



## Aphid and episodic O<sub>3</sub> injury in arugula plants (*Eruca sativa* Mill) grown in open-top field chambers

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### ARTICLE INFO

#### Article history:

Received 2 October 2008

Received in revised form 4 August 2009

Accepted 10 August 2009

Available online 3 September 2009

#### Keywords:

Ozone

Aphids

Plant–insect interactions

### ABSTRACT

Aphid attacks and tropospheric ozone (O<sub>3</sub>) cause stress and considerable damage in both wild and cultivated plants. Induced defense responses to aphid attacks and O<sub>3</sub> exposure share signaling pathways with common points. We investigated the plant–aphid interaction under O<sub>3</sub> exposure using open-top O<sub>3</sub> chambers. Ozone leaf injury was lower in aphid-infested plants than in aphid-free plants, although herbivore damage was not evident. Aphid population growth was strongly affected by previous exposure to O<sub>3</sub> but no direct effect of O<sub>3</sub> was observed. The possibility that during O<sub>3</sub> episodes, herbivores may reduce O<sub>3</sub> damage on host plants and that the offspring of the exposed aphids have lower population growth rates opens new and intriguing questions about potential effects of future increased tropospheric O<sub>3</sub> levels on plant–insect interactions.

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### 1. Introduction

Aphids damage crop plants due to photo-assimilate consumption and the toxic action of salivary enzymes. Their effect on growth is mainly due to the reduction of nutrient mass flow into the primary apical zone (Pollard, 1973). Harper and Kaldy (1982) found that the pea aphid (*Acyrtosiphon pisum*) infestation reduced growth, stem elongation and dry mass yield of alfalfa (*Medicago sativa*) under field conditions. Aphid-induced reduction in elongation rates results from a complex effect on nutrient fluxes, i.e. reversed nitrogen deposition rates of the growth zone, converted from a sink into a source tissue (Girousse et al., 2005). In response to herbivore damage, plants may express biochemical, physiological or morphological changes known as induced responses, some of which are activated by salivary enzymes (Karban and Myers, 1989). The signal is transmitted throughout the plant, establishing a communication among different plant tissues and conferring systemic acquired resistance (SAR) (Mudgett, 2005). In turn, plant responses may be in some cases beneficial to herbivores, resulting in induced susceptibility (Sauge et al., 2006). For example, avoidance of activation of defense responses by the greenbug aphid through induction of antioxidant proteins was observed in sorghum (Zhu-Salzman et al., 2004). Production of reactive oxygen species (ROS), particularly H<sub>2</sub>O<sub>2</sub>, has repeatedly been associated with defense activation in diverse plant–pathogen and plant–insect interactions (Bruxelles and

Roberts, 2001; Scheel, 2002). Under normal circumstances, the production of ROS-detoxifying enzymes would appear to represent an attempt of the plant to cope with oxidative damage resulting from elevated H<sub>2</sub>O<sub>2</sub> levels. However, their induction after an aphid attack results in an increased aphid population overwhelming the plant anti-stress system (Moran et al., 2002).

The oxidative burst associated with insect attacks is similar to that observed in plants exposed to tropospheric ozone (O<sub>3</sub>). This gas is the leading component of photochemical air pollution (Wohlgemuth et al., 2002; Baier et al., 2005), and its level is expected to triple within the next 40 years (Chameides et al., 1994). O<sub>3</sub>-induced ROS accumulation is concentrated in the periveinal regions of the leaves (Schraudner et al., 1998; Wohlgemuth et al., 2002). It affects cell membranes and produces defined lesions on the adaxial side of the leaf. Symptoms produced by O<sub>3</sub> were originally described in tobacco (*Nicotiana tabacum* L.), and characterized as weather flecks (Wohlgemuth et al., 2002). Some plant species can reduce O<sub>3</sub> damage by the production of volatile isoprenoids, and it has recently been demonstrated that O<sub>3</sub> episodes are actually able to stimulate isoprenoid emission by leaves (Fares et al., 2008).

