Ozone impedes the ability of a herbivore to find its host

Jose D Fuentes1, T’ai H Roulston2 and John Zenker1

1 Department of Meteorology, The Pennsylvania State University, University Park, PA 16802, USA
2 Department of Environmental Sciences, University of Virginia and Blandy Experimental Farm, 400 Blandy Farm Lane, Boyce, VA 22620, USA

E-mail: jdfuentes@psu.edu

Received 10 October 2012
Accepted for publication 4 March 2013
Published 26 March 2013
Online at stacks.iop.org/ERL/8/014048

Abstract
Plant-emitted hydrocarbons mediate several key interactions between plants and insects. They enhance the ability of pollinators and herbivores to locate suitable host plants, and parasitoids to locate herbivores. While plant volatiles provide strong chemical signals, these signals are potentially degraded by exposure to pollutants such as ozone, which has increased in the troposphere and is projected to continue to increase over the coming decades. Despite the potential broad ecological significance of reduced plant signaling effectiveness, few studies have examined behavioral responses of insects to their hosts in polluted environments. Here, we use a laboratory study to test the effect of ozone concentration gradients on the ability of the striped cucumber beetle (Acalymma vittatum) to locate flowers of its host plant, Cucurbita foetidissima. Y-tube experiments showed that ozone mixing ratios below 80 parts per billion (ppb) resulted in beetles moving toward their host plant, but levels above 80 ppb resulted in beetles moving randomly with respect to host location. There was no evidence that beetles avoided polluted air directly. The results show that ozone pollution has great potential to perniciously alter key interactions between plants and animals.

Keywords: air pollution, pollinators, floral volatiles

1. Introduction
Insects detect odor with olfactory neuron receptors (ORNs), which are located in their antennae. These receptors are capable of sensing plant-emitted volatile organic compounds (VOCs) in trace amounts, with as low as six molecules impinging on antennae (Angioy et al 2003). In many cases insects can detect a scent plume from distances greater than several hundred meters (Wenner et al 1991). However, in polluted air masses VOCs react with ozone, hydroxyl radical, and nitrate radicals, thereby reducing the integrity and the strength of the scents. In polluted environments it may be harder for the ORNs to be stimulated for insects located downwind from VOCs sources.

Olfactory responses to VOCs play several roles in plant–insect interactions. Most familiar of these functions is pollinator attraction. Pollinators follow both floral scents and visual cues to flowers and pollinate while gathering pollen and nectar. Floral scents can be either a learned stimulus that generalist pollinators associate with food (Arenas and Farina 2012) or an innate stimulus that specialist pollinators recognize as food upon emerging as adults (Dobson 1987). Either way, scent detection plays a key role in pollinator foraging efficiency and subsequently pollination. Similarly, herbivorous insects may follow the scent plumes of either floral (Theis and Adler 2012) or foliar (Bruce et al 2005) volatiles to locate their host plants. While most herbivores appear to respond to particular blends of chemical compounds rather than unique compounds (Bruce et al 2005), degradation of individual components in blends potentially renders host scent plumes unrecognizable. The potential disruption of plant–insect interactions is not limited to simple 2-way
interactions, however. Parasitoids that attack herbivores often use volatiles produced by damaged plants as cues to find their hosts (e.g. Penaflor et al 2011). Thus, interactions between plants and both their mutualists and antagonists depend on efficient signaling through VOCs.

