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Aphid individual performance may not predict population responses to elevated CO_2 or O_3

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Abstract

Changes in atmospheric composition affect plant quality and herbivore performance. We used the Aspen Free Air CO_2 Enrichment (FACE) facility to investigate the impacts of elevated carbon dioxide (CO_2) and ozone (O_3) on the performance of the aphid *Cepegillettea betulaefoliae* Granovsky feeding on paper birch (*Betula papyrifera* Marsh.). In Year 1, we simultaneously measured individual performance and population growth rates, and in Year 2 we surveyed natural aphid, predator and parasitoid populations throughout the growing season. Aphid growth and development (relative growth rate (RGR), development time, adult weight, embryo number and the birth weight of newborn nymphs) were unaffected by CO_2 and O_3. Aphid fecundity decreased on trees grown at elevated CO_2, O_3 and $CO_2 + O_3$. Neither nymphal performance nor adult size were reliable indicators of future fecundity at elevated CO_2 and/or O_3. Aphid populations protected from natural enemies were unaffected by elevated CO_2, but increased significantly at elevated O_3. Individual fecundity in elevated CO_2 and O_3 atmospheres did not predict population growth rates, probably because of changes in the strength of intraspecific competition or the ability of the aphids to induce nutrient sinks. Natural aphid, predator and parasitoids populations (Year 2) showed few significant responses to CO_2 and O_3, although CO_2 and O_3 did affect the timing of aphid and natural enemy peak abundance. Elevated CO_2 and O_3 affected aphid and natural enemy populations independently: no $CO_2 \times O_3$ interactions were observed. We conclude that: (1) aphid individual performance did not predict population responses to CO_2 and O_3 and (2) elevated CO_2 and O_3 atmospheres are unlikely to affect *C. betulaefoliae* populations in the presence of natural enemy communities.

Keywords: carbon dioxide, forests, global change, natural enemies, ozone, pests

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Introduction

The increases in atmospheric carbon dioxide (CO_2) and ozone (O_3) concentrations predicted to occur in the next 50–100 years (IPCC, 2001) are likely to affect the nutritional ecology of herbivorous insects (Brown, 1995; Bezemer & Jones, 1998). Since CO_2 and O_3 have strong and opposing effects on plant productivity (Makino & Mae, 1999; Krupa *et al*., 2001) and may alter both the nutritional and defensive composition of plant tissues (reviewed in Kangasjärvi *et al*., 1994; Lindroth, 1996), both gases may change the availability and quality of resources for herbivorous insects. Elevated CO_2 atmospheres reduce plant quality for most insect herbivores (reviewed in Bezemer & Jones, 1998; Coviella & Trumble, 1999), while elevated O_3 has more complex effects (reviewed in Brown, 1995). The few studies that have investigated the interactive effects of CO_2 and O_3 found no evidence that CO_2 modifies the effects of O_3 on herbivore performance (Heagle, *et al*., 1994; Herms *et al*., 1995; Kopper *et al*., 2001; Kopper & Lindroth, 2002), despite mounting evidence that CO_2 reduces the negative impacts of O_3 on plant productivity (Dickson *et al*., 1998; Donnelly *et al*., 2000).

Most investigations of herbivore responses to future global environmental change use short-term measures of growth and developmental rates or correlates of fecundity such as pupal or adult mass (Bezemer & Jones, 1998) and assume that small adults will produce

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fewer offspring than large adults. However, changes in plant quality may affect size × fecundity relationships by altering the allocation of resources between somatic and reproductive tissues (reviewed in Leather, 1988; Awmack & Leather, 2002). In many insect orders, changes in plant quality may affect the relationship between female size and the number of offspring produced (e.g., Homoptera (Wojciechowicz-Zytko & van Emden, 1995), Orthoptera (Joern & Behmer, 1998), Lepidoptera (Parry *et al*., 2001), Coleoptera (Berrigan, 1991) and Hymenoptera (Berrigan, 1991)). Because both CO_2 and O_3 affect plant quality, it may not be possible to make reliable predictions about the future fecundity of herbivorous insects without directly measuring the number of offspring produced. Similarly, recent studies (Bezemer *et al*., 1999; Percy *et al*., 2002) suggest that the performance of individual herbivores may not predict population responses to elevated CO_2 or O_3.

Aphids are a very useful model system for comparisons between individual and population responses to global change, since their biology is well understood (Dixon, 1998) and they develop rapidly and may have several generations in a single growing season. Several recent studies have investigated the independent effects of both CO_2 (Salt *et al*., 1996; Docherty *et al*., 1997; Bezemer *et al*., 1998, 1999; Diaz *et al*., 1998; Awmack & Harrington, 2000; Hughes & Bazzaz, 2001) and O_3 (Whittaker *et al*., 1989; Salt & Whittaker, 1995) on aphid populations. We are not aware of any investigations linking the interacting effects of CO_2 and O_3 on the performance of individual herbivores to the performance of aphid populations.

