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Divergent pheromone-mediated insect behaviour under global atmospheric change

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Abstract

While the effects of global atmospheric changes on vegetation and resulting insect populations('bottom-up interactions') are being increasingly studied, how these gases modify interactions among insects and their natural enemies ('top-down interactions') is less clear. As natural enemy efficacy is governed largely by behavioural mechanisms, altered prey finding and prey defence may change insect population dynamics. Here we show that pheromone-mediated escape behaviours, and hence the vulnerability of insects to natural enemies, are divergent under atmospheric conditions associated with global climate change. *Chaitophorus stevensis*, a common aphid on trembling aspen trees, *Populus tremuloides*, have diminished escape responses in enriched carbon dioxide (CO_2) environments, while those in enriched ozone (O_3) have augmented escape responses, to alarm pheromone. These results suggest that divergent pheromone-mediated behaviours could alter predator–prey interactions in future environments.

Keywords: air pollution, atmospheric change, bottom-up, carbon dioxide, greenhouse gas, ozone, pheromone, predator–prey, top-down

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Introduction

Carbon dioxide (CO_2) and tropospheric ozone (O_3) levels have increased 31% and 35%, respectively, since the mid-1800s (IPCC, 2001). Vegetative growth responses of forest and agricultural plants to CO_2 and O_3 have been well studied, with the consensus emerging that elevated CO_2 and O_3 levels are stimulatory and inhibitory, respectively (Saxe *et al*., 1998; Ceulemans *et al*., 1999; Karnosky *et al*., 2003). As elevated concentrations of these gases also alter the nutritional and defensive characteristics of plants, their effects can cascade through ecosystems and impact higher trophic levels, such as insect herbivores and their natural enemies (Percy *et al*., 2002). Chewing insects, such as lepidopteran larvae, generally respond to nutrient dilution in CO_2-enriched foliage by increasing consumption, typically resulting in no change or modest decreases in growth (Lindroth, 1996; Bezemer & Jones, 1998; Coviella & Trumble, 1999). Performance of the same insects may improve under elevated O_3 (Kopper & Lindroth, 2003). Sucking insects, such as aphids, however, are variable in their responses to plants grown under elevated CO_2 and O_3 (Holopainen, 2002).

In contrast to plant-mediated ('bottom-up') effects on insect herbivores, we know exceedingly little about natural enemy-mediated ('top-down') effects under changing atmospheric conditions (Hunter, 2001). Initial reports suggest that parasitoids and predators are more abundant and/or efficacious under elevated CO_2 levels (Stiling *et al*., 1999; Percy *et al*., 2002), but are negatively affected by elevated O_3 (Gate *et al*., 1995; Percy *et al*., 2002). While the mechanisms resulting in altered natural enemy efficacy under changing climactic conditions are unknown, behavioural responses by both natural enemies (e.g. host finding) and pest insects (e.g. prey dispersal) are recognized as important for understanding predator–prey population dynamics (Hassell, 1978; Mangel & Roitberg, 1992). Indeed, top-down processes are believed to be a key determinant for population sizes of insects that have a high predation risk and limited escape/defensive behaviours (Denno *et al*., 2003). Thus, discerning whether insect behaviours may be altered in response to changing atmospheric conditions is critically important for elucidating the mechanisms likely to underlie changes in insect populations in future environments.

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Here we describe a field experiment, investigating the effects of elevated levels of CO_2 and O_3 on pheromone-mediated dispersal behaviour of the phloem-feeding aphid, *Chaitophorus stevensis*. As aphids are sedentary, group-feeding insects, a principal anti-predator defence is clone-mate dispersal in response to alarm pheromone emission to areas with lower predation risk (Kislow & Edwards, 1972; Mondor *et al*., 2000). By measuring aphid dispersal rates, we assess whether pheromone-mediated behaviours may be altered under future atmospheric conditions.

