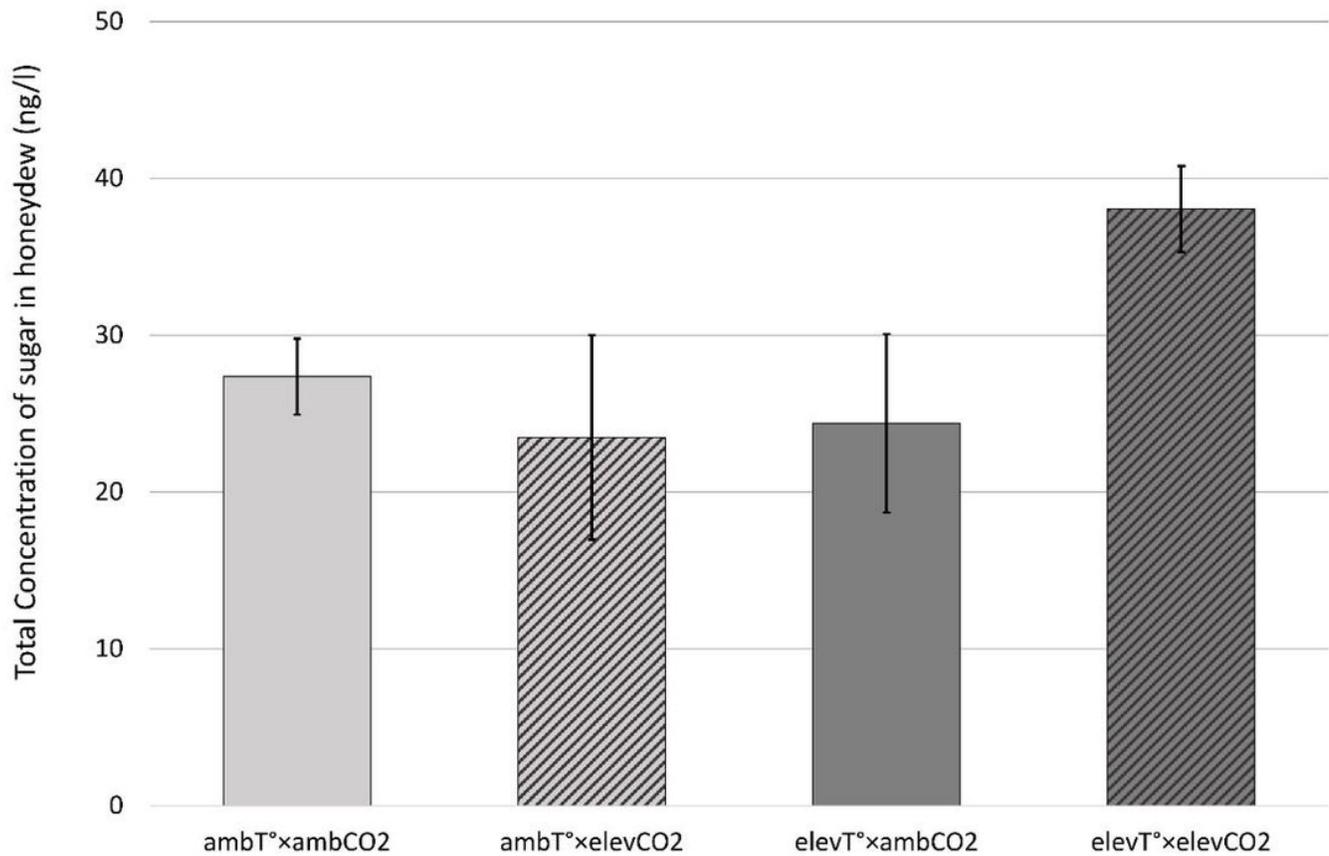


Figure 2

Total concentration of sugars in grams per liter of honeydew emitted by *A. fabae* aphids, under the four tested environmental conditions (n=6 replicates). “elev.” stands for elevated and “amb.” for ambient. Each bar plot represents mean \pm SE. ANOVA tests were performed with non-significance at the level $\alpha=0.05$

