Elevated carbon dioxide plus chronic warming causes dramatic increases in leaf angle in tomato, which correlates with reduced plant growth

Dileepa Madushanka Jayawardena1 | Scott Alan Heckathorn1 | Deepesh Raj Bista1 | Jennifer Kay Boldt2

1 Department of Environmental Sciences, University of Toledo, Toledo, Ohio, USA
2 Agricultural Research Service, United States Department of Agriculture, Toledo, Ohio, USA

Correspondence
Scott Alan Heckathorn, Department of Environmental Sciences, University of Toledo, 2801 W Bancroft Street, Toledo, OH 43606. Email: scott.heckathorn@utoledo.edu

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Abstract
Limited evidence indicates that moderate leaf hyponasty can be induced by high temperatures or unnaturally high CO2. Here, we report that the combination of warming plus elevated CO2 (eCO2) induces severe leaf hyponasty in tomato (Solanum lycopersicum L.). To characterize this phenomenon, tomato plants were grown at two levels of CO2 (400 vs. 700 ppm) and two temperature regimes (30 vs. 37°C) for 16–18 days. Leaf hyponasty increased dramatically with warming plus eCO2 but increased only slightly with either factor alone and was slowly reversible upon transfer to control treatments. Increases in leaf angle were not correlated with leaf temperature, leaf water stress, or heat-related damage to photosynthesis. However, steeper leaf angles were correlated with decreases in leaf area and biomass, which could be explained by decreased light interception and thus in situ photosynthesis, as leaves became more vertical. Petiole hyponasty and leaf-blade cupping were also observed with warming + eCO2 in marigold and soybean, respectively, which are compound-leaved species like tomato, but no such hyponasty was observed in sunflower and okra, which have simple leaves. If severe leaf hyponasty is common under eCO2 and warming, then this may have serious consequences for food production in the future.

KEYWORDS
climate change, global warming, heat stress, hyponasty, leaf cupping, leaf shape, petiole angle, Solanum lycopersicum

INTRODUCTION

Unfavourable abiotic environmental stimuli, such as supraoptimal temperature, water logging, and low light, can cause upward bending of leaves (petioles and/or blades) in certain species—a phenomenon known as “hyponasty” (Koini et al., 2009; Polko, Voesenek, Peeters, & Pierik, 2011; van Zanten, Voesenek, Peeters, & Millenaar, 2009; Vasseur, Pantin, & Vile, 2011; Voesenek, Colmer, Pierik, Millenaar, & Peeters, 2006). Leaf hyponasty is a slow-changing growth response, in contrast to heliotropic movements of leaves, which are readily reversible diurnal changes in leaf angle in response to light (King, 1997; Yu & Berg, 1994). Leaf hyponasty is caused by the differential growth of abaxial and adaxial surfaces of the leaf (Hayes & Lippincott, 1976; Palmer, 1985; Polko et al., 2011), and the extent of hyponasty is regulated by hormones (auxin, ethylene, abscisic acid, and gibberellins) and phytochromes (Koini et al., 2009; Millenaar et al., 2005; Polko et al., 2011; van Zanten et al., 2009). In nature, leaf hyponasty has been observed in response to suboptimal or supraoptimal light (Falster & Westoby, 2003; King, 1997; Polko et al., 2011; Werner, Ryel, Correia, & Beyschlag, 2001) and to escape submergence (Voesenek et al., 2006). Leaf hyponasty has also been observed in growth chambers, in response to high temperatures and very high CO2 (Dhawan, Bassi, & Spencer, 1981; Koini et al., 2009; Millenaar et al., 2005; van Zanten et al., 2009).
Petiole hyponasty in response to high temperatures has been studied in some detail, mainly using Arabidopsis thaliana as the model species to elucidate the signalling pathways involved (Koini et al., 2009; Millenaar et al., 2005; van Zanten et al., 2009); however, none of these studies investigated the potential consequences of hyponasty on plant growth or reproduction. Notably, Arabidopsis thaliana accessions from habitats with strongly fluctuating diurnal temperatures showed the least high-temperature-induced hyponasty, which indicates that hyponasty is a trait subject to natural selection (van Zanten et al., 2009). Petiole hyponasty of sunflower has occurred in response to elevated CO2 (eCO2), but it was induced using an unrealistically high CO2 level (10,000 ppm), and interestingly, it was accompanied by an increase in ethylene production (Dhawan et al., 1981). To our knowledge, only one study to date has investigated how leaf angle is affected by eCO2 plus high temperature, and this study observed no significant effect of high temperature and CO2 on the blade angle of the grasses, Lolium perenne L. and Festuca arundinacea Schreb. (Teughels, Nijss, van Hecke, & Impens, 1995). However, in a previous study examining the effects of high temperatures and/or CO2 on nutrient uptake in tomato (Solanum lycopersicum L. cv. Big Boy), we noted anodotally the presence of dramatic leaf hyponasty in the high-temperature plus high-CO2 treatment, relative to the other treatments, and this hyponasty correlated with large decreases in root nitrogen (N) uptake and plant growth (Jayawardena et al., 2017).

