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# Impact of plant and aphid stress history on infestation in arugula plants

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**Abstract**

Plants can activate inducible defence mechanisms against pests, pathogens, or chemical elicitors, such as ozone, mediated by reactive oxygen species (ROS), particularly hydrogen peroxide ($H_2O_2$). An unfavourable balance between ROS production and the plant antioxidant capacity seems to be responsible for the resulting susceptibility of the plant to insect attack. Arugula plants [*Eruca sativa* Mill. (Brassicaceae)] and green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), were used in this study to test the hypothesis that the growth of an aphid population depends on both plant and insect stress history. We investigated the impact of density and duration of a previous aphid infestation, and the time lag before re-infestation, on aphid population growth. In a second experiment, we assessed the effect on aphid population growth of previous ozone exposure of arugula plants in open top chambers receiving a continuous $O_3$ fumigation of 100–120 p.p.b., 90 min per day during 3 days. A third experiment was conducted to study the effect of aphid density during a previous infestation on the population growth on an uninfested host. Both previous herbivory and ozone changed the oxidative status of plant tissues and facilitated aphid population growth, which increased with the duration and density of a previous infestation by aphids. Colonization success also depended on the aphids' own history. Aphids coming from high-density populations and/or longer infestation periods produced larger populations on an (initially) uninfested plant. Pest outbreaks in a polluted environment might be expected to be modulated by the hosts' spatial-temporal heterogeneity related to the ozone exposure and previous herbivory.

## Introduction

Aphids are herbivores with extremely rapid population growth and they are of ecological as well as agronomical importance, mainly because of their role as virus vectors (Thaler, 1999; Underwood & Rausher, 2000). The behavioural patterns and enormous reproductive potential of aphids ensure their wide dispersal among populations of plants that host viruses (Swenson, 1968). Plants can activate inducible defence mechanisms against aphids, which can be systemically expressed or locally confined to infested plant parts (Nombela et al., 2008). It is well established that plant responses to herbivory may affect aphid-feeding behaviour (Montllor et al., 1983; Hays et al., 1999) resulting in changes in aphid fitness (Wool & Hales, 1996). However, plants may also alter herbivore performance during a later attack (Karban & Myers, 1989). Sauge et al. (2006) have measured behavioural and performance parameters of the success of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (green peach aphid) on plants previously infested by conspecifics as compared to the uninfested control using different *Prunus persica* L. Batsch genotypes. Some of the plant genotypes responded to previous herbivory by reducing fitness and/or preference of the aphids. On the contrary, opposite effects induced by a previous infestation, making the plant more attractive and/or susceptible to herbivory by the same species, were also observed (Prado & Tjallingii, 1997; Thaler et al., 2001).

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Plant responses to herbivore attack have been categorized into: (1) tolerance where essential plant resources such as carbon, nitrogen, and sulphur are moved to structures unavailable to insects, such as roots, or remobilized to meristems; (2) direct defence through the production of toxic or anti-nutritive compounds; and (3) indirect defence in which chemical cues are released to recruit predators and parasitoids to control herbivore numbers (Kerchev et al., 2012). None of these defence mechanisms can successfully explain facilitation of a subsequent aphid attack after an earlier infestation. In turn, increasing evidence points to the involvement of reactive oxygen species (ROS) in the plant response to insect infestation (including aphids), and the hormone-mediated ability of the insects to bypass plant-defence responses (Kusnierczyk et al., 2008; Goggin et al., 2010). Reactive oxygen species consist of free radicals such as superoxide radicals ($O_2$-), hydroxyl radicals (HO-), or hydrogen peroxide ($H_2O_2$). Under optimal growth conditions, ROS are mainly produced at a low level in organelles such as chloroplasts, mitochondria, and peroxisomes (Miller et al., 2010). However, during biotic or abiotic stress their rate of production is dramatically elevated. For example, linolenic acid degradation caused by aphid salivary enzymes, triggers its synthesis (Orozco-Cardenas & Ryan, 1999; Gatehouse, 2002). Reactive oxygen species attack lipids, proteins, and nucleic acids, causing lipid peroxidation, protein denaturation, and DNA mutation (Noctor & Foyer, 1998; Yu & Rengel, 1999). As a result, ROS may negatively affect the digestive system of insects through membrane damage (Smith & Boyko, 2007), or via the oxidative modification in polyphenols and the generation of highly reactive quinines in the food, which act as feeding deterrents (Kerchev et al., 2012). It has been demonstrated that ROS play important roles not only in direct defence against aphid herbivory but also in signalling (Walling, 2000; Kerchev et al., 2012). Aphid feeding upregulates a range of genes that are either responsive to ROS or are required for ROS metabolism (de Vos et al., 2005), activating the salicylic acid (SA)-dependent signalling pathways of plant responses to aphid attack (Walling, 2000; Boyko et al., 2006).

