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Use of insect exclusion cages in soybean creates an altered microclimate and differential crop response

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ABSTRACT

Insect exclusion cages are commonly used in agricultural and ecological studies to examine plant-insect interactions in a field setting while maintaining control over insect populations. However, these insect cages can unintentionally alter the climate inside of the cage and impact plant physiology, growth and yield as well as insect populations. This can subsequently affect interpretations of experimental results obtained from caged experiments. To address this concern, we measured meteorological variables in conjunction with soybean physiology, growth, and yield over a two-year period. In a 2011 field study in southern Wisconsin, we compared photosynthetic rates, leaf area index (LAI), soil environmental conditions, and various components of yield for plants grown inside and outside of an industry standard insect cage (Lumite 32 × 32 mesh). Inside of cages, several variables were higher ($P < 0.05$) including surface (0–6 cm) soil moisture (38%), stomatal conductance (42%), and total plant biomass (30%), while LAI was 20% lower ($P < 0.001$) inside of the cages. During the 2012 growing season, we measured wind speed, wind gusts, solar radiation, air temperature and relative humidity inside of cages compared to open field conditions. We found that wind speed and solar radiation were 89% and 42% lower, respectively, and air temperature, relative humidity and vapor pressure deficit were not significantly affected. There was also a significant ($P < 0.0001$) effect of the time of day on differences in wind speed and radiation between cages and open field plots. Our findings suggest that commonly used insect cages significantly alter the microclimate inside of the cage, and create a radiation regime in which the amount of direct and diffuse radiation received by plants is altered compared to the open field. Plant physiological processes and growth are affected by these environmental changes, adding a confounding factor when comparing caged to open field plants. Because the effects are likely a function of the type of cage, and mesh size and color, we recommend that future studies more thoroughly measure the microclimate for a variety of common cage types used in experiments.

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1. Introduction

Insect exclusion cages are widely used in entomological and agronomic studies to examine plant-insect interactions while allowing for control over pest populations and their natural enemies (Kidd and Jervis, 2005). While exclusion cages are a useful tool, their use can lead to significant modifications of natural environmental conditions inside of the cages (Buntin, 2001; Hand and Keaster, 1967; Woodford, 1973), and subsequently can influence the impact of insects as well as plant responses (Kidd and Jervis, 2005). These effects need to be better understood to determine

Abbreviations: CAD, cumulative aphid days; RUE, radiation use efficiency; VPD, vapor pressure deficit; LAI, leaf area index; A_{400}, photosynthetic rate at 400 ppm atmospheric CO_2; A_{max}, maximum photosynthetic rate; VWC, volumetric water content; PWP, permanent wilting point; LUE, light use efficiency.

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whether the creation of an unintentional and altered microenvironment has consequential effects on plant physiology (i.e., photosynthesis and stomatal conductance), plant phenological development and growth (i.e., leaf area index and plant height), carbon allocation, soil environmental (moisture and temperature) conditions, and whether these effects could introduce confounding factors in controlled experiments and change the interpretation of results.

Fine mesh cages are commonly used to manipulate insect abundance on plants while excluding additional influences, such as natural enemies or additional plant pests, and offer an attractive alternative to using insecticidal treatments in field experiments (Lawson et al., 1994). The porous, mesh material is used to construct a cage perimeter and block insects from either entering or leaving. But this physical barrier can also affect plants in other ways by reducing wind speed, solar radiation, and evaporative water loss (Buntin, 2001; Hand and Keaster, 1967; Moller et al., 2010; Simmons and Yeargan, 1990; Tanny, 2013; Woodford, 1973). Wind speed can affect stomatal conductance and evapotranspiration by modifying the boundary layer resistance near the leaf surface (Campbell and Norman, 1998), and in certain conditions, water use efficiency may be altered when soil water becomes limiting to plant growth. Reductions in solar radiation inside cages could effectively reduce photosynthesis and evapotranspiration and lead to increased plant available soil moisture and cooler soil temperatures. For example, in more arid regions of the world, insect-proof screen houses or agricultural screening in general is often used to intentionally reduce radiation load and wind speed when growing food crops so that water loss through evapotranspiration is decreased and water is conserved (Moller et al., 2004; Tanny, 2013).

The mesh cage material may also lead to an increase in the ratio of diffuse (scattered) to direct beam radiation, altering radiation use efficiency (RUE) and photosynthesis (Alton et al., 2007; Gu et al., 2002; Healey and Rickert, 1998; Knohl and Baldocchi, 2008; Moller et al., 2004). While a reduction in overall radiation intensity (i.e., photosynthetically active radiation or PAR) may decrease photosynthesis, some plants may adapt to lower PAR levels and have relatively high photosynthetic capacity (Boardman, 1977a,b; Healey et al., 1998). The air temperature may also be altered inside of cages due to reduced solar radiation and reduced mechanical air mixing attributed to a decrease in wind speed; a reduction in mixing would be more pronounced when the surface roughness length is higher. Changes in air temperature can also alter the biological rate of insect development inside of the cages, as well as photosynthesis and plant phenological development (Campbell and Norman, 1998). All of the aforementioned factors could confound studies of insect effects on plants because cages contribute to a modified environmental setting. Therefore, the true impact of pest presence on plants may be difficult to ascertain because pests and the insect cages themselves simultaneously affect plant biology and ecology.

Recently, exclusion cages have been used to study the impacts of the soybean aphid (*Aphis glycines* Matsumura) on plant biology and ecology where common natural enemies can reduce aphid populations and damage soybean (*Glycine max* L.) (Beckendorf et al., 2008; Catangui et al., 2009; Costamagna and Landis, 2006; Costamagna et al., 2008, 2007; Liu et al., 2004). Exclusion cages are frequently used in entomological and agronomic research and even the earliest published studies suggested that microclimatic conditions would likely be different between caged and open environment settings (Hand and Keaster, 1967; Lawson et al., 1994; Simmons and Yeargan, 1990; Woodford, 1973) (Table 1). However, there are only a few published studies that have reported how insect cages could have confounded interpretation of experimental results (Beckendorf et al., 2008; Desneux et al., 2006; Fox et al., 2004; Lawson et al., 1994; Rhainds et al., 2007; Simmons and Yeargan, 1990). One published study reported unintended effects such as increased plant biomass, yield, and a change in the overall health of plants (Simmons and Yeargan, 1990). Other more recent studies have measured either air temperature (Desneux et al., 2006) or relative humidity and air temperature (Fox et al., 2004) (Table 1), although neither of these studies made continuous measures of these variables over long time periods. Fox et al. (2004) concluded that there may be small effects of increased temperature on aphid populations inside of cages; however, because they did not measure wind speed or solar radiation inside of cages compared to open field conditions, and only made periodic measurements of air temperature, it is difficult to know if differential plant responses may have influenced aphid growth and population dynamics.

To more fully address the question of how insect cages influence the interpretation of plant-insect interactions, we report data from two companion experiments in 2011 and 2012. The experiments were designed to (1) quantify how exclusion cages influence the microclimatic conditions inside cages by collecting meteorological data (i.e., wind speed, solar radiation, air temperature, and relative humidity) over the majority of a growing season, and to (2) assess how a changed microclimate within cages affects soybean physiology, growth, and yield, as well as soil environmental conditions, compared to ambient conditions. We focused on testing the following hypotheses: (1) exclusion cages reduce wind speed and solar radiation, but increase air temperature, humidity, and soil moisture; and (2) soybean LAI, total biomass, and grain yield are lower inside of exclusion cages due to a consistently reduced intensity of solar radiation.

2. Methods and materials

2.1. Site description

All measurements were performed at the University of Wisconsin-Madison's Arlington Agricultural Research Station (Arlington, WI, 43.5°N lat., 89.5°W long.) during the 2011 and 2012 growing seasons. Soils at this site are classified as Plano silt loams (fine-silty, mixed, superactive, mesic typic Argiudolls), which are highly productive soils formed under the former Empire Prairie and part of the North American prairie–savanna ecotone before it was converted to agricultural land use in the mid-1800s. For the 1981–2010 period, mean annual air temperature was 6.8 °C and mean annual precipitation was 869 mm. The region typically receives 324 mm of precipitation during summer (June–August), with an average air temperature of 17.5 °C (NOAA, 2011). The average air temperature during the summers of 2011 and 2012 were both significantly above the climatological average (+3.2 °C in 2011 and +4.3 °C in 2012), and only 43% of normal summer precipitation was received in each year. In particular, August of 2011 (38.4 mm) and June (7.4 mm) through mid-July of 2012 were very dry periods and contributed to extensive drought conditions in the region. The growing degree days (GDD; base 10 °C) accumulated in 2011 and 2012 were 1299 °C and 1548 °C, respectively, which were both higher than the long-term (1981–2010) average of 1260 °C.