Despite the vast existing literature on the effects of O<sub>3</sub> on plants and the potential impact on food–web interactions, few studies have investigated plant–insect interactions under elevated O<sub>3</sub> conditions, and even fewer have focused on its impact on crop insect pest outbreaks. Previous studies have demonstrated points of convergence in the signaling circuitry of plants exposed to O<sub>3</sub> and aphid feeding. This “crosstalk” is manifested in common molecular or metabolic responses (Bostock, 2005). It seems clear that O<sub>3</sub>-induced production of ROS could serve as a general alarm

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signal leading to cell death (apoptosis) and expression of defense genes (Rao et al., 2000) to many plant pathogens (Yalpani et al., 1994; Sharma et al., 1996; Langebartels et al., 2002) and insects (Moran et al., 2002). However, studies about aphid responses to elevated O<sub>3</sub> produced variable results (Awmack et al., 2004), with both decreases (Sharma et al., 1996; Von Tiedemann and Firsching, 2000; Holopainen, 2002) or increases (Percy et al., 2002; Kopper and Lindroth, 2003; Awmack et al., 2004) in insect growth or oviposition rate in comparison to those on control plants grown under filtered air, being recorded. It appears that subtle variations in the experimental design and in the particular combination of stressors might influence the outcome of the above results. For example, O<sub>3</sub> effect on aphid population development depends on shoot elongation during O<sub>3</sub> exposure (Holopainen and Kössi, 1998). The induction patterns associated with phloem feeding organisms on the *Brassicaceae* family are still under study, but have been shown to share many aspects of generalized stress responses, which may include a combination of plant defenses through abortion of infested organs, plant protection associated with tolerance, and induced susceptibility of the host plant by the aphids (Sauge et al., 2006). Areas of overlap in aphid induction of plant defense responses with other forms of biotic and abiotic stresses in this plant family are likely (Moran et al., 2002).

The parallels of expression between O<sub>3</sub>-induced and aphid-induced responses in plants, including the increased oxidative stress common to both stimuli, prompted us to test the hypothesis that aphid-infection prior to O<sub>3</sub> exposure might reduce O<sub>3</sub> injury. We tested this hypothesis on arugula (*Eruca sativa* (P. Mill.)), a member of the *Brassicaceae* family. We also studied whether aphids (*Myzus persicae*) from populations that developed on arugula plants exposed to episodes of O<sub>3</sub> contamination were able to establish new infestations on unstressed plants.

## 2. Materials and methods

### 2.1. O<sub>3</sub> exposure in open-top chambers

Experiments were conducted at the IFEVA, Facultad de Agronomía, University of Buenos Aires experimental field (34°35'S, 58°29'W). Average maximum and minimum temperatures during the experimental period were 27.9 and 16.8 °C, respectively. Six 8 m<sup>3</sup> "open-top" chambers with crystal PVC walls mounted on a metal structure and tropospheric O<sub>3</sub> level regulation were used (Hogsett et al., 1985; Lefohn et al., 1986). Open-top chambers are a proven exposure technology in air pollution field research, developed to provide an environment that closely resembles ambient conditions, overcoming the limitations to the conduct of experiments under natural field conditions that was a feature of early O<sub>3</sub> studies (Heagle et al., 1973; Rogers et al., 1983).

Three chambers were used for the control treatment (ozone-free ambient air) and three for the O<sub>3</sub> treatment (charcoal filtered air with added O<sub>3</sub>). O<sub>3</sub> chambers received a daily fumigation of 90 min that increased O<sub>3</sub> concentration to 100–120 ppb. O<sub>3</sub> was generated by a spark discharge-type O<sub>3</sub> generator (Hogsett et al., 1985) and ambient air was pumped through an activated charcoal filter. Ozone was continuously monitored using a Model 450 Ozone Monitor API-Teledyne Instrument (Teledyne Advanced Pollution Instrumentation San Diego, CA).

### 2.2. Plants and aphids

Two arugula seeds were planted in each pot filled with a 50:25:25 soil:peat moss:sand mixture and kept in a glasshouse during 4 weeks. Seedlings were thinned to one per pot after 5 days and were well watered throughout the experiment. Aphids (*M. persicae*) were cultured on 2-week-old arugula plants in a

glasshouse under controlled temperature conditions (24–26 °C). New plants were added at weekly intervals as older, damaged, plants were removed.

### 2.3. Experimental procedures

Two experiments, each repeated twice, were carried out to investigate the effects of aphid/host plant interactions in response to O<sub>3</sub> exposure. In the first experiment, 4 weeks after planting four arugula plants were infested with six aphids per plant, and placed in each open-top chamber (both ozone and control), together with four uninfested plants. All plants from every treatment were covered with individual voile bags to prevent aphid migration between plants. The number of aphids per plant was recorded every other day. Plant lesions and chlorotic leaf symptoms were visually estimated as percent reduction of green tissue. Plants were harvested after 10 days; roots were carefully washed and separated from the shoots, and both were dried at 70 °C and weighed.