The aim of this study was to investigate the synergistic effects of eCO2 and chronic warming on leaf hyponasty in tomato, a model warm-season herbaceous eudicot. Because anthropogenic activities are increasing atmospheric CO2 concentration and increases in CO2 are contributing to global warming (IPCC, 2007, 2014), it is necessary to investigate the effects of warming and eCO2 on leaf hyponasty, singly and in combination, in order to understand how climate change will impact it. Tomato is the second most important vegetable crop in the world based on production per hectare, and behind China, the United States is the second largest producer of tomatoes in the world (FAO: UN, 2017; USDA: ERS, 2016). Hence, any adverse effects of climate change on tomato growth and productivity caused by leaf hyponasty would have a large economic impact. If increases in leaf hyponasty are common among other species, in addition to tomato, under high CO2 and warming, the potential consequences for food production would be even greater.

2 | METHODS

2.1 | Growth conditions and treatments

Seeds of tomato (Solanum lycopersicum L.) cultivars Early Girl (EG, an indeterminate hybrid with lobed leaflets), Brandywine (BW, an indeterminate heirloom with “potato-type” leaflets), and H3406 (an indeterminate hybrid with lobed leaflets) were grown and treated as described in Jayawardena et al. (2017). Briefly, seeds were germinated in trays filled with calcined clay, placed in a greenhouse and watered daily with tap water. At 14 and 18 days after sowing, seedlings were provided modified Hoagland’s nutrient solution wherein all chemicals were at quarter strength except KNO3, which was at full strength. Nutrient concentrations of the full-strength solution were 2 mM MgSO4, 1 mM KH2PO4, 1 mM K2HPO4, 2 mM CaCl2, 1.5 mM KNO3, 71 μM Fe-DTPA (diethylene-triamine-penta-acetic acid), 10 μM MnCl2, 50 μM H2BO3, 6 μM CuSO4, 6 μM ZnSO4, and 1 μM Na2MoO4; pH = 6.0. When seedlings reached the first-adult-leaf stage, similar-sized seedlings were transplanted into cylindrical pots filled with calcined clay (10-cm diameter × 40-cm length polyvinyl chloride pipes; one plant per pot, 16 pots per cultivar). At 2 and 4 days after transplanting, 250 mL of quarter-strength Hoagland’s nutrient solution was added to each pot. When plants reached the second-adult-leaf stage, all three cultivars were transferred to growth chambers at the same time.

A 2 × 2 factorial design was used with two levels of CO2 (400 vs. 700 ppm) and two temperature settings (day temperatures of 30°C [near optimum] vs. 37°C [supraoptimum], with night temperatures 5°C less than day temperatures). The four treatments were imposed using four growth chambers (model E36HO, Percival Scientific Inc., Perry, IA). Plants were initially kept inside the growth chambers for 24 hr under 500 μmol m−2 s−1 photosynthetically active radiation (PAR) with a 14-hr (0600–2000 hr) photoperiod at a near-optimum growth temperature of 30/25°C (day/night) and ambient CO2 (400 ppm) and humidity, to acclimatize to the new environment. Temperatures of the high-temperature-treatment chambers were gradually increased over 3 days to avoid potential heat shock, and once high-temperature-treatment chambers reached 37°C, CO2 treatments were started. Plants were thereafter provided 500 mL of full-strength Hoagland’s nutrient solution every other day (completely flushing the pots with new solution and then allowing excess solution to drain). During this period, plants were rotated periodically (every few days) inside the chambers to avoid position effects, and plants were switched between chambers once per week to avoid chamber effects. The relative humidity (RH%) and the estimated vapour-pressure deficit (VPD) of the chamber with near-optimal temperature plus ambient CO2 were 56.5% and 1.847 kPa, respectively, at midday. The high-temperature-treatment chamber had a RH% of 54.5% and a VPD of 1.932 kPa. The RH% and VPD of both of the other chambers (eCO2 plus near-optimal temperature and eCO2 plus supraoptimal temperature) were 64.5% and 1.507 kPa, respectively. Plants were harvested 16 to 18 days (depending on cultivar) after the start of treatments. When harvesting, plants (n = 4) were separated into leaves, stems, and roots, weighed to obtain fresh mass, and then placed in an oven at 70°C for 72 hr to obtain dry mass.