2.2. Soybean experiment

A paired cage/non-caged experiment was established in May 2011, whereby a 1.6 ha field was chisel plowed on May 12 and cultivated before planting. Soybean variety Dairyland 2011RR (Dairyland Seed, West Bend, WI) was planted on May 31 at a rate of 72.8 kg ha^{-1} (65 lbs acre^{-1}) and a depth of 3.8 cm (1.5 in) with 19 cm (7.5 in) row spacing. Plants were sprayed on July 7 with Roundup® PowerMAX (Monsanto, St. Louis, MO) at a rate of 1.75 L ha^{-1} (24 oz acre^{-1}). Insect cages (Lumite Inc., Alto, GA) with dimensions of 2 m × 2 m × 2 m (length × width × height) and a 32 × 32 mesh

Table 1
Previous studies that have measured the impact of insect exclusion cages on microclimate or plant growth.

Author(s)	Year	Plant	Insect	Cage effect on microclimate or plant growth
Hand and Keaster	1967	Not stated	None	Reductions in rainfall, solar radiation, wind speed and evaporation. No effects on air temperature or relative humidity
Woodford	1973	Potato and cauliflower	None	Reduced wind speed and solar radiation. Slight difference in cumulative temperature; less variation in temperature inside of the cages
Simmons and Yeargan	1990	Soybean	Green stink bug	Increased vegetative and reproductive plant growth and increased seed quality inside of cages; no effect on yield; reduced apparent effect on defoliation on yield; no effect on soil water; increased leaf area in the cages
Lawson et al.	1994	Apple trees	Common pests	Reduction in solar radiation, evaporation, air and soil temperature. Increased shoot elongation of apple trees, and reduction in fruit color intensity
Fox et al.	2004	Soybean	Soybean aphid	Minimal effects on air temperature, relative humidity or soybean growth
Desneux et al.	2006	Soybean	Soybean aphid	No significant impact on growing degree-days and air temperature
Rhainds et al.	2007	Soybean	Soybean aphid	Minimal effects on temperature over a 7-day measurement period
Collins et al.	2008	Citrus trees	Queensland fruit fly *Bactrocera tryoni* Froggatt	Cage shading intensity and color had significant effects on air temperature and solar radiation, but not relative humidity

size (0.5 mm × 0.5 mm) were used to evaluate micrometeorological conditions inside of cages. Twelve insect cages were erected on June 13 2011 when soybean were at development stage V0, and remained in place until harvest occurred. Insect cages were positioned in two parallel rows of six cages each whereby the rows were approximately 6 m apart, and individual cages were spaced 3 m apart. During installation, soil was tilled around the perimeter of insect cages to allow for mesh material to be buried approximately 30 cm belowground, which served as a physical barrier to outside plant encroachment and for added cage stability. Mowing of weeds to a height of approximately 10 cm around insect cages took place in early August during the experiment, and weeds were periodically hand pulled from around the perimeter of each cage plot. Twelve additional 2 m × 2 m open-air study plots were established in an adjacent part of the experimental field, orientated in the same manner as the caged plots (e.g., two parallel rows of six plots each with similar spacing dimensions). Cage and open study plots were not interspersed because we did not want the physical presence and close proximity of cages influencing wind speed and direction, thereby contaminating open field microclimate conditions.

To ensure that insect damage was not a significant factor to plant growth and crop yields, we measured soybean aphid abundance in each plot on a weekly basis. Weekly aphid counts on all plants in 2011 were collected by visual observation from the time of introduction on July 5 through September 9 to determine cumulative aphid days (CAD) in each study plot.

2.3. Biophysical measurements

Leaf area index (LAI; m^2 of single sided leaf area per m^2 of ground area) of each study plot was measured weekly during the soybean reproductive stage (July 28–September 8) using a LI-COR LAI-2000 plant canopy analyzer (LI-COR Inc., Lincoln, NE) under diffuse light conditions (e.g., sunrise or sunset). Leaf area index measurements were replicated twice within each plot. For the caged study plots, above and below canopy measurements were collected entirely inside of each cage. In all study plots, two above canopy readings and four below canopy readings were used to quantify LAI.

Leaf gas exchange (photosynthesis) measurements were collected approximately weekly from August 2nd through September 7th using a LI-COR LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE) coupled with a LED light source and CO_2 injection system. Assimilation (A) vs. internal CO_2 concentration (C_i) and light response curves (A vs. varying levels of PAR) were performed on two upper canopy, fully expanded trifoliate leaves for each plot, to ensure similar leaf age for the duration of the experiment. For A–C_i response curves, plants were illuminated with 2000 μmol quanta m^{-2} s^{-1} while CO_2 concentration inside the leaf chamber increased from 0 μmol CO_2 mol^{-1} to 1000 μmol CO_2 mol^{-1}. For light response curves, chamber CO_2 concentration remained constant at 400 μmol CO_2 mol^{-1} and light intensity decreased from 2000 μmol quanta m^{-2} s^{-1} to 0 μmol quanta m^{-2} s^{-1}. All gas exchange measurements were collected between 0900 and 1700 h local time. Ambient rates of photosynthesis (A_{400}) were calculated using the value measured at 400 μmol CO_2 mol^{-1} in the chamber head, with light levels above a saturation level of 1460 μmol quanta m^{-2} s^{-1}.

Three replicates each of leaf temperature, 10 cm soil temperature, and 0–6 cm volumetric water content were taken in each plot at the time that leaf gas exchange measurements were collected. Measurements of leaf temperature were taken on the underside of top canopy leaves with a Fluke 574 precision infrared thermometer (Fluke Corp., Everett, WA). Soil temperature was measured at a 10 cm depth using a hand-held temperature probe (HANNA Instruments, Smithfield, RI), and soil moisture was measured for a 0–6 cm depth with a Dynamax TH300 'Big Stick' Soil Moisture Probe (Dynamax, Houston, TX).

For each plant, five leaf reflectance spectra were also collected at 350–2500 nm from one upper and lower leaf selected at random using an ASD FieldSpec3 full-spectrum Spectroradiometer (Analytical Spectral Devices, Boulder, CO). All leaf spectra were collected concurrent with gas exchange measurements. Leaf percent nitrogen (N), percent carbon (C), percent cellulose and percent acid dissolvable lignin by dry weight were obtained by applying methods and partial least squares regression (PLSR) coefficients reported in Serbin et al. (2014) to averaged leaf spectra for each leaf.

Plots were harvested by hand on September 16th before plant senescence occurred, but after pod fill was completed (R6 stage). All plants within plots were cut at the soil surface, transported back to a UW-Madison lab and dried at 60 °C for 48 h. After drying, individual plant components (i.e., seed, pod, leaf, and stem) were hand separated and weighed. Specific leaf area (SLA; m^2 leaf area kg^{-1} of dry matter) was determined from samples collected during harvest using a 5 cm^2 of leaf area that was cut out from ten replicated

upper canopy and lower canopy leaves for each plot. Harvest index was also calculated using the ratio of dry seed weight to total above ground biomass.

2.4. Micrometeorology experiment

During the week of June 11 2012, four 3 m × 3 m × 2.8 m (length × width × height) insect cages (Lumite, Inc., Alto, GA) were erected in the field in a square pattern, approximately 9 m apart from each other when soybean plants were in the V_1 stage. These cages were slightly larger than those used in the 2011 experiment to accommodate the placement of a weather station inside, but were made of the exact same material and mesh size. Soybean variety Dairyland 1808R2Y (Dairyland Seed, West Bend, WI) was planted on May 23 at a rate of 89.6 kg ha^{-1} (80 lbs acre^{-1}) with 19 cm (7.5 in) row spacing and a 5 cm (2 in) depth. Plants were sprayed with Roundup® Powermax (Monsanto, St. Louis, MO) on June 22 at a rate of 1.75 L ha^{-1} (24 oz acre^{-1}). Plants were sprayed again on July 25 with Dimethoate 400 (Loveland Products, Loveland, CO) at a rate of 1.17 L ha^{-1} (16 oz acre^{-1}).