Changes in the performance of individual insects may not predict population responses if other factors limiting population growth rates are also affected by changes in plant quality at elevated CO_2 and/or O_3. Changes in the strength of intraspecific competition may affect aphid population growth rates at elevated CO_2 (Bezemer *et al*., 1999; Awmack & Harrington, 2000). Natural enemy performance may also decrease (Roth & Lindroth, 1995), increase (Stiling *et al*., 1999) or may be unaffected (Bezemer *et al*., 1998; Stacey & Fellowes, 2002). We are not aware of any studies investigating the impacts of O_3 on competition, but two studies (Gate *et al*., 1995; Dahlsten *et al*., 1997) found negative effects of O_3 on natural enemy performance, and Holton (2001) showed that parasitoid performance in $CO_2 + O_3$-enhanced atmospheres depended on host plant genotype.

Reliable predictions of the impacts of future global change on insect populations therefore require that we (1) understand changes in individual performance (2) can scale up individual performance to predict population responses, (3) understand the impacts of CO_2 and O_3 on top-down regulation by natural enemies and (4) understand the consequences of interactions between the effects of CO_2 and O_3 on herbivore and natural enemy populations. No study has yet combined investigations of the impacts of CO_2 or O_3 on individual insects with studies of insect populations, and related the results to surveys of natural populations exposed to natural enemies. The development of Free Air CO_2 Enrichment (FACE) facilities (Hendrey *et al*., 1993) has allowed natural invertebrate communities to be exposed to elevated CO_2 (and in some cases, O_3) atmospheres. However, although FACE facilities have been extensively used to investigate changes in plant communities (e.g. Garcia *et al*., 1998; Norby & Cotrufo, 1998; Reich *et al*., 2001), only one study has investigated the impacts on higher trophic levels (Hansen *et al*., 2001). In this paper, we describe a set of experiments conducted at the Aspen FACE site in Wisconsin, USA (Dickson *et al*., 2000) to investigate the independent and interacting effects of elevated CO_2 and O_3 on the performance of the aphid *Cepegillettea betulaefoliae* Granovsky reared on paper birch (*Betula papyrifera* Marsh.). At the individual scale (Study 1), we investigated the effects of CO_2 and O_3 on aphid growth and developmental rates, adult weight, embryo number and the cumulative fecundity of adult aphids. We compared these data with a simultaneous investigation of the effects of elevated CO_2 and O_3 on aphid populations, using aphids protected from natural enemies with mesh sleeves (Study 2), and, the following year, with a survey of natural aphid, predator and parasitoid populations (Study 3).

Materials and methods

Site description

The Aspen FACE site, situated in Harshaw, Wisconsin (45.6° lat., 89.5° long., Dickson *et al*., 2000) consists of twelve 30 m diameter FACE rings arranged in a fully replicated and blocked factorial design, with two levels of CO_2 and two levels of O_3. Three rings receive ambient air ('Control' i.e. ambient CO_2 and O_3), three rings receive elevated CO_2 ('CO_2' = 570 ppm CO_2, equivalent to ambient + 200 ppm), three rings receive an average of 50–60 ppb O_3 (approximately 1.5 times the background ambient levels of 30–40 ppb O_3) and the final three rings receive 570 ppm CO_2 and 50–60 ppb O_3. CO_2 and O_3 are released during daylight hours. O_3 is not released during fog or rain events, since natural O_3 production normally occurs as a result of photochemical reactions in the troposphere. O_3 fumigation follows a dynamic profile, established from a moderately polluted city (Leelenaw, Michigan, USA) and

varies according to the prevailing climatic conditions (Dickson *et al*., 2000). Each FACE ring was planted with trembling aspen (*Populus tremuloides* Michx.), sugar maple (*Acer saccharum* Marsh.) and paper birch (*Betula papyrifera* Marsh.) in 1997. CO_2 and O_3 exposure commenced in 1998. These experiments were carried out during the 1999 and 2000 growing seasons when the trees were between two and four metres tall. All the studies described below used the same seven trees in each FACE ring to estimate the variation in aphid performance within each ring, although 'ring' was used as the replicate in all statistical analyses.