2.2 | Plant measurements

Prior to harvest, leaf angle, leaf surface temperature (Tleaf), steady-state photosynthesis as net CO2 exchange per unit leaf area (Pn), stomatal conductance to water vapour (Gs), and specific leaf area were measured for all cultivars. Leaf cupping, Photosystem II (PSII) efficiency of light-adapted leaves (Fv/Fm′), and xylem pressure potential (Ψx) were measured only in the cultivar EG, and Pn, light-response curves were generated for each treatment of this cultivar.

To measure leaf angle (i.e., the angle between a horizontal plane and the leaf petiole), the angles between the stem and petioles of
the third, fourth, and fifth fully expanded leaves below the apical meristem which were developed and expanded while exposed to the treatment conditions were measured using a protractor. Each value was subtracted from 90, and then the three values were averaged to obtain a single value per plant. Leaf cupping (EG only) was measured as the ratio between the length of the leaflet after it was flattened along the midrib from leaf base to tip versus the length of the leaflet while curved (and before flattening); hence, a fully flat leaf would have a cupping value of 1, and this value would increase as cupping, or leaflet curvature, increased. Leaf cupping was measured in three leaflets (terminal + the next two opposite leaflets closest to the terminal leaflet) of the fourth fully expanded leaf from top of the plant. Adaxial leaf surface temperature of the terminal leaflet of the third recently expanded fully illuminated leaf was measured using a RadioShack Infrared digital thermometer (General Wireless Operations Inc. Fort Worth, TX); three measurements were taken and averaged for each leaflet.

To assess heat-related damage to photosynthesis, PSII efficiency of light-adapted leaves (Fv'/Fm') was measured using a portable fluorometer (model OS1-FL, Opti-Sciences Inc., Hudson, NH), by providing a saturating light pulse over 0.8 s (EG only). Chlorophyll fluorescence measurements were made on recently expanded fully illuminated leaflets (terminal leaflet of the third leaf below the apical meristem) receiving steady state irradiance 17 days after the start of treatments. These same leaflets were used to measure in situ Pn and Gs using a portable gas-exchange system (model LI-6400, LiCOR, Lincoln, NE), as described in the study of Wang et al. (2008). During these measurements, the air temperature and CO2 concentration in the gas exchange cuvette matched the growth chamber in which the plant was growing, and light intensity was 500 μmol m−2 s−1 PAR. Because incident light levels on leaves of plants in the growth chambers were not equal, due to changes in leaf angle with temperature and/or CO2, these measurements of Pn are an index of photosynthetic capacity. Measurements were made within 60 s of enclosing a leaflet in the cuvette, so that Pn and Gs reflected conditions within the growth chamber rather than the cuvette. In separate measurements, Pn-light-response curves were generated on individual plants (EG only), by enclosing a leaflet (same as above) in the cuvette for an extended time period and adjusting the light intensity. For these Pn-light-response curves, temperature and CO2 in the cuvette were also set to mimic growth chamber conditions. The leaflet was equilibrated at a light intensity of 2,000 μmol m−2 s−1 PAR until Pn stabilized, and thereafter, it was decreased to 1,500, 1,000, 500, 250, 120, 60, 30, and 0 μmol m−2 s−1 PAR in intervals; Pn was measured at each light intensity after ca. 2 min.

Specific leaf area (leaf surface area per unit dry mass) was calculated using leaf discs cut (using a cork borer) from the terminal leaflets of the third, fourth, and fifth leaves below the apical meristem of the plant. Total leaf area was calculated using specific leaf area and leaf dry mass data. Photosynthesis per plant (plant Pn) was calculated using Pn per unit leaf area (see above) and total leaf area per plant. To determine whether plants were water stressed or not, Ψw of the fourth leaf below the apical meristem of EG plants was measured using a pressure chamber at midday (PMS Instruments Co., Corvallis, OR).

