

Effects of elevated CO₂ and O₃ on leaf damage and insect abundance in a soybean agroecosystem

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Abstract By altering myriad aspects of leaf chemistry, increasing concentrations of CO₂ and O₃ in the atmosphere derived from human activities may fundamentally alter the relationships between insect herbivores and plants. Because exposure to elevated CO₂ can alter the nutritional value of leaves, some herbivores may increase consumption rates to compensate. The effects of O₃ on leaf nutritional quality are less clear; however, increased senescence may also reduce leaf quality for insect herbivores. Additionally, changes in secondary chemistry and the microclimate of leaves may render plants more susceptible to herbivory in elevated CO₂ and O₃. Damage to soybean (*Glycine max* L.) leaves and the size and composition of the insect community in the plant canopy were examined in large intact plots exposed to

elevated CO₂ (~550 μmol mol⁻¹) and elevated O₃ (1.2*ambient) in a fully factorial design with a Soybean Free Air Concentration Enrichment system (SoyFACE). Leaf area removed by folivorous insects was estimated by digital photography and insect surveys were conducted during two consecutive growing seasons, 2003 and 2004. Elevated CO₂ alone and in combination with O₃ increased the number of insects and the amount of leaf area removed by insect herbivores across feeding guilds. Exposure to elevated CO₂ significantly increased the number of western corn rootworm (*Diabrotica virgifera*) adults (foliage chewer) and soybean aphids (*Aphis glycines*; phloem feeder). No consistent effect of elevated O₃ on herbivory or insect population size was detected. Increased loss of leaf area to herbivores was associated with increased carbon-to-nitrogen ratio and leaf surface temperature. Soybean aphids are invasive pests in North America and new to this ecosystem. Higher concentrations of CO₂ in the atmosphere may increase herbivory in the soybean agroecosystem, particularly by recently introduced insect herbivores.

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Introduction

The increase in atmospheric CO₂ derived from the combustion of fossil fuels and changes in land use (Forster et al. 2007) accelerates the rate of plant photosynthesis, often causing an increase in plant productivity (Drake et al. 1997; Ainsworth et al. 2002; Long et al. 2004), whereas the increasing concentration of tropospheric O₃ (Prather et al. 2001) typically has the opposite effect (Fuhrer 2003; Morgan et al. 2003). Changes in the concentration of these gases in

the troposphere also affect many aspects of leaf structure and chemistry that indirectly affect productivity by changing the relationship between plants and insect herbivores. The magnitude and direction of these indirect effects vary widely (Kopper et al. 2001; Percy et al. 2002; Fuhrer 2003; Kopper and Lindroth 2003; Stiling et al. 2003). To date, only a few experiments have examined how these elements of global change alter the behavior of natural insect populations in agricultural ecosystems (Fuhrer 2003). Understanding how insect feeding behavior is altered by elevated CO₂ and O₃ will be important for predicting crop productivity as well as identifying insect species likely to become pests in the future (Herms et al. 1995; Baker et al. 2000).

Growth in elevated CO₂ generally increases the carbon-to-nitrogen ratio (C:N) of plant tissues (Lincoln et al. 1984; Heagle et al. 1998; Rogers et al. 2004; Hamilton et al. 2005), reducing the nutritional quality for protein-limited insects (Coviella and Trumble 1999). Insects may increase their food intake to compensate for reduced leaf nitrogen content (Coviella and Trumble 1999; Kopper et al. 2001; Holton et al. 2003), although this is not always the case (Kopper and Lindroth 2003; Knepp et al. 2005). Depending on the species and its life stage, nitrogen may not be limiting (Gratton and Denno 2003; Heisswolf et al. 2005), and insects also may respond to other primary and secondary compounds that change in elevated CO₂ (Coviella and Trumble 1999; Ziska et al. 2001; Agrell et al. 2005). Because it reduces stomatal conductance, elevated CO₂ also increases canopy temperature (Rogers et al. 2004; Yoshimoto et al. 2005). Insect generation times are strongly related to temperature (Bale et al. 2002) and rising canopy temperature may shorten the generation time of many pests, increasing the number of generations per season and further exacerbating crop losses (Bale et al. 2002; Fuhrer 2003).

Depending on the dose and duration of exposure, elevated O₃ has been shown to increase or decrease susceptibility to herbivores. By diverting assimilates away from the synthesis of plant defenses, elevated O₃ can increase the susceptibility of plants to insect attack (Endress and Post 1985; Chappelka et al. 1988; Sandermann et al. 1998; Long and Naidu 2002; Percy et al. 2002). In contrast, O₃ also may function as an abiotic elicitor of plant defenses and possibly deter folivorous insects (Sandermann et al. 1998). When plants are exposed to both gases simultaneously, elevated CO₂ reduces the effects of O₃ on photosynthesis (Herms et al. 1996; McKee et al. 2000; Isebrands et al. 2001; Heagle et al. 2003). The combined effect of these gases on leaf chemistry and insect performance has been explored in aspen trees (Kopper et al. 2001; Kopper and Lindroth 2003), but how these gases together affect folivorous insects in an agro-ecosystem is unknown.

Although many invasive insects are tolerant of a wide range of environmental conditions, elevated CO₂ and the

accompanying increases in global temperatures may facilitate the spread of non-native insect pests, particularly in northern and mid-latitudes (Pimentel et al. 2000; Strand 2000). Already, many ecosystems are affected by non-native insects and at least 40% of insect pest species in agricultural fields in the United States are non-indigenous (Pimentel et al. 2000; Strand 2000; Bale et al. 2002). The absence of natural enemies may contribute to the success of non-native pests, but the vulnerability of agro-ecosystems is exacerbated by their lack of genetic diversity for resistance to multiple stressors (Bale et al. 2002; Baker et al. 2005). Currently, ~\$13.9 billion of the potential value of crops is lost annually to non-native insect pests (Pimentel et al. 2000; USDA 2005). This figure will likely rise in the future as trade between the United States and countries with similar climate such as China continues to increase (Baker et al. 2005).

The objective of this research was to quantify the effect of elevated CO₂ and O₃, applied singly and in combination, on insect abundance and the loss of leaf area to herbivores in an intact soybean canopy. The Soybean Free Air Concentration Enrichment (SoyFACE) experiment provides elevated CO₂ and O₃ over an intact soybean crop and unfettered access to insect herbivores without altering other aspects of canopy microenvironment (Long and Naidu 2002). Previously, Hamilton et al. (2005) demonstrated that growth under elevated CO₂ increased herbivory of soybean by Japanese beetles (*Popillia japonica*), and increased damage to foliage was associated with elevated concentrations of soluble sugars that serve as powerful feeding stimulants. Using similar methods, we investigated the effects of inter-annual variation in insect populations on the amount of defoliation in soybean grown in elevated CO₂ and O₃. We predicted that leaf area consumed by herbivores in elevated CO₂ would increase in concert with an increase in leaf C:N. Because both elevated CO₂ and O₃ may reduce the nutritional value of leaves, we expected their effects on herbivory to be additive when both gases are elevated simultaneously.

Materials and methods

Site description

This study was conducted at the SoyFACE facility at the University of Illinois, Urbana-Champaign (40°02' N, 88°14' W, 228 m above sea level; www.soyface.uiuc.edu). Each experimental plot was surrounded by pipes that injected CO₂ or O₃ at a supersonic velocity from 300- μ m pores above the canopy (Miglietta et al. 2001). The target CO₂ concentration was 550 μ mol mol⁻¹; the actual average concentrations for 2003 and 2004 were 552 and 550 μ mol mol⁻¹, respectively, while the ambient plots were ~370 μ mol mol⁻¹ CO₂. The average daily (8 h) O₃

concentration was 60 nmol mol⁻¹, and the elevated O₃ plots were fumigated to 20% above ambient levels. The elevated CO₂ plus elevated O₃ plots received both gases simultaneously. One-minute average CO₂ and O₃ were $\pm 20\%$ of the target for > 95% of the time. The target concentrations for CO₂ and O₃ represent the predicted atmospheric levels by 2050 (Prather et al. 2001; Prentice et al. 2001). A more detailed description of the SoyFACE facility can be found in Dermody et al. (2006).