Insect rearing

All three studies used aphids from trees in the FACE rings (i.e. aphids that had developed at ambient or elevated CO_2 and/or O_3). No aphids were moved between FACE rings. The first two studies used aphids from cultures (initiated using wingless fourth instar aphids that had developed in each FACE ring) maintained on a newly expanded birch shoot in each ring and protected from natural enemies with mesh sleeves (45 cm length × 45 cm width). Since the development of aphid embryos begins in the ovarioles of their grandmothers (Dixon, 1998), these two studies used the grand-daughters of these initial aphids, ensuring that the aphids used had spent their entire development in the appropriate CO_2 and/or O_3 treatment. The third study surveyed natural aphid infestations throughout the growing season and did not use aphids from cultures.

Study 1. Measurement of individual performance

In each ring, seven groups of five 24 h old 1st instar nymphs (84 in total, the progeny of wingless adult aphids from the cultures described above) were used to investigate the impacts of elevated CO_2 and/or O_3 on aphid performance. The average birth weight of each group of five aphids was measured to the nearest microgram using a Mettler Toledo MT5 microbalance (Mettler, Columbus, Ohio, USA). Each group of aphids was then confined to the underside of the youngest fully expanded leaf on the youngest south-facing branch of each of seven trees, mimicking the location of natural aphid populations (C. S. Awmack, unpublished results). Aphids were attached to the leaves using light foam-based clip cages (Awmack, 1997) modified from MacGillivray & Anderson (1957), which allowed maximum air circulation and caused minimal damage to the leaf surface (no marks were visible when the cage was removed from the leaf). These groups of aphids remained on the same branch throughout the experiment and were moved to new leaves as they became available, as birch exhibits indeterminate growth and natural aphid populations move to new leaves throughout the growing season (C. S. Awmack, personal observation). The aphids were observed daily until the final adult moult, when they were re-weighed to calculate the relative growth rate (Radford, 1967) (an estimate of the aphids' ability to convert phloem sap to biomass) using the formula RGR (relative growth rate) = $[\ln(W2)-\ln(W1)]/D$, where $W1$ = birth weight, $W2$ = final weight (both in micrograms) and D = development time (the number of days from birth to the final adult moult).

One randomly selected adult from each clip cage was then dissected and the number of mature embryos (those with red eye pigments) was counted under a dissecting microscope at × 5 magnification to determine potential fecundity (Traicevski & Ward, 2002). A second randomly selected individual from each group was returned to each tree (again, to a new leaf on the same shoot) and was used to determine total fecundity (the total number of nymphs produced by the adult). All nymphs produced by this adult were counted and removed every 24 h to prevent any negative effects of crowding on fecundity. The clip cages were moved to the next new leaf on the branch every 4–5 days. The average birth weight of each nymph was calculated by removing and weighing all the nymphs produced during the second 24 h of reproduction.

Study 2: Aphid population responses to elevated CO_2 and O_3

The investigation of aphid population growth rates (Study 2) commenced two days after Study 1, and used the same seven trees in each FACE ring. Groups of five 24 h old aphids were added to the three youngest leaves and the terminal bud of the branch nearest the branch used in Study 1. Each shoot was thoroughly searched before the aphids were added, and all aphids, predators, parasitoids, eggs, etc. were removed. The aphid populations were protected from natural enemies using fine mesh sleeves (approximately 45 cm length × 45 cm width). After 28 days (approximately twice the development time of *C. betulaefoliae*, and coincident with the final measurements of individual aphid performance in Study 1), all aphids were removed from the sleeves and counted.

Study 3: Responses of aphid communities to elevated CO_2 and O_3

Study 3 consisted of a survey of the naturally occurring aphid populations, and investigated the effects of elevated CO_2 and O_3 on aphids exposed to the

predators and parasitoids inhabiting the FACE rings. A preliminary study (carried out in 1999) using trees that were not used for the measurement of individual performance showed that aphids and natural enemies were found only on the terminal shoots of the upper branches (C. S. Awmack, 1999 unpublished results). As only 7 birch trees in each FACE ring were available for entomological studies, surveys of natural populations were postponed until the following year (2000). This survey, conducted 13 times throughout the growing season, used one upper branch on each tree. The number of aphids and natural enemies (predominantly predators such as lacewings and ladybird beetles and parasitic Hymenoptera) on the terminal five short shoots on each branch were noted every 7–10 days from mid-June (when the first aphids were observed) until leaf fall at the end of September. The natural enemy data consisted of sightings of adult insects (particularly lacewings and ladybirds) and counts of egg masses produced by predators (particularly ladybirds, stinkbugs and lacewings). These egg masses were counted as '1' regardless of the number of eggs, because they represented a single visit by a predator. Parasitized aphid 'mummies' were included in the natural enemy counts and not the aphid counts. Eggs that had hatched and parasitized aphids from which the adult parasitoid had emerged were ignored on subsequent sampling dates.

