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Direct effect of ozone pollution on aphids: revisiting the evidence at individual and population scales

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Abstract

Most of our knowledge about the consequences of tropospheric ozone pollution on aphid–plant interaction assumes the absence of a direct effect of ozone on aphids. However, the biological effects of ozone encompass changes in reactive oxygen species signalling, oxidative stress accumulation, and the immune response of both plants and animals. The main objective of this work was to evaluate the direct effect of ozone on *Metopolophium dirhodum* (Walker) aphids (Hemiptera: Aphididae), linking mechanisms that operate at individual scale (oxidative stress parameters and aphid-bacteria symbiosis persistence) with their consequences at population scale (mortality, dispersion, and population growth). Two experiments were conducted in open-top chambers with three contrasting ozone exposure regimes (6 h at 0.5 ± 0.3, 50 ± 5, or 150 ± 10 p.p.b.) and artificial diets to evaluate the direct effect of ozone on aphids. Exposure of aphids to ozone increased insect mortality compared to the control treatment. However, the populations established from the surviving insects of each treatment increased similarly. Consistently, aphid symbiosis with *Buchnera aphidicola* Munson et al. (Proteobacteria) and *Hamiltonella defensa* Moran et al. (Enterobacteriaceae) persisted in all treatments. Ozone also affected aphid behaviour. The proportion of insects dispersing from diet cages was lower in the ozone treatments than in the control treatment. In addition, lipid peroxidation was higher at 150 p.p.b. than at 50 p.p.b. treatment, although not different from that in the control. The reduction in aphid dispersion coupled with increased mortality suggests that direct exposure to ozone could lower aphid efficiency as virus vectors. These results could be partially associated with mechanisms operating at individual scale (accumulation of oxidative damage). Overall, these experiments encourage reconsidering the impact of the direct effects of ozone on aphids when assessing the consequences of this component of global change on plant–aphid interactions.

Introduction

Ozone in the troposphere is both a pollutant and a greenhouse gas (IPCC, 2007; Booker et al., 2009). The background concentration of ozone has doubled during the past century (Vingarzan, 2004) and ozone-related global yield reductions vary between 2.2 and 5.5% in maize, 3.9–15% in wheat, and 8.5–14% in soybean, thus imposing a new scenario for food production (Avnery et al., 2011). These losses are associated with the accumulation of oxidative damage symptoms in the foliage, reductions in photosynthesis, and premature senescence of crops (Wilkinson et al., 2012). The combination of ozone effects on plants and insects may modify the impact of insect pests on crops. Aphids constrain crop yield by feeding damage and virus transmission (Ng & Perry, 2004; van Emden & Harrington, 2007). Feeding damage not only involves photoassimilate removal, but it also alters source-to-sink transport in plant tissues (Goggin, 2007). However, the consequences of ozone pollution on aphid–plant interaction are far from being understood.

Simultaneous exposure of plant and aphids to ozone may result in a complex interaction of effects, leading to

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variable results. Up to date, aphids have shown either positive, negative, or neutral effect response to ozone exposure (Warrington, 1989; Brown et al., 1992; Jackson, 1995; Holopainen & Kossi, 1998; Holopainen, 2002; Awmack et al., 2004; Menéndez et al., 2010; Mondor et al., 2010). In most of these investigations, the exposure of aphids and plants to ozone was simultaneous and the explanation of the results was centred on plants nutritional quality change: premature senescence, nutrient relocation from ozone-damaged tissues, and premature lignification (Holopainen, 2002). However, this approach excludes aphids as an active component of the plant–insect interaction, with the ability to avoid plant defences and modify source–sink relationships in the translocation of nutrients (Goggin, 2007; Giordanengo et al., 2010). In addition, the simultaneous exposure overlooks the possibility that ozone may directly and negatively affect aphids and their interaction with plants. Therefore, direct effects of ozone on aphids may be relevant to understand the idiosyncratic responses of aphids under ozone pollution and the potential effect of ozone pollution on the impact of aphids as crop pests.