Reactive oxygen species accumulation in plants is not an exclusive consequence of aphid attack. The equilibrium between the production and the scavenging of ROS may be perturbed by various biotic and abiotic stress factors such as pathogens, salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, herbicides, and air pollution (Bhattacharjee, 2005). Such disturbances in equilibrium lead to sudden increases in intracellular levels of ROS that can cause significant damage to cell structures (Miller et al., 2010). Moreover, some studies have shown convergence in the signalling pathways of plants exposed to $O_3$ and aphid feeding. This 'crosstalk' among signalling pathways results in the same response at metabolic or molecular levels (Bostock, 2005). It is not surprising therefore that ROS have been unequivocally shown to participate in the plant response to insects, including aphids. However, to date very little information is available concerning how ROS generation or ROS/hormone crosstalk are affected by insect herbivory, and the potential importance of redox signalling mechanisms in plant–insect interactions.

Plants themselves may also be negatively affected by the ROS they produce. Reactive oxygen species are non-targeting molecules and therefore their accumulation may result in self-harm, if surplus ROS are not neutralized (Sadd & Siva-Jothy, 2006). To counteract these risks, both plants and insects are equipped with an arsenal of enzymatic and non-enzymatic mechanisms, tightly controlling unwanted ROS accumulation (Moran et al., 2002; Apel & Hirt, 2004). Plants must find a balance between producing ROS for defence and producing ROS-detoxifying enzymes to help stabilize plant tissue damage due to oxidative degradation (Thompson & Goggin, 2006). Similarly, it is a well-established fact that the gut lumen of herbivorous insects has high activities of a range of antioxidant enzymes which provide insects with varying capacity to detoxify radicals within the gut lumen (Ahmad, 1992; Barbehenn et al., 2001). As a result, the balance between ROS and antioxidant production determines the oxidative status of both plants and aphids, which in turn might influence aphids' ability to infest new plants. In this way, infestation ability would increase when aphids are exposed to higher antioxidant levels, produced by themselves or their host plant either during a present or a previous infestation event. Part of the inconsistent outcomes of studies on the influence of previous infestations on aphid performance might be understood if not only the plant history was considered but also the history of the infesting aphids in terms of host and aphid redox status.

The objective of this research was to further our understanding of the factors controlling aphids' population growth. We hypothesized that the increase in the number of individuals in an aphid colony will depend on the stress history of both the aphids and their host, that is on both their oxidative status. We report results on: (1) the effects of previous infestations of *M. persicae* of different density and duration, and of the time lag before re-infestation, on the growth of a new aphid colony of the same species on arugula plants, *Eruca sativa* Mill (Brassicaceae); (2) the effect of exposure of the host plant to an ozone-contaminated environment previous to infestation; and (3) the performance of aphid individuals previously feeding on

hosts with different levels of insect density and feeding duration.

## Materials and methods

### Plant material

Arugula plants were grown from seeds of a commercial variety (Semillera Guasch, Bahia Blanca, Buenos Aires, Argentina) in 12-cm plastic pots in soil mix (50% ground, 25% peat moss, 25% sand), in a greenhouse under a long-day photoperiod of L16:D8, with temperature varying between 12 and 15 °C. After 5 days, the seedlings were thinned to one plant per pot. Plants were well watered throughout the experiment. One-month-old seedlings were used in the experiments.

### Aphids

The green peach aphid, *M. persicae*, is a serious pest affecting peach (*P. persica*) and other crops (Sauge et al., 2006). It is one of the most biologically diverse and widely distributed aphid species, and also the most important herbivore pest of arugula in Argentina and other temperate regions (Andorno et al., 2007). The aphid colony was established from *M. persicae* individuals collected from a naturally occurring infestation of arugula plants in the University of Buenos Aires experimental station (34°35′27″S, 58°28′49″ W). Apterous aphids were continuously reared on arugula seedlings at 20 ± 4 °C. New plants were added to the culture at weekly intervals as older damaged plants were removed.

### Experimental procedures

We conducted three experiments to assess the impact of plant and aphid history on aphid population growth on an uninfested new host. In the first two experiments, the effect of plant history was evaluated by applying an acute green peach aphid infestation or ozone exposure to plants prior to aphid infestation. In the third experiment, the effect of aphid density during previous infestation on aphid population growth was evaluated.