Materials and methods

Field site and gas treatments

The Aspen Free-Air CO_2 Enrichment (FACE) site, consisting of 12, 30 m diameter rings, is located near Rhinelander, WI, USA (45.7° latitude, 89.7° longitude). The 32 ha site was constructed, and trees planted inside each ring, in 1997. Each FACE ring is divided by a walkway system into three sectors: (1) mixed trembling aspen (*Populus tremuloides* Michx.) genotypes, (2) trembling aspen and sugar maple (*Acer saccharum* Marsh.), and (3) trembling aspen and paper birch (*Betula papyrifera* Marsh.). These stands have been exposed to ambient or elevated levels of CO_2 and/or O_3 from 1998 until present. The experiment consists of a randomized complete block design of three blocks of four treatments: (1) control ($367 \pm 15\,\mu L\,L^{-1}$ CO_2 and $38 \pm 13\,nL\,L^{-1}$ O_3), (2) elevated CO_2 ($+CO_2$, $537 \pm 77\,\mu L\,L^{-1}$), (3) elevated O_3 ($+O_3$, $51 \pm 22\,nL\,L^{-1}$) and (4) elevated CO_2 and O_3 ($+CO_2 + O_3$, $537 \pm 77\,\mu L\,L^{-1} + 51 \pm 22\,nL\,L^{-1}$, respectively). CO_2 levels are elevated to represent levels predicted for 2060, while O_3 levels follow a diurnal pattern based on O_3 readings currently experienced in urban areas of the southwestern Great Lakes region of the USA (Dickson *et al*., 2000). A computer-controlled trace gas monitoring system continually adjusts the ambient and elevated concentrations of gases delivered to forest stands through vertical vent pipes surrounding each ring.

Insect behaviour bioassays

Experiments were conducted on young *C. stevensis* colonies, feeding on trembling aspen leaves. These young aphid colonies had developed within the respective gas treatments, as colonies were composed entirely of (wingless) apterous individuals except for the winged (alate) individuals initiating the colonies, and were typical of early season colonies, being widely dispersed both within and among trees. Furthermore, *C. stevensis* have limited dispersal, remaining on trees within individual FACE rings (Percy *et al*., 2002).

Individual aspen leaves, containing a single aphid colony (25 ± 2 aphids), were carefully abscised from the tree and held in the same orientation as when on the tree (i.e. adaxial side up). Removing the leaf allowed careful observation of all aphids on the leaf, which was not possible directly on the tree, because of the 'trembling' of the leaves. Prior to applying one of two treatments, which were assigned at random using paper chits, to a particular colony, the numbers of each aphid developmental stage (first, second instars; third, fourth instars; and adults) in the colony were counted. After the initial count, an apterous aphid in the colony was selected at random for one of the following treatments: (1) no alarm pheromone (control) (an aphid was prodded lightly on the thorax, so as to not produce a visible pheromone droplet) or (2) alarm pheromone (treatment) (an aphid was prodded more heavily on the thorax and induced to emit a visible pheromone droplet) (Mondor & Roitberg, 2003).

Arboreal aphids seldom drop from their feeding sites, because of an inability to regain such sites thereafter. Consequently, aphids exhibiting any dispersal reactions in response to pheromone emission as well as those exhibiting the most extreme dispersal response, walking down the petiole and off the leaf, were recorded over 5 min. As recorded, these two behaviours are not mutually exclusive, i.e. aphids dispersing off the leaf are also included in the general dispersal response category. We chose not to make these categories mutually exclusive; so that we could better compare the effect of altered atmospheric conditions on total vs. extreme dispersal responses. Five minutes were chosen because aphid alarm pheromone has the largest effect on aphid colonies during the first few minutes following emission (Montgomery & Nault, 1977). Five subsamples of each treatment ($n = 5$ droplets, 5 no droplets) per ring ($n = 12$ rings) were conducted ($n = 120$ total). Aphid colonies were always selected from different trees and used just once during the experiment. Bioassays were conducted from 09:00 to 16:00 hours, inside treatment rings.