Data analysis

As FACE studies generally have low numbers of true replicates (e.g. $N = 3$ for each fumigation treatment at Aspen FACE), some authors have argued that data should be presented as significant if $P < 0.1$ (Filion *et al.*, 2000) to avoid Type II errors. In this paper, we will use the traditional value of alpha ($P < 0.05$) to determine significance, but we present exact P values throughout the text, and describe P values between 0.05 and 0.1 as marginally significant.

All data were analysed using analyses of variance in Procedure 'Mixed' in SAS (Littell *et al.*, 1996). Because Aspen FACE incorporates a 2×2 factorial experiment (2 levels each of CO_2 and O_3), the statistical model used CO_2 and O_3 as fixed treatment effects, with the three blocks and tree(block) as random effects.

The effects of elevated CO_2 and O_3 on the performance of individual aphids were analyzed using separate ANOVAs for each fitness parameter. The effects of elevated CO_2 and O_3 on aphid populations protected from natural enemies (Study 2), and on the abundance of natural aphid populations (Study 3) were analysed after the data had been $\log10(N + 1)$ transformed to normalize variance. Because natural enemy abundance was generally low, all natural enemies were grouped into a single category ('Natural enemies') rather than being analysed separately as 'predators', 'parasitoids', etc. The data from Study 2 were analysed using the model described above, while the data from Study 3 used a repeated measures ANOVA, with time (year) as the repeated measures term. The latter model also used a spatial (power) term, which fits a time-series covariance structure in which the correlations decline as a function of time, and takes into account the fact that the time intervals between sampling dates are not necessarily equal. This term also corrects for any autocorrelation among sampling dates (Littell *et al.*, 1996).

Results

Study 1. Measurement of individual performance

Elevated CO_2 and O_3 did not significantly affect the growth and developmental rates of individual *C. betulaefoliae* (Table 1, Fig. 1). The RGR of aphids reared at elevated CO_2 and/or O_3 did not differ from the control aphids (Fig. 1a), while aphid development times showed a small (and only marginally significant) increase at elevated CO_2, but not at elevated O_3 (Fig. 1b). Elevated CO_2 and O_3 did not affect the reproductive fitness of individual aphids as neither adult weight nor potential fecundity (embryo number) were significantly affected (Fig. 1c and d). CO_2 and O_3 had no effect on the allocation of resources to the next-generation nymphs, since the birth weight of the nymphs produced by these adults (G2 weight) was unaffected (mean birth weight (μg), ± SE): control (109 ± 17), CO_2 (95 ± 19), O_3 (119 ± 14) and $CO_2 + O_3$ (129 ± 23).

The fecundity (Fig. 2) of the individual aphids did not reflect either their adult weight or the number of embryos in their reproductive tract (Fig. 1c and d), since elevated CO_2 and O_3 had strong negative effects on the cumulative fecundity of *C. betulaefoliae*. The decrease in reproductive rates became more apparent towards the end of the adult's reproductive life, suggesting that females were unable to maintain embryo maturation throughout their lifetime. The fecundity of similarly sized aphids therefore differed according to the CO_2 or O_3 treatment that they experienced during their development.

Study 2: Aphid population responses to elevated CO_2 and O_3

Aphid populations protected from natural enemies using mesh sleeves did not show the same responses to CO_2 and O_3 as did individual aphids (Fig. 3).

Table 1 Results of analysis of variance (F and P values) of the impacts of elevated CO_2 and O_3 atmospheres on performance of the aphid *Cepegillettea betulaefoliae*

	RGR		Adult weight		Development time		Embryo number		G2 weight	
Treatment	$F_{1,4}$	P	$F_{1,4}$	P	$F_{1,6}$	P	$F_{1,4}$	P	$F_{1,4}$	P
CO_2	0.52	0.512	0.30	0.612	4.24	0.085	0.10	0.769	0.01	0.935
O_3	0.39	0.624	0.03	0.877	0.10	0.760	0.37	0.576	1.40	0.301
$CO_2 \times O_3$	0.67	0.462	0.10	0.773	0.04	0.851	0.01	0.939	0.41	0.556

See Materials and methods for a detailed description of the model used in the analysis and Fig. 1 for a graphical representation of the data.
MRGR, mean relative growth rate; RGR, relative growth rate; G2 weight, average birth weight of nymphs produced by the adults.