The first experiment was done using a factorial design with three factors: pre- infestation size [0 (= control), 2, or 20 aphids)], duration of pre-infestation (24 or 72 h), and time lag before re-infestation (0, 24, or 72 h) with five replicates for each combination (Figure 1). Plants were randomly assigned to the different treatments and were arranged according to a randomized complete block design. Previous infestation was performed by placing apterous adults (2 or 20) on a single leaf on a plant and removing them and their progeny 24 or 72 h later. Plants corresponding to each pre-infestation treatment were separated and randomly assigned to one of the three infestation time lags. Five new adults were placed on the same plants by manually transferring them onto the newest expanded leaf (immediately, 24, or 72 h after pre-infestation ended). Aphid population growth was then assessed in plants that had been previously infested by *M. persicae* (pre-infested) or not (control). Colonies were allowed to grow for 11 days, after which aphids were counted. In both cases, pre-infestation and new infestation, aphids were allowed to roam freely over the entire plant. Aphid colony growth was evaluated by daily counting through direct observation on the infested plants. Counting was done with the help of a hand-held tally counter without disturbing the colonies.

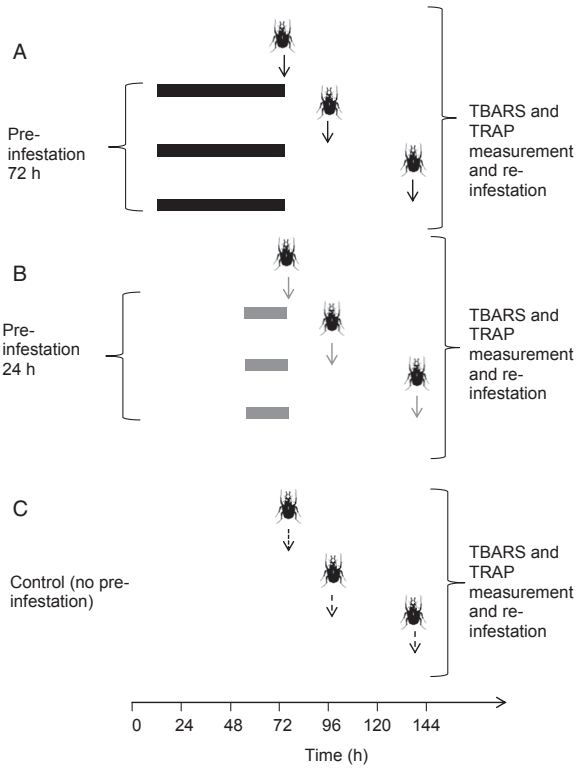


**Figure 1** Experimental procedures for experiment 1. Arugula plants were pre-infested with 2 or 20 *Myzus persicae* aphids for (A) 72 h, (B) 24 h, or (C) they were not pre-infested (control). Plants were sampled for oxidative damage with the thiobarbituric acid reactive substances (TBARS) assay and the antioxidant potential (TRAP) in leaves immediately after the pre-infestation period, 24 h later, and 72 h later. At the same time, plants were re-infested with five adults, and aphids in each treatment were counted 11 days later.

A subset of plants from each group was harvested after pre-infestation ended to determine the oxidative damage caused by herbivory-induced ROS and the production of antioxidants in arugula plants before the new infestation. Whole-shoot tissues of four plants per treatment were

harvested and immediately placed in liquid nitrogen keeping them separate, and transferred into a freezer at −80 °C, where they remained until analysis. The chain reaction that occurs during lipid oxidation mainly leads to the formation of peroxides and secondary products such as aldehydes. One way to assess the damage caused by lipid peroxidation is through a technique called 'colorimetric thiobarbituric acid reactive substances (TBARS) assay'. TBARS, mainly malondialdehyde, are formed as a by-product of lipid peroxidation (i.e., as degradation products of fats) and they can be detected using thiobarbituric acid as a reagent (Yagi, 1976). Thereafter, higher TBARS values indicate greater lipid damage. Batches of plants from each treatment were placed in 1% phosphoric acid and then homogenized for 5 min at 0 °C. The homogenates were filtered through two layers of cheesecloth and centrifuged at 3 000 ***g*** for 15 min. The pellets were discarded and the supernatants were used to assay the studied oxidative stress marker. Also, we estimated the antioxidant potential in leaves using the TRAP (total peroxyl radical-trapping antioxidant capability) technique developed by Wayner et al. (1985). This test is based on the generation of peroxyl radicals from 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH). After adding AAPH to a biological fluid, oxidation reactions are monitored by measuring oxygen consumption using an oxygen electrode. The initial lag phase before increased oxygen consumption is proportional to the antioxidant capacity of the sample, and the assay is standardized against known concentrations of the water soluble vitamin E analogue trolox. A low total antioxidant capacity is indicative of oxidative stress or increased susceptibility to oxidative damage (Young, 2001).