At present, little research has been carried out about the effects of air pollution on plant signaling. Preliminary research (McFrederick et al 2008, Pinto et al 2010) shows that VOCs involved in plant–insect interactions are reactive with pollutants such as ozone. Since the industrial revolution, changes in ambient pollution levels may have reduced the range at which insects detect VOCs emanating from their host (McFrederick et al 2009, 2008, Pinto et al 2010). Manipulative experiments, however, have been scarce. Pinto et al (2007a) found that ozone completely destroyed some types of herbivore induced VOCs in Brassica oleracea and Phaseolus lunatus. Parasitoids, however, were still able to locate their hosts, presumably because other VOCs not degraded by ozone provided sufficient signal. In a follow-up study using Brassica oleracea as the plant host, Pinto et al (2007b) found that ozone reduced the attractiveness of a parasitoid to herbivore-induced volatiles, but again some volatile-based signals remained to induce a lesser response in parasitoids. Himanen et al (2009) found that ozone substantially reduced the ability of parasitoids to orient to herbivorous hosts on transgenic Brassica napus. In lima beans, damage by herbivores causes plants to increase production of extra floral nectar that recruits natural enemies to their defense (Blande et al 2010). The damaged plants also release VOCs that stimulate extra floral nectar production in neighboring, undamaged plants. In ozonated environments, damaged plants still produce VOCs, but the distance at which neighboring plants are induced to produce extra floral nectar is reduced (Blande et al 2010), presumably because of the degradation of VOCs. Thus, from previous laboratory studies, there is evidence that degradation of VOCs, as predicted, does occur, but that there is some system specific variation in the susceptibility of the key VOC profile to degradation by ozone. As of yet, there are no studies on either herbivore or pollinator detection of host plants under ozone elevated conditions.

In the current laboratory study we test whether a specialist herbivore, the striped cucumber beetle (SCB), can still orient to host flowers when the floral volatile stream of its host is exposed to increasing ozone concentrations. Specifically, we ask:

(1) Do host flower volatiles, relative to clean air, remain attractive to SCBs under an increasing ozone concentration gradient?
(2) Do SCBs distinguish between two host flower volatile streams when one volatile stream is reacted with an increasing ozone gradient?
(3) Do SCBs respond to ozone itself, independent of host flower volatiles?

Together, this set of questions tests whether the interaction between a specialized herbivore and its host is likely to be affected by air pollution. It adds important information to a small set of studies on degradation of plant signaling and provides the first data on herbivore response to degraded signals.

2. Methods and materials

2.1. Study system

The striped cucumber beetle, Acalymma vitatum (F.) is a specialist herbivore of plants in the family Cucurbitaceae and uses floral volatiles to find its host plant (Andrews et al 2007). It is considered an important agricultural pest of cucurbit crops because of its role as a disease vector (Ellers-Kirk and Fleischer 2006). The beetles were collected daily from flowers of Cucurbita pepo in a community garden at Blandy Experimental Farm in Boyce, Virginia and maintained without food in individual vials in the laboratory. The flowers used to generate the floral volatiles for testing came from Cucurbita foetidissima plants grown in a herbivore-free enclosure, located ~25 m away from the laboratory.

2.2. Experimental approach

We used Y-tube olfactometry to assess the behavioral response of SCBs when presented a choice between two movement pathways, each carrying an air stream of similar velocity but different volatile profiles. All air streams were composed of clean air that was either left unchanged or treated with different combinations of floral volatiles and ozone. While this approach does not allow us to determine which volatiles are being degraded by ozone and which are key in the response of our study insect, it does provide a strong test of the effect of ozone exposure to the entire floral volatile profile on the ability of an insect that relies on that profile to find its host.

