Oecologia
DOI 10.1007/s00442-012-2261-8

PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Elevated CO_2 interacts with herbivory to alter chlorophyll fluorescence and leaf temperature in *Betula papyrifera* and *Populus tremuloides*

Paul D. Nabity · Michael L. Hillstrom · Richard L. Lindroth · Evan H. DeLucia

Received: 12 February 2011 / Accepted: 15 January 2012

Abstract Herbivory can influence ecosystem productivity, but recent evidence suggests that damage by herbivores modulates potential productivity specific to damage type. Because productivity is linked to photosynthesis at the leaf level, which in turn is influenced by atmospheric CO_2 concentrations, we investigated how different herbivore damage types alter component processes of photosynthesis under ambient and elevated atmospheric CO_2. We examined spatial patterns in chlorophyll fluorescence and the temperature of leaves damaged by leaf-chewing, gall-forming, and leaf-folding insects in aspen trees as well as by leaf-chewing insects in birch trees under ambient and elevated CO_2 at the aspen free-air CO_2 enrichment (FACE) site in Wisconsin. Both defoliation and gall damage suppressed the operating efficiency of photosystem II (ΦPSII) in remaining leaf tissue, and the distance that damage propagated into visibly undamaged tissue was marginally attenuated under elevated CO_2. Elevated CO_2 increased leaf temperatures, which reduced the cooling effect of gall formation and freshly chewed leaf tissue. These results provide mechanistic insight into how different damage types influence the remaining, visibly undamaged leaf tissue, and suggest that elevated CO_2 may reduce the effects of herbivory on the primary photochemistry controlling photosynthesis.

Keywords Plant–insect interactions · Photosynthesis · Thermography · FACE · Climate change

Communicated by Carlos Ballaré.

P. D. Nabity · E. H. DeLucia (✉)
Department of Plant Biology, University of Illinois,
265 Morrill Hall, 505 South Goodwin Avenue,
Urbana-Champaign, IL 61801, USA
e-mail: delucia@illinois.edu

P. D. Nabity · E. H. DeLucia
Institute for Genomic Biology, University of Illinois,
1206 West Gregory Drive, Urbana-Champaign, IL 61801, USA

M. L. Hillstrom · R. L. Lindroth
Department of Entomology, University of Wisconsin,
237 Russell Labs, 1630 Linden Drive, Madison,
WI 53706-1598, USA

Introduction

Arthropod herbivory alters ecosystem productivity, especially in outbreak years (Cyr and Pace 1993), and recent evidence suggests that the manner of feeding (e.g., chewing, phloem feeding) may alter how herbivory affects productivity (Zvereva et al. 2010; Patankar et al. 2011). Reductions in productivity result, in part, from damage-specific alterations of photosynthetic capacity. Direct tissue consumption (loss of photosynthetic area) by defoliators and direct cellular disruption or altered osmotic potential (extracted photosynthate) by piercing–sucking insects differentially affect photosynthesis (see Welter 1989). Insect feeding also selectively impairs remaining leaf tissue through direct and indirect alterations of leaf physiology (Nabity et al. 2009). Within the few systems (parsnip, soybean, *Arabidopsis*, various hardwood trees) evaluated thus far, the general response is a suppression of leaf photosynthesis beyond the area of arthropod feeding.

As arthropod herbivores feed, tissue damage may suppress photosynthesis in remaining tissues by severing vasculature (Aldea et al. 2006; Tang et al. 2006), altering sink/source relationships (Dorchin et al. 2006), autotoxicity (Gog et al. 2005), and defense-induced downregulation of photosynthetic genes (Bilgin et al. 2010). Remaining

Published online: 23 February 2012

tissues also may respond to the type of damage with varying degrees of physiological impairment; a 5% reduction in leaf area by a chewing herbivore resulted in a 20% reduction in the photosynthetic capacity of remaining parsnip leaf tissue (Zangerl et al. 2002), whereas gall damage to understory hardwood trees reduced photosynthesis in remaining tissues equal to the area removed (Aldea et al. 2006). Understanding these effects on physiology among damage types and within model systems where genomic information is readily accessible may help link the observed global genomic downregulation of photosynthetic genes (Bilgin et al. 2010) to physiological impairment in visibly unaltered remaining leaf tissues.

Atmospheric carbon dioxide is rising steadily (~ 2 µl l^{-1} $year^{-1}$, http://www.esrl.noaa.gov/gmd/ccgg/trends/) and generally enhances the photosynthesis of forest ecosystems (Saxe et al. 1998; Leakey et al. 2009; Lindroth 2010). Despite this enhancement, elevated CO_2 levels also increase damage rates by herbivores in aspen and birch (Couture et al. 2011). This increase in damage typically coincides with increased abundance of phloem-feeding herbivores and decreased chewing and galling herbivore abundance in field sites fumigated with CO_2 (Hillstrom and Lindroth 2008; Hillstrom 2010). Because increased CO_2 stimulates photosynthesis and reduces stomatal conductance, thereby improving leaf water status, the predicted increases in atmospheric CO_2 may modulate the effect of herbivory on photosynthesis. The propagation of fungal damage beyond visible lesions into remaining, visibly undamaged tissues is reduced under elevated CO_2 (McElrone et al. 2010); however, there are no examinations of how herbivores may alter the photosynthesis of trees grown under future CO_2 concentrations.