For the second experiment, six 'open top' chambers (OTCs) with tropospheric $O_3$ level regulation were used, built at IFEVA, Facultad de Agronomía, University of Buenos Aires campus. The 8-$m^3$ chambers had a metal structure with crystal PVC walls (Hogsett et al., 1985). Three chambers were used for the control treatment (charcoal-filtered ambient air) and three for the $O_3$ treatment (charcoal-filtered air with added $O_3$). Ozone was generated by a spark discharge-type $O_3$ generator (Hogsett et al., 1985). Ozone level inside the OTC was continuously monitored using a Model 450 Ozone Monitor API-Teledyne Instrument (Teledyne Advanced Pollution Instrumentation, San Diego, CA, USA). Five uninfested plants were placed in each chamber. Plants corresponding to the $O_3$ treatment were exposed to ozone in OTC calibrated to deliver a continuous flow of 100–120 p.p.b. $O_3$, during 90 min through daylight time. This treatment was applied for three consecutive clear sky days in spring. Control plants were placed in OTCs with charcoal-filtered air during the same period. TBARS and TRAP were assessed as described above, immediately after ozone treatment and 3 days later. After the 3rd day of ozone exposure, six adult aphids were placed on each plant and colony growth was assessed as described before.

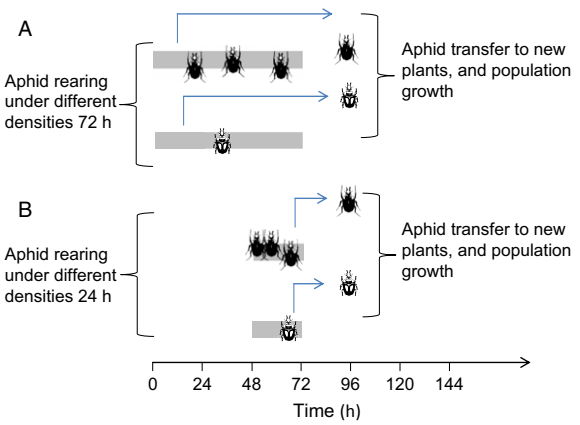


**Figure 2** Experimental procedures for experiment 3. Arugula plants were infested with high (50–100 aphids) or low (2–10 aphids) densities of *Myzus persicae* for (A) 72 h or (B) 24 h. Five aphids from each treatment combination were transferred to uninfested plants, where populations grew for 11 days.

The third experiment consisted of a factorial combination of two initial infestation densities (low: 2–10 aphids; high: 50–100 aphids) and two feeding times (24 and 72 h), with five replicates per combination. After the initial infestation, five adult aphids of each treatment were placed on herbivore-free plants, and aphid colony growth was assessed as described above (Figure 2).

#### Statistical analysis

To test for main effects and interactions of pre-infestation density and duration, time lag before re-infestation on TBARS and TRAP in experiment 1, and ozone exposure and time after exposure in experiment 2, separate analyses of variance (ANOVA) were performed for each parameter, followed by Tukey tests. Aphid populations in each experiment were analysed separately. The effect of pre-infestation density and duration, time lag before re-infestation, and their interactions were analysed using ANOVA, after checking for ANOVA assumptions (normality and homogeneity of variance), followed by a Tukey test A repeated measures one-way ANOVA was used to compare aphid colony growth on ozone-exposed and control plants in experiment 2, with sampling date (six dates) as repeated measure, followed by a Tukey test. Data for insect variables measured at the individual plant level were averaged (n = 5 plants per chamber) to avoid pseudo-replication in testing for differences between ozone and control chambers. We thus report means and standard errors based on

three chambers per ozone treatment. The effect of aphid initial density and feeding time on the growth of aphid colony started by the same individuals on new plants in experiment 3 was analysed with repeated measures ANOVA, with sampling date (six dates) as repeated measure, followed by Duncan's Multiple Range test. All analyses were performed using Infostat Professional v. 2008 (Di Rienzo et al., 2008).

## Results

### Aphid population growth on plants with a different history

Injury (accounted for by TBARS) and total antioxidant levels (accounted for by TRAP) were affected by initial aphid pre-infestation density (N) (2 or 20 individuals) and duration (D) (24 or 72 h), and time before re-infestation (T) (ANOVA, TBARS significant terms: N*D*T, $F_{4,36} = 5.13$, $P = 0.002$; N*D, $F_{4,36} = 17.88$, $P<0.001$; N*T, $F_{2,36} = 12.86$, $P<0.001$; N, $F_{2,36} = 22.82$, $P<0.001$; T, $F_{1,36} = 65.23$, $P<0.001$; D, $F_{2,36} = 23.95$, $P<0.001$; TRAP significant terms: N*D, $F_{4,36} = 11.22$, $P<0.001$; N*T, $F_{2,36} = 4.53$, $P = 0.017$; N, $F_{2,36} = 3.41$, $P = 0.04$; T, $F_{1,36} = 41.68$, $P<0.001$; D, $F_{2,36} = 12.01$, $P<0.001$) (Table 1). Plants exposed to the lowest pre-infestation density showed significantly higher injury than control plants immediately after the pre-infestation treatment (0 h), when the feeding time was the longest (72 h), and 24 h after the pre-infestation treatment, when feeding time was the shortest (24 h). Similarly, low injury levels were expressed in plants in the 72-h pre-infestation treatment after 24 h, and for both pre-infestation feeding periods after 72 h (Table 1).

