Insect Science

Insect Science (2012) 00, 1–11, DOI 10.1111/j.1744-7917.2012.01515.x

ORIGINAL ARTICLE

Impact of elevated CO_2 and increased temperature on Japanese beetle herbivory

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Abstract To examine how the major elements of global change affect herbivory in agroecosystems, a multifactorial experiment was conducted where soybeans were grown at two levels of carbon dioxide and temperature, including those predicted for 2050, under otherwise normal field conditions. Japanese beetles (*Popillia japonica* Newman) were enclosed on foliage for 24 h, after which the beetle survivorship, total and per capita leaf consumption, and leaf protease inhibitor activity were measured. The direct effect of temperature on beetle consumption and survivorship also was measured under controlled environmental conditions. No differences in total foliage consumption were observed; however, beetles forced to feed at elevated temperature in the field demonstrated greater per capita consumption and reduced survivorship compared to beetles feeding at ambient temperature. Survivorship was also greater for beetles that consumed foliage grown under elevated CO_2, but there were no interactive effects of CO_2 and temperature, and no differences in leaf chemistry were resolved. Leaf consumption by beetles increased strongly with increasing temperature up to ~37° C, above which increased mortality caused a precipitous decrease in consumption. An empirical model based on the temperature dependence of leaf consumption and flight suggests that the 3.5°C increase in temperature predicted for 2050 will increase the optimal feeding window for the Japanese beetle by 290%. Elevated temperature and CO_2 operating independently have the potential to greatly increase foliage damage to soybean by chewing insects, such as *Popillia japonica,* potentially affecting crop yields.

Key words carbon dioxide, FACE (free-air concentration enrichment), *Glycine max*, herbivory, Japanese beetles, *Popillia japonica*, temperature dependence

Introduction

Prior to the Industrial Revolution, the atmospheric carbon dioxide concentration was stable at ~270 μL/L for over 1 000 years (IPCC, 2001), and reconstructions of paleo-CO_2 concentrations indicate the atmosphere was well below 300 uL/L for more than 20 million years (Pearson and Palmer, 2000). With the increase of anthropogenic CO_2 emissions, the global concentration of CO_2 has increased to 390 μL/L today and is expected to nearly double from preindustrial levels by the end of the century (IPCC, 2001; NOAA, 2001). As a result of increasing CO_2 concentrations, the mean global temperature is expected to increase between 2–4°C, although the magnitude of this increase will vary regionally (IPCC, 2007; Meehl *et al.*, 2007). By 2050, the temperature in the central United States, where the majority of the soybean crop is cultivated, is expected to increase ~3°C (Hayhoe *et al.*, 2010; Lin *et al.*, 2010). In addition to directly affecting yields, elevated CO_2 and temperature may affect crop

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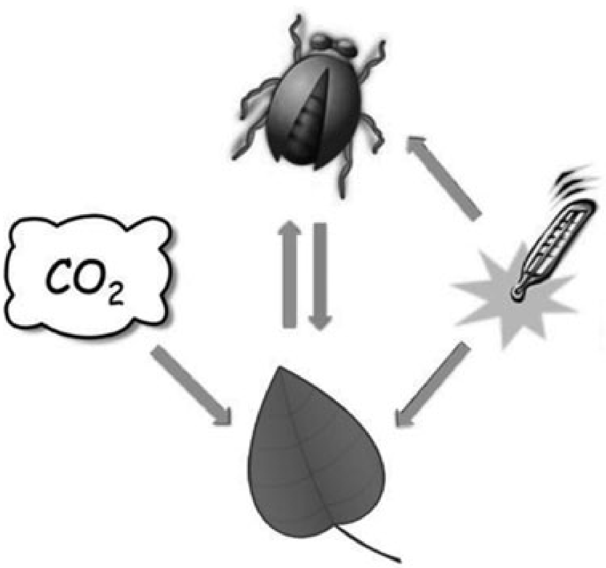

Fig. 1 A conceptual model illustrating the interactions between predicted increases in CO_2 and temperature on leaf consumption of soybean by Japanese beetles. At concentrations predicted for 2050, CO_2 does not directly affect Japanese beetles. However, elevated CO_2 alters plant chemistry that in turn increases palatability of foliage, Japanese beetle lifespan, and fecundity. Elevated temperature also can affect leaf attributes that influence nutritional suitability and, additionally, can directly affect insect metabolism.

production by altering susceptibility to insect herbivores (Zvereva & Kozlov, 2006; O'Neill *et al.*, 2008). Although the effect of elevated CO_2 on folivorous insects typically manifests itself indirectly by altering leaf chemical and physical properties affecting nutritional suitability, elevated temperature may also directly affect herbivory by altering insect metabolism (Fig. 1).