Weather stations were positioned within each cage at the center point. Two additional weather stations were placed in ambient conditions outside of the cages; one was located 9 m south of the block of cages, and one in center (between) of the four insect cages. This design was intended to mimic how paired caged and open study plots might be situated to study the impacts of soybean aphids on soybean plants. Each weather station contained a suite of meteorological instrumentation that was mounted to 2.4 m tall metal *t*-posts. A solar radiation sensor (silicon pyranometer model #S-LIB-M003; Onset Computer Corp., Bourne, MA) with a spectral response over the 300–1100 nm wavelength band was attached to a light sensor bracket at a height of 1.7 m above the soil surface facing south. A wind speed smart sensor (Onset Computer Corp., Bourne, MA) was attached at a 1.9 m height, and a 12-bit temperature/relative humidity smart sensor (Onset Computer Corp., Bourne, MA) was enclosed in a solar radiation shield (model #RS3; Onset Computer Corp., Bourne, MA) and mounted at 1.7 m. Measurements were collected every 15 min to form hourly averages and recorded using a HOBO® (Onset Computer Corp., Bourne, MA) micro station data logger from June 26 through September 14. Wind gust values were recorded as the highest 3-s sustained wind speed during the 15-min logging interval. Vapor pressure deficit (difference in vapor pressure between ambient and saturated air) was calculated using average hourly temperature and relative humidity (Campbell and Norman, 1998).

2.5. Statistical analyses

Statistical analyses were performed using the R software package version 0.97.449 (R Development Core Team, 2014) and JMP Pro (v. 11.0) (SAS, 2013). We explored the effect of cages on annual measures of plant productivity using the generalized least squares function in the nlme package for R (Pinheiro et al., 2015). This approach was chosen because it provided the ability to build linear models that accounted for differences in variances across caged and open plots. The significance of cage effects was determined by *F*-statistics derived from analysis of variance.

Several biophysical and physiological variables were measured periodically during the growing season. We explored the effect of cages on periodic measures using the generalized additive mixed-effects model (GAMM) function in the mgcv package for R (Wood, 2006). This approach was chosen because it provided the ability to model a smooth non-parametric effect of time and accommodated repeated measurements per plot and per sample date. Smoothing parameters for temporal effects were determined by optimization. Repeated measurements per plot and per sample date were accommodated using a random intercepts model with measurement nested within plot. The importance of the parametric cage effect was determined using a Wald chi-squared test. Although temporal effects were pervasive in the data, we only report cage effects because these were the focus of our study.

Micrometeorological data were obtained hourly throughout the growing season. For the purposes of determining daytime and nighttime averages for meteorological variables, a solar radiation value of 1.0 W m^{-2} was used as the day/night threshold. We explored the effect of cages on continuous, hourly micrometeorological measures using the GAMM functions in the mgcv package for R. This approach was chosen because it provided the ability to model a smooth non-parametric effect of time, accommodated repeated measurements per plot, and allowed for modeling of temporal autocorrelation in model residuals that was not accounted for by other effects. Smoothing parameters for temporal effects were determined by optimization. Repeated measurements per plot were accommodated using random intercepts terms. Temporal autocorrelation in residuals was modeled with a continuous, first-order, autoregressive (AR1) error model. For five response variables, it appeared that the cage effect varied by hour in the day. In these cases, an additional parametric hour effect, and an hour by day interaction, was added to the model. The importance of parametric cage and hour effects was determined using a Wald chi-squared test.

In our analyses of the cage effect on plant growth, physiology, and soil environmental conditions, only replicated caged ($n = 3$) and non-caged ($n = 11$) plots that were determined to have not experienced a reduction in yield due to the presence of high soybean aphid populations were used (Perillo, 2014). This requirement was meant to eliminate the confounding effects on yield of large and persistent soybean aphid populations during the reproductive phase. The result was such that the CAD reached (Perillo, 2014) in these plots were well below the reported economic threshold (10,000 CAD) that is expected to cause a yield decrease according to previously published research (Ragsdale et al., 2007). We eliminated one non-cage plot and nine caged plots from our analysis because they had extremely high CAD in the range of 114,000–341,000 CAD (Perillo, 2014).

3. Results

3.1. Cage effects on microclimate

Daytime and nighttime average wind speeds were 89% (1.06 m s^{-1}) and 96% (0.42 m s^{-1}) lower inside cages, respectively, than outside ($P < 0.0001$) when compared across the entire measurement period (81 days with 1268 15-min observations during the day and 648 at night) (Table 2). Maximum 1-h average wind speeds within cages reached 2.0 m s^{-1} (Fig. 1) with maximum instantaneous gusts of 4.9 m s^{-1} (Fig. 1), whereas outside of cages the maximum 1 h average wind speeds recorded were 6.1 m s^{-1} (Fig. 1) with maximum gusts of 10.0 m s^{-1} (Fig. 1). Daytime and nighttime average wind gusts were 76% (2.3 m s^{-1}) and 89% (1.3 m s^{-1}) lower inside cages, respectively, than outside ($P < 0.0001$) (Table 2). The cumulative distributions of caged versus open wind speeds were remarkably different (Fig. 2); approximately 69% of the hourly average daytime wind speed observations within cages were less than 0.1 m s^{-1}, whereas only 13% of all observations outside cages were recorded at or below 0.1 m s^{-1} (Fig. 2). We determined that for a 1 m s^{-1} average wind speed to occur inside of the Lumite 32 × 32 mesh insect cages, an average wind speed of 4.3 m s^{-1} is required outside (Fig. 1).

Average solar radiation was 42% lower (102 W m^{-2}; $P < 0.0001$) inside the cages than outside (Table 2) across all daytime

Table 2
Mean values of caged and ambient micrometeorology measurements from 2012. Given are the arithmetic means ± standard errors (SE). Daytime measurements were classified as occurring when solar radiation readings were ≥1 W m^{-2}. *P*-values are derived from Wald tests of generalized additive mixed model terms with dF = 1.

Quantity	Units	Cages (±SE)	Ambient (±SE)	X^2_1	*P*-value
Daytime wind speed	m s^{-1}	0.133 (0.035)	1.19 (0.061)	305.2	<0.0001
Nighttime wind speed	m s^{-1}	0.018 (0.019)	0.432 (0.032)	165.7	<0.0001
Daytime wind gusts	m s^{-1}	0.73 (0.069)	3.03 (0.12)	366.2	<0.0001
Nighttime wind gusts	m s^{-1}	0.16 (0.037)	1.45 (0.063)	415.4	<0.0001
Daytime solar radiation	W m^{-2}	140.8 (9.3)	243.2 (16.1)	40.5	<0.0001
Daytime relative humidity	%	68.1 (1.2)	69.2 (2.0)	0.317	0.573
Nighttime relative humidity	%	82.5 (1.1)	81.3 (1.9)	0.390	0.532
Daytime VPD	kPa	1.161 (0.054)	1.118 (0.094)	0.204	0.651
Nighttime VPD	kPa	0.449 (0.040)	0.494 (0.069)	0.419	0.518
Daytime air temperature	°C	23.2 (0.38)	23.1 (0.66)	0.055	0.815
Nighttime air temperature	°C	18.4 (0.18)	18.8 (0.56)	0.566	0.452
Daily maximum temperature	°C	29.3 (0.27)	29.0 (0.46)	0.392	0.531
Daily minimum temperature	°C	15.5 (0.26)	15.6 (0.45)	0.091	0.763
Diurnal temperature range	°C	13.8 (0.18)	13.4 (0.32)	1.637	0.201

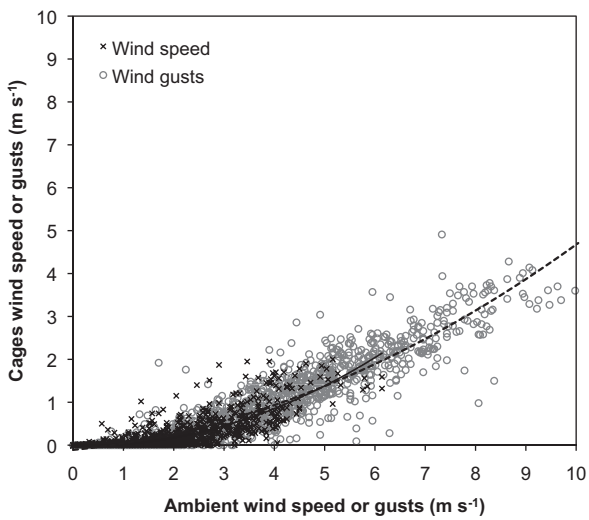

Fig. 1. Relationship between ambient (non-caged) and caged wind speed and wind gusts during measurement period in 2012. Wind gusts were calculated using the highest 3-s wind speed during each 15-min measurement interval. Graphs include both daytime and nighttime measurements, and model fits (solid line for wind speed and dashed line for wind gusts) are with a 2nd degree polynomial function.