2.3 Daily and diurnal leaf angle measurements

A second independent experiment was conducted as above using “Early Girl” to measure daily and diurnal changes in leaf petiole angle in response to eCO2 and warming. Plants (n = 4) were treated for 14 days using the above-mentioned 2 × 2 factorial design (400 vs. 700 ppm CO2 and 30 vs. 37°C). Petiole angle of the second leaf from the bottom was measured daily (this was the only measurable leaf at the beginning of the experiment). On day 7 and 14, petiole angle of the same leaf was measured in 6-hr intervals (0600, 1200, 1800, 2400 hr). After 14 days, high-CO2 and high-temperature treatments were stopped, and all plants were returned to the control conditions (400 ppm CO2 and 30°C). Potential recovery of leaf angle after high-CO2 and high-temperature treatments was assessed by measuring petiole angle of the fourth leaf from the bottom (not second leaf because it was senescing) daily from day 14 to day 20.

2.4 Investigation of other species

We examined four additional warm-season species to determine if warming and eCO2 induced leaf hyponasty in other species, as in tomato (Solanaceae); two with compound leaves like tomato [soybean (Glycine max cv. Chiba Green, Fabaceae) and marigold (Tagetes erecta cv. Antigua Orange, Asteraceae)], and two with simple leaves [sunflower (Helianthus annuus cv. Mammoth Russian, Asteraceae) and okra (Abelmoschus esculentus cv. Clemson Spineless, Malvaceae)]. Plants were grown and treated in a 2 × 2 factorial design as above. Soybean, marigold, sunflower, and okra plants (n = 4) were harvested 16, 20, 17, and 21 days after the start of temperature and CO2 treatments, respectively, and dry mass was measured. Prior to harvest, leaf angles of marigold, sunflower, and okra and leaf cupping of soybean were measured.

![FIGURE 1 Tomato (Solanum lycopersicum cv. Early Girl) and soybean (Glycine max cv. Chiba Green) plants grown at ambient CO2 (400 ppm) and 30°C (near optimal) daytime temperature versus elevated CO2 (700 ppm) and 37°C (warming) daytime temperature for 14 days](image-url)
Data were analysed using two-way (two levels of CO₂ × two levels of temperature) analysis of variance (ANOVA) using JMP 13 software (SAS Institute Inc., Cary, NC). Tukey’s post hoc test was used for multiple comparisons only if ANOVA results were significant \((P < 0.05)\) for the interaction term or for both temperature and CO₂ terms. Model assumptions of independence, normality, and equal variance were checked with residual versus fitted, normal Q–Q, and S–L plots, respectively, using R version 3.1.2 (R Core Team [2013], Vienna, Austria). Data were transformed if they did not meet the model assumptions (only biomass data were log transformed). Figures were generated using SigmaPlot 12.5 (Systat Software Inc., Chicago, IL). Results presented in figures are untransformed means and standard errors of means.

### RESULTS

Both petiole angle (Table S1) and leaflet angle (Figure 1) increased with the combination of chronic warming plus eCO₂ compared with the other treatments. eCO₂ slightly but significantly increased petiole angle and warming also significantly increased petiole angle (Figure 2 a–c). The combined effect of eCO₂ and warming increased petiole angle ca. two-fold in all three cultivars more than the effect of eCO₂ or warming alone (i.e., the effects of warming and eCO₂ were additive).

Leaf, stem, root, and total plant dry mass of all three cultivars generally decreased with warming (Table S1, Figure 2d–f). In contrast, the effect of eCO₂ on leaf, stem, root, and total plant dry mass was dependent on temperature. Specifically, biomass increased (significantly) or tended to increase (nonsignificantly), with eCO₂ in all cultivars at
30°C, whereas the opposite occurred at 37°C, and in all cultivars, the lowest biomass was observed with warming plus eCO2.

As with biomass, total leaf area per plant of all three cultivars decreased with warming, and effects of eCO2 were dependent on temperature (Table S1, Figure 2g–i). For example, eCO2 did not affect leaf area at 30°C, whereas at 37°C, eCO2 decreased leaf area in H3406 and EG (marginal significance), with no change in BW. In all three cultivars, the lowest leaf area was observed with warming plus eCO2.