Statistical analyses

As individual assays within each FACE ring were subsamples rather than true replicates, and because subsamples contained different numbers of aphids, overall proportions of aphids of each developmental stage (first, second instar; third, fourth instar; and adults) dispersing in response to alarm pheromone were calculated. These proportions were obtained by dividing the total number of dispersers by the total

number of aphids, summed across the five subsamples for each treatment for each ring. These proportions were then transformed ($x' = \arcsin\sqrt{x}$) to achieve normality (Zar, 1984). As aphid responses to the no pheromone (control) treatment were extremely low ($6.1 \pm 2.1\%$) across all gas levels, we removed this treatment from the analysis to better clarify the effects of aphid dispersal in response to alarm pheromone. Data were subsequently analysed using two, three factor split-plot ANOVAs (JMP IN 5.1, SAS Institute, 2005). The two whole-plot factors were CO_2 and O_3 (fully crossed) with ring block incorporated as a random blocking variable (Dickson *et al.*, 2000). Aphid developmental stage (first, second instar; third, fourth instar; and adults) was incorporated as a subplot factor, within gas treatments. All interactions between CO_2, O_3, and aphid developmental stage were incorporated into the model as subplot interactions. The response variables in the two analyses were the proportion of aphids: (1) exhibiting any dispersal responses and (2) dispersing entirely from the leaf, in response to alarm pheromone emission.

Results

Pooled across instars, aspen aphids showed divergent responses to alarm pheromone under elevated CO_2 and O_3. Significantly fewer aphids exhibited any dispersal responses ($F_{1,8} = 9.45$, $P = 0.015$) or dispersed entirely from the leaf ($F_{1,8} = 10.11$, $P = 0.013$), in response to alarm pheromone under elevated CO_2 relative to ambient CO_2 (Figs 1 and 2). Conversely, elevated concentrations of O_3 increased dispersal responses to alarm pheromone, both in total ($F_{1,8} = 21.16$, $P = 0.002$) and from the leaf itself ($F_{1,8} = 28.61$, $P < 0.001$) (Figs 1 and 2). There were no significant $CO_2 \times O_3$ interactions for either general dispersal ($F_{1,8} = 0.49$, $P = 0.74$) or dispersal from the leaf ($F_{1,8} = 1.40$, $P = 0.27$).

Pooled across gas treatments, neither total dispersal ($F_{2,16} = 1.66$, $P = 0.22$) nor dispersal from the leaf ($F_{2,16} = 1.90$, $P = 0.18$) differed by instar. Similarly, though adult aphids appeared to respond much less under augmented CO_2 concentrations, we observed no instar $\times$ CO_2 interactions (total dispersal, $F_{2,16} = 1.29$, $P = 0.30$; dispersal from leaf, $F_{2,16} = 1.46$, $P = 0.26$) or instar $\times$ $CO_2 \times O_3$ interactions (total dispersal, $F_{2,16} = 2.69$, $P = 0.099$; dispersal from leaf, $F_{2,16} = 0.049$, $P = 0.95$), for either type of dispersal. There were, however, significant instar $\times$ O_3 interactions for both total dispersal ($F_{2,16} = 10.22$, $P = 0.001$) and dispersal from the leaf ($F_{2,16} = 18.10$, $P = 0.0001$). Dispersal behaviour did not differ appreciably in immature instars under atmospheres containing ambient vs. enriched levels of O_3 (control and $+CO_2$ vs. $+O_3$ and $+CO_2 + O_3$ treatments), but was vastly divergent in adults. In fact, 0% of adults dispersed from the leaf under $+CO_2$ while 100% dispersed under $+O_3$ (Figs 1 and 2).

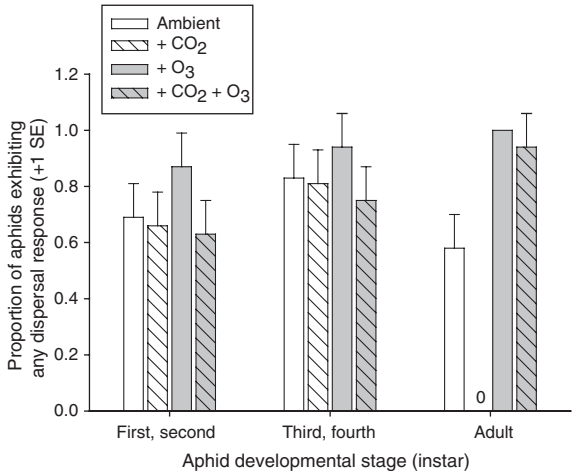

Fig. 1 Proportions of three aphid developmental stages exhibiting any dispersal responses in response to aphid alarm pheromone emission (means + 1 SE). See text for detailed explanations of significant effects.