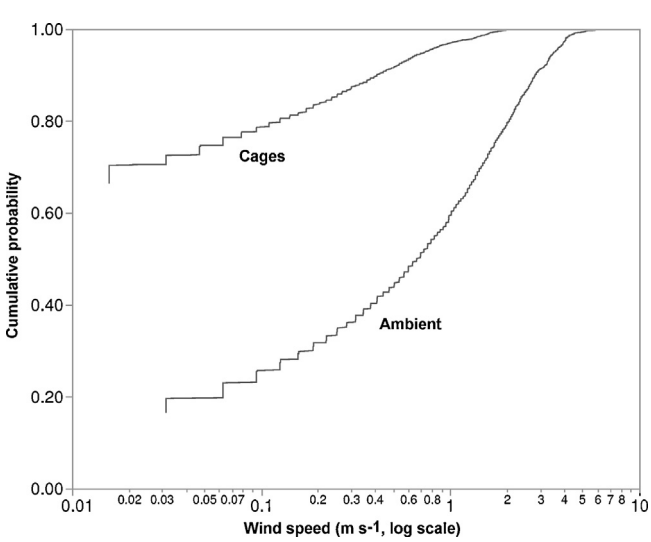

Fig. 2. Cumulative distribution function for caged and ambient daytime wind speed during measurement period in 2012.

observations; however, the magnitude of the difference was a function of the time of day and sky conditions (e.g., cloud cover; Fig. 3), as well as the day of year (Fig. 4a). Over the entire length of the experiment, soybean plants within cages received 53% less total accumulated solar radiation than in ambient conditions (Fig. 5; slope = 0.53). On average, the peak daily difference in radiation received between caged and open observations occurred at 1347 local time (Fig. 4b), or approximately 45 min after solar noon when radiation was highest, with the smallest differences occurring near sunrise and sunset under diffuse sky conditions (Fig. 3). The time of day when the peak solar radiation difference occurred did not vary over the course of the experiment (Fig. 4b; $P = 0.765$); however, the absolute difference in radiation between caged and open plots declined linearly as a function of total incoming radiation (Fig. 4a; $P = 0.016$) over the course of the experiment which was attributed to seasonal changes in sun angle. The maximum solar radiation recorded in the cages was 507 W m^{-2} compared to 932 W m^{-2} in the open plots.

During daytime hours, ambient VPD was higher, and at night VPD was higher in the cages; however, the differences were not significant (Table 2). Average air temperature was not significantly different within insect cages compared to ambient temperatures during either daytime or nighttime across all observations (Table 2).

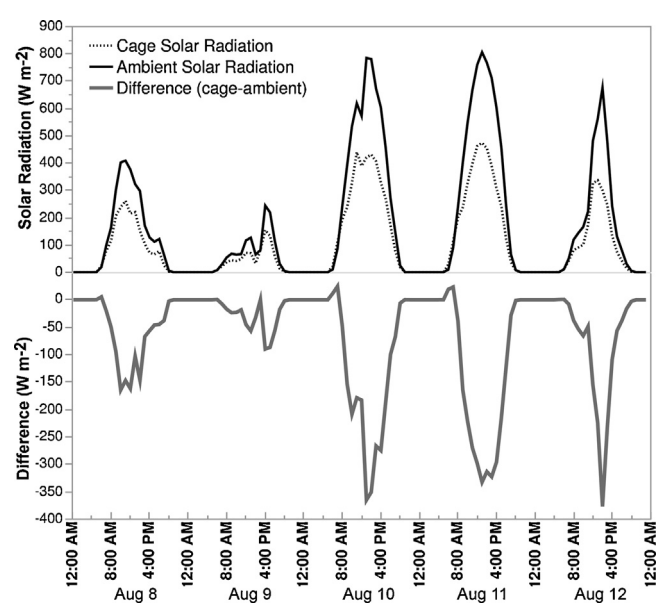

Fig. 3. Hourly averaged solar radiation for caged and ambient plots during August 8–12, 2011, and the hourly differences. Cloudy sky conditions prevailed on August 8–9, and full sun (clear sky) conditions occurred on August 10–11.

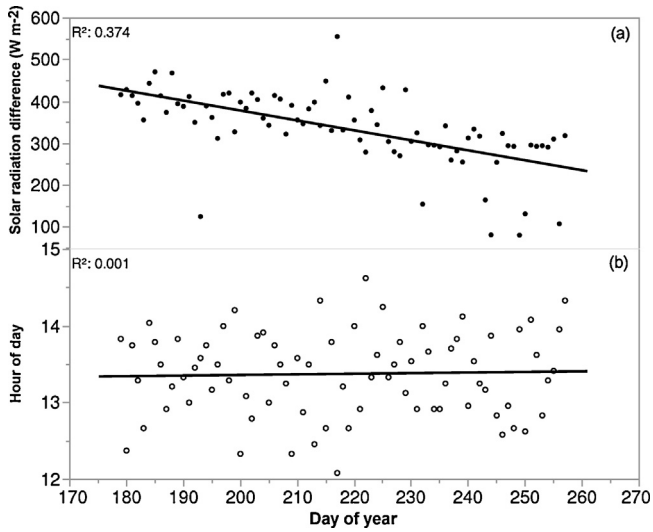

Fig. 4. (a) Difference in daily peak solar radiation between ambient conditions and cages (ambient-cages) and (b) the local time (hours) the peak difference occurred for each day during the experiment.

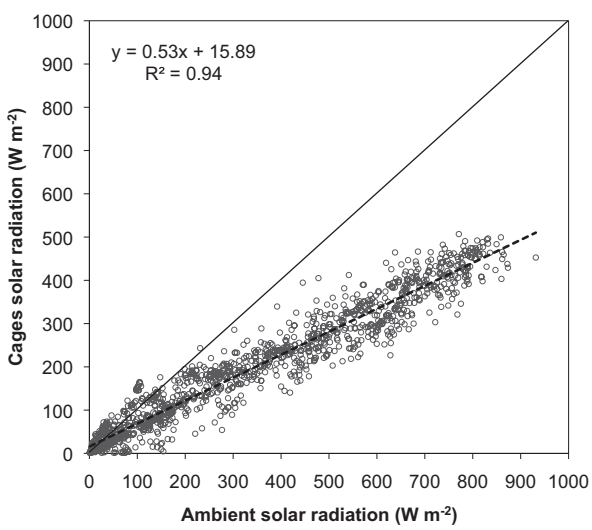

Fig. 5. Scatter plot of hourly average solar radiation measured within cages compared to ambient conditions. Only daytime radiation values (>1 W m^{-2}) are included.

The slope of linear regression for air temperature between caged and open plots was 0.99 (R^2 = 0.98, P < 0.0001), whereas for VPD the slope was 0.96 (R^2 = 0.98, P < 0.0001). Daily average maximum and minimum temperatures as well as diurnal temperature range were not significantly different between caged and open plots (Table 2). However, there was a weak (R^2 = 0.031), but significant (P < 0.0001) positive relationship between the magnitude of the difference in air temperatures (i.e., cages – ambient conditions) and the ambient air temperatures.

3.2. Diel patterns

Over the course of the measurement period, the differences between cage and ambient conditions exhibited consistent diel patterns for each of the four variables measured (Fig. 6). All variables studied showed an influence of time of day on their magnitude (Table 3), which was an expected result. However, all meteorological variables measured also had a significant interaction between time (hour of day) and the environmental setting – the cage effect (Table 3).