The evaluation of the direct effects of ozone on aphids requires the isolation of the aphids from the plants they feed upon. This can be accomplished by feeding the aphids on artificial diets during ozone exposure. Although this technique has allowed to demonstrate that ozone has no direct effect on aphids' individual growth rate (Brown et al., 1992; Jackson, 1995), more recent work has shown that population growth rate cannot be predicted from individual growth rate (Holopainen & Kossi, 1998; Awmack et al., 2004; Mondor et al., 2010). Therefore, alternative targets for ozone effects on aphids need to be identified.

Reactive oxygen species (ROS) represent an interesting interface for the study of aphid–plant interaction under ozone pollution. Oxidative damage is the consequence of the overaccumulation of ROS, free radicals produced as by-products of oxidation–reduction (REDOX) reactions (Dowling & Simmons, 2009). At low concentration and specific moments of life history, ROS have beneficial effects on plants and animals. They are important signalling molecules involved in immune defence (Nappi & Ottaviani, 2000; Apel & Hirt, 2004), growth and development, and acclimation to the environment (Gechev et al., 2006; Costantini et al., 2010). Therefore, living organisms benefit from ROS as long as they remain capable of controlling their spread and accumulation. This control is mainly accomplished by antioxidants, which can be lipid-soluble molecules (α-tocopherol, β-carotene), water-soluble molecules (glutathione and ascorbate), or enzymatic antioxidants (superoxide dismutase, catalase, peroxidase, and enzymes belonging to the ascorbate-glutathione cycle) (Alexieva et al., 2003). If ROS accumulation overcomes these detoxification mechanisms, cumulative oxidative damage to DNA, RNA, and proteins may impair cell function or lead to cell death (Dowling & Simmons, 2009). Insects have previously shown to be susceptible to oxidative stress derived from ozone exposure (Cross et al., 2002; Holmstrup et al., 2011) and plant defence against aphid feeding by accumulating ROS, leading to direct aphid oxidative injury (Smith & Boyko, 2007). In addition, aphids benefit from feeding on plants with enhanced antioxidant content (Kerchev et al., 2012). Because aphids are susceptible to oxidative stress and ozone concentration inside the crop canopy reaches damaging levels (Jaggi et al., 2006), tropospheric ozone pollution may have a direct effect on aphids as well as the ability to modify aphid–plant interaction.

Aphid-bacteria symbiosis is another means to gain understanding on the variable effects of ozone on aphids. Most aphid species maintain an obligate symbiosis with *Buchnera aphidicola* Munson et al. (Proteobacteria), a gamma-proteobacterium which provides essential amino-acids lacking in plant phloem sap and is vertically transmitted from mother to offspring (Douglas, 1989; Munson et al., 1991). This symbiosis is essential for aphid population growth, as the lack of these bacteria leads to lower reproductive output and slower growth of nymphs, which are also smaller in adulthood and produce no offspring, or a few offspring that usually die without growing or developing (van Emden & Harrington, 2007). In addition, aphids can harbour facultative or secondary endosymbionts which are not generally required for survival or reproduction, but are mutualistic in the context of various ecological interactions, such as protection from parasitoid wasps, fungal pathogens, heat shock, and expansion of host plant range (Oliver et al., 2010). Although *B. aphidicola* has previously shown to be susceptible to heat stress and facultative endosymbionts may decrease the effect of this environmental stress (Montllor et al., 2002), these symbioses have not been studied in the context of ozone pollution. However, the effects of ozone pollution on aphid fecundity and aphid population growth (Awmack et al., 2004; Menéndez et al., 2010) resemble those of *B. aphidicola* symbiosis breakdown, reinforcing the importance of considering aphid-endosymbiont association in the context of ozone pollution experiments.

This work is aimed at better understanding aphid–plant interaction in ozone-polluted environments. Our main objective is to evaluate the direct effect of ozone on aphids, linking mechanisms that operate at individual scale with their consequences at population scale. We hypothesised that direct ozone exposure causes oxidative stress

accumulation in aphids, which can further compromise aphid-bacteria symbiosis. At population scale, these effects translate into increased mortality, reduced dispersion ability, and reduced population growth.