Changes in the concentration of atmospheric CO_2 induce changes in plant nutritional quality and defensive chemistry, and these factors play an essential role in insect feeding (Fig. 1). The increase in carbon-to-nitrogen ratio typically observed under elevated CO_2 decreases the nutritional quality of the foliage for insects (Ainsworth *et al.*, 2002, 2007), which may in turn result in compensatory feeding and increased levels of damage (Lincoln *et al.*, 1993; Coviella & Trumble, 1998; Bezemer & Jones, 1998; Whittaker, 1999; Stitt & Krapp, 1999). Lincoln *et al.* (1984) found increased feeding by soybean loopers on leaves grown under elevated CO_2, and the total amount of insect damage to soybean leaves was greater in large plots exposed to elevated CO_2 compared to ambient air, in otherwise normal environmental conditions (Hamilton *et al.*, 2005; Dermody *et al.*, 2008). Japanese beetles (*Popilla japonica* Newman) prefer soybean grown under elevated CO_2 (Hamilton *et al.*, 2005) and Japanese beetles feeding on foliage grown under elevated CO_2 live longer and produce more offspring than those feeding on ambient-grown foliage (O'Neill *et al.*, 2008). In addition to higher leaf sugar concentrations that stimulate feeding by Japanese beetles (Ladd, 1986), plants grown under elevated CO_2 are more poorly defended (Ainsworth *et al.*, 2002; O'Neill *et al.*, 2008). Cysteine protease inhibitor (CystPI) was downregulated in soybean plants exposed to elevated CO_2 (Casteel *et al.*, 2008; Zavala *et al.*, 2009). CystPI is a small protein that inhibits insect digestive proteases from breaking down the plant tissue and obtaining essential nutrients (Habib & Fazili, 2007; Chen, 2008). Other aspects of plant nutritional quality that are altered in soybeans and other plants include a decrease in water concentration, increases in leaf toughness, and increased allocation of photosynthates to phenolic compounds (Coviella & Trumble, 1998; Ainsworth *et al.*, 2002; Zvereva & Kozlov, 2006).

Temperature also alters the chemistry of foliage and consequently its nutritional quality (Fig. 1). The optimal temperature for soybean photosynthesis is 28–32°C, and the highest foliar sugar concentrations are found in this range (Vu *et al.*, 2001). Plant nitrogen also varies with temperature and typically reaches maximum levels at 24°C (Rufty *et al.*, 1981; Hungaria & Franco, 1993). It has been hypothesized that increased temperatures will increase photosynthesis and decrease allocations toward plant defenses (Herms & Mattson, 1992), but this hypothesis has received little empirical support and in some cases the opposite pattern has been observed (Dury *et al.*, 1998; Bidart-Bouzat & Imeh-Nathaniel, 2008). Leaf chemical defenses in soybean have not been examined under variable temperature, but the expression of defense genes in soybean seeds is known to vary with temperature (Upchurch & Ramirez, 2011).

Temperature not only plays an indirect role in altering herbivory by changing plant constituents, but it also exerts direct pressure on herbivory and insect development (Fig. 1) (Uvarov, 1931; Ayres & Scriber, 1994; Lombardero *et al.*, 2000). Leaf consumption typically increases with increasing temperature (Yang & Joern, 1994), and this response can be modified by changes in the nutritional quality of food (Kingsolver & Woods, 1998; Coggan *et al.*, 2011). Expected changes in temperature will affect species differently, as favored temperature ranges are species-specific (Bale *et al.*, 2002). Within a favored temperature range, increases in temperature accelerate the rate of development, potentially leading to more generations per year and an expansion in geographical range (Parmesan *et al.*, 1999; Bale *et al.*, 2002). For

Japanese beetles, an introduced species that feeds on over 300 plant species, the effect of temperature on development depends on life stage (Ludwig, 1928; Potter & Held, 2002). The optimum temperature for eggs to hatch is 30°C, whereas the optimum temperature for pupation is 30–32°C (Ludwig, 1928). The effect of temperature on larval development is variable (Ludwig, 1928). In the adult stage, flight activity and feeding occur from 29–35°C, typical of midday summer temperatures (Kreuger & Potter, 2001; Potter & Held, 2002).