As previously discussed, differences in solar radiation were a function of the ambient radiation magnitude, and therefore, the peak differences occurred in early afternoon (Fig. 6a). Wald tests confirmed that a significant interaction between time (i.e., hour or the day) and environment (i.e., cages vs. ambient conditions) occurred for radiation differences, suggesting that the rate with which radiation changed over the course of a day was different for each environmental setting (Table 3). As well, the variability of the radiation differences was much smaller during the morning hours and increased significantly after local noon. Wind speed differences were smallest during the nighttime hours, reaching a minimum value right before about sunrise (e.g., 0500 h local time), and gradually increased in magnitude through the late afternoon before beginning a decline that coincided with approximate sunset times (e.g., 2000 h local time) (Fig. 6b). Wald tests also confirmed that a significant interaction between time and environment occurred for wind speed (and wind gust) differences, suggesting that the rate with which wind speed (and gusts) changed over the day was different for each environmental setting (Table 3). For VPD and air temperature differences, the Wald tests showed that there was also a significant interaction of time of day and environmental setting (Table 3). Vapor pressure deficit (Fig. 6c) and air temperature (Fig. 6d) differences were at a minimum during nighttime hours, and rapidly increased after sunrise, reaching a maximum difference in late morning to early afternoon. For VPD differences, the least amount of variability in those differences was observed for the nighttime hours through approximately 3–4 h after sunrise (e.g., around 0900 h local time).

3.3. Cage effects on soybean productivity and biophysical responses

Total aboveground soybean biomass was increased by 30% inside of the cages (P = 0.055) and yield was 23% higher (P = 0.098) (Table 4). The harvest index (HI) inside of cages was slightly (5.9%) lower than ambient conditions (P = 0.022; Table 4). The seed weight (measured as the weight of 100 seeds), pod weight, ratio of seed to pod weight, and specific leaf area (SLA) for bottom leaves were not significantly effected by the insect cages (Table 4). The specific leaf area for top leaves was 24% lower inside of cages (P = 0.041; Table 4).

Average LAI over the measurement period was 17% (1.09 m^2 m^{-2}) lower in the cages (P < 0.001; Table 5). The average difference in LAI ranged from 0.55 m^2 m^{-2} on July 28 to 2.04 m^2 m^{-2} greater outside the cages on September 7 (Fig. 7). The LAI reached maximum values in mid-August and declined thereafter for both caged and open plots (Fig. 7). Leaf temperatures were not significantly different over the study period (Table 4), averaging 23.5 °C inside of cages and 23.8 °C in open plots (Table 5, Fig. 8a). Leaf temperatures were generally 3–8 °C warmer than air temperature and responded similarly to daily air temperature fluctuations in both open and caged plots (Fig. 8a).

Average 10 cm soil temperature inside of the cages was 19.0 °C and 19.3 °C in open plots (Fig. 8a), and over the entire measurement period, these were significantly different (Table 5; P = 0.0016). Average surface (0–6 cm) soil volumetric water content (VWC), was 38% higher inside of the cages (P < 0.001) when compared across the entire measurement period (Table 5; Fig. 8b). Average VWC inside of the cages was 0.19 m^3 m^{-3} with a maximum plot measurement of 0.29 m^3 m^{-3}, whereas the average VWC in open plots was 0.138 m^3 m^{-3} with a maximum plot measurement of 0.27 m^3 m^{-3}. On four measurement days during the soybean reproductive stage (8/8, 8/18, 8/29, 9/7), open plots had VWC readings below permanent wilting point (PWP) for the Plano silt loam soil (0.13 m^3 m^{-3}; Campbell and Norman, 1998), which contributed to the average VWC during the entire measurement period remaining below PWP

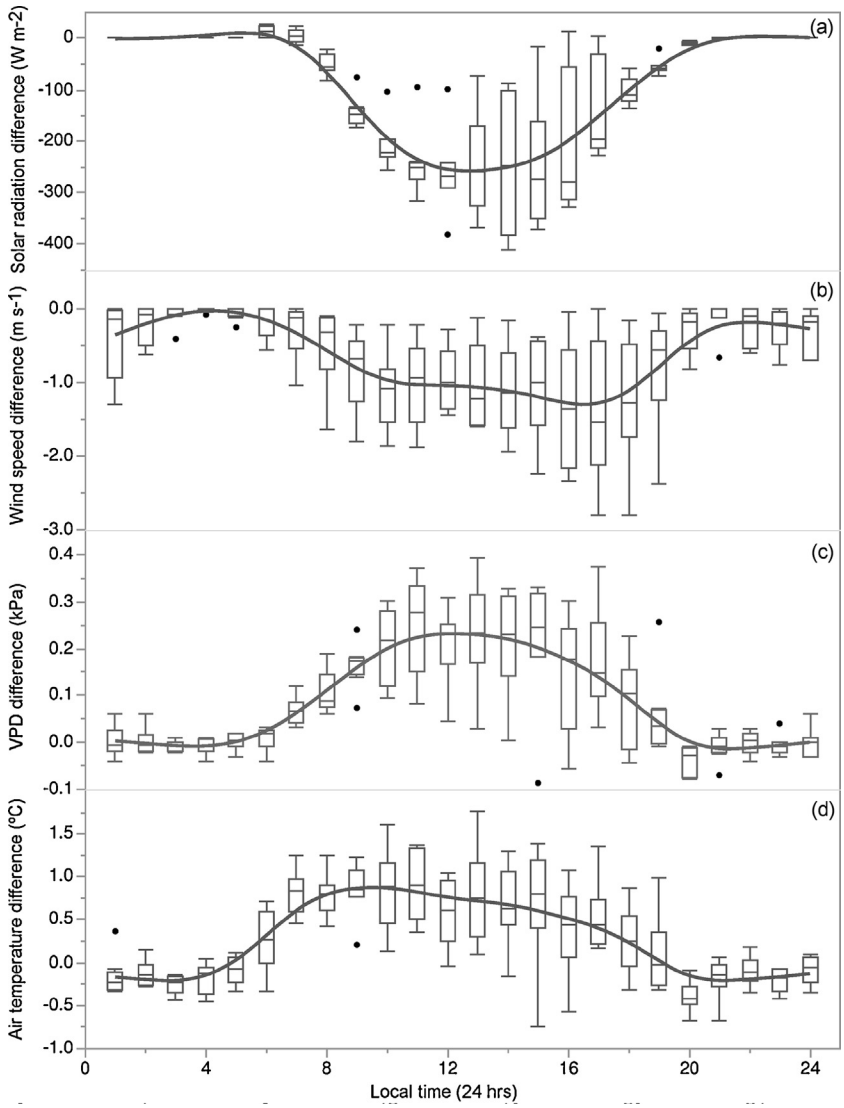

Fig. 6. Box plots (median, quartiles, and outliers shown) depicting the statistical hourly differences between ambient plots and cages (ambient-cages) during a representative week of data collected (July 28–August 3, 2012) for (a) solar radiation, (b) wind speed, (c) vapor pressure deficit, and (d) air temperature. Solid lines represent a cubic spline ($P < 0.05$) fit to the hourly data. The local time of 24 h represents midnight.

Table 3
Generalized additive mixed model summary for the effects of environmental setting (ambient open air vs. cages) and time of day and their interaction on solar radiation, wind speed, wind gusts, air temperature, and vapor pressure deficit. *P*-values are derived from Wald tests with dF = 1.

Source of variation	Solar radiation *P*	Wind speed	Wind gusts	Air temperature	VPD
Environment (E)	<0.0001	<0.0001	<0.0001	NS[a]	NS
Time of day (*T*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
E × *T*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] NS: non significant; $P > 0.1$.

Table 4
End of growing season soybean growth measurements from the 2011 paired caged-ambient study. Reported are the arithmetic means ± standard errors (SE). *P*-values are derived from *F*-tests with numerator dF = 1 and denominator dF = 12.

Quantity	Units	Cages (±SE)	Ambient (±SE)	$F_{1,12}$	*P*-value
Aboveground biomass	$g\,m^{-2}$	95.6 (9.2)	73.5 (10.4)	4.50	0.055
Yield	$g\,m^{-2}$	30.5 (2.9)	24.8 (3.2)	3.22	0.098
Harvest index	Fraction	0.32 (0.003)	0.34 (0.006)	6.91	0.022
Weight of 100 seeds	g	13.5 (0.24)	13.89 (0.27)	1.86	0.198
Pod mass	$g\,m^{-2}$	14.2 (2.8)	10.66 (3.0)	1.37	0.265
Seed and pod mass	$g\,m^{-2}$	45.5 (4.8)	35.9 (5.4)	3.08	0.105
Seed to pod ratio		2.19 (0.10)	2.44 (0.16)	2.26	0.159
Specific leaf area (top leaves)	$g\,m^{-2}$	30.1 (3.73)	39.6 (4.2)	5.23	0.041
Specific leaf area (bottom leaves)	$g\,m^{-2}$	27.9 (2.13)	28.2 (2.6)	0.008	0.931

Table 5
Repeated, periodic measurements of soybean growth and physiology, leaf tissue chemistry, and soil environmental conditions from the 2011 paired caged-ambient study. Reported are the arithmetic means ± standard errors (SE). *P*-values are derived from Wald tests of GAMM terms with dF = 1.