Chlorophyll fluorescence imaging is a powerful tool for making high-resolution, spatially resolved measurements of component processes of photosynthesis in damaged leaf tissues (Oxborough 2004; Aldea et al. 2006). Measurement of the quantum efficiency of photosystem II (ΦPSII) is particularly useful in this regard because it is highly correlated with the rate of CO_2 uptake in intact leaves (Genty and Meyer 1994; Baker and Oxborough 2005; Tang et al. 2006) and relates directly to carbon assimilation, an ecologically important trait. Using this approach, Aldea et al. (2006) identified spatial patterns in fluorescence and thermal images with some degree of host specificity; defoliation typically reduces ΦPSII in remaining leaf tissue along cut edges, whereas some gall-formers reduce ΦPSII well beyond visible feeding damage. However, no studies have investigated how arthropod herbivory affects remaining leaf tissue under elevated CO_2 or among herbivore damage types within one plant species to minimize the variability introduced by species. The objective of this research was to determine if elevated CO_2 modulates the effect of herbivory on the spatial pattern of photosynthesis as indicated by variations in ΦPSII in two tree species growing under otherwise natural environmental conditions. Because elevated CO_2 may enhance photosynthesis and water use efficiency, and alter the production of secondary chemicals, we hypothesized that elevated CO_2 will attenuate the effects of herbivore damage. We tested this hypothesis at the aspen free-air CO_2 enrichment (FACE) site in northern Wisconsin. We utilized aspen trees of a single clone to minimize genetic variability, and examined multiple insect herbivores inflicting three different damage types.

Materials and methods

Experimental design

Research was conducted during the summers of 2008–2009 at the aspen free-air CO_2 enrichment (Aspen FACE) site in north-central Wisconsin, USA (W 89.5°, N 45.7°). This 32 ha site contained twelve 30 m diameter plots with the following treatment combinations: ambient, elevated CO_2 (ambient + 200 µl l^{-1}), elevated O_3 (1.5 × ambient), and elevated CO_2 plus elevated O_3. Three plots were designated to each fumigation treatment and blocked from north to south across the site. Only ambient and elevated CO_2 plots were used for this experiment.

Each plot contained five aspen (*Populus tremuloides* Michx.) genotypes in addition to birch (*Betula papyrifera* Marsh.) and sugar maple (*Acer saccharum* Marsh.); however, we examined only one aspen genotype and birch. We selected aspen genotype 216 for our study because it responds strongly to CO_2 enrichment (Noormets et al. 2001). Aspen genotype 216 and birch were used during 2008, and only aspen was examined again in 2009. For both aspen and birch, defoliation damage of unknown age and herbivore source was examined in 2008. In addition, two other insect damage types were evaluated on aspen: skeletonizing damage by larval sawflies (*Phyllocolpa* sp.), where feeding occurs within the folded edges of leaves, and leaves galled by midge flies (*Harmandia* sp; see Fig. 1). To control for time after chewing damage occurred, we conducted a 24 h feeding trial of gypsy moth (*Lymantria dyspar* L.) on aspen. In each plot, three branches with full sun exposure on three different trees were enclosed in fine mesh bags; each bag contained 20–30 undamaged leaves on determinant shoots. Ten fifth-instar larvae were placed in each bag and allowed to feed overnight. Measurements of chlorophyll fluorescence and leaf temperature were made the following day.

To assess how elevated CO_2 alters the effects of herbivore damage, we identified leaves from each tree species within each plot for each damage type and randomly