Materials and methods

Aphid rearing

Metopolophium dirhodum (Walker) (Hemiptera: Aphididae) specimens were collected from a spontaneous population growing on *Lolium multiflorum* Lam. and other Poaceae at the experimental field of IFEVA (Instituto de Fisiología y Ecología Vinculado a la Agricultura, Faculty of Agronomy, University of Buenos Aires, Argentina; 34°35′S, 58°29′W). Laboratory colonies were established on wheat plants [*Triticum aestivum* L. (Poaceae) cv. Cronox (Don Mario, Chacabuco, Argentina)] at 22 ± 1 °C and L12:D12 photoperiod. The microbiota of both wild and experimentally reared *M. dirhodum* have been characterised at the beginning of this work, confirming the presence of *B. aphidicola*, and revealing the co-occurrence of the secondary symbiont *Hamiltonella defensa* Moran et al. (Enterobacteriaceae) in every analysed individual (Telesnicki et al., 2012).

Experimental procedures

Two experiments were conducted in 'open-top' chambers located at IFEVA to evaluate the direct effects of ozone on aphids. In both experiments, aphids were fed artificial diets during ozone exposure to isolate direct effects of ozone from indirect (plant-mediated) effects. Aphids received a single exposure to ozone during the first 6 h of daylight. These experiments were conducted between April and June 2011. Average minimum and maximum temperatures during this period were 8.4 and 23.6 °C.

Ozone exposure

Six 8-m^3 'open-top' chambers with crystal PVC walls mounted on a metal structure and ozone level regulation were used for the experiments (Hogsett & Tingey, 1985; Lefohn et al., 1986). Ozone was generated from charcoal-filtered ambient air by a spark discharge-type ozone generator and ambient air was pumped through an activated-charcoal filter. Ozone concentration inside the open-top chambers was continuously monitored using a Model 450 Ozone Monitor API-Teledyne Instrument (Teledyne Advanced Pollution Instrumentation, San Diego, CA, USA). Ozone and filtered air were mixed in different proportions to obtain three contrasting ozone exposure conditions. Two chambers received activated-charcoal-filtered air and were used for the control treatment (0.5 ± 0.3 p.p.b.). The remaining chambers received ozone mixed with activated-charcoal-filtered air in two proportions, yielding two chambers with an intermediate mean ozone concentration (50 ± 5 p.p.b.) and two chambers with a high mean ozone concentration (150 ± 10 p.p.b.).

Artificial diet and diet cages

The artificial diet consisted of an aqueous solution of 20 amino acids (10 mM Ala, 16 mM Arg, 20 mM Asn, 10 mM Asp, 3.3 mM Cys, 10 mM Glu, 10 mM Gln, 10 mM Gly, 10 mM His, 6 mM Ile, 6 mM Leu, 10 mM Lys, 5 mM Met, 3 mM Phe, 7 mM Pro, 10 mM Ser, 12 mM Thr, 4 mM Trp, 2 mM Tyr, and 7 mM Val) and sucrose (440 mM). This diet was provided to the insects inside a double-layer parafilm sachet mounted on a 55-mm-diameter Petri dish, bordered with a rigid plastic mesh stripe and covered with polyester voile (Figure 1). As the experiment was designed to evaluate the effect of ozone on insects' lipid peroxidation and the total reactive antioxidant potential, an artificial diet formulation without added vitamins and minerals was chosen deliberately to avoid diet*ozone interaction effect. This diet has been proven to be nutritionally sufficient to allow aphid reproduction (Kim & Jander, 2007). These artificial diet devices were placed 40 cm away from the ozone source at 20 cm height. Permeability to ozone was verified with semi-quantitative ozone test strips (MN OZONE test strips; Macherey-Nagel, Bethlehem, PA, USA) to ensure that aphids were actually exposed to contrasting ozone conditions inside the diet cages. Ozone test strips were placed inside the diet cages and outside of the diet cages within

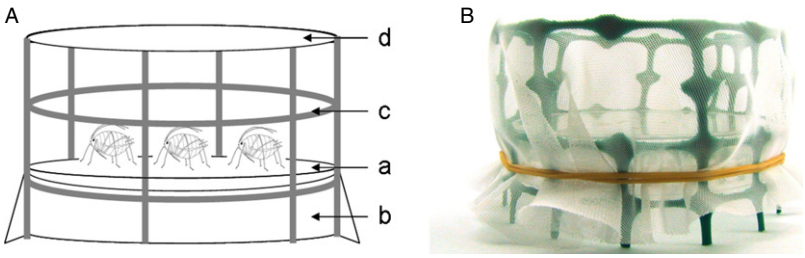

Figure 1 (A) Diagram and (B) photograph of the diet cages employed for aphid feeding during ozone exposure. The artificial diet sachet (a) was provided over a 55-mm-diameter Petri dish (b), bordered with a rigid plastic mesh stripe (c), and covered with polyester voile (d).