Total antioxidant potential (TRAP) showed a pattern opposite to the injury levels: the lowest antioxidant values were obtained from plants assayed immediately after pre-treatments, whereas 24 h after pre-treatments, plants from both treatments showed about a three-fold increase in antioxidant levels (Table 1). Plants corresponding to the 24-h feeding period reached a similar value to that of control plants, which was exceeded in the 72 h treatment. Antioxidant levels for each pre-infestation treatment (24 and 72 h) stayed constant over time (Table 1).

Plants exposed to high-initial aphid pre-infestation density (20 individuals) showed injury levels that were equal to or lower than that of control plants (Table 1). Injury levels of the 24-h pre-infestation treatment decreased to the lowest level when plants were assayed 72 h later. In this high-initial-aphid-density treatment, antioxidant levels also behaved in a pattern opposite to injury levels. Compared to control plants, levels of antioxidants were similarly high when the plants had similarly low levels of injury.

**Table 1** Mean (± SD) thiobarbituric acid reactive substances (TBARS) and total peroxyl radical-trapping antioxidant capability (TRAP) in arugula leaves after various pre-infestation periods (h) with *Myzus persicae*, and herbivore density (0, 2, or 20 aphids). Plants were harvested immediately after pre-infestation ended (0 h), and after 1 (24 h) or 3 (72 h) days (n = 4; n represents separate supernatants from plant tissues)

| Initial pre-infestation density | Duration of pre-infestation (h) | Time after pre-infestation (h) | TBARS ($\mu M\ g^{-1}$) | TRAP ($\mu M\ g^{-1}$) |
|---|---|---|---|---|
| Control | 24 | 0 | 5.1 ± 0.8 | 180 ± 15 |
| | | 24 | 5.0 ± 0.2 | 184 ± 14 |
| | | 72 | 6.6 ± 0.9 | 181 ± 11 |
| | 72 | 0 | 5.0 ± 0.1 | 190 ± 17 |
| | | 24 | 4.8 ± 0.3 | 195 ± 3 |
| | | 72 | 4.9 ± 0.7 | 189 ± 6 |
| 2 | 24 | 0 | 4.3 ± 2.4 | 81 ± 4** |
| | | 24 | 8.4 ± 0.2*** | 203 ± 12 |
| | | 72 | 2.8 ± 0.3*** | 200 ± 28 |
| | 72 | 0 | 6.1 ± 0.7** | 171 ± 9 |
| | | 24 | 1.4 ± 0.4*** | 255 ± 15* |
| | | 72 | 2.1 ± 0.4** | 276 ± 15* |
| 20 | 24 | 0 | 5.5 ± 0.5 | 172 ± 20 |
| | | 24 | 5.4 ± 0.5 | 182 ± 12 |
| | | 72 | 1.6 ± 0.6*** | 187 ± 21 |
| | 72 | 0 | 1.6 ± 0.4*** | 224 ± 14* |
| | | 24 | 1.7 ± 0.9*** | 236 ± 18* |
| | | 72 | 2.0 ± 0.3*** | 224 ± 16** |

Means were compared with their corresponding control (i.e., zero pre-infestation) (Tukey's test: *0.01<P<0.05, **0.001<P<0.01, ***P<0.001).

*Myzus persicae* population growth was increased by density ($F_{1,61} = 7.56$, $P<0.01$) and duration ($F_{2,61} = 4.25$, $P = 0.02$) of pre-infestation and reduced by the time lag before re-infestation ($F_{2,61} = 5.90$, $P<0.01$). Pre-infestation with a low density of aphids enhanced aphid population growth compared with control only if the new infestation occurred immediately after removal of the first one, and with the longest duration of pre-infestation (72 h) (Figure 3A). When re-infestation occurred immediately after pre-treatment, pre-infestation with 20 aphids (high density) produced a significantly higher subsequent aphid number compared to control, non-pre-infested plants, irrespective of duration of pre-infestation (Figure 3B). Twenty-four hours later, pre-infestation resulted in an increased population size only if it lasted 72 h (longer period); pre-infestation had no effect on subsequent aphid population size if re-infestation occurred after 72 h (Figure 3B).