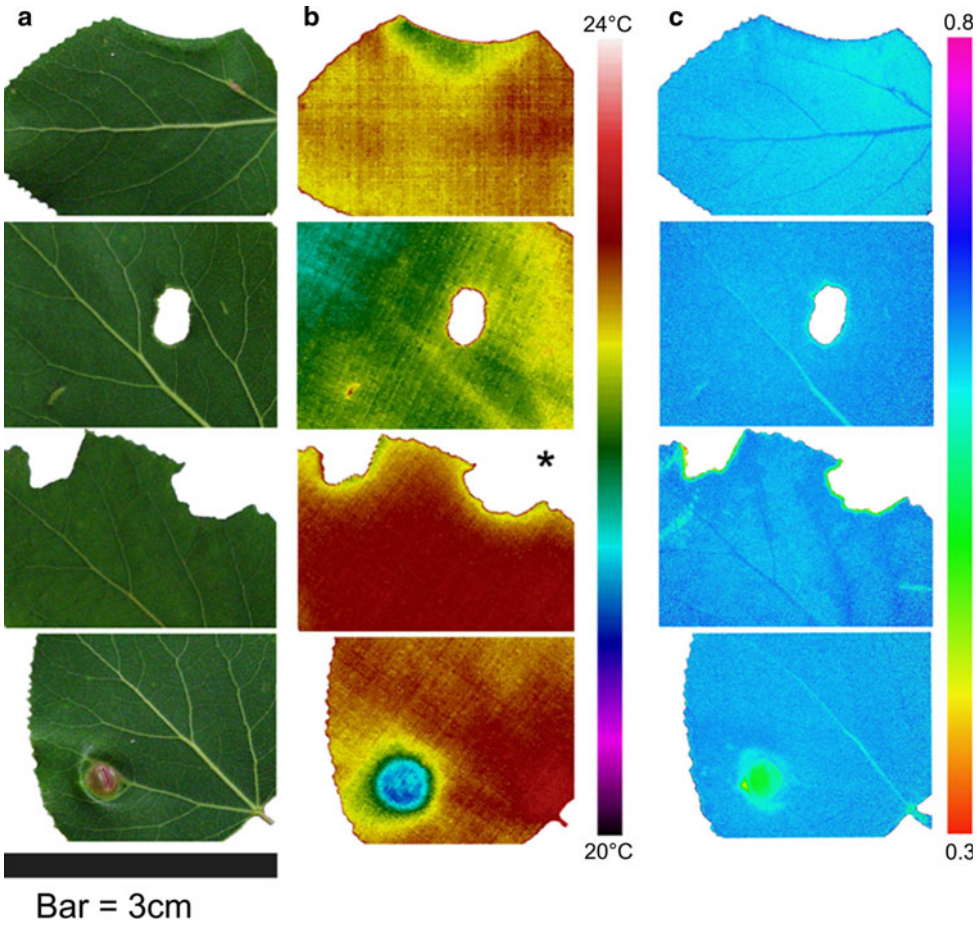

Fig. 1 Representative true-color reflected light images (**a**), false-color thermal images (**b**), and false-color images of fluorescence (ΦPSII efficiency, **c**) for damage from leaf folds (*Phyllocolpa* larvae, *top row*), defoliation (unknown age and herbivore, *second row*), 24 h defoliation (*Lymantria dispar*, *third row*), and gall (*Harmandia* sp., *bottom row*) on aspen (*Populus tremuloides*). All images are represented to scale, with the exception that 24 h defoliation damage (*) is on the same false-color scale from 20 to 26°C

selected four leaves for each species/damage type combination. All leaves were on determinant shoots. The spatial patterns of temperature and photosynthetic electron transport were measured by thermography and chlorophyll fluorescence, respectively. Data from these cohorts represented subsamples for each plot and were subsequently pooled by plot ($n = 3$).

Thermography

To quantify the effects of herbivory on the spatial pattern of water loss, thermal images of each damage type were taken using an infrared camera (ThermaCAM Infrared Camera, FLIR Systems, Portland, OR, USA). Branchlets were excised and rapidly transported back to a nearby field laboratory. Individual leaves were then excised from branchlets under degassed water, their petioles were placed in a water-filled 2 ml tube, and they were allowed to equilibrate to steady-state, light-adapted conditions (150 µmol m^{-2} s^{-1} PFD at 25°C). Excised leaves were imaged at constant light and temperature (150 µmol m^{-2} s^{-1} PFD at 25°C) and at ambient CO_2.

Chlorophyll fluorescence

To quantify the effects of different damage types on the efficiency of primary photochemistry, the spatial pattern of the operating efficiency of photosystem II (ΦPSII) was measured with an imaging chlorophyll fluorometer (Walz Imaging PAM, Walz GmbH, Effeltrich, Germany). Branchlets and leaves were collected and maintained in a field laboratory under the conditions described above. Leaves were allowed to equilibrate to environmental conditions within the field laboratory until a steady state was reached. This occurred when the fluorescent yield (continuously monitored by the camera) no longer fluctuated in false-color pixel intensity, thereby indicating stable electron transport across the leaf surface. Once a steady state was reached, fluorescense was recorded for a 2 × 3 cm area of the leaf surface centered on the herbivore damage. ΦPSII was calculated from an initial image of minimum fluorescence in a light-adapted state (F') and an image of fluorescence following a 1 s saturating pulse (ca. 2,500 µmol m^{-2} s^{-1}; F'_m) using the formula: $\Phi PSII = (F'_m - F')/F'_m$. Although the leaves selected in this experiment were from the sunlight

canopy and were typically exposed to higher ambient light conditions, the assessment of pulse amplitude modulated (PAM) fluorescence was made under low irradiance to optimize the imaging device at its highest resolution and to enhance the correlation between fluorescence and photosynthetic rate (Longstaff et al. 2002). Light-adapted fluorescence was used over dark-adapted fluorescence because the former provides a more accurate quantification of quantum yield, and therefore a stronger proxy for photosynthesis (Baker 2008).

The entire process of collecting tissue from the canopy, allowing leaves to reach steady-state temperature and fluorescence yield, and imaging both thermal and ΦPSII spatial patterns spanned <30 min per leaf.

Image analysis

True-color reflected-light images were taken with a digital camera (PowerShot SD1000, Canon, Lake Success, NY, USA) for each leaf using a camera mounted a fixed distance from the leaf and with a standard within each image to accurately calculate leaf area. The total leaf area in pixels was calculated for each false-color fluorescence and thermal image using the appropriate software (ImagingWin v2.32, Heinz Walz GmbH, Effeltrich, Germany; ThermaCam Quick Report, FLIR Systems, Portland, OR, USA). This area in pixels was then compared to the calculated leaf area to quantify the number of false-color pixels occurring in visibly damaged tissue (the galled region, the leaf fold, or the approximate leaf area defoliated), regions of undamaged tissue (with an equivalent thermal or fluorescent signature to undamaged control leaves), and regions in-between, where visible damage ceased but altered fluorescence or thermal signatures occurred (hereafter "propagated damage"). This process allowed accurate calculation of the true dimensions covered by each false-color pixel. False-color pixels were then counted for each region and converted to distances/areas. Propagated damage was quantified as any pixel intensity deviating >5% from undamaged tissue within the same leaf. Because chewing herbivores remove tissue, there is minimal visible damage (a cut edge), so we calculated propagated damage as the distance of pixels from the cut edge, which deviated >5% from undamaged tissue within the same leaf.