the same open-top chamber, at the same distance from the ozone source as was employed during the experiments. Aphids were caged in the diet devices 12 h prior to ozone exposure and kept inside the aphid rearing chambers until the beginning of the experiment.

In the first experiment, we placed eight-diet cages containing 25 aphids in each open-top chamber. Artificial diets have variable success in preventing the insects from escaping, as diet composition is a limited substitute for plant phloem sap, which may encourage the search for an alternative food source (Sadeghi et al., 2009). Therefore, the proportion of escaped aphids may be considered as an estimate of aphid dispersion. The aphids were recounted in four diet cages randomly selected from each open-top chamber and the proportion of escaped aphids was calculated from the missing individuals at the end of ozone exposure. Insect mortality was defined as the proportion of insects found dead inside the diet cages. The surviving insects from all diet cages were grouped to obtain two samples from each open-top chamber; they were immediately frozen with liquid nitrogen and kept under −70 °C to asses oxidative stress parameters.

The second experiment was aimed at testing the effect of ozone exposure on the aphid success of subsequent plant infestation. Aphids were exposed to ozone during the first 6 h of daylight as in the first experiment. After ozone exposure, 10 aphids were transferred to each wheat plant (four plants per treatment) and recounted after 1, 5, and 30 days from ozone exposure. Wheat plants with aphids were covered with a plastic mesh cylinder and a voile bag to avoid aphids from escaping and later kept at 22 ± 1 °C and L12:D12 photoperiod, without ozone exposure. Aphid samples for diagnostic PCR of endosymbiotic bacteria were taken immediately after treatment and on the following day. Additional samples were obtained from the offspring of the exposed aphids. All samples were stored at −70 °C until DNA extraction.

Oxidative stress biomarkers

Lipid peroxidation of aphid tissue was assessed by the thiobarbituric acid reactive species technique (TBARS) following a protocol modified after Łukasik et al. (2009). The reaction mixture was modified from the original paper as follows: aphid homogenate (1 g tissue in 9 ml of 30 mM phosphate buffer with 120 mM KCl, pH 7.4), 4% butylated hydroxytoluene (BHT) in ethanol, 20% trichloroacetic acid (TCA), and 0.7% thiobarbituric acid (TBA) was incubated at 100 °C for 1 h. This reaction yields a malondialdehyde-TBA (MDA-TBA) complex, which has a specific absorption peak at 532 nm ($\varepsilon = 1.56$ mM^{-1} cm^{-1}). Antioxidant potential was estimated by total reactive antioxidant potential (TRAP) of tissue homogenates (Lissi et al., 1995). Protein content was assessed following the protocol by Lowry et al. (1951), with bovine serum albumin (BSA) as a standard.

PCR detection of endosymbiotic bacteria

Aphids were processed individually for total DNA extraction by using a CTAB-based protocol modified after Doyle & Doyle (1987). DNA concentration and purity were assessed in a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington DE, USA). Primers for *Buchnera* detection were AAGCTTGCTTTCTTGTCGGC GA and CTTCTGCGGGTAACGTCACAAA, which yield a 423 bp product corresponding to the *rrs* gene of the 16S subunit of the ribosome. Primers employed for *H. defensa* detection were the *Hamiltonella*-specific forward primer PABSF and general reverse primer 16SB1 (Darby et al., 2001; Douglas et al., 2006). Reactions were performed on an Eppendorf Mastercycler® (Eppendorf, Hamburg, Germany) thermal cycler under the following conditions: for *Buchnera*: 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; for *H. defensa*: 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The products of amplification were separated in a 1% (wt/vol) agarose gel stained with ethidium bromide. As *Buchnera* is exclusively associated to aphids, DNA extracted from *Epinotia* spec. moths was included as a negative control in the *Buchnera* reactions. In the *H. defensa* PCR, the negative control consisted of total DNA extracted from the aphid *Schizaphis graminum* (Rondani), which is known not to contain secondary symbionts (Sandström et al., 2001).