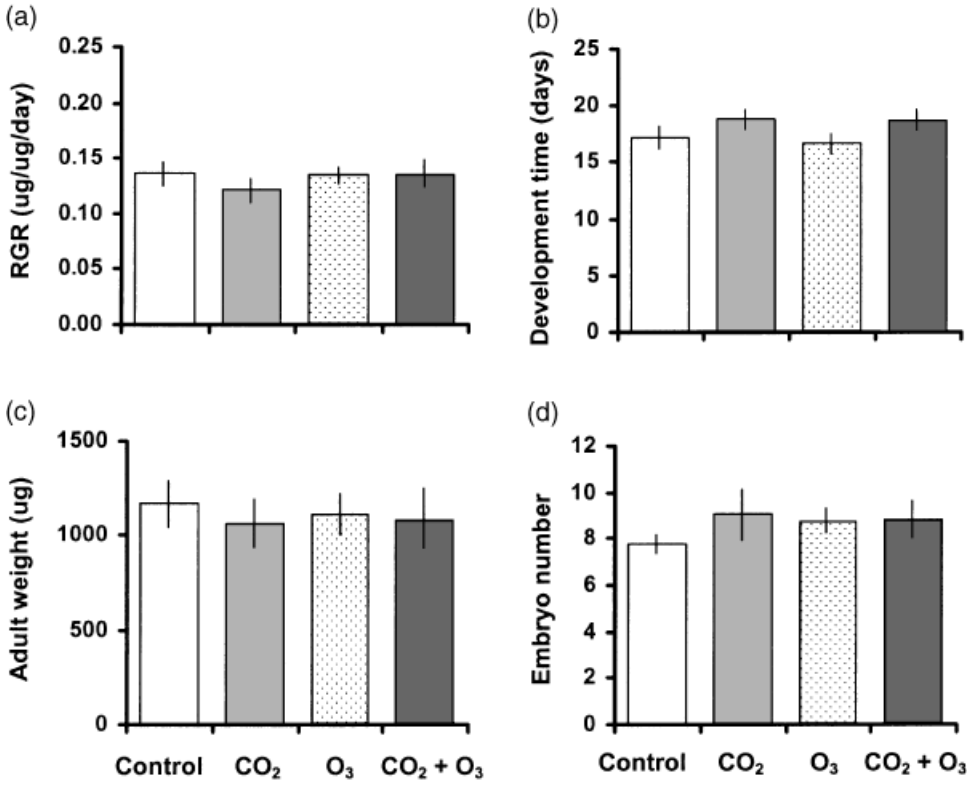


Fig. 1 Performance of individual *Cepegillettea betulaefoliae* reared on *Betula papyrifera* at the Aspen FACE site. Control = ambient CO_2, ambient O_3, CO_2 = elevated (570 ppm) CO_2, ambient O_3, O_3 = elevated (1.5 × ambient) O_3, ambient CO_2, $CO_2 + O_3$ = elevated CO_2, elevated O_3. (a) relative growth rate, (b) development time, (c) adult weight and (d) embryo number (potential fecundity). All data are shown ± 1 SEM and are the means of data from three replicate FACE rings.

Population responses did not reflect individual growth and developmental parameters (Fig. 1) or individual fecundity (Fig. 2). Aphid populations were marginally significantly smaller at elevated CO_2 ($F_{1,6} = 4.66$, $P = 0.074$), but increased at elevated O_3 ($F_{1,6} = 11.86$, $P = 0.014$). Elevated CO_2 did not modify the impacts of elevated O_3 on aphid populations (i.e. no significant CO_2*O_3 interaction: $F_{1,6} = 0.81$, $P = 0.403$).

Study 3: Aphid community responses to elevated CO_2 and O_3

The effects of elevated CO_2 and O_3 on communities of aphids and their natural enemies were more complex when populations were followed throughout the growing season the following year (Fig. 4). Aphid and natural enemy abundance both varied throughout the

growing season (significant time (year) interaction: aphids, $F_{12,960} = 40.97$, $P = < 0.0001$, natural enemies, $F_{12,960} = 3.50$, $P < 0.0001$). Aphid abundance was significantly affected by elevated CO_2 on some sampling dates towards the end of the growing season (significant $CO_2 \times$ date interaction, $F_{12,\ 960} = 1.88$, $P = 0.033$). Elevated O_3 alone did not modify aphid abundance (O_3 main effect: $F_{1,6} = 1.50$, $P = 0.267$) but on some sampling