2.3. Experimental apparatus

Two separate air streams were used as inputs into the test system: filtered ambient air versus filtered ambient air admixed with 0–120 ppb ozone (figure 1). Flow of clean air was carried out by a pump system (Stimulus Controller CS-55, Syntech Inc., Germany). A 2.5 l reaction chamber was placed downstream of the ozone input to facilitate mixing of floral volatiles with ozone. The flower was placed upstream of the ozone rather than in the reaction chamber to avoid direct reactions between ozone and plant tissue. An ozone generator (Model 165, Thermo Scientific Inc., Pittsburg, PA) produced the desired ozone mixing ratios. Ozone levels were monitored using an ozone gas analyzer (Model 202, 2B Technologies Inc., Boulder, CO). Ozone analyzer and generator had their own mass flow controller to regulate the airflows. Airflows of each stream varied with the experimental setup to account for diversions in the system (e.g., half the ozone output went to the ozone analyzer), but the output airflow through each arm of the Y-tube was 1 l min⁻¹. To regulate the airflow through one of the arms of the Y-tube, a stainless steel needle valve was placed between the flower chamber and the Y-tube. To conduct the insect experiments, a clean glass Y-tube with a
15 cm long trunk and two 7 cm long arms was connected to the system using 1/4′′ Teflon tubing. Three experimental setups were used for the three experiments. We used two different setups to test if ozone influenced a cucumber beetle’s movement toward its host flower. First, we compared insect response to clean air versus floral volatiles + ozone to test if an increasing ozone gradient made a host floral stream indistinguishable from clean air. In this setup, half the airflow went directly to the Y-tube and half was diverted into a flower chamber then a reaction chamber, where the floral volatile stream mixed approximately 1:1 with the ozone stream before passing to the Y-tube (figure 1(A)). The air stream out of the reaction chamber and into the Y-tube was 1 l min$^{-1}$ and included ozonated air plus volatiles. The second setup testing whether ozone influenced floral recognition compared insect response to two air streams coming from the same flower chamber, one subsequently mixing with ozone, one going directly to the Y-tube (figure 1(B)). This tested whether increasing amounts of ozone would lead to increasing amounts of preference for the non-ozonated stream. Although this setup diluted the ozonated floral stream 1:1 at all ozone mixing ratios while the non-ozonated stream was not diluted, there was no preference for the undiluted stream at low ozone levels. Thus, when volatiles were well above the detection threshold, a difference in concentration of this magnitude apparently did not result in a preference on its own. In a study of chemo-orientation behavior of eastern tent caterpillars, Peterson and Fitzgerald (1991) found that two attractive scent trails could differ by an order of magnitude without caterpillars showing a preference for the higher concentration, while a 4:1 dilution gradient would induce preference at lower concentrations. To test if ozone itself repels cucumber beetles, we compared insect response between clean air and 20–120 ppb ozone (figure 1(C)).

2.4. Experimental protocol

Cucumber beetles werestarved for at least one hour before they were used for trials. The insects were then placed in the mouth of the tube at which point a rubber stopper (with a hole to allow air flow) was used to cap the Y-tube. The Y-tube was also covered with a small blanket to calm the insect and reduce visual cues, thus promoting navigation through olfaction rather than vision. The Y-tube was rotated 180° about its axis after every other trial to prevent directional biases caused by background conditions in the laboratory. Insects were observed for five minutes. A choice was recorded for that insect if it crossed the halfway point of an arm of the Y-tube. If no choice was made after 5 min then that trial was discarded. Each experiment testing insect response to ozonated air with host flower volatiles was carried out for 30 completed trials, while the response to ozone itself was carried out for 20 trials. The Y-tube was thoroughly cleaned out and dried with ethanol every five trials.

2.5. Statistics

A chi squared test comparing the proportion of choices made to each treatment under a given ozone level was carried out using the software package R (R Development Core Team, 2005, Vienna, Austria), assuming an expected ratio of 50%.

3. Results

Striped cucumber beetles chose to move toward their host flower over 80% of the time when presented with the choice of clean air versus air with host flower volatiles (figure 2). As ozone levels increased, however, they chose their host flower
Figure 2. Y-tube choices by striped cucumber beetles when presented with ozonated floral volatiles versus clean air. Asterisks denote statistical significance (* indicates $p < 0.05$).

Figure 3. Y-tube choices by striped cucumber beetles when presented with ozonated floral volatiles versus non-ozonated floral volatiles. Asterisks denote statistical significance (* indicates $p < 0.05$, ** $p < 0.01$).

less frequently. At ozone mixing ratios of 80 ppb and above they no longer chose their host flower significantly more often than clean air (figure 2). When presented with ozonated versus non-ozonated host flower volatiles, their choices were random at low ozone mixing ratios but became increasingly biased toward non-ozonated air with host flower volatiles as ozone levels increased (figure 3). At ozone levels of 80 ppb and above they significantly chose non-ozonated air with host flower volatiles. The SCBs showed no aversion to ozone itself at any tested levels (figure 4).