Reflectance measurement

To further explore possible mechanisms for how physiological damage may propagate from developing galls, spectral reflectance was measured on galls, visibly undamaged tissue on the same leaf, and adjacent undamaged leaves. Galls were selected because of the greater alteration in thermal spatial patterns than other damage types and because the damaged tissue remained on the leaf over time (24 h defoliation damage resulted in large alterations in thermal spatial patterns, but this effect was not sustained in older tissue). Leaf reflectance was measured in June 2009 on undamaged and galled leaves excised from equivalent nodes on determinate shoots in both elevated CO_2 and ambient plots. Four leaves for each damage type were removed from branchlets from different trees and combined for a single average of each damage type for each plot ($n = 3$). Leaves were irradiated by an internal tungsten halogen light source in a field lab, as previously described, and upwelling irradiance (300–1,100 nm) was recorded using a portable spectroradiometer (UniSpec Spectral Analysis System, PP Systems, Haverhill, MA, USA). The spectroradiometer was equipped with a visible/near-infrared detector of <10 nm Raleigh resolution and 3.3 nm bin size (<0.3 nm accuracy). Measurements were taken in a field laboratory with the fiber optic probe pointed downward to measure upwelling irradiance under ambient conditions. Prior to each measurement, a reference scan was performed by pointing the probe downward on a white reference standard (PP Systems).

Reflectance data (R) were used to calculate the photochemical reflectance index, water index, and normalized difference vegetation index. The photochemical reflectance index (PRI) was calculated as $(R_{531} - R_{570})/(R_{531} + R_{570})$; this index responds to the composition of xanthophyll pigments and correlates with ΦPSII and net CO_2 assimilation (Gamon et al. 1997). The water index (WI) measures tissue water content and was calculated as R_{900}/R_{970} (Penuelas et al. 1997). The normalized difference vegetation index $NDVI_{750}$ assesses foliar chlorophyll content and was calculated as $(R_{750} - R_{705})/(R_{750} + R_{705})$ (Sims and Gamon 2002).

Statistics

This study employed a split plot design with blocking where whole-plot treatments consisted of CO_2 level (ambient and elevated) and subplot consisted of damage type (defoliation, gall, leaf fold, undamaged). We analyzed aspen separately from birch because damage to the two tree species was produced by different insects, and thus treatments of herbivore type were asymmetrically applied. The model for each analysis used was: $Y_{ijkl} = \mu + B_i + C_j + e_{ij} + D_k + CD_{jk} + e_{ijk}$, where fixed effects included CO_2 level (C_j), damage type (D_k), and their interaction term (CD_{jk}). Block (B_i), whole plot error (e_{ij}), and subplot error (e_{ijk}) were random effects. Only aspen was examined again in 2009. Analysis of variance (ANOVA; PROC MIXED, SAS v.9.2, SAS Institute, Cary, NC, USA) was used for all comparisons. Because low replication ($n = 3$) increases the probability of type II errors but increasing α increases

the probability of type I errors (Filion et al. 2000), we considered P values $0.05 \leq 0.10$ to be marginally significant and ≤ 0.05 to be significant.

Results

Arthropod damage altered ΦPSII and leaf surface temperature in visibly damaged tissue and reduced ΦPSII of remaining tissues adjacent to feeding damage; however, the extent that damage propagated into remaining leaf tissue depended on the type of injury (Tables 1, 2; Fig. 1). Defoliation and gall formation increased the spatial heterogeneity of leaf temperature and ΦPSII, but skeletonizing damage by larval sawflies did not. Defoliation reduced ΦPSII ~5–7% for ~1 mm from chewed edges and increased the temperature of the chewed edge in both aspen and birch (Figs. 2, 3). Aspen leaves with 24 h larval gypsy moth defoliation damage responded with larger reductions in ΦPSII efficiency (~14%) and enhanced evaporative cooling along the chewed edge; propagated damage reduced the temperature ~0.5°C up to 4 mm away from the damage (Table 1). At its maximumm, gall damage reduced ΦPSII >10% relative to undamaged tissues, but the distance that damage (defined as pixels deviating >5% from undamaged tissue on the same leaf) propagated into remaining leaf tissue was small (0.3 ± 0.1 mm). Gall damage enhanced evaporative cooling and reduced temperature by 1.2 ± 0.2°C; this cooling effect propagated 2.5 ± 0.2 mm into adjacent nongalled tissues. Gall damage reduced the spectral reflectance for all wavelengths between 400 and 700 nm with the exception of a peak near 550 (i.e., xanthophylls). Gall damage also reduced PRI and increased water content (WI; Table 3).

Elevated CO_2 reduced the distance that defoliation damage in birch and 24 h defoliation damage in aspen altered ΦPSII in adjacent tissues. (Table 2; Fig. 2). Elevated CO_2 also reduced the cooling effect of gall formation on remaining leaf tissue (Fig. 3). Although birch responded to ambient and elevated CO_2 with similar leaf temperatures and distances that damage propagated to those seen for aspen, these effects were not statistically resolved. Elevated CO_2 also marginally reduced the potential chlorophyll content of undamaged leaves (NDVI; Table 3).