Total plant photosynthesis of all three cultivars was significantly affected by the interaction of CO2 × temperature (Table S1, Figure 2j–l). At 30°C, eCO2 significantly increased total plant photosynthesis of EG and BW, but did not affect H3406. At 37°C, eCO2 significantly decreased total plant photosynthesis of H3406 but did not affect EG or BW. At ambient CO2, warming significantly reduced total plant photosynthesis of BW and H3406. As with biomass and leaf area, eCO2 decreased or tended to decrease, total plant photosynthesis at 37°C, such that it was lowest with warming plus eCO2.

In all three cultivars, photosynthetic capacity (Pn per unit leaf area at 500 μmol m−2 s−1 PAR) was affected significantly only by CO2 and increased at eCO2 (Table S1, Figure 3a–c). In contrast, stomatal conductance (Gs) of all three cultivars was minimally affected by eCO2 or warming, singly or in combination (Figure 3d–f). Leaf water potential (Ψw), measured only in EG, decreased slightly with warming but was unaffected by eCO2 (Figure 3d). Individually, CO2 and temperature each increased Tleaf of all three cultivars, and there was an additive effect of warming and eCO2 on Tleaf in EG and BW (Table S1, Figure 3g–i).

In EG, PSII efficiency of light-adapted leaves (Fv′/Fm′) was not affected by warming, and it tended to increase with eCO2 (marginally significant; Table S1, Figure 4a). Leaflet cupping in EG was significantly affected by the interactive effect of CO2 and temperature, with the combined effect of eCO2 and warming increasing leaflet cupping to a modest extent, compared with the other three treatments, which had no effect (Table S1, Figure 4b).

In the second independent experiment, plants of the eCO2 plus warming treatment displayed the highest leaf angle throughout the treatment period, followed by warming only, eCO2 only, and control treatments, respectively. Petiole angles in the warming, eCO2, and (especially) warming plus eCO2 treatments increased gradually over the course of several days of treatment (Figures 5 and 6a). In the warming and eCO2 treatments, petiole angles reached a plateau after ca. 120 hr, before declining as leaves aged; however, they did not plateau until ca. 280 hr with warming plus eCO2. Petiole angles of leaves did not fluctuate diurnally, as shown by more frequent sampling between 162–180 and 330–348 hr (= 0600 to 2400 HR). Once warming and high-CO2 treatments were stopped and all plants were thereafter grown under control temperatures and ambient CO2,
petiole angles of elevated-CO$_2$- and temperature-treated plants gradually declined, reaching or nearly reaching control levels within 140 hr (Figure 5, Figure 6a,b).

From the $P_n$-light-response curves of the plants from the second experiment (Figure 7), it is apparent that eCO$_2$ increased $P_n$ at 30°C, relative to ambient CO$_2$ at 30°C, at light intensities greater than 100 μmol m$^{-2}$ s$^{-1}$ PAR. Warming also increased $P_n$ under ambient CO$_2$ at light greater than ca. 200 μmol m$^{-2}$ s$^{-1}$ PAR. The eCO$_2$ plus warming treatment had the lowest $P_n$ values at low irradiance levels (<300 μmol m$^{-2}$ s$^{-1}$). Notably, plants growing at 30°C and ambient CO$_2$ held their leaflets approximately horizontal and thus received ca. 500 μmol m$^{-2}$ s$^{-1}$ PAR in the growth chambers during the photoperiod, whereas plants growing at 37°C and eCO$_2$ held most of their leaflets at angles of 70° or higher and thus received light levels of 300 μmol m$^{-2}$ s$^{-1}$ PAR or less in the growth chambers. Hence, the large increase in petiole and leaflet angle with warming plus eCO$_2$ would have reduced in situ $P_n$ per unit leaf area by ca. 23% or more (Figure 7).

The combination of warming plus eCO$_2$, compared with warming or eCO$_2$ alone, also increased petiole angle in marigold, especially in younger leaves, and it increased leaflet cupping in soybean (petiole angle was not affected; Figure 8). In contrast, petiole angle did not increase with either warming, eCO$_2$, or their combination in sunflower and okra. In soybean and marigold, which have compound leaves, biomass did not increase in elevated, compared with ambient, CO$_2$ at 37°C, as it did at 30°C; yet in sunflower and okra, which have simple leaves, biomass increased in elevated, compared with ambient, CO$_2$ at both 30 and 37°C. As in tomato, blade cupping did not change in magnitude during the day (not shown).