4. Discussion

As plant-emitted volatiles are transported across landscapes they can undergo chemical reactions with ozone and other oxidants. Since the late 1800s, ground-level ozone levels have increased (Marenco et al 1994). In the United States, with the continued changes in airborne pollutants and in some cases increases in ozone levels (e.g. Cooper et al 2010, Fiore et al 2002) the modified air chemistry can perniciously interfere with the volatiles’ ecological roles. Increased levels of air pollution weaken the scent signals because of enhanced chemical reactions. Such chemical transformations are likely to have negative effects on both emitting plants and intended recipients of scent signals. While air pollution levels in major urban centers in the United States are declining (Parrish et al 2011), airborne pollutants in rural environments are increasing and may continue to increase in response to enhanced long-range transport of polluted air masses from Asia (e.g. Cooper et al 2010). Such elevated pollution levels in the rural atmosphere will have detrimental implications for insect communities that rely on plant signaling to locate food.

The levels of ozone tested in this study (0–120 ppb) are routinely observed during the summer time in locales such as the mid-Atlantic region of the United States (Parrish et al 2011). With some regions projected to have 24 h mean levels above 70 ppb by the year 2100 (Sitch et al 2007), plant–insect interactions may be substantially affected. We found that the ozone mixing ratio of approximately 80 ppb represented the threshold level above which the herbivore could not distinguish its host plant from clean air. In their study of interplant signaling of lima beans, Blande et al (2010) also found 80 ppb to be the ozone level above which ecological effects on signaling could be distinguished. Our finding that cucumber beetles did not distinguish their host plant from clean air in the presence of ozone indicates that ecological interactions that utilize insect recognition of plant VOCs could collapse, or at least become extremely inefficient, as a long distance cue is removed from the environment. Our finding that ozone itself did not influence movement behavior indicates that the primary driver of our results is the chemical destruction of floral VOCs. While we did not find any effect of ozone on insect response at lower ozone mixing ratios, it is possible that our experimental design underestimates the effect of ozone. Our study insect was located very close to the source of volatiles, and the volatiles were directed to the insect through an enclosed tube. This volatile stream was likely more concentrated than those found at some distance from host plant patches in nature due to division of the volatile stream by wind currents and diffusion of the volatile compounds in open environments. With a less concentrated volatile stream, lower ozone mixing ratios could be sufficient to lower the concentration below detection.
Although elevated ozone levels may reduce herbivore damage to some plants, they could disrupt mutualistic interactions at the same time. In the case of cucurbits, the striped cucumber beetle uses the chemical compound indole as a key attractant (Andrews et al 2007). Indole is reactive with ozone (Atkinson et al 1995), and thus less likely to attract cucumber beetles in polluted environments. Squash bees, important pollinators of squash and pumpkins (Julier and Roulston 2009), do not use indole as an attractant but do use cinnamaldehyde emitted by flowers. Cinnamaldehyde is less reactive than indole, and thus polluted environments could reduce the odor plume of indole more than cinnamaldehyde. Both insects also respond positively to 1,2,4-trimethoxybenzene, which reacts with ozone to an intermediate extent. Thus, in this example, the cues of the pest may be more affected by ozone than the cues of the mutualist, but both respond to at least one reactive compound. Across different plant–insect systems, there is likely to be a range of outcomes for plant productivity in polluted environments, depending on the different ecological interactions present and the extent to which different floral attractants are impacted by air pollutants. Clearly, both laboratory and field studies on multiple plants and insects are needed to demonstrate the generality of the results presented here and to understand the range of potential ecological outcomes.

We have focused here only on the degradation of VOCs by ozone, but we must acknowledge that ozone also has direct effects on plant health and productivity independent of VOC emission. These effects range from various measures of plant reproductive output and fertility (Leisner and Ainsworth 2012) to direct damage of leaves and other plant organs (e.g. Novak et al 2007). Thus, the potential problems of reduced plant signaling exacerbate other ozone-induced effects. While plant species and genotypes are known to vary in ozone sensitivity (Staszak et al 2004), reductions in plant signaling may prove to be more uniform given the widespread use of the same VOCs, in different combinations, among plants (Bruce et al 2005). Further assessment of the risks posed to plant–insect interactions through signal degradation are needed before further anthropogenic activity increases tropospheric pollutants to levels that routinely interfere with plant signaling.