Discussion

Arthropod herbivory increased the spatial heterogeneity of photosynthesis and water use in remaining leaf tissue across tree species, but it did so in a damage-specific manner. Although background levels of herbivory are typically low (<16%), with the exception of outbreak years, even low levels may alter biomass (Wolf et al. 2008; Patankar et al. 2011). Our data indicated that fresh defoliation damage immediately impaired ΦPSII in remaining aspen tissues, and that this reduction in ΦPSII attenuated with time (Table 2; Fig. 2). Older damage also reduced ΦPSII and transpiration in remaining tissues, suggesting that the leaf never completely recovered from the original herbivore attack (Tables 1, 2; Fig. 2). Another common damage type—gall formation—reduced ΦPSII, but this reduction was localized and did not propagate from the gall into the surrounding leaf tissue. In contrast to ΦPSII, lower leaf temperatures indicated that transpiration was enhanced in undamaged leaf tissue surrounding galls (Fig. 2). Because as little as a 0.5°C change in leaf surface temperature can indicate a 10% shift in stomatal conductance (Jones 1999) and ΦPSII correlates strongly with photochemistry (Genty and Meyer 1994), these subtle alterations in remaining leaf tissue may alter carbon gain at larger scales. When summed at the leaf level, the area of apparently healthy tissue with lower ΦPSII can be equal to the area with visible herbivore damage (Aldea et al. 2006; Zangerl et al. 2002). Thus, relying on visible damage may underestimate the effect of herbivory on photosynthesis and plant productivity.

Elevated CO_2 increased leaf temperature in aspen which, in turn, interacted with damage type to reduce the cooling effect of immediate defoliation damage and gall formation. Although the values of birch leaf temperature under ambient and elevated CO_2 were similar to aspen, the effect of CO_2 was not statistically resolvable at the low replication common among FACE sites (Filion et al. 2000). Elevated CO_2 reduced the distance that damage to ΦPSII propagated into remaining tissue in birch and 24 h defoliation in aspen. These results support the hypothesis that elevated CO_2 can mitigate the effect of herbivory on photosynthesis.

The mechanisms underlying suppressed ΦPSII and altered transpiration vary with the type of herbivore damage. Defoliation damage may sever vasculature and initiate a cooling effect through evaporative water loss from cut tissues; however, defoliation damage may also initiate water movement from the vascular tissue into the apoplast, where it evaporates at the equilibrium point between water vapor and liquid, often at a distance from the cut edge (Aldea et al. 2005). In instances where no reduction in ΦPSII occurred concomitantly with an enhanced cooling effect, as with 24 h defoliation damage >1 mm from cut edges in aspen, apoplastic water transfer may have triggered reductions in leaf temperature without altering photosynthesis. Given that apoplastic water movement is driven by the equilibrium point between vapor and liquid, we would expect ambient temperatures and not CO_2 concentrations to influence the equilibrium point. Our results

Table 1 Mean (±SE) leaf temperature for damaged and undamaged tissues grown under ambient and elevated CO_2, and the distance that the change in temperature propagated into adjacent tissue (part-table A)

Species	Damage type	Undamaged temp (°C)		Damaged temp (°C)		Propagation (mm)	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
A							
Aspen	Leaf fold	22.0 ± 0.3	23.7 ± 0.2	21.8 ± 0.2	23.5 ± 0.2	ND	ND
	Defoliation	22.0 ± 0.3	23.9 ± 0.2	22.3 ± 0.3	23.9 ± 0.3	0.5 ± 0.0	0.3 ± 0.1
	Gall	21.8 ± 0.3	23.7 ± 0.2	20.2 ± 0.3	22.8 ± 0.3	2.2 ± 0.4	2.7 ± 0.2
Birch	Defoliation	22.4 ± 0.4	23.5 ± 0.3	22.6 ± 0.4	23.9 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
Aspen	24 h defoliation	25.3 ± 0.2	25.0 ± 0.2	24.6 ± 0.2	24.4 ± 0.2	3.9 ± 0.3	4.4 ± 0.4

P values	Temperature	Propagation
B		
Aspen		
CO_2	0.04	0.50
Damage	<0.01	<0.01
CO_2 × damage	0.03	0.14
Birch[b]		
CO_2	0.16	0.20
Damage	0.20	NA
CO_2 × damage	0.66	NA
Aspen (24 h feeding experiment)		
CO_2	0.49	0.33
Damage	<0.01	NA
CO_2 × damage	0.78	NA

Damage by leaf folding did not alter the spatial pattern of temperature within a leaf. A summary of the *P* values for the main effects of CO_2 fumigation, different damage types, and their interaction is also included (part-table B)

ND propagated distance was not detectable

agree with this notion, as 24 h defoliation damage in both ambient and elevated CO_2 propagated equal distances into remaining leaf tissue. However, elevated CO_2 reduced the propagated distance of damage to ΦPSII efficiency. Spectral reflectance suggested that aspen leaves grown under elevated CO_2 had lower chlorophyll content, reduced ΦPSII, but elevated water content (Table 3). Thus, it is possible that the increases in leaf mass per unit area (i.e., increased thickness) in aspen species grown under elevated CO_2 (Liberloo et al. 2007) and the observed increase in water content under elevated CO_2 enhanced leaf tolerance to desiccation induced by defoliation damage. This tolerance, in turn, may have reduced the suppression of ΦPSII at cut edges.

Gall formation transforms host morphology and physiology at some cost to the host in order to enhance the fitness of the attacking parasite (Stone and Schonrogge 2003). Leaf galls may enhance net photosynthesis by reducing respiration (Dorchin et al. 2006), yet structural and chemical changes often yield tissues that are deficient in photosynthetic proteins and pigments (e.g., Yang et al. 2007). Gall formation in aspen generated tissues with reduced ΦPSII and enhanced transpiration relative to undamaged tissues. Whereas other hardwood species show that damage to ΦPSII propagated away from the gall into remaining nongalled leaf tissues (e.g., *Carya glabra*; Aldea et al. 2006), aspen galls reduced ΦPSII only minimally outside of the galled structure. Higher spectral reflectance between 400 and 700 nm, with the exception of a peak near 550 (i.e., xanthophylls), suggests reduced absorbance for all photosynthetically active pigments. This finding, in addition to the observed lower N content in gall tissue compared to nongalled leaf tissue of galled leaves (unpublished data), suggests that a lack of photosynthetic pigment protein complexes contributed to reduced ΦPSII, but that this reduction was limited to the actual area occupied by the gall.