The experiment consisted of four randomized blocks within a continuous crop of soybean. Each block contained four 20-m diameter octagonal plots (total area 314 m²) exposed to ambient air, elevated CO₂, elevated O₃ or elevated CO₂ plus elevated O₃. Each block was replicated four times. Soybean (*Glycine max* cv. 93B15, Pioneer Hi-Bred, Johnston, IA, USA) was planted at 0.38-m row spacing in each year. This variety is typical of those grown in commercial production.

Leaf area removal

Leaf damage was measured in July and August of 2003 and 2004. The July measurements coincided with the expected peak levels of Japanese beetle (*Popillia japonica*). The August measurements coincided with peak levels of bean leaf beetle (*Cerotoma trifurcata*) and soybean looper (*Pseudoplusia includens*).

To estimate the loss of leaf area to herbivory, 100 plants were randomly selected in each experimental plot and a single leaf was randomly harvested from the top four trifoliates of each plant. Leaves were selected by first randomly selecting the row, then the plant and then finally the nodal position. Randomization was performed in the field by choosing from a set of numbers from 1–30 for row and plant, and from 1–3 for each leaflet on a soybean node. Leaves were cut at the base and photographed against a calibrated background with a high-resolution digital camera (Coolpix 950; Nikon, Melville, NY, USA). The digital photographs were analyzed for tissue lost to herbivory using image analysis software (Scion Image, Beta Version 4.0.2; Scion Corp., Frederick, MD, USA). The size of the leaf was measured and the area before damage was estimated by reconstructing the perimeter. The amount of damage was then converted to a percentage of the estimated original leaf area.

Insect censuses

Insect censuses were conducted to identify species potentially responsible for leaf damage. To minimize disturbance, insect censuses were conducted two days prior to collecting leaves for assessment of leaf area removal. A modified Allen vacuum (Stihl Model BG 85 Blower, Stihl Inc. Virginia

Beach, VA, USA) was fitted with mesh bags (1.4 mm diameter) and each plot was vacuumed for a total sampling time of five minutes per plot. The mesh bags captured the major leaf chewers at SoyFACE. The contents of the mesh bags were freeze-dried for later identification. Similar methods have been employed for other crops (Zalom et al. 1993).

During an outbreak in 2003 we quantified the density of soybean aphids (*Aphis glycines*) on plants grown in ambient and elevated CO₂ and O₃. In each experimental plot, 50 leaves were selected randomly, using the same method employed for measurement of herbivory (see above), from the top two trifoliates and handled carefully to avoid dislodging aphids. Leaves were taken immediately to a field laboratory, where the number of aphids on a randomly selected 2-cm² plot on each leaf was counted, with the condition that the midrib and edge of each leaf were excluded from selection. Aphids were classified as alate (winged), apterous (wingless), or dead. The majority of aphids were actively feeding and apterous, indicating a healthy reproducing population (Rice et al. 2005).

Leaf chemistry and temperature

To determine if leaf carbon and nitrogen levels were altered by elevated CO₂ or O₃, 2-cm² discs from six leaves were sampled within two days of the measurements of area loss. Leaves were chosen randomly using the same method as those employed for leaf sampling, i.e. by first randomly selecting the row, then the plant. The nodal position was however kept constant between treatments. Randomization was performed in the field by choosing from a set of numbers from 1–30 for row and plant, and from 1–3 for each leaflet on a soybean node. The leaf discs from each experimental plot were dried, ground to a fine powder using an automatic grinder (BioSpec products, Bartlettville, Oklahoma, USA) and pooled. Leaf carbon and nitrogen levels were determined with an elemental CHN analyzer (ECS 4010, COSTECH Analytical, USA).

Leaf temperatures were measured in ambient and elevated CO₂ plots in 2004 with two non-contact digital infrared thermometers (RadioShack, Fort Worth, Texas, USA). The thermometers were cross-calibrated over non-biological (metal and concrete) and biological (leaves) surfaces. The surface temperature of 25 randomly selected leaves at the top of the canopy was measured in each plot by holding the thermometers 10 cm above the leaf surface; each leaf was measured at two locations for a total of 50 individual leaf temperature measurements per plot. To avoid confounding time with treatment, the measurement of temperature in each ring was paired (e.g. an ambient plot was measured at the same time as an elevated CO₂ plot). Measurements were performed in blocks. Measurements

on the same leaf were averaged and the experimental plot was treated as the independent replicate.

Statistical analysis

To fulfill the assumptions of ANOVA, the data on leaf area removed by insects were square-root transformed before analysis. Data were separated by year and analyses were performed on the plot means; date and treatment were fixed effects and blocks were included as a random component ($n = 4$; SAS version 8; SAS Institute, Cary NC). A repeated measure ANOVA (PROC MIXED) was used to test for treatment effects on percentage leaf area removed by insects. Values are presented as least squares means with standard errors. The total number of insects and the numbers of individuals of the five main insect species (Japanese beetles, potato leafhoppers, western corn rootworm, soybean nodule fly and multicolored Asian lady beetle (*Harmonia axyridis*) were analyzed in a mixed model ANOVA with treatment and date as fixed effects and blocks as a random component. Leaf carbon and nitrogen contents were analyzed in a mixed model repeated measures ANOVA. A mixed model ANOVA (PROC MIXED; SAS, The SAS Institute; Version 8.1, Cary, NC.) was used to test for treatment effects on leaf temperature. Treatment and day of measurement were fixed effects and blocks were random effects. Post hoc linear contrasts were performed to elucidate treatment effects within interaction terms. All comparisons were made between treatments and the control. Given the limitation on sample size inherent in all FACE experiments, we sought to avoid type II errors by recording results significant at both $P \leq 0.05$ and at 0.1 as in Hamilton et al. (2004) and Dermody et al. (2005).

Results

Exposure to elevated CO_2 increased the area of soybean leaves removed by insect herbivores in 2004 but not in 2003 (August 2003, ambient vs. CO_2 , F-stat = 0.5, $P \leq 0.47$, $n = 4$, August 2004, ambient vs. CO_2 , F-stat = 5.4, $P \leq 0.1$, $n = 4$, Fig. 1). No effect of elevated O_3 on leaf area removed was evident in either year ($P > 0.1$). There was, however, a significant interaction between elevated CO_2 and O_3 in July 2004 (F-stat = 4.4, $P \leq 0.1$, $n = 4$, Fig. 1). The amount of leaf area removed was significantly greater for plants exposed simultaneously to elevated CO_2 plus elevated O_3 , but the absence of a main effect of O_3 suggests that this response was driven by exposure to elevated CO_2 .