Acknowledgments

The US National Science Foundation (NSF) supported this research (grant number ATM 0939455). JZ received support from NSF to participate in this research (grant number GEO 0914597).

References

Andrews E S et al 2007 Pollinator and herbivore attraction to Cucurbita floral volatiles J. Chem. Ecol. 33 1682–91
Angiyo A M et al 2003 Extreme sensitivity in an olfactory system Chem. Senses 28 279–84
Arenas A and Farina W M 2012 Learned olfactory cues affect pollen-foraging preferences in honeybees Apis mellifera Anim. Behav. 83 1023–33
Atkinson R et al 1995 Atmospheric and indoor chemistry of gas-phase indole, quinoline, and isoquinoline Atmos. Environ. 29 3423–32
Blande J D et al 2010 Air pollution impedes plant-to-plant communication by volatiles Ecol. Lett. 13 1172–81
Bruce T J A et al 2005 Insect host location: a volatile situation Trends Plant Sci. 10 269–74
Cooper O R et al 2010 Increasing springtime ozone mixing ratios in the free troposphere over western North America Nature 463 344–8
Dobson H E M 1987 Role of flower and pollen aromas in host-plant recognition by solitary bees Oecologia 72 618–23
Ellers-Kirk C and Fleischer S J 2006 Development and life table of Acalyphra vittatum (Coleoptera: chrysomelidae), a vector of Erwinia tracheiphila in cucurbits Environ. Entomol. 35 875–80
Fiore A M et al 2002 Background ozone over the United States in summer: origin, trend, and contribution to pollution episodes J. Geophys. Res.—Atmos. 107 ACH 11–1–25
Himanen S J et al 2009 Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (Brassica napus) New Phytol. 181 174–86
Julier H E and Roulston T H 2009 Wild bee abundance and pollination service in cultivated pumpkins: farm management, nesting behavior and landscape effects J. Econ. Entomol. 102 563–73
Leisner C P and Ainsworth E A 2012 Quantifying the effects of ozone on plant reproductive growth and development Glob. Change Biol. 18 606–16
Marenco A et al 1994 Evidence of a long-term increase in tropospheric ozone from Pic du Midi data series: consequences: positive radiative forcing J. Geophys. Res.—Atmos. 99 16617–32
McFrederick Q S et al 2008 Air pollution modifies floral scent trails Atmos. Environ. 42 2336–48
McFrederick Q S et al 2009 Effects of air pollution on biogenic volatiles and ecological interactions Oecologia 160 411–20
Novak K et al 2007 Ozone air pollution effects on tree-ring growth, delta C-13, visible foliar injury and leaf gas exchange in three ozone-sensitive woody plant species Tree Physiol. 27 941–9
Parrish D D et al 2011 Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? J. Chem. Ecol. 37 683–94
Pinto D M et al 2007a Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? J. Chem. Ecol. 33 683–94
Pinto D M et al 2007b The role of ozone-reactive compounds, terpenes, and green leaf volatiles (GLVs), in the orientation of Cotesia plutellae J. Chem. Ecol. 33 2218–28
Pinto D M et al 2010 Plant volatile organic compounds (VOCs) in ozone (O3) polluted atmospheres: the ecological effects J. Chem. Ecol. 36 22–34
Sitch S et al 2007 Indirect radiative forcing of climate change through ozone effects on the land-carbon sink Nature 448 791–4
Staszak J et al 2004 Genetic differences of Pinus ponderosa Doug. ex Law. trees tolerant and sensitive to ozone Water Air Soil Pollut. 153 3–14
Theis N and Adler L S 2012 Advertising to the enemy: enhanced floral fragrance increases beetle attraction and reduces plant reproduction Ecology 93 430–5
Wenner A M et al 1991 Recruitment, search behavior, and flight ranges of honey bees Ann. Zool. 31 768–82