Aspen galls enhanced transpiration in gall tissues, thereby reducing leaf temperatures in adjacent tissues. Because no tissues had been cut, as with defoliation damage, the movement of water to the developing gall was likely driven by evapotranspiration through stomata, the only other openings in the leaf surface. We observed increased leaf temperatures across all tissue types under

Table 2 Mean (±SE) ΦPSII for damaged and undamaged leaf tissues grown under ambient and elevated CO_2, and the distance that the damage propagated into adjacent tissue (part-table A)

Species	Damage type	Undamaged ΦPSII		Damaged ΦPSII		Propagation (mm)	
		Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
A							
Aspen	Leaf fold	0.616 ± 0.004	0.626 ± 0.004	0.605 ± 0.008	0.619 ± 0.004	ND	ND
	Defoliation	0.615 ± 0.003	0.611 ± 0.006	0.568 ± 0.005	0.569 ± 0.005	0.81 ± 0.05	0.73 ± 0.05
	Gall	0.619 ± 0.004	0.616 ± 0.005	0.551 ± 0.016	0.542 ± 0.018	0.14 ± 0.08	0.43 ± 0.20
Birch	Defoliation	0.601 ± 0.012	0.602 ± 0.013	0.569 ± 0.008	0.571 ± 0.008	0.80 ± 0.04	0.59 ± 0.05
Aspen	24 h Defoliation	0.609 ± 0.008	0.619 ± 0.006	0.525 ± 0.007	0.523 ± 0.008	0.94 ± 0.03	0.86 ± 0.02

	ΦPSII	Propagation
B		
Aspen		
CO_2	0.66	0.65
Damage	<0.01	0.07
CO_2 × damage	0.69	0.44
Birch		
CO_2	0.44	<0.01
Damage	0.04	NA
CO_2 × damage	0.32	NA
Aspen (24 h feeding experiment)		
CO_2	0.88	0.03
Damage	<0.01	NA
CO_2 × damage	0.98	NA

Damage by leaf folding did not alter the spatial pattern of ΦPSII within a leaf. A summary of the *P* values for the main effects of CO_2 fumigation, different damage types, and their interaction is included (part-table B)

ND propagated distance was not detectable

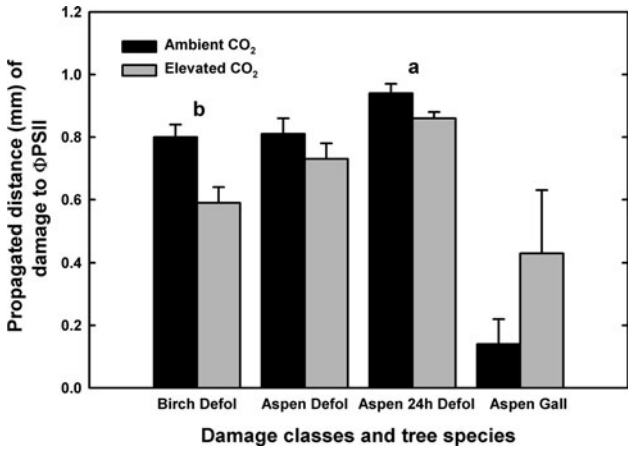

Fig. 2 The mean (±SE) distance that damage to ΦPSII propagated into remaining tissues adjacent to defoliated edges and gall formation ($n = 3$ per damage type). Values for ΦPSII reduced by >5% relative to remaining tissues were designated as damaged. Significant deviation between fumigation treatments is indicated by "*a*" for $P < 0.05$ or "*b*" for $P < 0.01$. Only aspen leaf fold damage did not propagate into the remaining tissue, i.e., it was not detectable (ND) and therefore was not included; however, all other damage types did propagate into remaining tissues ($P < 0.01$)

elevated CO_2 in June. This result agrees with other studies on tree response to elevated CO_2 (see Wittig et al. 2007 for a synthesis), and suggests that elevated CO_2 reduced stomatal aperture, thereby reducing transpiration and increasing leaf temperature. Thus, it is likely that the reduced stomatal aperture on galls and the surrounding leaf area attenuated the cooling effect of gall formation. Gall formation by *Harmandia* species in aspen typically occurs adjacent to major veins (pers. observation) and distorts xylem elements in other aspen species (Hyde 1922), possibly to facilitate transport to a developing sink. While the degree to which this alters the nutrient flux to the developing parasite is unknown, the increased temperature relative to ambient conditions suggests that insect development may occur faster under predicted future shifts in climate.