Increased foliar damage to plants exposed to elevated CO_2 in August 2004 corresponded with greater numbers of insects (Fig. 2). Total numbers of insects were also higher

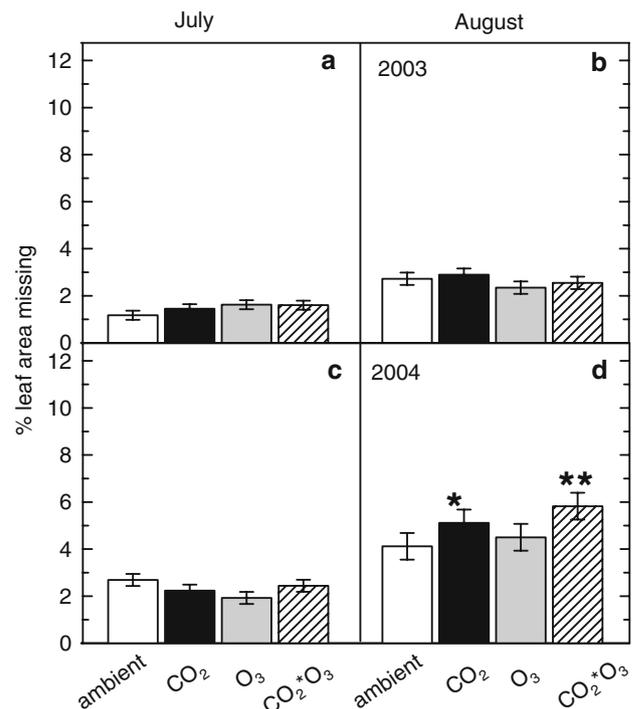


Fig. 1 Amount of leaf tissue removed by herbivores as a percentage of the original leaf area, for soybean grown in ambient air (open bars), $550 \mu\text{mol mol}^{-1} \text{CO}_2$ (black bars) and $1.2 \times$ ambient O_3 (grey bars) and elevated $\text{CO}_2 * \text{O}_3$ (striped bar). The top two panels (a and b) correspond to July and August 2003 and the lower two (c and d) to July and August 2004. Each bar represents the least squared mean \pm standard error ($n = 4$) calculated from the repeated measures ANOVA. Significant differences ($P \leq 0.05$) between elevated CO_2 or O_3 and ambient air are indicated by **, * indicates differences significant at $P \leq 0.1$

in elevated CO_2 and $\text{CO}_2 * \text{O}_3$ in August 2004. This result was driven primarily by increases in the number of western corn rootworms in elevated CO_2 plots (ambient: 15 ± 11 rootworms; elevated CO_2 : 57 ± 13 rootworms, F-stat = 5, $P \leq 0.04$, $n = 4$). There was no detectable effect of elevated CO_2 or elevated O_3 on the size of the insect population in 2003 (e.g. July, 2003, ambient: 17 ± 3 insects; elevated CO_2 : 18 ± 3 insects, F-stat = 0.1, $P \leq 0.9$, $n = 4$; Fig. 2).

The composition of the insect community, including folivores and predators, varied from July to August. Japanese beetles (*P. japonica*) and potato leafhoppers (*E. fabae*) were abundant in July (Fig. 2). By August the numbers of individuals of these species had declined and western corn rootworms became dominant. Soybean nodule flies were present in July 2004 in all treatments (Fig. 2). Other species present in August but in low numbers (≤ 5 individuals per plot) included minute pirate bugs (*Orius tristicolor*), soybean loopers (*Pseudoplusia includens*), cucumber beetles (*Diabrotica undecimpunctata howardii*) and bean leaf beetles (*Cerotoma trifurcata*). Of these,

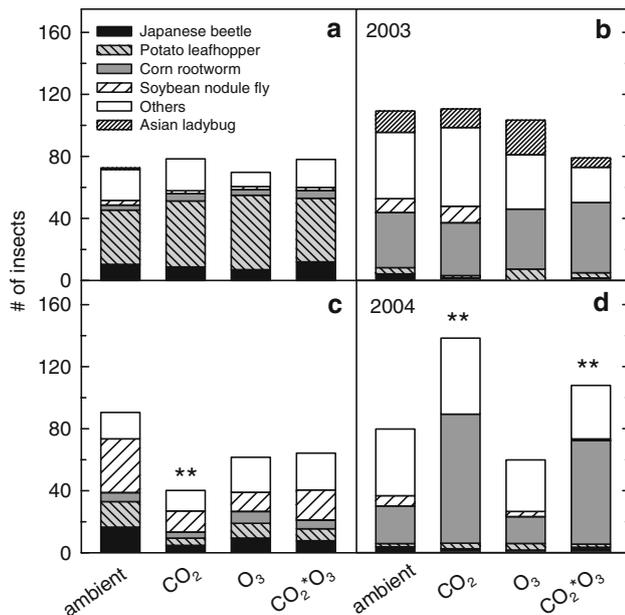


Fig. 2 Total number of insects captured in soybeans grown in ambient air, 550 $\mu\text{mol mol}^{-1}$ CO₂, 1.2 \times ambient O₃ and elevated CO₂*O₃. Each bar represents the pooled mean of all insect species separated into each of the dominant species. The black represents Japanese beetles, dark grey and striped represent potato leafhoppers, grey represents western corn rootworm, striped represents soybean nodule fly, fine stripes represent Asian lady beetle, and white bars include other species which generally numbered less than five individuals per experimental plot. The top two panels (a and b) correspond to July and August 2003 and the second two (c and d) to July and August 2004. Significant differences ($P \leq 0.05$) between elevated CO₂ or O₃ and ambient air are indicated by **, * indicates differences significant at $P \leq 0.1$

Japanese beetles and soybean loopers are folivores and probably were responsible for the greatest proportion of leaf area loss. Because of their low numbers, it is unlikely that various predators contributed to observed differences in leaf damage among the treatments.

During an outbreak in August 2003, soybean aphids responded strongly to elevated CO₂ but not to O₃. Aphid densities were almost twice as high on leaves in elevated CO₂ and CO₂*O₃ plots compared to leaves grown in ambient air (Fig. 3).

In three out of four time periods, exposure to elevated CO₂ reduced the nitrogen content (F-stat = 22.8, $P \leq 0.01$, $n = 4$) and increased the C:N of leaves relative to those grown in ambient air (F-stat = 5.4, $P \leq 0.03$, $n = 4$, Table 1). When CO₂ was combined with O₃ this effect was dampened; there was a trend toward lower leaf nitrogen and higher C:N in the CO₂*O₃ treatment but it was statistically significant only in July 2004 (Table 1). Apart from an isolated effect on C:N in August 2004, elevated O₃ had no detectable effect on these aspects of leaf chemistry (Table 1).

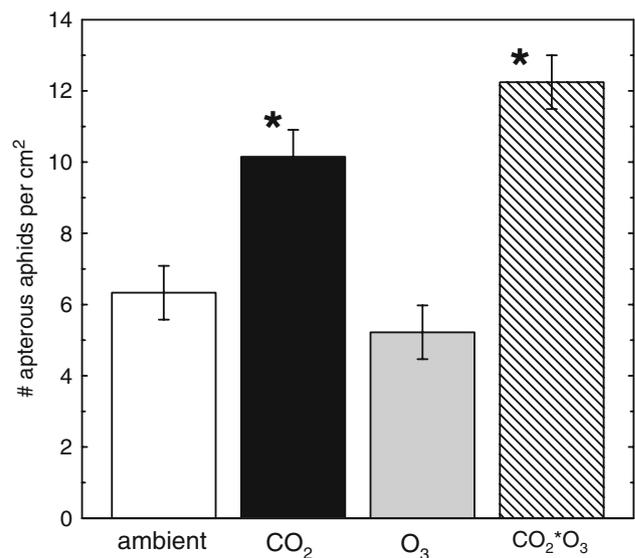


Fig. 3 The density of apterous aphids on soybean leaves in 2003 grown in ambient air (open bars), 550 $\mu\text{mol mol}^{-1}$ CO₂ (black bars) and 1.2 \times ambient O₃ (grey bars) and elevated CO₂*O₃ (striped bar). Each bar represents the least squared mean \pm standard error ($n = 4$) calculated from the repeated measures ANOVA. Significant differences ($P \leq 0.05$) between elevated CO₂ or O₃ and ambient air are indicated by *

Table 1 N content (mg g^{-1}), C content (mg g^{-1}) and C:N ratio of soybean leaves measured in mid-July and mid-August of 2003 and 2004.