Ozone pre-exposure increased TRAP values compared to that of control plants ($F_{1,16} = 4.48$, $P = 0.05$) although

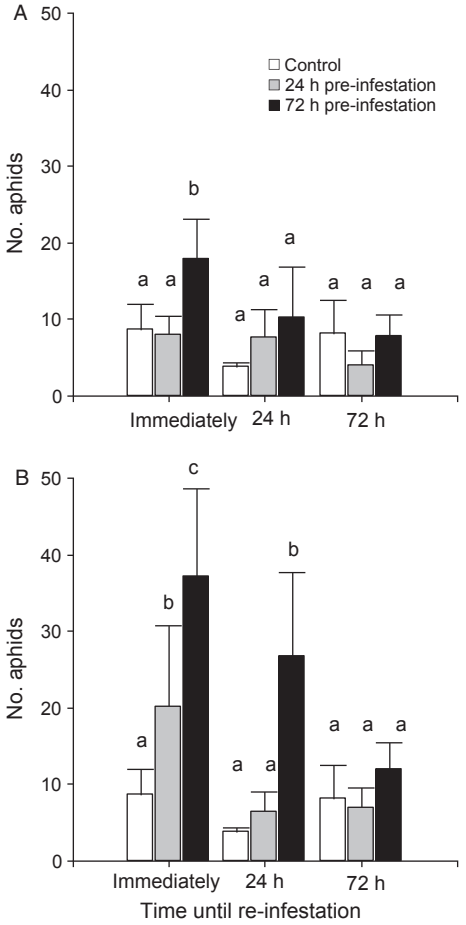


**Figure 3** *Myzus persicae* population size (mean number of aphids + SE; n = 5), in plants with no pre-infestation (control) or pre-infested for 24 or 72 h. Density of pre-infestation was (A) low (2 aphids), or (B) high (20 aphids). Plants were re-infested immediately, 24 h, or 72 h after pre-infestation ended. Means within a treatment (i.e., within a time after pre-infestation) capped with the same letters are not significantly different (Tukey's test: P>0.05).

oxidative injury levels (TBARS) did not differ among treatments ($F_{1,16}$ = 1.06, P = 0.31) (Table 2). Aphid population growth rate on plants pre-treated with ozone was higher than that of control plants throughout the 1st week ($F_{1,91}$ = 4.16, P = 0.11) (Figure 4). On day 7, aphid density on the treated plants was two-fold higher than on control plants. After that period, population growth rate increased on control plants and the difference between treatments was reduced to about 20% halfway along the 2nd week (Figure 4).

#### Aphid population growth started by aphids with a different history

Aphid population growth depended on the density at which aphids had been previously reared, and on the duration of pre-infestation ($F_{3,45}$ = 17.51, P<0.001). After 9 days, the highest aphid population growth was observed when it was started by aphids coming from high-density populations and with the longest pre-infestation time (Figure 5). When pre-infestation lasted 72 h, the number of aphids from the high-density pre-infestation treatment was almost two-fold higher compared to the low-density pre-infestation treatment, and five-fold higher than when pre-infestation lasted 24 h, regardless of the pre-infestation density (Figure 5).

**Table 2** Mean (± SD) thiobarbituric acid reactive substances (TBARS) and total peroxyl radical-trapping antioxidant capability (TRAP) in arugula leaves immediately (0 h) after ozone exposure and 72 h later. Ozone plants were exposed to the pollutant for 3 days and then moved to control chambers for three more days until the second TBARS and TRAP measurement (n = 3; n represents separate supernatants from plant tissues harvested in each ozone or control chamber)

| Treatment | Time after end of exposure (h) | TBARS ($\mu M\ g^{-1}$) | TRAP ($\mu M\ g^{-1}$) |
|---|---|---|---|
| Control | 0 | 1.2 ± 0.2 | 54 ± 3 |
| | 72 | 6.2 ± 1.3 | 54 ± 7 |
| Ozone | 0 | 0.7 ± 0.1 | 63 ± 1* |
| | 72 | 5.1 ± 0.8 | 67 ± 7* |

Means were compared with their corresponding control (Tukey's test: *P<0.05).