Quantity	Units	Cages (±SE)	Ambient (±SE)	X^2_1	*P*-value
Leaf area index (LAI)	$m^2\ m^{-2}$	5.43 (0.18)	6.52 (0.20)	28.66	<0.001
Leaf temperature	°C	23.5 (0.37)	23.8 (0.42)	0.618	0.432
Stomatal conductance	μmol $H_2O\ m^{-2}$ leaf area s^{-1}	0.54 (0.074)	0.38 (0.084)	3.21	0.015
Ambient photosynthesis (@400 ppm CO_2)	μmol $CO_2\ m^{-2}\ s^{-1}$	24.2 (0.76)	23.8 (0.91)	0.174	0.677
Maximum photosynthesis	μmol $CO_2\ m^{-2}\ s^{-1}$	31.1 (2.1)	28.7 (2.5)	0.87	0.356
Leaf nitrogen	%	3.73 (0.09)	3.52 (0.11)	3.75	0.061
Leaf carbon	%	46.5 (0.10)	47.0 (0.12)	21.1	<0.001
Leaf lignin (acid dissolvable)	%	18.7 (0.87)	21.4 (1.1)	6.21	0.018
Leaf cellulose	%	13.2 (0.35)	14.7 (0.43)	12.3	0.001
Soil temperature (10 cm)	°C	19.0 (0.09)	19.3 (0.10)	10.19	0.0016
Soil–water content (0–6 cm)	$m^3\ m^{-3}$	0.191 (0.007)	0.138 (0.007)	49.8	<0.001

(Fig. 8b). In contrast, the surface VWC in the caged plots never reached the PWP (Fig 8b).

3.4. Cage effects on physiological responses

Photosynthetic rates at 400 ppm CO_2 (A_{400}) were not significantly altered inside of the cages (Table 5; Fig. 9a). However, leaf stomatal conductance values were on average 42% higher inside of cages, with the most significant differences occurring in late July and early August (Table 5; Fig. 9b). Average maximum photosynthesis (A_{max}) was 8% higher inside of the caged plots; however, the difference was not significant across all measurements collected or on any individual measurement date. Leaf carbon, cellulose, and acid dissolvable lignin were all significantly lower inside of cages, but leaf nitrogen was slightly higher, although the significance level for N was just above $P = 0.05$ (Table 5).

We measured soybean leaf light response curves (PAR vs. assimilation) when leaf temperatures were near 25 °C (+/−1.0 °C) to determine whether caged plants may have adapted to the reduced light environment inside of the cages, and increased their photosynthetic rates at lower light levels. Using a square root transformation on the PAR values in the statistical model fitting exercise (i.e., rectangular hyperbola with R^2 values of 0.89 and higher), we found that on the three days where light response curves were measured in both open and caged plots (August 18, 22 and 29) there was no significant difference between caged and open plants ($P = 0.85$, $P = 0.59$, and $P = 0.22$, respectively) based on how rates of photosynthesis responded to a continuum of PAR levels.

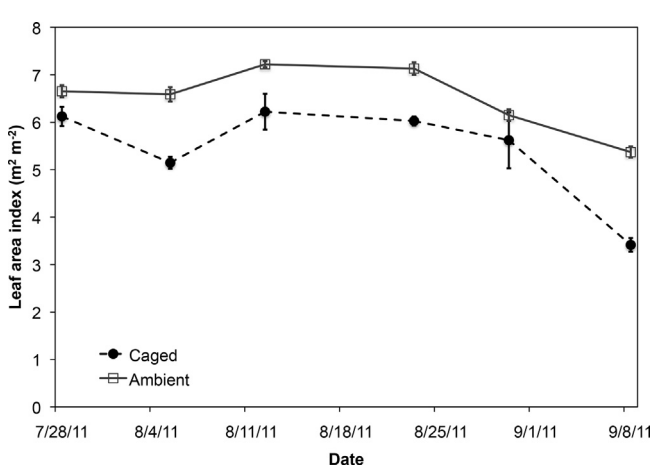

Fig. 7. Periodic measurements of leaf area index (LAI; m^2 one-sided leaf area per m^2 ground area) for caged and ambient plots in 2011 with standard error bars.

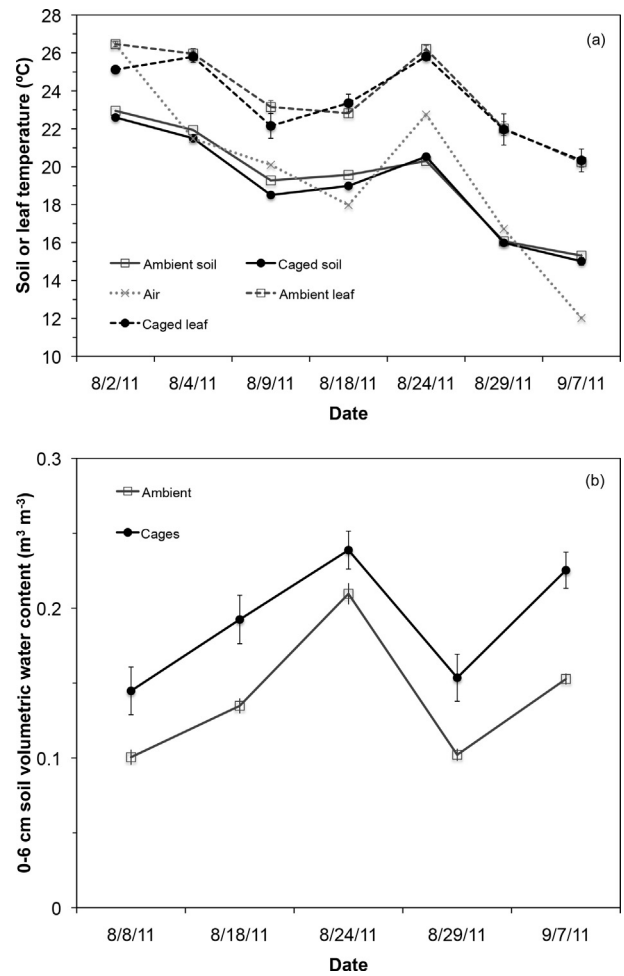

Fig. 8. Measurements of (a) 10 cm soil temperature and leaf temperature and (b) 0–6 cm volumetric water content for caged and ambient plots in 2011 plotted with standard error bars. Measurements were collected between the hours of 0900 and 1700 each day. The daily average air temperature from the Arlington Agricultural Research Station in Arlington, WI is plotted for reference.

4. Discussion

4.1. Cages create an altered microenvironment

The microenvironment that was created inside of insect cages was substantially altered by an 89% reduction in average daytime wind speed compared to ambient, open-air conditions. Previous studies that have investigated the impact of insect cages on microenvironments have reported 49% and 85% reductions in average wind speed (Hand and Keaster, 1967; Woodford, 1973). The