In the second experiment a group of naturally infested arugula plants with 20–30 aphids was exposed to O<sub>3</sub>, 4 h a day during 3 consecutive days, while another group of infested plants remained in the control chambers. Immediately after O<sub>3</sub> exposure nymphs were transferred to 3-week-old plants that had never been either infested or exposed to O<sub>3</sub> before. Six aphids were placed on each plant and counted 5, 10 and 15 days after the beginning of the experiment.

### 2.4. Production of antioxidants

An experiment was set up to determine the production of antioxidants by aphid-infested arugula plants. Aphid induction of antioxidants in leaves was estimated through two parameters of oxidative stress: thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation (Yagi, 1976), and total radical-trapping antioxidant potential (TRAP) (Lissi et al., 1992). Each plant was originally infested with 20 aphids, and the aphid population was later allowed to increase without restriction. Leaf samples were harvested and frozen immediately after aphid infestation, and 3 and 6 days later. The chain reactions that occur during lipid oxidation lead to peroxide formation, and secondary products like aldehydes. A colorimetric technique was used by which the thiobarbituric acid joined to malondialdehyde (MDA) forms a Schiff base with an absorption peak at 532 nm. The TRAP index reflects the free radicals that react with a solution of antioxidants and so it is a measure of the quantity of antioxidants in a sample.

### 2.5. Statistics

Percent of green tissue and shoot–root rate of plants growing under the different treatments were analyzed by means of split-plot design analysis of variance (ANOVA). Main-plot effects were the atmospheric treatments (O<sub>3</sub>-free vs. elevated). The subplot factor was herbivory (presence vs. absence of aphids). All interactions between ozone and herbivory were included as subplot interactions. As experimental replication of the fumigation treatment involves an entire open-top chamber, individual plants within each chamber are not true replicates. As a result, the plant response variables for each treatment pot were calculated. Then we obtained the mean value of plant biomass and percentage of green tissue per chamber on each sampling date. The effect of O<sub>3</sub> exposure on aphid population growth was analyzed by repeated measures ANOVA, and one way ANOVA was used to compare antioxidant production of aphid-infested and uninfested plants. InfoStat Professional, Argentina (InfoStat, 2008) was used for all statistical analyses.

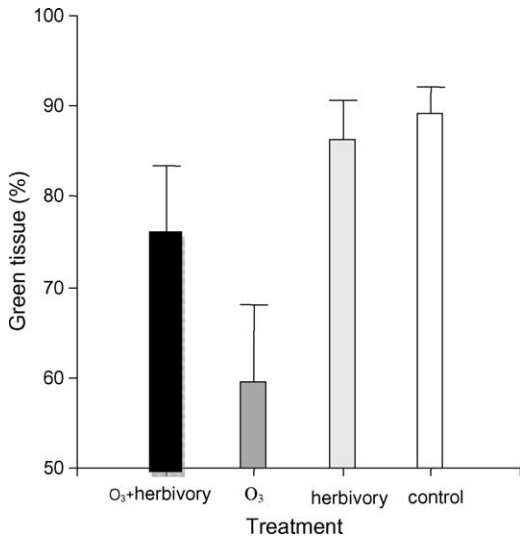


Fig. 1. Leaf damage caused by aphid infestation and O<sub>3</sub> exposure, estimated as remaining percentage of green tissue (mean + SE).

3. Results

All plants exposed to O<sub>3</sub> developed typical O<sub>3</sub> injury symptoms (necrotic patches and mottling on the leaf surfaces). The reduction in green leaf tissue due to O<sub>3</sub> damage was lower in aphid-infested plants (interaction  $P = 0.03$ ) (Fig. 1, Table 1). However, the proportion of green tissue in leaves of infested plants did not differ from the control ( $P = 0.10$ ) (Fig. 1, Table 1).

Plant size and allometry were affected by both biotic and abiotic stress factors. O<sub>3</sub> exposure and aphid feeding produced a 4–5-fold reduction in plant biomass, mainly due to a decrease in root growth (Table 1). As a result, shoot–root ratios of aphid-infested plants were twice that of the control (Fig. 2, Table 1).

The growth of the aphid population was similar in O<sub>3</sub> and O<sub>3</sub>-free environments ( $P > 0.05$ ) (Fig. 3). However, aphid population growth in ozone-free environments was strongly influenced when aphids came from plants that had been exposed to ozone ( $P = 0.0059$ ).