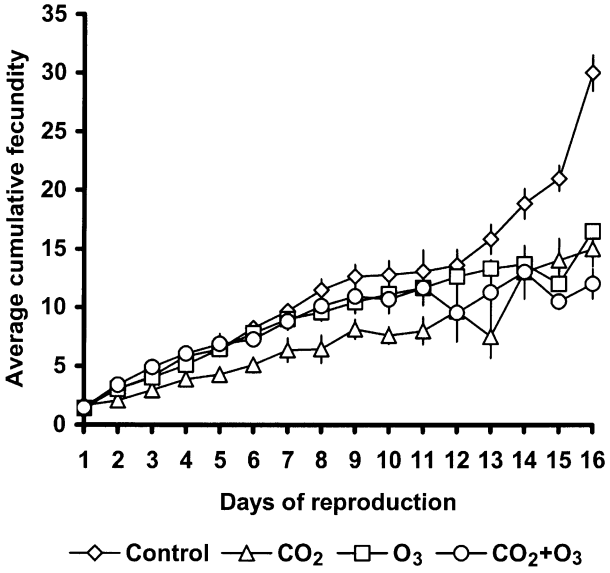


Fig. 2 Average cumulative fecundity of *Cepegillettea betulaefoliae* reared on *Betula papyrifera* at the Aspen FACE site. Control = ambient CO_2, ambient O_3, CO_2 = elevated (570 ppm) CO_2, ambient O_3, O_3 = elevated (1.5 × ambient) O_3, ambient CO_2, $CO_2 + O_3$ = elevated CO_2, elevated O_3. All data are shown ± 1 SEM and are the means of three replicate FACE rings. Average fecundity declined at elevated CO_2 on day 13 because a female with a particularly high reproductive rate died, reducing the average for the entire replicate.

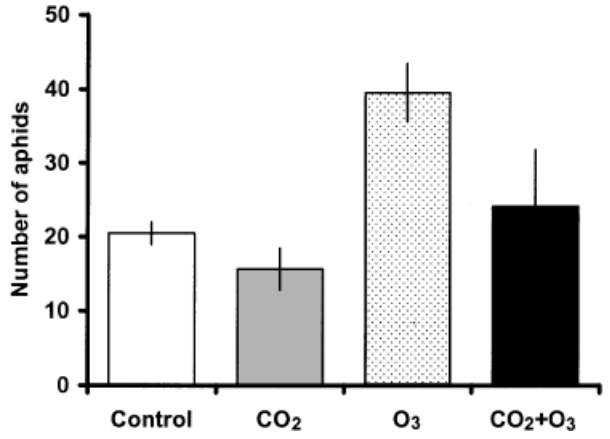
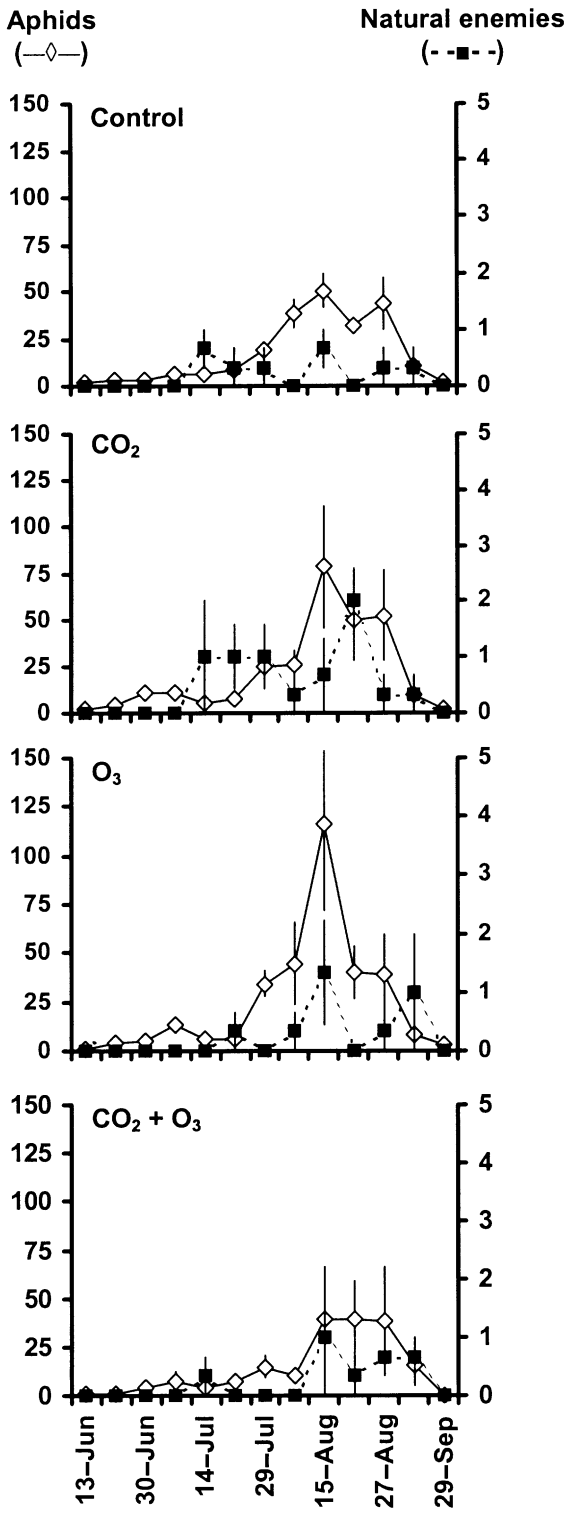