Year	Month	Treat	N (mg g^{-1})	C (mg g^{-1})	C: N
2003	July	Ambient	57.77 (1.57)	446.73 (1.77)	7.75 (0.21)
		CO ₂	53.62 (2.83)	451.96 (2.56)	8.50 (0.42)
		O ₃	60.93 (2.85)	444.29 (1.14)	7.34 (0.34)
		CO ₂ *O ₃	55.17 (0.59)	448.54 (2.58)	8.13 (0.10)
2003	August	Ambient	58.64 (0.87)	459.93 (0.83)	7.85 (0.13)
		CO ₂	52.51 (1.67)	456.36 (1.12)	8.72 (0.30)
		O ₃	56.80 (0.46)	458.84 (1.71)	8.08 (0.05)
		CO ₂ *O ₃	55.39 (2.80)	461.22 (3.23)	8.39 (0.41)
2004	July	Ambient	51.58 (0.87)	452.72 (5.26)	8.78 (0.12)
		CO ₂	47.67 (1.23)*	453.48 (2.48)	9.53 (0.26)*
		O ₃	49.67 (1.46)	442.14 (2.05)	8.92 (0.24)
		CO ₂ *O ₃	41.37 (2.11)	441.70 (9.07)	10.74 (0.48)
2004	August	Ambient	46.11 (2.44)	463.90 (1.22)	10.15 (0.57)
		CO ₂	44.33 (0.70)	457.03 (5.42)	10.31 (0.15)
		O ₃	51.18 (0.77)	459.97 (8.69)	8.98 (0.05)
		CO ₂ *O ₃	44.47 (2.1)	457.72 (5.21)	10.35 (0.44)

Soybean was grown in ambient air, 550 $\mu\text{mol mol}^{-1}$ CO₂, 1.2 \times ambient O₃ and CO₂*O₃. Leaf discs were sampled from the same section of the canopy in which herbivory was surveyed. Leaves were sampled within two days of photographing the leaf area removal by herbivores. The least squared means of 4 experimental plots are presented with standard error of the mean in parentheses. Significant differences ($P \leq 0.05$) between elevated CO₂ or O₃ or CO₂*O₃ and ambient air are in bold, * indicates differences significant at $P \leq 0.1$

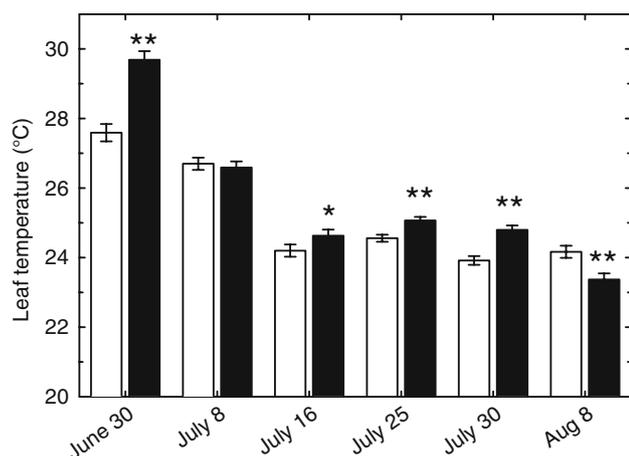


Fig. 4 Mean temperature of leaf surfaces in a soybean canopy grown in ambient air (open bars) and 550 $\mu\text{mol mol}^{-1}$ CO_2 (black bars). Leaf temperature was measured between 11 am and 2 pm on June 30th, July 8th, July 16th, July 25th, July 30th and August 8th in 2004. Each bar represents the least squared mean \pm standard error ($n = 4$) calculated from the repeated measures ANOVA. Significant differences ($P \leq 0.05$) between elevated CO_2 or O_3 and ambient air are indicated by **, * indicates differences significant at $P \leq 0.1$

With the exception of August, when senescence of the soybean canopy was more advanced in the ambient plots (Dermody et al. 2006), and July 8th, exposure to elevated CO_2 increased leaf temperature (F-stat = 25.1, $P \leq 0.01$, $n = 4$, Fig. 4). The average increase was $\sim 1^\circ\text{C}$ across all measurement dates.

Discussion

In August 2004, plants grown under elevated CO_2 had significantly more leaf damage and in 2003 the populations of chewing insects and phloem-feeding aphids were greater than for plants grown in ambient air (Figs. 1 and 2). In the year prior to this study, Hamilton et al. (2005) also observed a substantial increase in leaf damage caused by greater numbers of Japanese beetles in these same elevated- CO_2 plots. Taken together, these results suggest that future increases in atmospheric CO_2 will increase the susceptibility of soybean to insect pests. However, in a cool and wet July 2004, the total number of insects was reduced by growth in elevated CO_2 , indicating how climate variability may complicate any predictions of the effects of elevated CO_2 and O_3 on insect pests. Damage to soybean foliage was caused primarily by increased populations of non-indigenous insects (e.g. Japanese beetles and soybean aphid) that have only recently appeared in Midwest fields (Potter and Held 2002; Ragsdale et al. 2004; Rutledge et al. 2004). The spread of invasive pests is an increasingly important threat to agro-ecosystems, and the increased

abundance of these species on plants grown under elevated CO_2 suggests that these two elements of global change may act synergistically to reduce crop yields in the future.

Growth in elevated CO_2 changes many facets of plant metabolism that potentially alter the feeding behaviour of insects. In this study, increased numbers of insects and leaf area loss to herbivory were associated with greater leaf C:N in elevated CO_2 (Table 1, Figs. 1 and 2). Most phytophagous insects are limited by nitrogen and greater C:N may have stimulated herbivory. Additionally, nitrogen metabolite pools can change following exposure to elevated CO_2 (Stitt and Krapp 1999); for instance Ainsworth et al. (2007) measured decreased levels of ureides, specifically allantoin in developing leaves growing in elevated CO_2 at SoyFACE. As a storage form of N, allantoin is not an optimal N source for folivorous insects on soybean (Wilson and Stinner 1984), so it is possible that increased susceptibility of leaves to herbivory in elevated CO_2 reflected a preference of insects for these lower ureide levels. The nutritional requirements of insects differ depending on life stage, with actively growing larval forms often limited by nitrogen and reproducing adults limited by energy sources (Cloutier et al. 2000; Jacob and Evans 2000; Kopper et al. 2001; Wheeler 2003; Scheirs et al. 2004). As reproductive adults, Japanese beetles and western corn rootworm may respond more strongly to the substantial increase in soluble carbohydrates evident in soybean leaves grown under elevated CO_2 (Hamilton et al. 2005). Insect herbivores also may have responded to changes in leaf secondary compounds in plants grown in elevated CO_2 . Specifically, increased lifespan and fecundity of Japanese beetles are associated with higher levels of individual flavonols (quercetin and kaempferol) that have potent antioxidant properties (B.F. O'Neill, unpublished), as well as with reduced levels of defensive protease inhibitors (Zavala et al. 2008) in leaves grown under elevated CO_2 . The relative contribution of changes in primary versus secondary metabolism on herbivory of soybean under elevated CO_2 is not yet known.