At the end of the experimental period the level of aphid infestation of the O<sub>3</sub>-challenged arugula plants was one half of that observed for the aphid population growing on control plants (Fig. 4).

After 3 days of aphid infestation, the level of TBARS in infested leaves was higher than that of the control plant tissues ( $P < 0.05$ ) but returned to control levels 6 days after the start of infestation ( $P > 0.05$ ) (Table 2). TRAP from aphid-infested plant tissues remained always significantly lower ( $P = 0.01$ ) than those from control plants; three and 2-fold lower for the shortest and the longest aphid-infection period, respectively (Table 1). No damage was observed on the foliage of aphid-infested plants.

4. Discussion

The response induced by aphid attack strongly decreased the level of tissue injury resulting from several episodes of O<sub>3</sub>

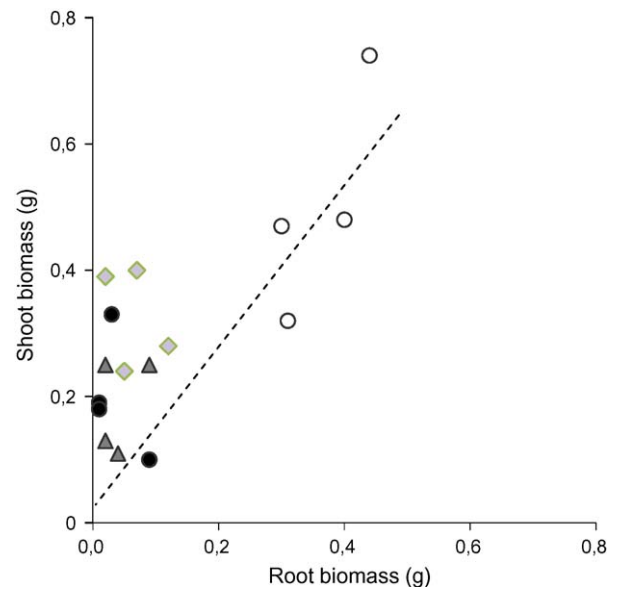


Fig. 2. Aphid infestation (◇), O<sub>3</sub> exposure (△), and aphids + O<sub>3</sub> (●) modulate shoot/root biomass relationship of arugula plants. The dashed line represents the fitted linear regression model for the control plants (○) ( $R^2 = 0.66$ ).

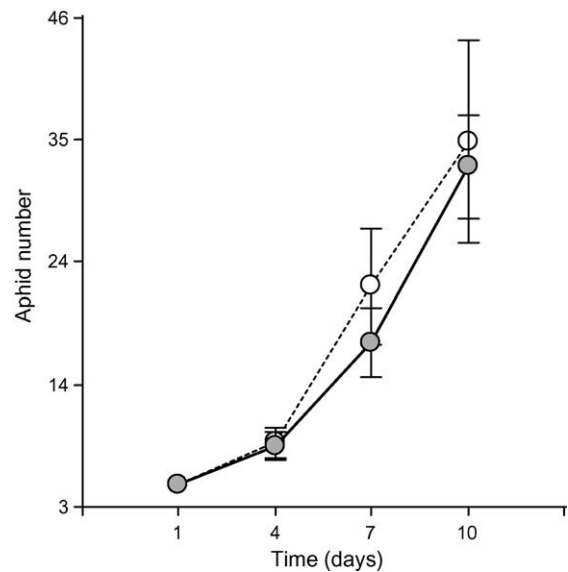
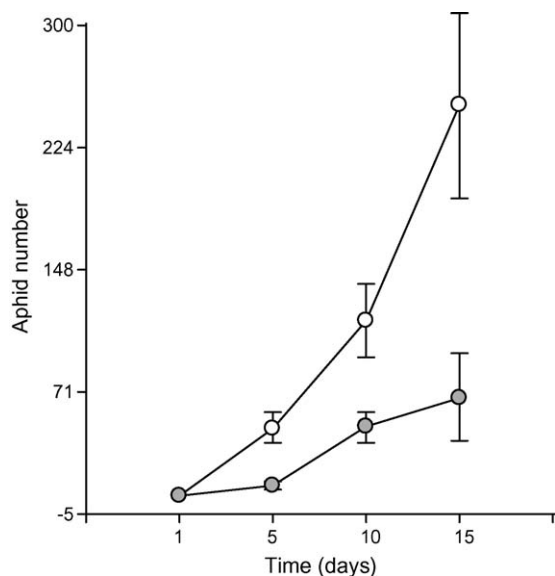