Statistical analysis

The first experiment was analysed using a nested design with ozone exposure as a fixed effect with three levels and the open-top chambers as a random effect with six levels. The second experiment was analysed using a nested factorial design with three factors: ozone exposure (fixed effect, three levels), open-top chamber (random effect, six levels), and time (fixed effect, three levels). Normality of all variables was evaluated applying Shapiro–Wilks test and Q-Q plot and the homogeneity of the variance was evaluated using Levene's test. TBARS and TRAP values were log transformed. Because chamber effect was not significant, all chambers under the same treatment were pooled. Tukey's least significant difference (LSD) test was used to compare means among treatments. Statistical analyses were performed using InfoStat (Infostat, 2008).

Results

Diet cages were permeable to ozone and ozone test strips inside diet cages reached the same ozone level as the ones

placed outside the diet cage, within the same open-top chamber. Also, these semi-quantitative ozone test strips adequately reflected ozone concentration detected by the quantitative ozone monitoring system. Direct exposure of aphids to ozone increased insect mortality in both ozone doses compared to the control treatment ($F_{2,21} = 10.01$, $P = 0.0006$; Figure 2A). In the second experiment, the populations established from the surviving insects of each treatment increased similarly (Table 1). The proportion of winged aphids at the final recount did not differ among treatments (Table 1).

Ozone also affected aphid behaviour. The proportion of insects dispersing from diet cages was highest in the control treatment, whereas both ozone treatments reduced insect dispersion ($F_{2,21} = 4.15$, $P = 0.028$; Figure 2B).

Lipid peroxidation (TBARS) was affected by exposure to ozone ($F_{2,8} = 9.58$, $P = 0.0075$); it was significantly lower at 50 p.p.b. than at 150 p.p.b. or the control (Figure 3A). No significant effect of ozone treatment was detected on aphid TRAP ($F_{2,8} = 4.10$, $P = 0.060$; Figure 3B).

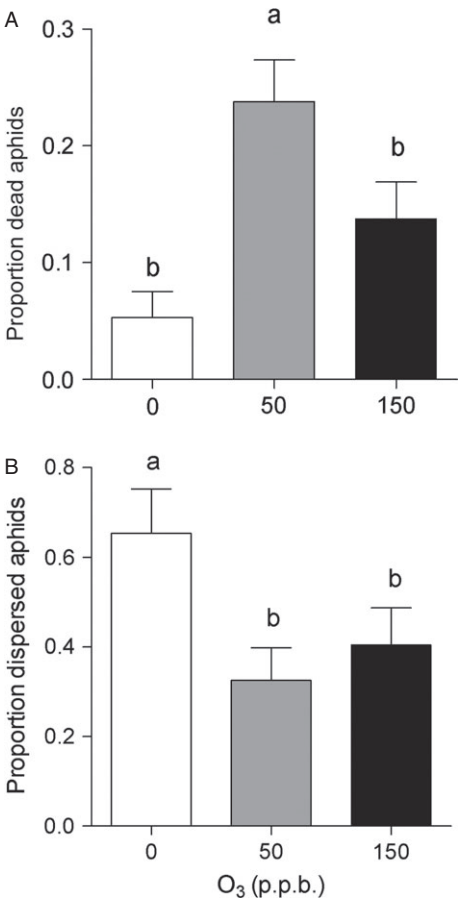

Figure 2 Mean (+ SEM; n = 8) (A) mortality and (B) dispersion of caged aphids, feeding on artificial diets, after direct exposure to ozone. Means capped with different letters are significantly different (Tukey's LSD test: $P<0.05$).