4 | DISCUSSION

To the best of our knowledge, this is the first study to show that eCO$_2$ plus chronic warming can induce dramatic leaf hyponasty and to show that the large increases in leaf angle induced by warming + eCO$_2$ correlate with decreases in plant growth in tomato. Individually, warming and eCO$_2$ caused modest petiole and/or blade-cupping hyponasty, although the effects of warming + eCO$_2$ were as large as, or larger than, the separate effects of warming and eCO$_2$ added together. This warming + eCO$_2$ hyponasty increased in magnitude over several days
of treatment, and it decreased slowly upon a return to control temperatures and ambient CO₂. Interestingly, warming + eCO₂ leaf hyponasty was observed only in the compound-leaved species examined (tomato, marigold, soybean) and not in the simple-leaved species (sunflower, okra).

In tomato, the species we studied in detail, warming + eCO₂ leaf hyponasty correlated with significant reductions in growth, relative to warming alone (which also decreased growth) or to eCO₂ alone (which increased growth). In soybean and marigold, leaf hyponasty with warming + eCO₂ correlated with a lack of growth stimulation under eCO₂, compared with control temperatures. In tomato, leaf hyponasty caused by warming + eCO₂ was not correlated with chamber humidity or VPD or with Gₛ, Ψₛ, Fᵥ'/Fₘ', or Gₛ capacity, indicating this leaf hyponasty was not caused by water stress (based on Gₛ and Ψₛ), or heat-related damage to photosynthesis (based on Fᵥ'/Fₘ' and Gₛ). Leaf temperatures in all treatments were well below air temperatures because light intensity in the growth chambers was moderate (500 μmol m⁻² s⁻¹ PAR), the air was well-mixed with fans, and tomato has compound leaves with relatively narrow leaflets; hence, Tleaf for tomato never exceeded 30°C even at 37°C air temperature (with Tleaf in controls ca. 24°C).

Reductions in plant growth associated with warming + eCO₂-induced leaf hyponasty could be explained by reductions in in situ photosynthesis per unit leaf area. Because light in the growth chambers was provided by lamps mounted on chamber ceilings, light interception by leaves decreased dramatically (e.g., from 500 to 300 μmol m⁻² s⁻¹ PAR) when leaf angle increased with warming + eCO₂. In the field, the angle at which light impinges on leaves changes during the day, so that a horizontal leaf receives the highest light intensity during midday, and the lowest light intensity at sunrise and sunset, although a vertically-oriented leaf receives the highest light levels at midmorning and mid-afternoon (Ehleringer & Forseth, 1980). Despite these differences in light environment between growth chamber and field, if leaf angle changes from horizontal to vertical, total daily light interception by a leaf decreases (Ehleringer & Forseth, 1980). Hence, warming + eCO₂ leaf hyponasty would decrease total daily light interception in the field as well as in the growth chamber. However, ultimately, it will be necessary to investigate eCO₂ plus warming driven hyponasty under field conditions, where natural light regimes and canopy development influence leaf size, shape, and angle.

Petiole angle in tomato and blade cupping in soybean did not change significantly throughout a given day; hence, the leaf hyponasty observed in this study was not diurnal heliotropism. Rather, leaf hyponasty in existing leaves increased in magnitude over several days in response to warming and eCO₂. It increased over 11 days of treatment for warming + eCO₂ in tomato, before petiole angles began to decrease due to the senescence of leaves as they aged. Further, photosynthetic capacity (Pₛ, at 500 μmol m⁻² s⁻¹ PAR) and PSII efficiency (Fᵥ'/Fₘ') both tended to increase with increasing CO₂, as expected, but neither decreased with warming. Although Tleaf increased slightly with eCO₂ and more with warming and was generally highest in warming + eCO₂, these temperatures were not stressful, as indicated by the Pₛ, Fᵥ'/Fₘ', and Gₛ. Leaf temperatures in all treatments were well below air temperatures because light intensity in the growth chambers was moderate (500 μmol m⁻² s⁻¹ PAR), the air was well-mixed with fans, and tomato has compound leaves with relatively narrow leaflets; hence, Tleaf for tomato never exceeded 30°C even at 37°C air temperature (with Tleaf in controls ca. 24°C).