Fig. 3. Growth of the aphid population in O<sub>3</sub> (●) and O<sub>3</sub>-free (○) environments.

exposure, even though the growth of the aphid population was negatively influenced by the pollutant. Our assessment of plant responses to the interaction of both stressors was done under field conditions including daily and seasonal weather fluctuations. Although an agroecosystem and its environment cannot be completely recreated within the open-top chamber, plants and aphids can be grown under realistic field conditions (Strain and Thomas, 1992). The arugula plants used in our experiments were

Table 1 The effect of ozone exposure (O<sub>3</sub>) and aphid feeding (herbivory) on the growth of Arugula plants, as determined by split-plot ANOVAS.

Treatment	Total biomass (g)		Root biomass (g)		Shoot/root ratio		Green leaf tissue (%)	
	F	P	F	P	F	P	F	P
O <sub>3</sub>	33.07	0.001	63.50	0.0002	1.72	0.23	5.68	0.07
Herbivory	11.45	0.01	36.30	0.0009	5.91	0.05	2.7	0.10
O <sub>3</sub> × Herbivory	12.20	0.01	32.82	0.0012	0.01	0.09	467	0.03



**Fig. 4.** O<sub>3</sub> effect on aphid population growth. Aphids were exposed to O<sub>3</sub> (●) or O<sub>3</sub>-free (○) conditions and then transferred to the control plants.

**Table 2**

Parameters of oxidative stress measured in arugula plants after varying periods of aphid infestation. TBARS: thiobarbituric acid reactive substances and TRAP: total radical-trapping antioxidant potential.

Treatment	TBARS (μM/g leaf)	TRAP (μM)
Control	1,15 ± 0,2	111 ± 15
After 3 days infestation	4,65 ± 1,1 <sup>**</sup>	38 ± 7 <sup>**</sup>
After 6 days infestation	1,98 ± 0,1	53 ± 8 <sup>*</sup>

<sup>\*</sup> P < 0.05 (F-test).

<sup>\*\*</sup> P < 0.01 (F-test).

highly sensitive to O<sub>3</sub>. Exposed plants developed typical visible symptoms of injury consisting of necrotic patches and mottling on the leave surface. It could be argued however, that an important limitation of the OTCs is the elevation of air temperature that can occur within them, because reduced air movement in the chambers compared to open areas, can lead to greater solar heating (Olszyk et al., 1980). Thus the increased chamber temperatures can confound the responses to the primary variable of interest and compromise experimental objectives. Although the same temperature elevation should occur in all chambers (elevated ozone and control), the relative sensitivity of plants to ozone could be difficult to predict from their sensitivity determined in OTCs. Some experiments have addressed these critical questions, and no interactions were found at least for CO<sub>2</sub>-temperature (Norby et al., 1999). Nevertheless temperature monitoring during the experimental period revealed that differences between temperature inside and outside the chambers never exceeded 1.5 °C.

Aphid feeding did not cause any visible leaf damage. This is consistent with results of experiments performed with another species of the same plant family (*Arabidopsis thaliana*), which showed lack of necrosis or other symptoms after 72–96 h of *M. persicae* infestation (Moran et al., 2002). This could reflect the maintenance of a favorable oxidative-reduction condition in the plant by the aphid salivary sheaths (Miles and Oertli, 1993). Size and allometry of arugula plants were impacted by both biotic and abiotic stress factors. Stress reduced individual plant growth. In the aphid-infested plants in particular, the magnitude of the reduction was greater for roots than shoots, suggesting that compensation