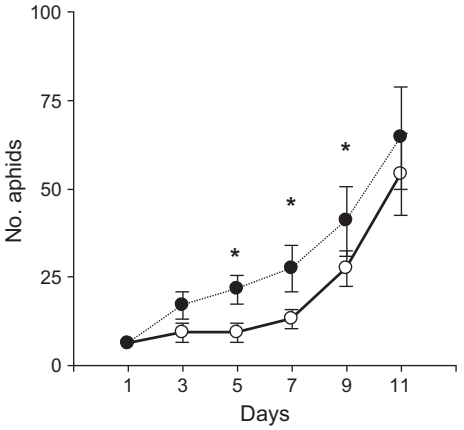


**Figure 4** *Myzus persicae* population size (mean number of aphids ± SE; n = 3) feeding on arugula plants previously exposed to ozone (●) or on unexposed control (○) plants. Plants corresponding to the ozone treatment were exposed to $O_3$ in open top chambers (OTCs) calibrated to deliver a continuous flow of 100–120 p.p.b. $O_3$, during 90 min through daylight time for three consecutive days. Control plants were placed in OTCs with charcoal-filtered air during the same period. Asterisks indicate significant differences between treatment and control plants (Tukey's test: P<0.05).

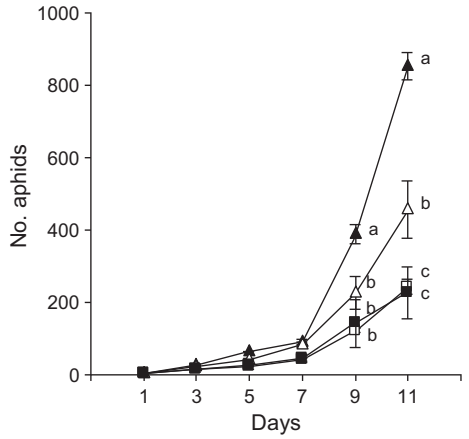


**Figure 5** *Myzus persicae* population size (mean number of aphids ± SE; n = 5) of insects coming from a 72-h period of pre-infestation with high (▲) or low (△) insect density, or a 24-h pre-infestation with high (■) or low (□) insect density. Means per day with different letters are significantly different among treatments (ANOVA followed by Duncan's test: $P<0.05$).

## Discussion

We showed that previous exposure of arugula to aphids or ozone increases new aphid colony expansion, and that these data correlate with the oxidative balance of the plants. We also demonstrated that the previous history of aphids (whether raised in dense or sparse colonies) impacts on subsequent colony expansion rates. These results concur with earlier studies showing that previous infestation could affect subsequent insect performance (Dugravot et al., 2007; Nombela et al., 2008). Both the density and the duration of a previous acute green peach aphid infestation dramatically increased the growth of the aphid population. Interestingly, the aphid population growth was enhanced only if the second colonization of the host occurred immediately after the first one was terminated. This suggests that metabolites with rapidly changing concentration were involved in the facilitation process, and concurs with previous studies with *Arabidopsis thaliana* (L.) Heynh. that uncovered a rapid systemic signal propagation dependent on the accumulation of ROS that was independent of ethylene, SA, or jasmonic acid signalling, pathways that can be triggered by wounding, heat, cold, high-intensity light, or salinity stress (Miller et al., 2009). Despite the fact that with our experimental procedures we were not able to directly test how the balance between ROS and antioxidant production determines the oxidative status of plants and aphids, our experimental data strongly support the idea that facilitation is controlled by the antioxidant potential of the plant. Plant injury level (accounted for by TBARS) of plants pre-infested for 72 h with a low density of aphids was higher than that of control plants and antioxidant level (TRAP) was similar to that of control plants immediately after pre-infestation ended. Twenty-four hours later, the TBARS level had decreased and TRAP had increased indicating that repair had occurred (i.e., healing involves elimination of ROS through antioxidant consumption). Instead, in the treatment with 24 h pre-infestation, injury levels continued to increase over the 24 h after the pre-treatment, and the total antioxidant level only reached control plant values. These differences between treatments in the dynamics of plant injury levels and total antioxidant levels are an indication that plants that facilitated aphid population growth (72 h feeding period) had an overall higher antioxidant potential than those with a shorter pre-treatment period (24 h). When aphid re-infestation was carried out 24 h after pre-treatment, high antioxidant consumption for repair was occurring in the plants with the lowest feeding time and therefore, no increase in TRAP was observed at 72 h sampling, but plant injury remained at lower values than control plants. This consumption may explain the lack of aphid population growth enhancements at the 24- and 72-h re-infestation times. Differential aphid population growth rates for the high pre-infestation density treatments can be similarly explained by the TRAP and TBARS levels at the re-infestation times. Because of the high-initial aphid density, antioxidants were probably triggered early, regardless of the length of the feeding time.