Table 1 Mean (± SD, n = 4) aphid population size (no. individuals per plant) after ozone exposure. After 6 h ozone exposure on artificial diets, aphids were transferred to wheat plants and recounted 1, 5, and 30 days later. The proportion of winged aphids was determined on day 30

Ozone (p.p.b.)	Days after exposure			
	1	5	30	Winged aphids
0	19 ± 6	30 ± 12	2056 ± 39	0.23 ± 0.01
50	14 ± 7	20 ± 14	2182 ± 563	0.23 ± 0.01
150	10 ± 7	15 ± 12	1405 ± 1162	0.20 ± 0.01
$F_{2,7}$	1.23	0.51	0.79	0.04
P	0.35	0.62	0.49	0.96

Both *Buchnera* and *H. defensa* symbionts were consistently detected after all treatments. PCR yielded fragments of the expected size in *M. dirhodum* regardless of the ozone dose and time since exposure, whereas no reaction was seen in the negative controls.

Discussion

This study demonstrates that ozone can exert a direct and negative effect on aphid individuals with consequences at the population scale. This effect did not correlate with oxidative stress markers at the individual scale. Ozone exposure increased aphid mortality, compared to the control. However, mortality at 50 p.p.b. was higher than at 150 p.p.b. To dismiss the possibility that aphid dispersion might have confounded ozone's effect on mortality (and lead to this mortality pattern), aphid mortality was additionally calculated as the proportion of dead insects over remaining insects instead of the initial numbers. Both calculations rendered a similar mortality pattern (not shown).

Two conclusions can be drawn from a comparison of the present results with previous work in which ozone toxicity to insects was evaluated. First, the direct effect of ozone on aphids tested on artificial diets depends on the variables under consideration, as it happens when plants and aphids are simultaneously exposed to ozone (Holopainen & Kossi, 1998; Awmack et al., 2004; Mondor et al., 2010). Although the direct effect of ozone pollution on the mean relative growth rates of aphids has already been dismissed (Brown et al., 1992; Jackson, 1995), aphid mortality patterns deserve further attention. Although Jackson (1995) reported that ozone fumigation did not affect aphid

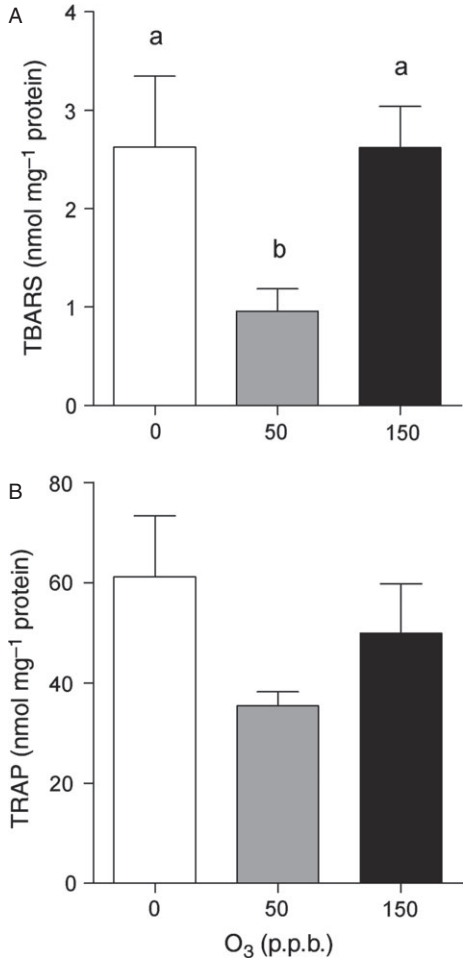

Figure 3 Oxidative stress biomarkers on aphids after direct exposure to ozone: mean (+ SEM; n = 4) level of lipid peroxidation (A, TBARS) and total reactive antioxidant potential (B, TRAP). Means capped with different letters are significantly different (Tukey's LSD test: $P<0.05$).

mortality, the mortality rate attributable to artificial diets was higher in her experiment than in ours. Second, insects may be sensitive to the direct effect of ozone at current pollution levels. In contrast with previous work in which, ozone toxicity to insects was evaluated (Holmstrup et al., 2011; Sousa et al., 2012), ozone doses applied in our experiments were three orders of magnitude lower.