growth and repair metabolism preventing leaf damage may compromise root growth. However, aphid-produced hormonal changes could be also responsible for higher shoot–root ratios in aphid-infested plants (Ilarduya et al., 2003). This could explain in part the reduction of O<sub>3</sub> damage on plants pre-infested with aphids, which increased the antioxidant production in those plants. Exposure to biotic or abiotic stress factors generally induces ROS production in cells that in turn activates an antioxidant system (Perl-Treves and Perl, 2002). Ilarduya et al. (2003) reported that aphids induced biochemical and physiological changes in the host plants that included ROS production and Gomez et al. (2004) argued that, depending on the aphid–plant combination, these changes included an increase in the foliar antioxidant response. In our study, ROS production in the aphid-infested plants is supported by the high TBARS levels. In turn the low TRAP levels detected on aphid-infested plants might be an indicator of antioxidant consumption and neutralization of the oxidative burst, which correlates with the absence of visible foliar damage in infested plants and the lower leaf injury in O<sub>3</sub>-exposed plants. Moreover, herbivores might increase the emission of volatile compounds with an O<sub>3</sub>-scavenging activity, contributing to the protection against O<sub>3</sub> damage. Some Mediterranean *Quercus* species emit isoprenoids such as monoterpenes, with antioxidant activity which allows these plants to scavenge O<sub>3</sub> at leaf level (Loreto et al., 2004; Fares et al., 2008). Production of those isoprenoids is stimulated by O<sub>3</sub> episodes (Fares et al., 2008). Herbivores, i.e. mites, can induce the emission of volatile organic compounds, similar to those induced by O<sub>3</sub> (Vuorinen et al., 2004). Arugula plants produce several aromatic compounds (Mastelic et al., 2008; Jirovetz et al., 2002); however, in our experiments arugula plants exposed to O<sub>3</sub> expressed unmistakable foliar injuries. This suggests that the volatiles produced by O<sub>3</sub> exposure did not have scavenging activity, or they were not produced in sufficient amount.

Aphid nymphs born from adults exposed to and feeding on O<sub>3</sub>-exposed plants were less capable of infesting new plants than those nymphs from the control plants. This could be a consequence of the direct effect of O<sub>3</sub> on aphids, an indirect effect through the plant response to O<sub>3</sub> or a combination of both. Because aphids were able to maintain population growth rates on O<sub>3</sub>-exposed plants similar to those growing on untreated control plants, it seems that host facilitation induced by aphids would hide the negative effect on the aphids caused by O<sub>3</sub>, which became apparent when the insects were transferred to unstressed plants. Previous studies have suggested that O<sub>3</sub> by itself produces no effect on insect ability to reproduce or feed (Agrell et al., 2005). This information and our results are consistent with the idea that the reduced ability of the insect population to colonize and infest new plants was the result of aphid–plant interaction rather than the direct effect of the O<sub>3</sub> on the individuals.

The integration of studies on plant performance, responses and insect behavior, coupled with the biochemical approach used here seems useful to better define plant–aphid interactions under O<sub>3</sub> stress. Our experiments bring new evidence that aphids may not only reduce O<sub>3</sub> damage on plants, but also that aphid attack intensity could be modified in areas with periods of high O<sub>3</sub> concentration. On the other hand, this model takes no account of the potential adaptation of aphids to atmospheric changes. It is possible that more O<sub>3</sub>-tolerant clones will prosper as the less-tolerant currently present decline. This information has implications for pest outbreaks and merits further investigation. It could be expected that the convergence of responses to different stressors in agricultural systems, inducing oxidative stress in plant tissues, might render a plant phenotype functionally adaptive or maladaptive to a particular stress. A better understanding of these interactions will provide insight into the role of

aphids in ecological communities, and will lead to the development of novel and durable ways to manage biotic and abiotic resistance traits for crops.

## 5. Conclusions

Our results provide evidence that plant responses to insect herbivore will be strongly influenced by future levels of tropospheric ozone. The outcome of plant–aphid–O<sub>3</sub> interactions will depend, at the molecular level, on the balance between ROS and antioxidant production, resulting from the shared responses to both stressors. This balance will depend not only on the relative magnitude, but also on the timing and order of the stress factor. Stressor interactions were evident also at the individual plant level and in aphid population growth. However, the stochasticity in the occurrence of high levels of ozone and aphid plant challenge makes it difficult to make inferences of the consequences of these interactions at individual and population levels.

## Acknowledgments

A.I. Menéndez holds a scholarship from University of Buenos Aires. This work was supported by grants PICT 20306 of the Agencia Nacional de Promoción Científica y Tecnológica of Argentina, and UBACyT G063 of the University of Buenos Aires.