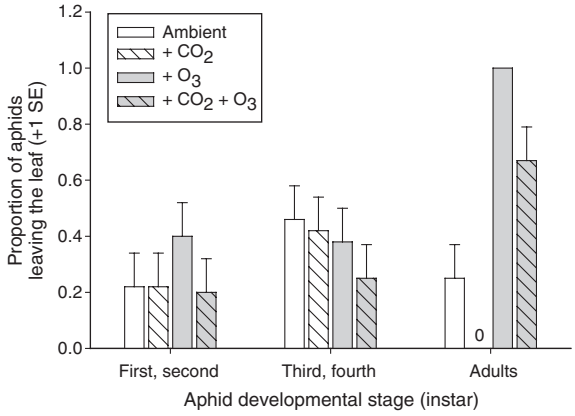

Fig. 2 Proportions of three aphid developmental stages dispersing from the leaf in response to aphid alarm pheromone emission (means + 1 SE). See text for detailed explanations of significant effects.

Discussion

Pheromones are utilized by insects for several purposes, including alarm signalling (Kislow & Edwards, 1972; Blatt *et al.*, 1998; Hunt *et al.*, 2003), kin recognition (Dani *et al.*, 2001; VanderMeer & Alonso, 2002), and sexual communication (Pickett *et al.*, 1992; Landolt & Phillips, 1997). The prevalence of chemical communication among organisms makes it imperative that we

understand how these chemical signals may be altered under atmospheric conditions that contribute to global climate change. Here, we showed that the principal means of defence for aphids, dispersal responses to alarm pheromone, decreases under elevated CO_2 but increases under elevated O_3. Thus, intraspecific olfactory communication may be radically altered in response to elevated concentrations of different greenhouse gases.

Divergent defensive behaviours in response to elevated levels of CO_2 and O_3 may, at least partially, contribute to altered insect population dynamics. As adults are more strongly affected by changing O_3 concentrations than are immature aphids, it is tempting to speculate that greater numbers of *C. stevensis* commonly observed under elevated O_3 (Percy *et al*., 2002) are attributable to increased adult defensive behaviour. Population sizes of insects that are sedentary and group-living are often determined by the abundance and efficacy of natural enemies (Walker & Jones, 2001; Denno *et al*., 2003). Thus, augmented or reduced escape/defensive behaviours under changing atmospheric conditions may, along with other contributing factors, culminate in altered abundances and spatial distributions of pest insects.

Thus far, the mechanism resulting in differential dispersal responses to alarm pheromone under altered atmospheric conditions remains unknown. There are undeniable changes in host plant quality within different gas treatments, and aphid dispersal is a well-known trade-off between predation risk and host plant quality (Dill *et al*., 1990; Losey & Denno, 1998). Glasshouse experiments, however, suggest that an altered ability of aphids to produce and/or respond to alarm pheromone may contribute to altered defensive behaviours under changing climactic scenarios (Awmack *et al*., 1997). Preliminary evidence also suggests that exposure to atmospheric pollutants, such as O_3, can rapidly destabilize pheromone structure and resulting activity (Arndt, 1995). Intriguingly, in our experiment aphids exhibited increased dispersal responses to alarm pheromone under elevated O_3. Perhaps pheromone degradation was not a factor in our experiment because of the short-term nature of alarm pheromone activity. Atmospheric pollutants likely alter insect behaviour through a variety of factors including altered host plant quality, pheromone quality/quantity, and pheromone reception.

In summary, the existence of divergent pheromone-mediated behaviours in insects under conditions of global atmospheric change could have substantial implications not just from an ecological perspective, but also for how insect-derived compounds are used in pest management programmes. Understanding how global atmospheric change will enhance or negate not only bottom-up but also top-down effects on insect behaviour will vastly improve our ability to predict shifts in insect population dynamics and community interactions in future environments.

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