![Figure 6](image-url) Daily and diurnal leaf petiole angle changes of *Solanum lycopersicum* cv. Early Girl plants grown at ambient (400 ppm) versus elevated (700 ppm) CO₂ and 30°C (near optimal) versus 37°C (warming) daytime temperatures for 14 days (a), after which all plants were grown at 400 ppm CO₂ and 30°C for an additional 6 days (b). Each bar represents mean (n = 4) ± 1 standard error of mean of each treatment combination.

![Figure 7](image-url) Photosynthesis (Pₛ) light-response curves of *Solanum lycopersicum* cv. Early Girl treated with ambient (400 ppm) versus elevated (700 ppm) CO₂ and 30°C (near optimal) versus 37°C (warming) daytime temperatures. Each symbol represents mean (n = 3) ± 1 standard error of mean of each treatment combination.
leaves produced during warming + eCO2 treatment remained at elevated leaf angles until senescence. Also, leaf hyponasty was slowly reversible when warming and eCO2 treatments were stopped and plants were then grown at ambient CO2 and control temperatures. Based on these results, we conclude that increases in leaf angle and/or blade cupping with warming + eCO2 are growth responses.

The combination of warming + eCO2 induced leaf hyponasty in the three compound-leaved species examined (tomato, marigold, and soybean) but not in the two species with simple leaves (sunflower and okra). In tomato and marigold, both petiole and blade angle increased, and mild blade cupping occurred; however, the extent of the changes in leaf angle was greater for tomato. In soybean, blade cupping was dramatic with warming + eCO2, but leaflet angle increased only modestly (from lower than horizontal to ca. horizontal), and the angle of the main petiole did not change. These limited results suggest the possibility that warming + eCO2 leaf hyponasty occurs in compound-, but not simple-, leaved species. Based on results from this study, we are currently investigating the effects of warming and eCO2 on leaf hyponasty in other species, including other members of the Fabaceae and Solanaceae, as well as in other growth forms (e.g., woody species and rosette-leaved herbaceous species).

Though not investigated in this study, leaf hyponasty induced by warming + eCO2 was likely mediated by changes in the expression in hormones and other signalling compounds. Several plant signaling compounds are known to be involved in leaf hyponasty, especially ethylene, and both eCO2 and temperature are known to affect ethylene production (Koini et al., 2009; Millenaar et al., 2005; Polko et al., 2011; van Zanten et al., 2009). Further experiments will be required to elucidate the signalling pathways involved in warming + eCO2 leaf hyponasty, and this information may be useful in the development of genotypes with desirable hyponasty traits. However, if warming + eCO2 leaf hyponasty is maladaptive, which requires further
investigation to determine, then selection or development of genotypes that exhibit less hyponasty will improve plant performance with climate change.

Current global warming is mostly driven by increases in the concentration of atmospheric CO2 (IPCC, 2014). By itself, increases in CO2 benefit plants by increasing photosynthesis via decreases in stomatal opening (DaMatta, Grandis, Arenque, & Buckeridge, 2010; Leakey et al., 2009; Wang, Heckathorn, Wang, & Philpott, 2012). However, the benefits of increasing CO2 may sometimes be offset by the detrimental effects of heat stress, such as heat-related damage to photosynthesis (Wang et al., 2012). For example, heat stress exacerbates the decrease in foliar N concentration (%N) caused by growth in eCO2 (Wang et al., 2012). In tomato, we previously characterized the negative synergistic effects of eCO2 (700 vs. 400 ppm) and chronic warming (37 vs. 30°C) on shoot and root %N, which could be explained by decreases in root N uptake and assimilation associated with reductions in the concentration of N-uptake and -assimilatory proteins (Jayawardena et al., 2017). A reduction in whole-plant carbon gain and plant growth, if caused by leaf hyponasty, could also contribute to decreases in N uptake and assimilation with warming + eCO2, by reducing energy and carbon skeletons available to roots. However, it is also possible that warming + eCO2 leaf hyponasty has adaptive benefits under conditions where light levels are often higher than in this study, such as by reducing photo-inhibition during high temperatures. Hence, the net effect of warming + eCO2 leaf hyponasty on food quantity and nutritional quality remains to be determined.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.

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ORCID
Dileepa Madushanka Jayawardena https://orcid.org/0000-0002-0137-9302

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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