Fig. 3 Final populations of *Cepgillettea betulaefoliae* reared for 28 days on *Betula papyrifera* at the Aspen FACE site. All replicates initially contained five 1st instar aphids, protected from natural enemies with mesh bags. Control = ambient CO_2, ambient O_3, CO_2 = elevated (570 ppm) CO_2, ambient O_3, O_3 = elevated (1.5 × ambient) O_3, ambient CO_2, $CO_2 + O_3$ = elevated CO_2, elevated O_3. All data are shown ± 1 SEM and are the means of three replicate FACE rings.



dates, elevated O_3 modified the effects of elevated CO_2 on aphid abundance (significant $CO_2 \times O_3 \times$ date interaction $F_{12,960} = 2.13$, $P = 0.013$). Natural enemy abundance increased under elevated CO_2, but only on one sampling date towards the end of the growing season ($F_{1,6} = 6.91$, $P = 0.039$). Natural enemy abundance was unaffected by elevated O_3 ($F_{1,6} = 1.51$, $P = 0.265$).

Discussion

These data represent the first investigation of the long-term effects of elevated CO_2 and O_3 atmospheres on natural insect herbivore populations. The aphids used in this study were exposed to the most 'realistic' simulation of future elevated CO_2 and O_3 atmospheres currently possible. At the individual scale, elevated CO_2 and O_3 did not significantly affect growth rates, potential fecundity (embryo number) or offspring quality (Fig. 1, Table 1), but the cumulative fecundity of aphids was lower than that of aphids reared on the control trees (Fig. 2). The population-scale study (Study 2) showed that elevated CO_2 did not significantly affect aphid populations, while elevated O_3 had a strong positive effect. The surveys of aphid and natural enemy communities (Study 3) revealed complex time-dependent interactions with elevated CO_2 and no effects of elevated O_3. The data suggest firstly, that CO_2 and O_3 had few interacting effects on *C. betulaefoliae*, and secondly, that measures of individual fecundity did not predict population responses, and population responses did not predict community responses.

Previous studies show that the responses of other aphid species to elevated CO_2 or O_3 are also complex. Tree-feeding aphids show few significant responses to elevated CO_2 (Docherty *et al*., 1997), while crop-feeding species may respond positively (Awmack *et al*., 1997; Bezemer *et al*., 1998; Hughes and Bazzaz 2001; Zhang *et al*., 2001; Stacey & Fellowes, 2002), negatively (Newman *et al*., 1999) or not at all (Hughes & Bazzaz, 2001), and the same species may show different responses on different host plant species (Awmack *et al*., 1997; Bezemer *et al*., 1999). Aphid responses to O_3 also depend on the aphid and host plant species (Brown, 1995; Holopainen *et al*., 1995), plant phenology (Holopainen & Kössi, 1998), soil nutrient availability (Kainulainen *et al*., 2000) and the duration of exposure (Brown *et al*., 1992). *Cepegillettea betulaefoliae* performance on paper birch therefore does not confirm any general trends, since aphids, like many other species (Lawton, 2000), show idiosyncratic responses to elevated CO_2 and O_3.

The poor correlation between individual and population growth rates is intriguing. The growth, development, adult weight and potential fecundity of individual aphids was unaffected by elevated CO_2 or O_3 (Fig. 1). Size was not, therefore, a reliable predictor of future fecundity. As the newly moulted adults contained similar numbers of mature embryos (Fig. 1f), changes in resource allocation within the developing aphid (perhaps associated with nutrient stress (e.g. Brough & Dixon, 1990) do not explain these results. Aphids reared at elevated CO_2 and/or O_3 may either have been unable to maintain embryo maturation once their initial complement of mature embryos were born, or have resorbed the youngest undifferentiated embryos in response to low host plant quality (e.g. Ward & Dixon, 1982; Sequeira & Dixon, 1996). The decline in *C. betulaefoliae* reproductive output at elevated CO_2 and O_3 may therefore be related to either an inability to maintain embryo maturation or to the resorption of embryos to maintain adult survival. If these data reflect a more general trend (*i.e.* that adult weight and/or embryo number do not reflect future fecundity), studies that have used adult weight as a correlate of fecundity to predict the responses of other herbivore species to elevated CO_2 and O_3 atmospheres (reviewed in Brown, 1995; Bezemer & Jones, 1998) may be misleading.