Chronic exposure to O_3 did not affect the susceptibility of soybeans to herbivory (Fig. 1). Growth in elevated O_3 induces many biochemical changes in leaves, including multiple defence pathways; however, these effects are strongest in older leaves that develop at high levels of O_3 (Reid and Fiscus 1998; Sandermann et al. 1998; Booker et al. 2005; Riikonen et al. 2005). Many herbivorous insects preferentially feed on young upper canopy leaves (Bale et al. 2002; Potter and Held 2002), which show few symptoms of O_3 damage after chronic exposure (Ewert and Porter 2000; Morgan et al. 2004). This phenomenon may explain why our results contrast with those of Endress and Post (1985) and Chappelka et al. (1988), who, in closed systems and with acute exposure, found that leaf area

removal increased for soybean foliage grown in elevated O₃. In previous experiments, the effects of elevated O₃ on leaf chemistry and thus on herbivory may have been overestimated by artefacts associated with enclosed systems, e.g. unrealistically high concentrations of O₃ at the base of the canopy at the site of O₃ injection into the chamber (Long and Naidu 2002). FACE technology avoids these artefacts and allows a chronic treatment, more representative of the predicted scenario for major agricultural areas such as the Midwest United States (Prather et al. 2001). However, the effects of O₃ may be more severe in agricultural areas heavily influenced by cities (Ashmore et al. 2006). In aspen trees exposed to elevated CO₂ and O₃ in a FACE system, the effects of the treatments varied depending on which insect herbivore was examined; however, overall effects on leaf chemistry and insect performance were relatively small (Kopper et al. 2001; Lindroth et al. 2001; Kopper and Lindroth 2003).

The density of western corn rootworms was greater on soybean plants grown in elevated CO₂ relative to those in ambient air (Fig. 2). Western corn rootworm is the most important pest of corn in the United States, and, until recently, annually rotating corn with soybean was the principal management strategy to control its numbers (Onstad et al. 2003). Some populations of western corn rootworm circumvent this crop rotation strategy by ovipositing in soybean fields (Onstad et al. 2003; Crowder et al. 2005), and nutritional stress increases the oviposition rate of western corn rootworm (Mabry et al. 2004). Soybean foliage is not an optimal food for western corn rootworm, and higher C:N of soybeans in elevated CO₂ relative to ambient air may have increased nutritional stress, thereby increasing the number of eggs (Schroeder et al. 2006). Although CO₂ is an attractant for western corn rootworm larvae in soil, the adult rootworms are not attracted to CO₂ (Bernklau and Bjostad 1998; Hammack and Petroski 2004). Like many other folivorous insects, western corn rootworms are attracted to plants and stimulated to feed by green leaf volatiles (Hammack 2001; Pare et al. 2005; Pophof et al. 2005). Greater production of green leaf volatiles measured in soybeans grown in elevated CO₂ relative to ambient air (B.F. O'Neill, unpublished) may have contributed to the higher numbers of western corn rootworm. Elevated CO₂ also increased the emission of green leaf volatiles in other systems (Staudt et al. 2001; Vuorinen et al. 2005).

During an outbreak in 2003, the number of aphids per unit leaf area was considerably higher for soybeans grown under elevated CO₂ than in ambient air (Fig. 3). In addition to altering leaf chemistry, growth under elevated CO₂ may have improved the canopy micro-environment for aphids. By decreasing stomatal conductance, exposure to elevated CO₂ increases canopy temperature (Rogers et al. 2004),

which may in turn shorten the generation time of aphids (Bezemer et al. 1998; Holopainen and Kainulainen 2004; Newman 2004) and contribute to their higher densities on soybeans. Although the effect varied across the season, we measured an average increase of 1°C in leaf surface temperature in 2004 (Fig. 4). The exception in August was associated with a change in phenology; plants in the ambient plots had initiated canopy senescence at this time (Dermody et al. 2006) and presumably were operating at lower conductances and hence had greater leaf temperatures than those growing under elevated CO₂. In this system, continuous measurements over three growing seasons revealed that leaf temperature was consistently 0.2°C higher during the day and 0.5°C higher at mid-day under elevated CO₂ (Bernacchi et al. 2006).

Nutritional factors also may have contributed to increased aphid numbers in soybeans grown under elevated CO₂. Soybean aphids preferentially feed on young soybean leaves (Ragsdale et al. 2004), and in elevated CO₂ higher maximum leaf area index and delayed senescence contributed to an abundance of young leaf tissue (Dermody et al. 2006). Aphids rely on free amino acids in phloem fluid (Helden et al. 1994) and their response to elevated CO₂ may be governed by interactions with leaf nitrogen (Newman et al. 2003). Leaf nitrogen content is high in soybean (Dermody et al. 2006) and was probably sufficient to allow continued growth of aphid populations, even with increased leaf C:N in elevated CO₂ (Hoover and Newman 2004).

Soybean aphids were first recorded in the United States in 2000 (Wedburg 2000) and as newly invasive pests their populations are not regulated by specialist predators. However, soybean aphids are vulnerable to predation by generalists, including multicolored Asian lady beetles and minute pirate bugs (Fox et al. 2004). The multicolored Asian lady beetle responds rapidly to increased populations of soybean aphids and is a well known aphid predator in its native range (Koch 2003; Mignault et al. 2006). At SoyFACE, Asian lady beetles co-occurred with soybean aphid, although we were unable to detect any effects of elevated CO₂ and CO₂*O₃ on their populations. Asian lady beetles are becoming an integral part of the pest management strategy for soybean aphid (Rutledge et al. 2004), but the higher density of aphids in the elevated CO₂ plots suggests that the feeding activity of Asian lady beetles may not compensate for faster population growth rates of aphids in elevated CO₂ and CO₂*O₃. Aphid feeding can reduce instantaneous rates of leaf level photosynthesis by as much as 50% (Macedo et al. 2003) and can lower subsequent soybean yields by as much as 28% (Wang et al. 1996). Aphid outbreaks are highly episodic and difficult to compare across years; however, in 2003, aphid densities were highest when soybean was in the reproductive phase and at

its most vulnerable to herbivore damage (Haile et al. 1998). If this pattern is repeated in the future, the productivity gains expected in elevated CO₂ and CO₂*O₃ (Morgan et al. 2005) may be reduced by aphid outbreaks.

A variety of factors, including increased leaf C:N and warmer leaf surfaces, were associated with higher susceptibility of soybean to herbivory in elevated CO₂ and CO₂*O₃. In addition to the effects on leaf area removal, the fecundity of Japanese beetles that were fed on leaves grown in elevated CO₂ was greater than those that fed on leaves grown under ambient conditions (O'Neill et al. 2008). Greater fecundity suggests that the rate of population increase for this species will be even higher under elevated CO₂ than it is today. The effects of elevated CO₂ on aphid densities were particularly notable, given the capacity of this pest to reduce soybean yields (Wang et al. 1996; Rice et al. 2005). Predicting the response of insect herbivores to elevated CO₂ and O₃ is difficult, and will depend on feeding guild, life stage and adaptability of both the plant and insect to changing atmospheric conditions (Bezemer et al. 1998; Hughes and Bazzaz 2001; Newman et al. 2003; Chen et al. 2004). Because we were unable to quantify insect movement between plots and the role of diseases and predators in driving population dynamics, our results highlight the net effects of elevated CO₂ and O₃ on leaf area loss to herbivory. It is possible that insects preferentially move to elevated CO₂ plots given their preference for tissue grown in elevated CO₂ (Hamilton et al. 2005). Despite this limitation, our results suggest that greater insect damage may be one factor contributing to lower than expected yield gains when soybean is grown under elevated CO₂ in open-air FACE experiments compared to partially enclosed chambers that reduce the movement of pests (Long et al. 2006).