Arthropod herbivory can reduce plant productivity by removing photosynthetic leaf area. In addition, results from this study and others (Aldea et al. 2005, 2006; Patankar et al. 2011; Zangerl et al. 2002) indicate that in some cases damage to leaf surfaces causes a reduction in the quantum efficiency of photosystem II fluorescence (ΦPSII), which is

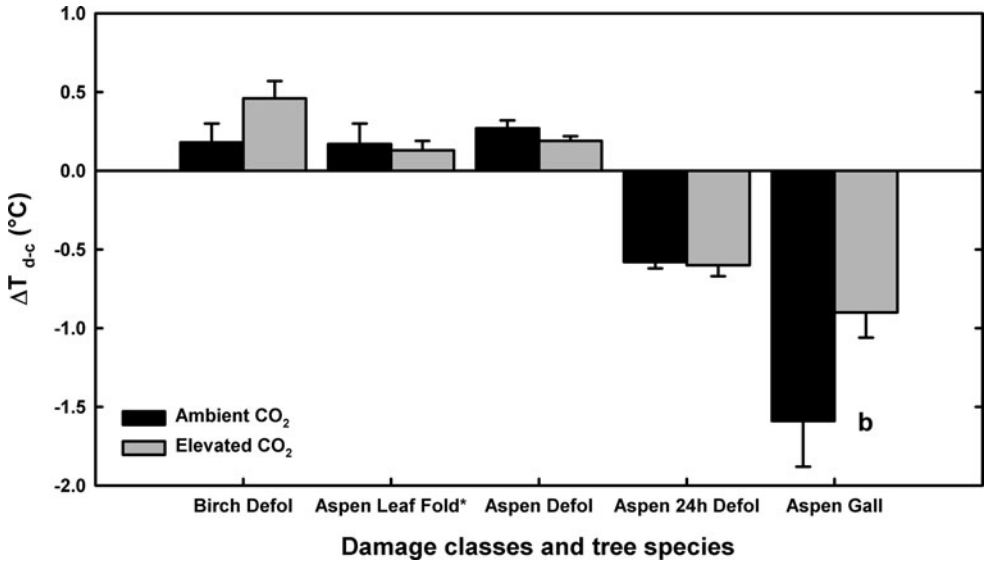

Fig. 3 The mean (±SE) difference in leaf temperature (ΔT) between visibly damaged (*d*) and remaining (*c*) tissue within the same leaf ($n = 3$ per damage type). Significant deviation between fumigation treatments for each damage type is indicated by "*a*" for $P < 0.05$ or "*b*" for $P < 0.01$. *Only aspen leaf fold damage did not differ in temperature from remaining tissue (i.e., ΔT did not differ from 0); all other damage types did ($P < 0.01$)

Table 3 Reflectance indices (unitless) were calculated from the spectral reflectances of gall damage and nearby undamaged leaves grown under ambient and elevated CO_2

Index	Ambient CO_2		Elevated CO_2		*P* value		
	Aspen leaf	Aspen gall	Aspen leaf	Aspen gall	CO_2	Damage	CO_2 × damage
PRI	0.09 ± 0.01	−0.05 ± 0.01	0.06 ± 0.01	−0.04 ± 0.01	0.89	<0.01	0.08
WI	1.03 ± 0.00	1.10 ± 0.01	1.03 ± 0.00	1.10 ± 0.02	0.98	<0.01	0.76
NDVI	0.32 ± 0.01	0.07 ± 0.01	0.29 ± 0.00	0.07 ± 0.01	0.07	<0.01	0.13

There was no significant difference in the indicies measured near the gall on a damaged leaf and on an undamaged leaf, so only data for nearby undamaged leaves are shown. A summary of *P* values for the main effects of CO_2 fumigation and damage as well as their interaction is included

highly correlated with the rate of carbon assimilation. These reductions in photosynthesis in the remaining leaf tissue following herbivory vary with the plant species under attack as well as the herbivore (Nabity et al. 2009). The mechanisms governing this reduction in photosynthesis are not well understood, but in some cases relate to disruptions in carbon and water transport. Insofar as growth under elevated CO_2 increases the rate of carbon uptake and reduces water loss by reducing stomatal conductance, it is expected that this element of global change would modulate the effect of herbivory on photosynthesis. Though measurement variation was high, growth under elevated CO_2 reduced the distance that herbivore-induced reductions in photosynthesis propagated away from the point of damage in aspen and birch, suggesting that—at least for these species—elevated CO_2 may reduce the impact of herbivory on photosynthesis. Arthropod herbivory directly alters productivity and will interact with the changing climate, albeit with a high degree of uncertainty, in hardwood forest ecosystems (Lindroth 2010).

Acknowledgments We thank the members of the DeLucia Laboratory for constructive remarks on an earlier version of this manuscript. We thank the University of Illinois Dissertation Travel Grant for providing travel support to and from Rhinelander, WI, USA, in 2009. Aspen FACE is principally supported by the Office of Science (BER), US Department of Energy, grant no. DE-FG02-95ER62125 to Michigan Technological University, and contract no. DE-AC02-98CH10886 to Brookhaven National Laboratory, the US Forest Service Northern Global Change Program and North Central Research Station, Michigan Technological University, and Natural Resources Canada—Canadian Forest Service. This work was supported by a US Department of Energy (Office of Science, BER) grant (DE-FG02-06ER64232) to E. H. DeLucia and a University of Wisconsin hatch grant (WIS04898) to R. L. Lindroth.