Although this negative effect of ozone at the population scale was clear, ozone effects at the individual scale remained elusive and did not support our initial hypotheses. The antioxidant potential remained practically unchanged and lipid peroxidation did not increase with ozone exposure. Antioxidant potential stability should not be interpreted as a lack of oxidative stress. Rather, it means that the consumption of antioxidants remained in a stationary equilibrium with the renewal of antioxidant power. Excessive accumulation of oxidative stress leads to the pathway of cell-tissue-organism death. In our experiment, lipid peroxidation showed an irregular pattern. Oxidative damage in aphids exposed to 50 p.p.b. of ozone was lower than in the control and the 150 p.p.b. treatment. It is possible that this pattern resulted from the complex combination of ozone-induced damage accumulation and antioxidant repair mechanisms. Also, it should be noticed that the oxidative stress biomarkers were estimated based on the surviving insects. Therefore, it is congruent that lipid peroxidation was the lowest in the 50 p.p.b. treatment, in which aphid mortality was the highest. Besides, the level of lipid peroxidation detected in this experiment was higher than that reported in the literature, where TBARS values rarely reach 1 nmol mg^{-1} protein in aphids (Łukasik et al., 2009, 2012). These differences could be associated with experimental conditions. Although the experiments reported by Łukasik et al. (2009, 2012) were performed in rearing chambers, the experiments presented herein were performed under field conditions. In our experiment, the biological effects have gone far beyond the oxidative stress perspective making it more reasonable to conclude in terms of population scales processes.

Previous efforts made to evaluate the direct effect of ozone on aphids found no effect of ozone on the individual relative growth rate of aphids when applying ozone at 100 p.p.b. (Jackson, 1995), which is comparable to the treatment applied in this experiment. However, individual growth rate may not be a good predictor for aphid population growth under gas pollution (Holopainen & Kossi, 1998; Awmack et al., 2004; Mondor et al., 2010). Gaseous pollutants may impose a trade-off between somatic and reproductive tissues that may uncouple the physiological effects found at the individual scale from those observed at the population scale (Mondor et al., 2010). In our experiments, the aphids' ability to build up new populations was not affected by ozone despite the oxidative damage and increased mortality under ozone exposure. Our aphid population was probably rather homogeneous, as it was founded from only few individuals. Nevertheless, it is possible that the aphids varied in their susceptibility to ozone. In this case, ozone exposure could have removed the most susceptible individuals. Also, if only the tolerant aphids survived, differences in their oxidative stress markers and further population growth would be unlikely.

Aphids are isolated from the plants in many situations during their life cycle (Dill et al., 1990; van Emden & Harrington, 2007). The dispersion of winged and wingless aphids, within a plant and among plants of the same field, frequently occurs by walking (van Emden & Harrington,

2007). This movement usually occurs when plant nutritional quality decreases, and in consequence dispersion may provide an adaptive advantage when resource quality is not optimal (Honek et al., 1998; Lombaert et al., 2006). However, ozone reduced the dispersion of aphids from the artificial diets. In addition, in the presence of natural enemies, aphid movement reduces parasitoidism (Dill et al., 1990). Therefore, it would be interesting to test whether ozone effect on aphid movement has implications for this trophic interaction.

Despite the negative effects of ozone on aphid survival and dispersion, the subsequent growth of aphid populations and the proportion of winged individuals were not affected by previous exposure to ozone. Taken together with the stability of antioxidant levels, these results do not support the hypothesis that successful colonisation of new plants depends on the antioxidant potential of insects. However, the experimental procedure might have favoured this result as the aphids were manually transferred to the target plants to ensure that all the populations were initiated with an equal number of founders.

Both endosymbionts previously identified in this aphid population persisted under all ozone conditions. The persistence of *B. aphidicola* is consistent with the similar population growth among treatments, given that a lower rate of population growth would be expected if aphids were free of *B. aphidicola*. However, it remains elusive whether *H. defensa* had a protective role with respect to *B. aphidicola* (Montllor et al., 2002; Moran et al., 2005; Wernegreen, 2012), because our experimental design did not include the experimental manipulation of this endosymbiont.

Overall, this study suggests that direct exposure to ozone could lead to a lower spread of viral diseases in crops. The detrimental effect of ozone on aphid dispersion and mortality merits further attention, to estimate the potential modification of their impact as crop pests. Also, a better understanding of aphids' antioxidant response to exogenous oxidative conditions imposed by ozone pollution seems to be critical. Antioxidant uptake and cycling may modify oxidative damage accumulation as well as modify aphids' nutritional interaction with their host plant.

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