Previous studies of the nutritional quality of the birch trees used in this study show that the trees grown at elevated CO_2, O_3 and $CO_2 + O_3$ had reduced tissue nitrogen concentrations compared with the control trees (Kopper *et al*., 2001). In this study, individual fecundity was therefore correlated with tissue nitrogen concentrations, although aphid population responses to CO_2 and O_3 (Fig. 3) were not. The reduced nutritional quality of these trees therefore did not explain the difference between the individual- and population-scale responses of *C. betulaefoliae* to elevated CO_2 and O_3. Because all the aphids used had been reared in the appropriate environment for at least three generations prior to the study, maternal effects from ambient-CO_2 and -O_3 environments can probably be ruled out. Docherty *et al*. (1997) also found that aphid performance at elevated CO_2 did not correlate with leaf amino acid concentrations, although Kainulainen *et al*. (2000) found a positive correlation between aphid performance and amino acid availability at elevated O_3.

Fig. 4 Populations of aphids and natural enemies on *Betula papyrifera* at the Aspen FACE site ($N = 1$ tree per ring). The natural enemies were predominantly predators and parasitoids. Control = ambient CO_2, ambient O_3, CO_2 = elevated (570 ppm) CO_2, ambient O_3, O_3 = elevated (1.5 × ambient) O_3, ambient CO_2, $CO_2 + O_3$ = elevated CO_2, elevated O_3. All data are shown ± 1 SEM and are the means of three replicate FACE rings. Since natural enemy abundance was low, all data were pooled prior to analysis.

Some aphid species perform better when they are reared in large aggregations (Eichorn, 1968; Way & Cammell, 1970; Dixon & Wratten, 1971) since they can induce nutrient sinks more effectively than aphids reared individually. Studies investigating the induction of sinks in free-living aphids (e.g. Way & Cammell, 1970) suggest that the benefits of aggregation only occur during the early stages of aphid population growth: after this, intraspecific competition due to crowding may reduce population growth rates. We suggest that short-term changes in either the ability of the aphids to induce nutrient sinks or the strength of intraspecific competition explain the differences between individual and population performance in this study. The surveys of natural populations (Study 3) may not have detected these responses because of competition with other herbivores (which were excluded in Studies 1 & 2), changes in natural enemy performance (rather than abundance) or differences in weather conditions between the two sampling years.

The low natural enemy densities observed in this system (Fig. 4) suggest that the strength of top-down regulation during this study was weak, relative to the impacts of CO_2 and O_3 on plant quality. Since *C. betulaefoliae* does not have an alternative host in the vicinity of the FACE site and more than 99% of the aphids encountered in the population surveys were wingless (C. S. Awmack, personal observation), the aphid populations in this study are unlikely to have been affected by immigration or emigration. The natural enemies, however, are more mobile and exploit aphid populations on the other plant species in the FACE rings (C. S. Awmack, personal observation). In contrast to the data presented here, similar natural enemy populations on the aspen trees inter-planted with the birch trees used in this study exhibited strong positive responses to elevated CO_2 and negative responses to elevated O_3 (Percy *et al*., 2002). The effects of elevated CO_2 and O_3 on the population dynamics of aphids and their natural enemies may therefore be influenced by both the aphid and plant species, even among species in the same ecosystem. Further studies are planned to investigate the abundance of insect herbivores and their natural enemies on a wider range of host plants within the FACE rings, and to investigate changes in the performance (rather than abundance) of the natural enemies in this system.

We conclude that the performance of *C. betulaefoliae* at elevated CO_2 and O_3 depended on whether the aphids were reared as individuals or in populations and hence that individual-based studies may not be reliable indicators of population responses to CO_2 or O_3. Scaling up data collected using individuals to predict the population responses of other herbivorous insects to CO_2 and O_3 may be problematic, as growth and developmental rates did not predict fecundity, and fecundity did not predict population growth. We found little evidence that elevated CO_2 modifies the impacts of elevated O_3 on aphid performance, despite the evidence that elevated CO_2 atmospheres reduce the damaging effects of O_3 on tree productivity at this site (Dickson *et al*., 1998). The responses of this aphid species to CO_2 and O_3 appear to be driven by changes in plant quality and the strength of intraspecific competition, and not by changes in the strength of top-down regulation, since natural enemy abundance showed few responses to CO_2 or O_3. Other herbivores, particularly those exploiting fast-growing host plants such as agricultural crops, may differ in their responses to CO_2 and O_3: a wider range of species needs to be investigated before generalizations can be made about the impacts of elevated CO_2 and O_3 on all aphid and natural enemy populations.

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