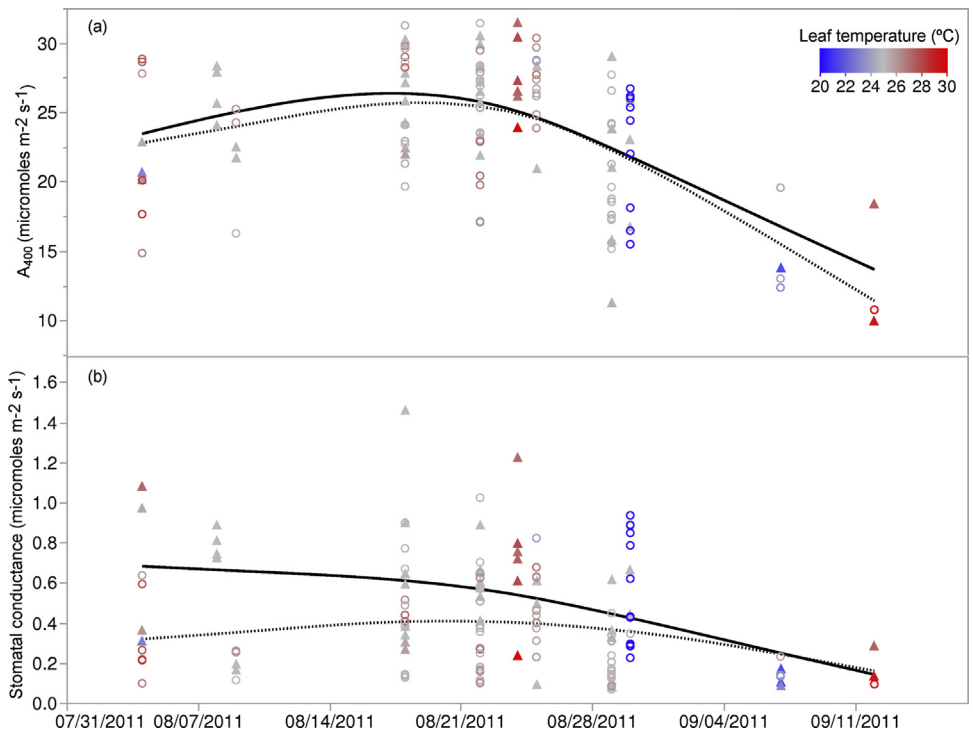

Fig. 9. Measurements of soybean (a) photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) at 400 ppm CO_2 (A_{400}) and (b) stomatal conductance (μmol H_2O m^{-2} s^{-1}), collected between the hours of 0900 and 1700 for caged and ambient plots. Ambient plot data are denoted by open circles and caged plot data are represented with solid triangles. Symbol colors represent leaf temperatures at the time of observation. Solid (cages) and dotted (ambient) lines represent cubic splines ($P < 0.05$) fit to each dataset.

relationship between caged and ambient wind speed is not consistent in the literature due to differences in mesh size, measurement height, duration of measurements, and quantity of measurements. While the cage size in the two previous studies was similar to the cages used in our experiment, the mesh sizes and materials used were different; Woodford (1973) used a white terylene voile material with a 200 μm mesh size and Hand and Keaster (1967) used a saran screen cage material with 20-mesh per 2.54 cm. While we found a non-linear relationship between ambient and cage wind speeds, Woodford (1973) found a linear relationship but Hand and Keaster (1967) did not find a consistent relationship. Both Woodford (1973) and Hand and Keaster (1967) had limited timeframes of reference, five days and 45 days (with daily accumulated winds speeds) respectively, and thus, a smaller number of data points. The longer timeframe, with more consistent data points in our study allowed us to calculate with high confidence the influence of commonly used insect cages on wind speed and wind gusts.

One of the potential impacts of lower wind speeds inside of the insect cages is a reduction in evapotranspiration (ET). The Penman–Monteith modeling approach (Campbell and Norman, 1998; Tanny, 2013) illustrates that reduced wind speed can decrease the rate of ET when stomatal conductance is high. Higher wind speeds increase the boundary layer conductance, which supports an increase in water transport away from leaves. In contrast, at lower wind speeds, vertical mixing is decreased, and the water vapor gradient is reduced and water is not evaporated as efficiently (Campbell and Norman, 1998). However, plant response to wind speed is complex given the simultaneous effects on leaf temperature, and the confounding influence of stomatal conductance (Campbell and Norman, 1998). In some environments, lower wind speeds can lead to improved plant health by reducing the rates at which water is lost from the soil and plant leaves, causing plants to become more water-use efficient and less prone to water stress. However, lower wind speeds can increase the heat load on leaves making it more difficult for them to remain at or below the air temperature, thereby potentially decreasing the rate of photosynthesis. This primarily occurs under more stressful environmental conditions such as full sun, drought, and high air temperatures (Campbell and Norman, 1998).

Insect cages significantly reduced solar radiation received by soybean plants, particularly during the mid morning to mid afternoon hours when radiation intensity was highest. We note that the Lumite mesh used in our study did not completely "shade" the plants; shadows were still cast on plants and soil and direct beam radiation was still transmitted through the cage material, although at a reduced intensity and with a likely corresponding change in the ratio of direct beam to diffuse radiation (Healey and Rickert, 1998). Previous work has shown comparable reductions in solar radiation beneath insect cages (Hand and Keaster, 1967; Lawson et al., 1994; Moller et al., 2010; Tanny, 2013; Woodford, 1973); however, mesh size can impact the reduction in radiation (Lawson et al., 1994). The slope of the cage vs. ambient radiation relationship (0.418) measured by Woodford (1973) was similar to slope of our relationship (0.53; Fig. 5). The overall effect of the cages was that the largest decrease in radiation occurred when solar radiation intensity is highest, which is a function of time of day, time of year, and sky condition. The peak difference between caged and ambient solar radiation occurred between 1300 and 1400 h local time, and the magnitude decreased over the growing season due to the decline in solar radiation intensity with time since the summer solstice. Variations on a daily basis, which were highest during the afternoon hours, are due to the effects of cloudiness as the total incoming solar radiation is reduced by clouds compared to days with clear skies and maximum atmospheric transmittance; the high variability from day to day in the afternoon hours (Fig. 6a) is likely due to variable cloudiness as clouds developed in response to daytime heating.

Temperature was not significantly altered inside of the cages. This is consistent with other cage studies that have documented minimal changes in temperature (Collins et al., 2008; Desneux et al., 2006; Fox et al., 2004; Hand and Keaster, 1967; Lawson et al., 1994; Woodford, 1973). While there was an expectation that temperature would be higher inside of cages due to a reduction in mixing with the ambient atmosphere, the reduction in radiation potentially compensated for decreased turbulent mixing due to reduced wind speeds. The VPD was not significantly impacted by the presence of cages, which is in agreement with previous studies that reported minimal to no effects of cages on relative humidity (Collins et al., 2008; Fox et al., 2004; Hand and Keaster, 1967). However, relative humidity is not the best meteorological variable to use when assessing connections between a microenvironment, plant stomatal response, and ET; instead, VPD is preferred because unlike relative humidity, the rate of ET is influenced by stomatal conductance and its sensitivity to VPD (Ocheltree et al., 2014). Moreover, rates of ET scale nearly linearly with VPD (Campbell and Norman, 1998).

4.2. Cages supported lower soil temperatures, higher surface soil moisture and stomatal conductance

Soil moisture was consistently higher inside the cages, which was likely due to lower ET attributed to lower wind speeds and reduced solar radiation (Hand and Keaster, 1967; Lawson et al., 1994). This effect has been shown in previous cage studies where rates of evaporation decreased between 17 % and 34 % (Hand and Keaster, 1967; Lawson et al., 1994). Lawson et al. (1994) suggested that rainfall collecting on the cages and running down the sides into the soil or the reduced radiation load (e.g., reduced ET) were able to compensate for decreased rainfall received inside of insect cages. In our study, lower LAI inside of the cages potentially contributed to decreased plant water demand and lower total transpiration, resulting in higher VWC inside of the cages. Stomatal conductance was likely higher inside of the insect cages due to increased availability of plant available water (Brady et al., 1975; Sanchez-Diaz and Kramer, 1971; Sionit and Kramer, 1976). The observed decrease in soil temperatures within cage plots was expected given a corresponding increase in soil moisture and significantly lower intensity of solar radiation.

Increased surface soil water inside of the cages likely supported the increased total above ground biomass by reducing water stress even though solar radiation and LAI were both lower. Decreased LAI inside of the cages was likely a result of stunted plant growth inside of the cages early in the season, potentially due to reduced solar radiation. Later in the season, when drier conditions prevailed, reductions in LAI, wind speed, and solar radiation inside of the cages likely contributed to a reduction in ET and conservation of additional surface soil water compared to open field plots (Tanny, 2013). Subsequently, during the reproductive stage of soybean growth (pod fill), plants inside the cages benefitted from higher VWC, likely supporting the increase in total plant biomass by the end of the season (Table 4). While Hand and Keaster (1967) reported that precipitation was 16% lower inside of their insect cages, increased surface soil moisture inside of our cages suggests that even if there was a reduction in precipitation inside cages, other environmental factors led to reduced ET – which is supported by higher surface VWC inside of cages compared to ambient (open field) conditions. In support of our findings here, Moller et al. (2004) reported that sweet pepper water use was 60% lower within an insect proof screenhouse compared to ambient conditions, which was attributed to a 40–50% reduction in radiation load and lower windspeed.