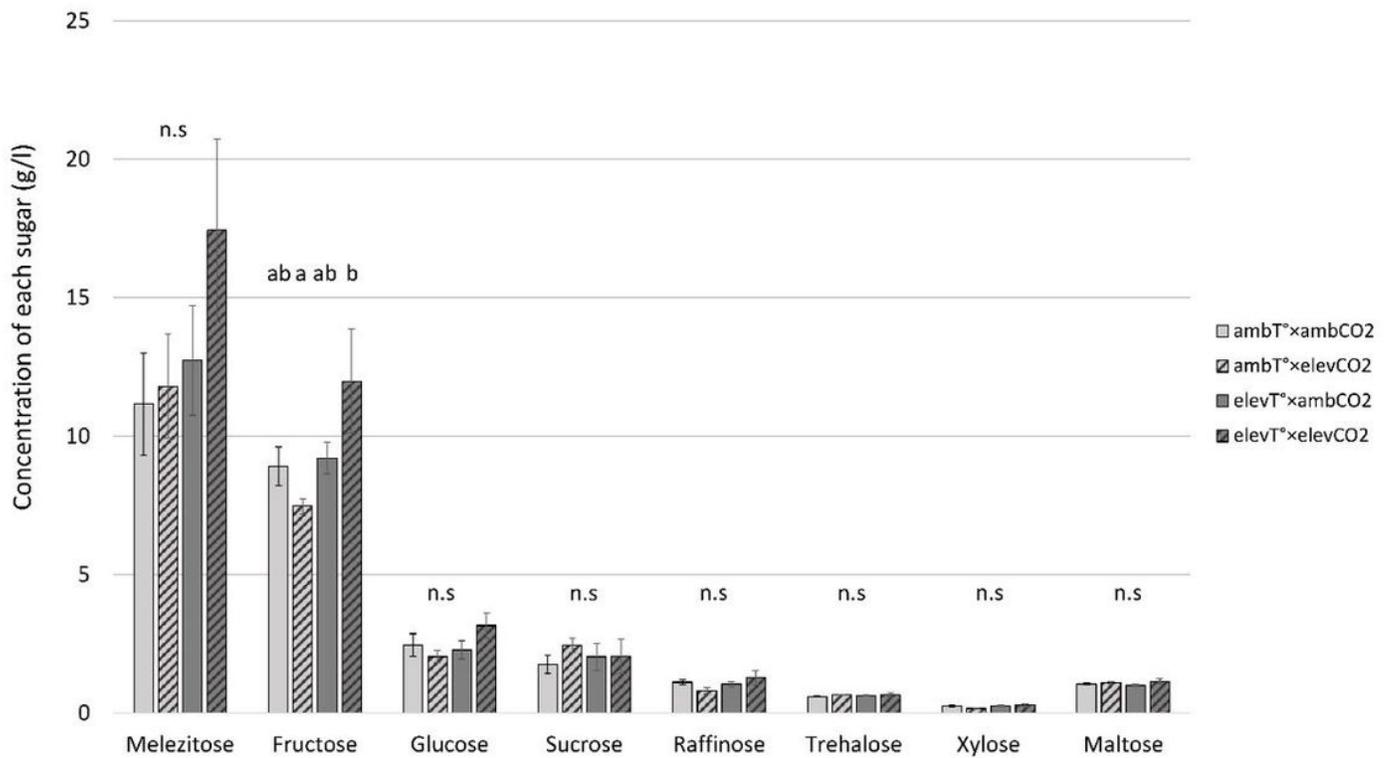


Figure 3

Concentration of each sugar detected in honeydew (in g/L) under the four environmental conditions (n=6 replicates). "elev." stands for elevated and "amb." for ambient. Each bar plot represents mean \pm SE. ANOVA tests were performed for each sugar. "n.s." stands for not-significant. Bars sharing no common letter were statistically different results (Tuckey post hoc tests)