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References

- Agrell J, Kopper B, McDonald E, Lindroth R (2005) CO₂ and O₃ effects on host plant preferences of the forest tent caterpillar (*Malacosoma disstria*). *Glob Chang Biol* 11:588–599. doi:10.1111/j.1365-2486.2005.00924.x
- Ainsworth EA, Davey PA, Bernacchi CJ, Dermody OC, Heaton EA, Moore DJ et al (2002) A meta-analysis of elevated [CO₂] effects on soybean (*Glycine max*) physiology, growth and yield. *Glob Chang Biol* 8:695–709. doi:10.1046/j.1365-2486.2002.00498.x
- Ainsworth EA, Rogers A, Leakey ADB, Heady LE, Gibon Y, Stitt M et al (2007) Does elevated atmospheric [CO₂] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? *J Exp Bot* 58:579–591. doi:10.1093/jxb/erl233
- Ashmore M, Toet S, Emberson L (2006) Ozone—a significant threat to future world food production? *New Phytol* 170:201–204. doi:10.1111/j.1469-8137.2006.01709.x
- Baker RHA, Sansford CE, Jarvis CH, Cannon RJC, MacLeod A, Walters KFA (2000) The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agric Ecosyst Environ* 82:57–71. doi:10.1016/S0167-8809(00)00216-4
- Baker R, Cannon R, Bartlett P, Barker I (2005) Novel strategies for assessing and managing the risks posed by invasive alien species to global crop production and biodiversity. *Ann Appl Biol* 146:177–191. doi:10.1111/j.1744-7348.2005.040071.x
- Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK et al (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob Chang Biol* 8:1–16. doi:10.1046/j.1365-2486.2002.00451.x
- Bernacchi CJ, Leakey ADB, Heady LE, Morgan PB, Dohleman FG, McGrath JM et al (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO₂ and ozone concentrations for 3 years under fully open-air field conditions. *Plant Cell Environ* 29:2077–2090. doi:10.1111/j.1365-3040.2006.01581.x
- Bernklau EJ, Bjostad LB (1998) Behavioral responses of first-instar Western corn rootworm (Coleoptera:Chrysomelidae) to carbon dioxide in a glass bead bioassay. *J Econ Entomol* 91:444–456
- Bezemer TM, Jones TH, Knight KJ (1998) Long-term effects of elevated CO₂ and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia* 116:128–135. doi:10.1007/s004420050571
- Booker FL, Miller JE, Fiscus EL, Pursley WA, Stefanski LA (2005) Comparative responses of container versus ground-grown soybean to elevated carbon dioxide and ozone. *Crop Sci* 45:883–895. doi:10.2135/cropsci2004.0198
- Chappelka AH, Kraemer ME, Mebrahtu T, Rangappa M, Benepal PS (1988) Effects of ozone on soybean resistance to the Mexican Bean Beetle (*Epilachna varivestis* Mulsant). *Environ Exp Bot* 28:53–60. doi:10.1016/0098-8472(88)90046-9
- Chen FJ, Wu G, Ge F (2004) Impacts of elevated CO₂ on the population abundance and reproductive activity of aphid *Sitobion avenae* feeding on spring wheat. *J Appl Entomol* 128:723–730. doi:10.1111/j.1439-0418.2004.00921.x
- Cloutier C, Jean C, Fournier M, Yelle S, Michaud D (2000) Adult Colorado potato beetles, *Leptinotarsa decemlineata* compensate for nutritional stress on oryzacystatin I-transgenic potato plants by hypertrophic behavior and over-production of insensitive proteases. *Arch Insect Biochem Physiol* 44:69–81. doi:10.1002/1520-6327(200006)44:2<69::AID-ARCH2>3.0.CO;2-6
- Coviella CE, Trumble JT (1999) Effects of elevated atmospheric carbon dioxide on insect-plant interactions. *Conserv Biol* 13:700–712. doi:10.1046/j.1523-1739.1999.98267.x
- Crowder DW, Onstad DW, Gray ME, Pierce CMF, Hager AG, Ratcliffe ST et al (2005) Analysis of the dynamics of adaptation to transgenic corn and crop rotation by Western corn rootworm (Coleoptera : Chrysomelidae) using a daily time-step model. *J Econ Entomol* 98:534–551
- Dermody O, Long SP, DeLucia EH (2006) How does elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently? *New Phytol* 169:145–155. doi:10.1111/j.1469-8137.2005.01565.x

- Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO₂. *Annu Rev Plant Physiol* 48:609–639. doi:[10.1146/annurev.arplant.48.1.609](https://doi.org/10.1146/annurev.arplant.48.1.609)
- Endress AG, Post SL (1985) Altered feeding preference of Mexican bean beetle *Epilachna varivestis* for ozonated soybean foliage. *Environ Pollut* 39:9–16. doi:[10.1016/0143-1471\(85\)90058-3](https://doi.org/10.1016/0143-1471(85)90058-3)
- Ewert F, Porter JR (2000) Ozone effects on wheat in relation to CO₂: modelling short-term and long-term responses of leaf photosynthesis and leaf duration. *Glob Change Biol* 6:735–750. doi:[10.1046/j.1365-2486.2000.00351.x](https://doi.org/10.1046/j.1365-2486.2000.00351.x)
- Forster P, Ramaswamy V, Artaxo P, Bernsten T, Betts R, Fahey DW et al (2007) Changes in atmospheric constituents and in radiative forcing. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Avery KB, Tignor M, Miller HL (eds) *Climate change 2007: the physical science basis*. Contribution of Working Group I to the fourth assessment report of the intergovernmental panel on climate change
- Fox TB, Landis DA, Cardoso FF, Difonzo CD (2004) Predators suppress *Aphis glycines* Matsumura population growth in soybean. *Environ Entomol* 33:608–618
- Fuhrer J (2003) Agroecosystem responses to combinations of elevated CO₂, ozone and global climate change. *Agric Ecosyst Environ* 97:1–20. doi:[10.1016/S0167-8809\(03\)00125-7](https://doi.org/10.1016/S0167-8809(03)00125-7)
- Gratton C, Denno RF (2003) Seasonal shift from bottom-up to top-down impact in phytophagous insect populations. *Oecologia* 134:487–495
- Haile FJ, Higley LG, Specht JE (1998) Integrated pest management: soybean cultivars and insect defoliation: yield loss and economic injury levels. *Agron J* 90:344–352
- Hamilton JG, Dermody OC, Aldea M, Zangerl AR, Rogers A, Berenbaum MR et al (2005) Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environ Entomol* 34:479–485
- Hammack L (2001) Single and blended maize volatiles as attractants for diabroticite corn rootworm beetles. *J Chem Ecol* 27:1373–1390. doi:[10.1023/A:1010365225957](https://doi.org/10.1023/A:1010365225957)
- Hammack L, Petroski RJ (2004) Field capture of Northern and Western corn rootworm beetles relative to attractant structure and volatility. *J Chem Ecol* 30:1809–1825. doi:[10.1023/B:JOEC.0000042403.88930.a7](https://doi.org/10.1023/B:JOEC.0000042403.88930.a7)
- Heagle AS, Miller JE, Pursley WA (1998) Influence of ozone stress on soybean response to carbon dioxide enrichment: III. yield and seed quality. *Crop Sci* 38:128–134
- Heagle AS, Miller JE, Pursley WA (2003) Growth and yield responses of potato to mixtures of carbon dioxide and ozone. *J Environ Qual* 32:1603–1610
- Heisswolf A, Obermaier E, Poethke HJ (2005) Selection of large host plants for oviposition by a monophagous leaf beetle: nutritional quality or enemy-free space? *Ecol Entomol* 30:299–306. doi:[10.1111/j.0307-6946.2005.00706.x](https://doi.org/10.1111/j.0307-6946.2005.00706.x)
- Helden AJ, Dixon AFG, Carter N (1994) Environmental factors and morphological discrimination between Spring and Summer migrants of the grain aphid, *Sitobion avenae* (Homoptera, Aphididae). *Eur J Entomol* 91:23–28
- Herns DA, Mattson WJ, Karowe DN, Coleman MD, Trier TM, Birr BA et al (1995) Variable performance of outbreak defoliators on aspen clones exposed to elevated CO₂ and O₃. In: Hom J, Birdsey R, O'Brian K (eds) *Proceedings 1995 meeting of the northern global change program*, Gene Technology Report NE-214, U. S. Department of Agriculture, Forest Service, North-eastern Forest Experiment Station, Radnor, PA, pp 43–55
- Herns DA, Mattson WJ, Karowe DN, Coleman MD, Trier TM, Birr BA et al (1996) Variable performance of outbreak defoliators on aspen clones exposed to elevated CO₂ and O₃. In: Hom J, Birdsey R, O'Brien K (eds) *Proceedings 1995 meeting of the northern global change program*, Pittsburgh, PA., pp 43–55
- Holopainen JK, Kainulainen P (2004) Reproductive capacity of the grey pine aphid and allocation response of Scots pine seedlings across temperature gradients: a test of hypotheses predicting outcomes of global warming. *Can J For Res* 34:94–102. doi:[10.1139/x03-203](https://doi.org/10.1139/x03-203)
- Holton MK, Lindroth RL, Nordheim EV (2003) Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO₂, O₃, and plant genotype. *Oecologia* 137:233–244. doi:[10.1007/s00442-003-1351-z](https://doi.org/10.1007/s00442-003-1351-z)
- Hoover JK, Newman JA (2004) Tritrophic interactions in the context of climate change: a model of grasses, cereal aphids and their parasitoids. *Glob Change Biol* 10:1197–1208. doi:[10.1111/j.1529-8817.2003.00796.x](https://doi.org/10.1111/j.1529-8817.2003.00796.x)
- Hughes L, Bazzaz FA (2001) Effects of elevated CO₂ on five plant-aphid interactions. *Entomol Exp Appl* 99:87–96. doi:[10.1023/A:1018921829430](https://doi.org/10.1023/A:1018921829430)
- Isebrands JG, McDonald EP, Kruger E, Hendrey G, Percy K, Pregitzer K et al (2001) Growth responses of *Populus tremuloides* clones to interacting elevated carbon dioxide and tropospheric ozone. *Environ Pollut* 115:359–371. doi:[10.1016/S0269-7491\(01\)00227-5](https://doi.org/10.1016/S0269-7491(01)00227-5)
- Jacob HS, Evans EW (2000) Influence of carbohydrate foods and mating on longevity of the parasitoid *Bathyplectes curculionis* (Hymenoptera:Ichneumonidae). *Environ Entomol* 29:1088–1095
- Knepp RG, Hamilton JG, Mohan JE, Zangerl AR, Berenbaum MR, DeLucia EH (2005) Elevated CO₂ reduces leaf damage by insect herbivores in a forest community. *New Phytol* 167:207–218. doi:[10.1111/j.1469-8137.2005.01399.x](https://doi.org/10.1111/j.1469-8137.2005.01399.x)
- Koch RL (2003) The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control and non-target impacts. *J Insect Sci* 3:1–16. doi:[10.1672/1536-2442\(2003\)003\[0001:TMOAAE\]2.0.CO;2](https://doi.org/10.1672/1536-2442(2003)003[0001:TMOAAE]2.0.CO;2)
- Kopper BJ, Lindroth RL (2003) Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* 134:95–103. doi:[10.1007/s00442-002-1090-6](https://doi.org/10.1007/s00442-002-1090-6)
- Kopper BJ, Lindroth RL, Nordheim EV (2001) CO₂ and O₃ effects on paper birch (Betulaceae : *Betula papyrifera*) phytochemistry and whitemarked tussock moth (Lymantriidae : *Orgyia leucostigma*) performance. *Environ Entomol* 30:1119–1126
- Lincoln DE, Sionit N, Strain BR (1984) Growth and feeding response of *Pseudoplusia includens* (Lepidoptera:Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. *Environ Entomol* 13:1527–1530
- Lindroth RL, Kopper BJ, Parsons WJ, Bockheim JG, Karnosky DF, Hendrey GR et al (2001) Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environ Pollut* 115:395–404. doi:[10.1016/S0269-7491\(01\)00229-9](https://doi.org/10.1016/S0269-7491(01)00229-9)
- Long SP, Naidu SL (2002) Effects of oxidants at the biochemical, cell and physiological levels with particular reference to ozone. In: Bell JNB, Treshow M (eds), *Air pollution and plants*. Wiley, London
- Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: Plants FACE the future. *Annu Rev Plant Biol* 55:591–628. doi:[10.1146/annurev.arplant.55.031903.141610](https://doi.org/10.1146/annurev.arplant.55.031903.141610)
- Long SP, Ainsworth EA, Leakey ADB, Nosberger J, Ort DR (2006) Food for thought: lower-than-expected crop yield stimulation with rising CO₂ concentrations. *Science* 312:1918–1921. doi:[10.1126/science.1114722](https://doi.org/10.1126/science.1114722)
- Mabry TR, Spencer JL, Levine E, Isard SA (2004) Western corn rootworm (Coleoptera: Chrysomelidae) behavior is affected by alternating diets of corn and soybean. *Environ Entomol* 33:860–871
- Macedo TB, Bastos CS, Higley LG, Ostlie KR, Madhavan S (2003) Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. *J Econ Entomol* 96:188–193