4.3. Soybean leaves did not acclimate to lower radiation, but cages can create a radiation regime that supports increased productivity

An analysis of light response curves for soybean leaves showed no differences between open and caged plots. Had light response curves showed increased photosynthetic rates for caged plants, this might have explained the increased biomass measured in the cages, suggesting soybean plants adapted to the lower light intensities inside of cages. A previous study of soybean reported that plants acclimated to two different light regimes (550 μmol photons m^{-2} s^{-1} and 950 μmol photons m^{-2} s^{-1}) and had different photosynthetic capacity when exposed to the same radiation intensity (Bunce, 1991; Bunce et al., 1977). In addition to these effects, Egli et al. (1985) reported that shading soybean plants with a black shade cloth during pod and seed development increased the duration of seed growth.

We found that A_{400} rates were not altered inside of the cages; therefore, there was likely no photosynthetic adaptation of the soybean plant to the lower light environment inside of the cages. Because caged plots had a reduced light environment and typically never reached light saturation levels (~650 W m^{-2} or 1500 μmol photons m^{-2} s^{-1}), the actual rate of photosynthesis for leaves near the top of the canopy (e.g., where the majority of sunlit leaves reside) may have been reduced inside of the cages compared to the open plots (Pons and Pearcy, 1994; Sinclair and Horie, 1989), particularly from mid morning to mid afternoon when radiation differences were at a maximum (Fig. 6a). However, the average PAR intensity on fully shaded leaves in the cage canopy environment may have been higher than that in open field plots due to differences in the ratio of direct beam vs. diffuse radiation as diffuse radiation has been shown to increase beneath screens used in cropping systems (Moller et al., 2010).

Cages may act similarly to clouds by not only decreasing the magnitude of total incident PAR on a canopy, but by also altering the ratio of diffuse to beam radiation (Gu et al., 1999; Moller et al., 2010). An increase in the fraction of diffuse radiation associated with cloud cover can lead to an increase in light use efficiency (LUE) by plants (Alton et al., 2007; Gu et al., 2002). An increase in LUE may compensate for lower levels of solar radiation inside of the cages, allowing plants to be as productive as those experiencing full sunlight. Diffuse radiation also does not attenuate as quickly as direct beam radiation, which allows for increased light penetration and intensities near the bottom of a plant canopy where photosynthesis is usually lower (Knohl and Baldocchi, 2008; Moller et al., 2010). In high LAI canopies (>6.0 m^2 m^{-2}) such as soybean, approximately 75% of leaf area is shaded assuming a spherical leaf angle distribution (light extinction coefficient = 0.5/cosine of sun zenith angle). Cages may cause an increase in the average diffuse and scattered light illumination on shaded leaves in the canopy (Campbell and Norman, 1998), which could support higher assimilation rates in caged plots because of the following two reasons: (1) there were no differences in light response curves between caged and open field plants; and (2) the majority of leaf area in higher LAI canopies is shaded. Therefore, cages could create a substantially different radiation regime than found in open field conditions that could support increased overall productivity (Moller et al., 2010).

4.4. Previous studies compared to new findings

Insect cages, similar to the ones in this study, are commonly used in field experiments to maintain control over insect populations. A few studies have performed a thorough investigation of the caged microclimate using meteorological instrumentation (Hand and Keaster, 1967; Woodford, 1973), while others have identified specific impacts on their experiment (Lawson et al., 1994;

Simmons and Yeargan, 1990) (Table 1). Some studies have reported no impacts or focused on the change in aphid populations (Chacon et al., 2008; Donaldson et al., 2007; Gardiner et al., 2009). However, most cage studies do not sufficiently identify how the outcome of their experiment may have been altered by the presence of the cages because they have not measured a full suite of meteorological and soil environmental variables to understand the confounding effects.

A few soybean aphid studies have measured temperature and relative humidity (Desneux et al., 2006; Fox et al., 2004; Rhainds et al., 2007), but did not identify a changing radiation regime and wind speed as being important and dominant controls on the microenvironment, photosynthesis, ET; from the current results, these clearly influence soil moisture dynamics, stomatal response, and productivity. Some studies have relied on citing previous results (e.g., no effects of cages), but the problem is more complex than represented by measures of temperature and relative humidity (Tanny, 2013). We concluded there were still important differences in the soil environment and plant growth caused by the Lumite cages used, largely attributed to a modified radiation regime and reduced wind speeds. Two recent studies (Beckendorf et al., 2008; Riedell et al., 2013) referenced another study (Bell and Baker, 2000) that suggested the amber colored Lumite mesh material is commonly used in field studies because it is known for good for light penetration and wind flow; however, this referenced study did not actually report radiation or wind speed measured directly within cages. The Bell and Baker (2000) study suggested that the Lumite "32 × 32" screen material was classified as having "low" air resistance, ranking near the bottom of many tested screen materials for exerting an influence on wind flow. Clearly, the results of our study demonstrated that the Lumite 32 × 32 screening significantly impedes air movement across plants and modifies the radiation regime and studies from arid regions using agricultural screening also refute this claim (Tanny, 2013). Therefore, we argue that these previous statements about Lumite being preferred because of good light penetration and wind flow should be questioned.

Lastly, the seasonal and diel patterns of differences in microclimate between caged and open plots (Figs. 4 and 6) suggest that the influence of cages is not consistent across time. These differences are likely a function of the time of day as well as calendar date. The latitude (e.g., sun angle and intensity of solar radiation) of a study site and the baseline climatology will likely influence the magnitude of differences between cages and ambient conditions.

5. Conclusions

Insect cages used in this study created distinctly different microenvironment compared to ambient conditions by inhibiting wind flow and changing the radiation regime, which impacted surface soil environmental conditions, stomatal conductance, LAI, total plant biomass and harvest index, and leaf tissue chemistry. However, insect cages are an important and often relied upon apparatus in field settings as part of plant-insect relation studies in agronomy and entomology; therefore, the unintended impacts of using insect cages should be quantified to understand how they influence experimental results. Measurements similar to the ones collected in this study (e.g., wind speed, solar radiation or PAR, air temperature, vapor pressure deficit, soil temperature and soil volumetric water content) are critical to provide context needed to qualify potential impacts on plant physiology and possibly even insect development. One of our key findings was that higher surface soil moisture inside of cages, due to reduced ET, supported increases in stomatal conductance that likely led to increased total plant biomass compared to plants grown in ambient conditions. In addition and may be more importantly, the insect cages also likely contributed to a significant change in the radiation regime, whereby shaded leaves in the caged canopy were exposed to a higher illumination level than those in the open field plots, which would have supported increased assimilation by plants.

While the outcome of our study may invoke concern for those using insect cages to estimate plant responses, we stress that the degree of impact that insect cages have is influenced by the physical properties of material used, as well as the ambient environmental conditions. The influence of cages is likely not static; in our mid latitude experiment, time of day and seasonal changes drive the magnitude of measured environmental differences between cages and ambient conditions, suggesting that a standard response or influence of cages on experiments is unlikely. Extreme environmental conditions that exist during experiments may also exacerbate the degree of influence that cages have on experiments. For example, the catastrophic US drought of 2012 might have put caged plants at a physiological advantage due the importance of any additional soil moisture on plant growth. In our study during a short-term (~4 week) drought in 2011, open field plots were subjected to surface soil moisture at the permanent wilting point for an extended time period, but this was not the case inside of the caged plots.

A reduction of LAI inside of cages, while at least partially attributed to lower solar radiation, was also potentially influenced by human disturbance while making repetitive (e.g., weekly) measurements of LAI or aphid counts throughout the growing season. This could also create a disturbance factor on plants that could potentially be confused with the impacts of insect/pest pressure if yield is the key effect being studied.

We have described numerous issues regarding use of insect cages, but also acknowledge that they are crucial to continued research. As such, we urge researchers to take a more thorough approach to accounting for the potential influences of cages that go beyond simplistic measures of how temperature and relative humidity change inside cages. A more complete understanding of the connections in the soil-plant-insect-atmosphere system is ultimately needed to determine whether insect cages are complicating the interpretation of plant growth or physiological responses to insect manipulation studies that use insect cages.

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