## References

- Agrell, J., Kopper, B., McDonald, E.P., Lindroth, R.L., 2005. CO<sub>2</sub> and ozone effects on host plant preferences of the forest tent caterpillar. *Global Change Biol.* 11, 588–599.
- Awmack, C.S., Harrington, R., Lindroth, L., 2004. Aphid individual performance may not predict population responses to elevated CO<sub>2</sub> or O<sub>3</sub>. *Global Change Biol.* 10, 1414–1423.
- Baier, M., Kandlbinder, A., Gollack, D., Dietz, K., 2005. Oxidative stress and ozone: perception, signaling and response. *Plant Cell Environ.* 28, 1012–1020.
- Bostock, R.M., 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. *Ann. Rev. Phytopathol.* 43, 545–580.
- Bruxelles, G.L., Roberts, M.R., 2001. Signals regulating multiple responses to wounding and herbivores. *Plant Sci.* 20, 487–521.
- Chameides, W.L., Kasibhatla, P.S., Yienger, J., Levy, H.I., 1994. Growth of continental-scale metro-agro-plexes, regional ozone pollution, and world food production. *Science* 264, 74–77.
- Fares, S., Loreto, F., Kleist, E., Wildt, J., 2008. Stomatal uptake and stomatal deposition of ozone in isoprene and monoterpene emitting plants. *Plant Biol.* 10, 44–54.
- Girousse, C., Moulia, B., Silk, W., Bonnemaïn, J.L., 2005. Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. *Plant Physiol.* 137, 1474–1484.
- Gomez, S.K., Oosterhuis, D.M., Rajguru, S.N., Johnson, D.R., 2004. Molecular biology and physiology foliar antioxidant enzyme responses in cotton after aphid herbivory. *J. Cotton Sci.* 8, 99–104.
- Harper, A.M., Kaldy, M.S., 1982. Effect of the pea aphid, *Acyrtosiphon pisum* (Hemiptera: Homoptera: Aphididae) on yield and quality of forage alfalfa. *Can. Entomol.* 114, 485–489.
- Heagle, A.S., Body, D.E., Heck, W.W., 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2, 365–368.
- Hogsett, W.E., Tingey, D.T., Colman, S.R., 1985. A programmable exposure control system for determination of the effects of pollutant exposure regimes on plant growth. *Atmos. Environ.* 19, 1135–1145.
- Holopainen, J.K., 2002. Aphid response to elevated ozone and CO<sub>2</sub>. *Entomol. Exp. Appl.* 104, 137–142.
- Holopainen, J.K., Kössi, S., 1998. Variable growth and reproduction response of the spruce shoot aphid, *Cinara pilicornis*, to increasing ozone concentrations. *Entomol. Exp. Appl.* 87, 109–113.
- Ilarduya, O.M., QiGuang, X., Kaloshian, I., 2003. Aphid induced defense response in Mi-1-mediated compatible and incompatible tomato interactions. *Am. Phytopathol. Soc.* 16, 699–708.
- InfoStat, 2008. Infostat version 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- Jirovetz, L., Smith, D., Buchbauer, G., 2002. Aroma compound analysis of *Eruca sativa* (Brassicaceae) SPME headspace leaf samples using GC-MS, and olfactometry. *J. Agric. Food Chem.* 50 (16), 4643–4646.
- Karban, R., Myers, J.H., 1989. Induced plant responses to herbivory. *Ann. Rev. Ecol. Syst.* 20, 331–348.
- Kopper, B.J., Lindroth, R.L., 2003. Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* 134, 95–103.
- Langebartels, C., Wohlgenuth, H., Kschieschan, S., Grün, S., Sandermann, H., 2002. Oxidative burst and cell death in ozone-exposed plants. *Plant Physiol. Biochem.* 40, 567–575.
- Lefohn, A.S., Hogsett, W.E., Tingey, D.T., 1986. A method for developing ozone exposures that mimic ambient conditions in agricultural areas. *Atmos. Environ.* 20, 361–366.
- Lissi, E., Pascual, C., Del Castillo, M., 1992. Luminol luminescence induced by 2,2'-Azo-bis (2-amidinopropane) thermolysis. *Free Radic. Res. Commun.* 17, 299–311.
- Loreto, F., Pinelli, P., Manes, F., Kollist, H., 2004. Impact of ozone on monoterpene emissions and evidence for an isoprene-like antioxidant action of monoterpenes emitted by *Quercus ilex* leaves. *Tree Physiol.* 24, 361–367.