- McKee IF, Mulholland BJ, Craigon J, Black CR, Long SP (2000) Elevated concentrations of atmospheric CO₂ protect against and compensate for O₃ damage to photosynthetic tissues of field-grown wheat. *New Phytol* 146:427–435. doi:10.1046/j.1469-8137.2000.00659.x
- Miglietta F, Peressotti A, Vaccari FP, Zaldei A, deAngelis P, Scarascia-Mugnozza G (2001) Free-air CO₂ enrichment (FACE) of a poplar plantation: the POPFACE fumigation system. *New Phytol* 150:465–476. doi:10.1046/j.1469-8137.2001.00115.x
- Mignault MP, Roy M, Brodeur J (2006) Soybean aphid predators in Quebec and the suitability of *Aphis glycines* as prey for three Coccinellidae. *BioControl* 51:89–106. doi:10.1007/s10526-005-1517-1
- Morgan PB, Ainsworth EA, Long SP (2003) How does elevated ozone impact soybean? A meta-analysis of photosynthesis, growth and yield. *Plant Cell Environ* 26:1317–1328. doi:10.1046/j.0016-8025.2003.01056.x
- Morgan PB, Bernacchi CJ, Ort DR, Long SP (2004) An in vivo analysis of the effect of season-long open-air elevation of ozone to anticipated 2050 levels on photosynthesis in soybean. *Plant Physiol* 135:2348–2357. doi:10.1104/pp.104.043968
- Morgan PB, Bollero GA, Nelson RL, Dohleman FG, Long SP (2005) Increase in above-ground net primary production and yield of soybean is less when [CO₂] is elevated in the open. *Glob Chang Biol* 11:1–10. doi:10.1111/j.1365-2486.2005.001017.x
- Newman JA (2004) Climate change and cereal aphids: the relative effects of increasing CO₂ and temperature on aphid population dynamics. *Glob Chang Biol* 10:5–15. doi:10.1111/j.1365-2486.2003.00709.x
- Newman JA, Gibson DJ, Parsons AJ, Thornley JHM (2003) How predictable are aphid population responses to elevated CO₂? *J Anim Ecol* 72:556–566. doi:10.1046/j.1365-2656.2003.00725.x
- O'Neill BF, Zangerl A, DeLucia EH, Berenbaum MR (2008) Longevity and fecundity of Japanese beetle, *Popillia japonica* (Newman), on foliage grown under elevated CO₂. *Environ Entomol* 37:601–607
- Onstad DW, Crowder DW, Isard SA, Levine E, Spencer JL, O'Neal ME et al (2003) Does landscape diversity slow the spread of rotation-resistant western corn rootworm (Coleoptera: Chrysomelidae)? *Environ Entomol* 32:992–1001
- Pare PW, Farag MA, Krishnamachari V, Zhang HM, Ryu CM, Kloepper JW (2005) Elicitors and priming agents initiate plant defense responses. *Photosyn Res* 85:149–159. doi:10.1007/s1120-005-1001-x
- Percy KE, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG et al (2002) Altered performance of forest pests under atmospheres enriched by CO₂ and O₃. *Nature* 420:403–407. doi:10.1038/nature01028
- Pimentel D, Lach L, Zuniga R, Morrison D (2000) Environmental and economic costs of nonindigenous species in the United States. *Bioscience* 50:53–65. doi:10.1641/0006-3568(2000)050[0053:EAECON]2.3.CO;2
- Pophof B, Stange G, Abrell L (2005) Volatile organic compounds as signals in a plant-herbivore system: Electrophysiological responses in olfactory sensilla of the moth *Cactoblastis cactorum*. *Chem Senses* 30:51–68. doi:10.1093/chemse/bji001
- Potter DA, Held DW (2002) Biology and management of the Japanese beetle. *Annu Rev Entomol* 47:175–205. doi:10.1146/annurev.ento.47.091201.145153
- Prather M, Ehhalt D, Dentener F, Derwent R, Dlugokencky E, Holland E et al (2001) Atmospheric chemistry and greenhouse gases. In: Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linder PJ, Dai X, Maskell K, Johnson CA (eds) *Climate change 2001: the scientific basis. Contribution of Working Group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, pp 183–240
- Prentice IC, Farquhar GD, Fasham MJR, Goulden ML, Heimann M, Jaramillo VJ et al (2001) The carbon cycle and atmospheric carbon dioxide. In: Houghton JT, Y.D., Griggs DJ, Noguer M, van der Linder PJ, Dai X, Maskell K, Johnson CA (eds) *Climate change 2001: the scientific basis. Contribution of Working Group I to the third assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, pp 241–280
- Ragsdale DW, Voegtlin DJ, O'Neil RJ (2004) Soybean aphid biology in North America. *Ann Entomol Soc Am* 97:204–208. doi:10.1603/0013-8746(2004)097[0204:SABINA]2.0.CO;2
- Reid CD, Fiscus EL (1998) Effects of elevated [CO₂] and/or ozone on limitations to CO₂ assimilation in soybean (*Glycine max*). *J Exp Bot* 49:885–895. doi:10.1093/jexbot/49.322.885
- Rice ME, O'Neal M, Pedersen P (2005) Soybean aphids in Iowa—2005. Iowa State University—University Extension, Ames
- Riikonen J, Holopainen T, Oksanen E, Vapaavuori E (2005) Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO₂ and O₃ in the field. *Tree Physiol* 25:621–632
- Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ et al (2004) Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment. *Plant Cell Environ* 27:449–458. doi:10.1111/j.1365-3040.2004.01163.x
- Rutledge CE, O'Neil RJ, Fox TB, Landis DA (2004) Soybean aphid predators and their use in integrated pest management. *Ann Entomol Soc Am* 97:240–248. doi:10.1603/0013-8746(2004)097[0240:SAPATU]2.0.CO;2
- Sanderman JH, Ernst D, Heller W, Langebartels C (1998) Ozone: an abiotic elicitor of plant defense reactions. *Trends Plant Sci* 3:47–50. doi:10.1016/S1360-1385(97)01162-X
- Scheirs J, Zoebisch TG, Schuster DJ, De Bruyn L (2004) Optimal foraging shapes host preference of a polyphagous leafminer. *Ecol Entomol* 29:375–379. doi:10.1111/j.0307-6946.2004.00600.x
- Schroeder JB, Gray ME, Ratcliffe ST, Estes RE, Long SP (2006) Effects of elevated CO₂ and O₃ on a variant of the Western corn rootworm (Coleoptera: Chrysomelidae). *Environ Entomol* 35:637–644
- Staudt M, Joffre R, Rambal S, Kesselmeier J (2001) Effect of elevated CO₂ on monoterpene emission of young *Quercus ilex* trees and its relation to structural and ecophysiological parameters. *Tree Physiol* 21:445–448
- Stiling P, Hunter MD, Rossi AM, Hymus GJ, Drake BG (2003) Elevated CO₂ lowers relative and absolute herbivore density across all species of a scrub-oak forest. *Oecologia* 134:82–87. doi:10.1007/s00442-002-1075-5
- Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant Cell Environ* 22:583–621. doi:10.1046/j.1365-3040.1999.00386.x
- Strand JF (2000) Some agrometeorological aspects of pest and disease management for the 21st century. *Agric For Meteorol* 103:73–82. doi:10.1016/S0168-1923(00)00119-2
- USDA (2005) Soy stats, a reference guide to important soybean facts & figures. American soybean association. United States Department of Agriculture, St. Louis, Missouri
- Vuorinen T, Nerg AM, Vapaavuori E, Holopainen JK (2005) Emission of volatile organic compounds from two silver birch (*Betula pendula* Roth) clones grown under ambient and elevated CO₂ and different O₃ concentrations. *Atmos Environ* 39:1185–1197. doi:10.1016/j.atmosenv.2004.09.077
- Wang SY, Boa XZ, Sun YJ, Chen RL, Zhai BP (1996) Study on the effects of the population dynamics of soybean aphid (*Aphis*

- glycines*) on both growth and yield of soybean. *Soybean Sci* 15:243–247
- Wedburg J (2000) Important update on aphids in soybeans. *Wis Crop Manager*. <http://ipcm.wisc.edu/wcm/pdfs/2000/00-22insect1.html>. Accessed 17 Aug 2000
- Wheeler GS (2003) Minimal increase in larval and adult performance of the biological control agent *Oxyops vitiosa* when fed *Melaleuca quinquenervia* leaves of different nitrogen levels. *Biol Control* 26:109–116. doi:10.1016/S1049-9644(02)00129-9
- Wilson KG, Stinner RE (1984) A potential influence of rhizobium activity on the availability of nitrogen to legume herbivores. *Oecologia* 61:337–341. doi:10.1007/BF00379631
- Yoshimoto M, Oue H, Kobayashi K (2005) Energy balance and water use efficiency of rice canopies under free-air CO₂ enrichment. *Agric For Meteorol* 133:226–246. doi:10.1016/j.agrformet.2005.09.010
- Zalom FG, Pickel C, Walsh DB, Welch NC (1993) Sampling for *Lygus hesperus* Knight (Hemiptera: Miridae) in strawberries. *J Econ Entomol* 86:1191–1195
- Zavala J, Casteel C, DeLucia EH, Berenbaum MR (2008) Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. *Proc Natl Acad Sci USA* 105:5129–5133. doi:10.1073/pnas.0800568105
- Ziska LH, Ghannoum O, Baker JT, Conroy J, Bunce JA, Kobayashi K et al (2001) A global perspective of ground level, ‘ambient’ carbon dioxide for assessing the response of plants to atmospheric CO₂. *Glob Change Biol* 7:789–796. doi:10.1046/j.1365-2486.2001.00436.x