References

Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, DeLucia EH (2005) Indirect effects of insect herbivory on leaf gas exchange in soybean. Plant Cell Environ 28:402–411

Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, Frank TD, DeLucia EH (2006) Comparison of photosynthetic damage from arthropod herbivory and pathogen infection in understory hardwood samplings. Oecologia 149:221–232

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113

Baker NR, Oxborough K (2005) Chlorophyll fluorescence as a probe of photosynthetic productivity. In: Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photo-synthesis (Advances in Photosynthesis and Respiration, vol 19). Springer, Dordrecht, pp 65–82

Bilgin DD, Zavala JA, Zhu J, Clough SJ, Ort DR, DeLucia EH (2010) Biotic stress globally down-regulates photosynthesis genes. Plant Cell Environ 33:1597–1613

Couture JJ, Meehan TD, Lindroth RL (2012) Atmospheric change alters foliar quality of host trees and performance of two outbreak insect species. Oecologia 168:863–876

Cseke LJ, Tsai CJ, Rogers A, Nelsen MP, White HL, Karnosky DF, Podila GK (2009) Transcriptomic comparison in the leaves of two aspen genotypes having similar carbon assimilation rates but different partitioning patterns under elevated [CO_2]. New Phytol 182:891–911

Cyr H, Pace ML (1993) Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. Nature 361:148–150

Dorchin N, Cramer MD, Hoffmann JH (2006) Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. Ecology 87:1781–1791

Filion M, Dutilleul P, Potvin C (2000) Optimum experimental design for free-air carbon dioxide enrichment (FACE) studies. Global Change Biol 6:843–854

Gamon JA, Serrano L, Surfus JS (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia 112:492–501

Genty B, Meyer S (1994) Quantitative mapping of leaf photosynthesis using chlorophyll fluorescence imaging. Aust J Plant Physiol 22:277–284

Gog L, Berenbaum MR, DeLucia EH, Zangerl AR (2005) Autotoxic effects of essential oils on photosynthesis in parsley, parsnip, and rough lemon. Chemoecology 15:115–119

Hillstrom ML (2010) Effects of elevated carbon dioxide and ozone on forest insect abundance, diversity, and community composition (Ph.D. dissertation). University of Wisconsin, Madison

Hillstrom ML, Lindroth RL (2008) Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. Insect Conserv Divers 1:233–241

Hyde KC (1922) Anatomy of a gall on *Populus trichocarpa*. Bot Gaz 74:186–196

Jones HG (1999) Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant Cell Environ 22:1043–1055

Leakey ADB, Ainsworth EA, Bernacchi CJ, Rogers A, Long SP, Ort DR (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot 60:2859–2876

Liberloo M, Tulva I, Raim O, Kull O, Ceulemans R (2007) Photosynthetic stimulation under long-term CO_2 enrichment and fertilization is sustained across a closed *Populus* canopy profile (EUROFACE). New Phytol 173:537–549

Lindroth RL (2010) Impacts of elevated atmospheric CO_2 and O_3 on forests: phytochemistry, trophic interactions, and ecosystem dynamics. J Chem Ecol 36:2–21

Longstaff BJ, Kildea T, Runcie JW, Cheshire A, Dennison WC, Hurd C, Kana T, Raven JA, Larkum AW (2002) An in situ study of photosynthetic oxygen exchange and electron transport rate in the marine macroalga *Ulva lactuca* (Chlorophyta). Photosynth Res 74:281–293

McElrone AJ, Hamilton JG, Krafnick AJ, Aldea M, Knepp RG, DeLucia EH (2010) Combined effects of elevated CO_2 and natural climatic variation on leaf spot diseases of redbud and sweetgum trees. Environ Pollut 158:108–114

Nabity PD, Zavala JA, DeLucia EH (2009) Indirect suppression of photosynthesis on individual leaves by arthropod herbivory. Ann Bot 103:655–663

Noormets A, McDonald EP, Dickson RE, Kruger EL, Sober A, Isebrands JG, Karnosky DF (2001) The effect of elevated carbon dioxide and ozone on leaf- and branch-level photosynthesis and potential plant-level carbon gain. Trees 15:262–270

Oxborough K (2004) Imaging of chlorophyll a fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. J Exp Bot 55:1195–1205

Patankar R, Thomas SC, Smith SM (2011) A gall-inducing arthropod drives declines in canopy photosynthesis. Oecologia 167:701–709

Penuelas J, Pinol J, Ogaya R, Filella I (1997) Estimation of plant water concentration by the reflectance water index WI (R900/R970). Int J Remote Sens 18:2869–2875

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436

Sims DA, Gamon JA (2002) Relationship between pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. Remote Sens Environ 81:337–354

Stone GN, Schonrogge K (2003) The adaptive significance of insect gall morphology. Trends Ecol Evol 18:512–522

Tang JY, Zielinski RE, Zangerl AR, Crofts AR, Berenbaum MR, DeLucia EH (2006) The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. J Exp Bot 57:527–536

Welter SC (1989) Arthropod impact on plant gas exchange. In: Bernays EA (ed) Insect–plant interactions. CRC, Boca Raton, pp 135–151

Wittig VE, Ainsworth EA, Long SP (2007) To what extent do current and projected increases in surface ozone affect stomatal conductance of trees? A meta-analytic review of the last three decades of experiments. Plant Cell Environ 30:1150–1162

Wolf A, Kozlov MV, Callaghan TV (2008) Impact of non-outbreak insect damage on vegetation in northern Europe will be greater than expected during a changing climate. Clim Change 87:91–106

Yang CM, Yang MM, Huang MY, Hsu JM, Jane WN (2007) Lifetime deficiency of photosynthetic pigment–protein complexes CP1, A1, AB1, and AB2 in two cecidomyiid galls derived from *Machilus thumbergiii* leaves. Photosynthetica 45:589–593

Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, DeLucia EH (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. Proc Natl Acad Sci USA 99:1088–1091

Zvereva EL, Lanta V, Kozlov MV (2010) Effects of sap-feeding insect herbivores on growth and reproduction of woody plants: a meta-analysis of experimental studies. Oecologia 163:949–996