- Mastelic, J., Jerkovic, I., Blazjevic, I., Radonic, A., Krstulovic, L., 2008. Hydrodistillation-adsorption method for the isolation of water-soluble, non-soluble and high volatile compounds from plant materials. *Talanta* 76, 885–891.
- Miles, P.W., Oertli, J.J., 1993. The significance of antioxidants in the aphid–plant interaction: the redox hypothesis. *Entomol. Exp. Appl.* 67, 273–285.
- Moran, P.J., Cheng, Y., Cassell, J.L., Thompson, G.A., 2002. Gene expression profiling of *Arabidopsis thaliana* in compatible plant–aphid interactions. *Arch. Insect Biochem. Physiol.* 51, 182–203.
- Mudgett, M.B., 2005. New insights to the function of phytopathogenic bacterial type III effectors in plants. *Ann. Rev. Plant Biol.* 56, 509–531.
- Norby, R.J., Wullschlegel, S.D., Gunderson, C.A., Johnson, D.W., Ceulemans, R., 1999. Tree responses to rising CO<sub>2</sub> in field experiments: implications for the future forest. *Plant Cell Environ.* 22, 683–714.
- Olszyk, D.M., Tibbitts, T.W., Hertsberg, W.M., 1980. Environment in open-top field chambers utilized for air-pollution studies. *J. Environ. Qual.* 9, 610–615.
- Percy, K.E., Awmack, C.S., Lindroth, R.L., 2002. Altered performance of forest pests under atmospheres enriched by CO<sub>2</sub> and O<sub>3</sub>. *Nature* 420, 403–407.
- Perl-Treves, R., Perl, A., 2002. Oxidative stress: An introduction. In: Inzé, D., Van Montagu, M. (Eds.), *Oxidative Stress in Plants*. Taylor and Francis, pp. 1–32.
- Pollard, D.G., 1973. Plant penetration by aphid feeding aphids (Hemiptera, Aphidoidea): a review. *Bull. Entomol. Res.* 62, 631–714.
- Rao, M.V., Koch, J.R., Davis, K.R., 2000. Ozone: a tool for probing programmed cell death in plants. *Plant Mol. Biol.* 44, 345–358.
- Rogers, H.H., Heck, W.W., Heagle, A.S., 1983. A field technique for the study of plant responses to elevated carbon dioxide concentrations. *J. Air Pollution Control Assoc.* 33, 42–44.
- Sauge, M.-H., Mus, F., Lacroze, J.-P., Pascal, T., Kervella, J., Poëssel, J.-L., 2006. Genotypic variation in induced resistance and reduced susceptibility in the peach-Myzus persicae aphid system. *Oikos* 113 (2), 305–313 (9).
- Scheel, D., 2002. Oxidative burst and the role of reactive oxygen species in plant–pathogen interactions. In: Inzé, D., Van Montagu, M. (Eds.), *Oxidative Stress in Plants*. pp. 137–153.
- Schraudner, M., Moder, W., Wiese, C.W., van, Camp, Inze, D., Langebartels, C., 1998. Ozone induced oxidative burst in the ozone biomonitor plant tobacco Bel W3. *Plant J.* 16, 235–245.
- Sharma, Y.K., León, J., Raskin, I., Davis, K.R., 1996. Ozone induced responses in *Arabidopsis thaliana*: the role of SA in the accumulation of defense-related transcripts and induced resistance. *Plant Biol.* 93, 5099–5104.
- Strain, B.R., Thomas, R.B., 1992. Field measurements of CO<sub>2</sub> enhancement and climate change in natural vegetation. *Water, Air, Soil Pollut.* 64, 45–60.
- Von Tiedemann, A.V., Firsching, K.H., 2000. Interactive effects of elevated ozone and carbene dioxide on growth and yield of leaf rust-infected versus non-infested wheat. *Environ. Pollut.* 108, 357–363.
- Vuorinen, T., Nerg, A.M., Holopainen, J.K., 2004. Ozone exposure triggers the emission of herbivore-induced plant volatiles, but does not disturb tritrophic signaling. *Environ. Pollut.* 131, 305–311.
- Wohlgenuth, H., Mittelstrass, K., Kschieschan, S., Bender, J., Weigel, H.-J., Overmyer, K., Kangasjarvi, J., Sandermann, H., Langebartels, C., 2002. Activation of oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. *Plant Cell Environ.* 25, 717–726.
- Yagi, K., 1976. A simple fluorometric assay for lipoperoxide involved in blood plasma. *Biochem. Med.* 15, 212–216.
- Yalpani, N., Enyedi, A.J., León, J., Raskin, I., 1994. Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance in tobacco. *Planta* 193, 373–376.
- Zhu-Salzman, K., Salzman, R.A., Ji-Eun, A., Hisashi, K., 2004. Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiol.* 134, 420–431.