Previous studies have reported ROS production induced by aphid attack and increased antioxidant levels that neutralize the oxidative damage (Lukasik, 2007; Lukasik et al., 2009). For example, in cabbage (*Brassica oleracea* L.) the level of antioxidants changed in response to aphid attack (Khattab, 2007). One of the components of the plant antioxidant system is ascorbic acid (AsA) (Hancock & Viola, 2005). Several lines of research have previously suggested that AsA plays a key role in plant–insect interactions, as its abundance in plants modifies insect infestation ability and/or plant susceptibility to insect feeding (Goggin et al., 2010). Several mechanisms have been suggested by which AsA modulates insect success on plants: effects may be mediated by AsA as an essential dietary nutrient, as an antioxidant in the insect midgut neutralizing harmful $H_2O_2$, or as a substrate for plant-derived AsA oxidase, which can lead to generation of toxic ROS. Ascorbic acid can also influence the efficacy of plant defences such as myrosinases and tannins, and alter insects' susceptibility to natural enemies (Goggin et al., 2010). Even though we did not quantify the antioxidant enzymes in plant tissues, the TRAP index reflects the free radicals that react with a solution of antioxidants and so it

is a measurement of the quantity of antioxidants in a sample (Lissi et al., 1992).

The role of the ROS-antioxidant balance in the facilitation of the aphid infestation is further clarified by the second experiment in which ozone exposure resulted in similar results. Acute episodes of high levels of ozone generate an oxidative environment that induces the production of ROS and antioxidant molecules in the exposed plant tissues (Kangasjärvi et al., 2005). In this experiment, as the oxidative episode ended, the level of antioxidants in the plant increased, remaining at relatively high levels for several days. This finding agrees with other fumigation experiments with acute ozone stress (100 p.p.b.), in which the antioxidant levels of leaves of different plant species change profoundly. For example, the ozone-injured leaves of *Trifolium repens* L. and *Centaurea jacea* L. had 6–8 times more total phenolic acids than uninjured leaves (Severino et al., 2007). These results indicate that ozone oxidative preconditioning may exert an influence on the antioxidant/pro-oxidant balance for preservation of cell redox state in plant tissues and an increase in endogenous antioxidant systems.

Ozone or pre-infestation-driven facilitation could be due to disruption of the defence system induced by ROS (Moloi & van der Westhuizen, 2006), as a consequence of high antioxidant levels in the plants' tissues neutralizing ROS (Foyer & Noctor, 2005; Khattab, 2007), or to aphid ingestion of antioxidants reducing the negative impact of plant toxins (Lukasik et al., 2009). Disregarding which of these mechanisms is responsible for aphid growth facilitation, in the third experiment, the insect population ability to challenge an unstressed host was determined by the origin of the founder aphid individuals. Both aphid crowding and feeding period enhanced antioxidants' potential in the host tissues (as measured in the first experiment). This suggests that the redox state of aphids, determined by a dynamic balance between ROS and antioxidant production by the host, could have also allowed a higher population growth on the new host if large amounts of antioxidants were transferred to the aphids while they were feeding on the first plant. Moreover, in a previous study, we found that the population growth rate of *M. persicae* feeding on arugula plants was not affected by exposure to acute high levels of ozone episodes (Menéndez et al., 2010). However, if individual aphids collected from populations exposed to ozone were placed on unstressed host plants, their ability to challenge the new host was severely reduced in relation to that of aphids obtained from plants growing in a similar environment but without ozone episodic exposure. As ozone-exposed aphids were collected immediately after the end of an acute ozone episode, it was speculated that the oxidative balance in the host plant controlling the aphid oxidative status was low due to the highly oxidative environment in which the population was growing. This might have caused a decrease in the insects' ability to withstand free radicals produced by the host in response to herbivory, when the population migrated to a new host with low antioxidant level. The influence of the host plant on the antioxidant defence mechanism of the insect has been clearly demonstrated in studies, in which the pro-oxidant status of host plants affected the level of antioxidants in pea aphid tissues (Lukasik, 2007; Lukasik et al., 2009). These studies demonstrated that the aphids have an AsA-recycling system for removing ROS, and that pro-oxidant allelochemicals in the plant diet may be eliminated by the insect at the expense of upregulation of antioxidative enzymes in response to increased oxidative stress. These findings are consistent with our hypothesis that increased concentrations of antioxidants in insect tissues form an important component of the defence of herbivorous insects against both exogenous and endogenous oxidative radicals.

In summary, our results suggest that the success of a *M. persicae* population seems to be related to a high level of antioxidants in the first attacked plants, likely transferred to the aphids before they change hosts, or in the new host with a history of stress, which paradoxically increases the ability of the aphids to neutralize the plant's defence response. This information could help resolve the controversy posed in the introduction about the factors determining insects' population growth, and provide relevant information to understand aphid outbreaks in plant populations. Moreover, our results shed light on the many questions relevant to pest outbreaks in the current scenario of global change. Pest outbreaks in a polluted environment might be expected to be modulated by the hosts' spatio-temporal heterogeneity related to the ozone exposure and previous herbivory, determining changes in the oxidative status of both the host plant and the insects.

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