The objective of this study was to examine how increases in CO_2 and temperature, two major elements of global climate change, will affect Japanese beetle herbivory on soybean and the plant defensive response to this herbivory. This research extends previous studies that have examined how CO_2 affects chemical defenses and nutritional suitability of this species (Fig. 1) (Hamilton *et al*., 2005; Casteel *et al*., 2008; O'Neill *et al*., 2008; Zavala *et al*., 2008, 2009; Dermody *et al*., 2008; Casteel, 2010), by examining how CO_2 may interact with temperature under field conditions. The United States is the world's leading soybean producer and exporter; changes in yield caused by herbivory can lead to substantial economic consequences (USDA, 2011; Allen & Boote, 2000).

Materials and methods

Physiological thermoregulation of stationary beetles

To determine the relationship between leaf and beetle temperature, images of Japanese beetles feeding on soybean foliage located in both the sun and shade were taken with a thermal camera (spectral range: 7.5 to 13 μm; Flir System ThermalCAM T400 (Flir Systems Inc., Wilsonville, OR, the United States). Measurements were made in the ambient plots in the soybean free-air concentration enrichment (SoyFACE) experiment (described below). A total of 38 independent images were obtained on two days in July 2007; images were collected during the peak feeding period of 1 000 to 1 500 CST. Average leaf temperature was calculated from four locations on the leaf surface near each beetle and integrated surface temperature of each beetle was calculated using proprietary software (Fig. 2; Flir System QuickReport). Data were fitted with a linear regression (Origin Pro 8.5, OriginLab, Northampton, MA, USA).

Effect of temperature on feeding activity

An experiment in controlled-environment chambers was conducted to determine the relationship between air temperature and the rate of leaf consumption by Japanese beetles (Fig. 1). Eight chambers (Environ GC Series Plant Growth Chambers; Environmental Growth Chambers, Chagrin Falls, OH, USA) were set to temperatures between 16°C and 40°C (day and night temperature were the same and the photoperiod was 17 h). Irradiance during the day and humidity in each chamber were controlled at 500 μmol/m^2/s (PPFD) and 60%, respectively. Japanese beetles were collected from the field using commercial pheromone traps (Bag-a-Bug, Spectrum Brand, Madison WI, the United States) and were starved for 24 h prior to the experiment. One beetle was placed in a Petri dish containing one leaf disk (4 cm diameter) on moistened filter paper. Leaf disks were collected from soybean grown under ambient temperature and CO_2 conditions. Each Petri dish (128 total) was randomly assigned to one of the eight chambers ($n = 16$ per chamber). After 24 h, leaf disks were photographed with a digital camera (Canon PowerShot G9, Canon Inc., Lake Success, NY, USA) and the leaf area consumed was calculated with pixel counting software (ImageJV1.40g by Wayne Rasband National Institutes of Health, USA). Statistical analysis and a linear fit were conducted using OriginPro 8.5.

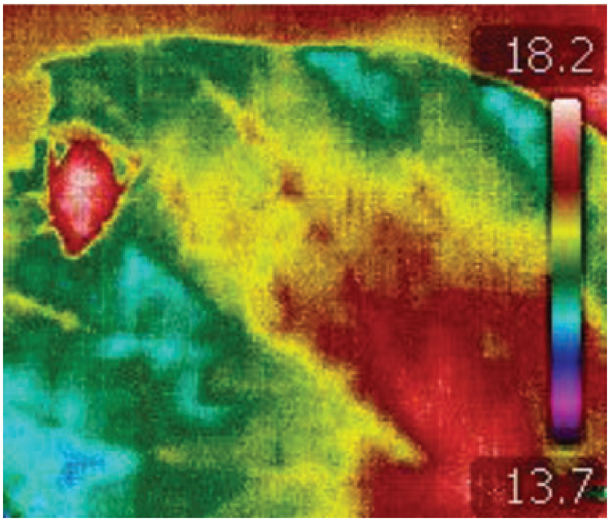

Fig. 2 A representative false-color image of surface temperature of a Japanese beetle feeding on a soybean leaf. Images were taken with a thermal camera (Flir System ThermalCAM T400, Boston, MA, the United States). The Japanese beetle (upper left) is located on a cut soybean leaf.

Effect of temperature on survivorship

To determine survivorship of Japanese beetles under different temperature regimes, Japanese beetles were collected as they emerged from a nearby turf field during the first and second week of July 2011. Beetle gender was determined according to Fleming (1972). Two beetles were placed in each of the 39 plastic containers (11.5 cm diameter; 18 cm height) filled with ~3 cm of fine-grain sifted sand. Containers were covered with ventilated lids and randomly assigned to three different controlled environment chambers ($n = 13$, per chamber) set to air temperatures of 26°C, 31°C, and 36°C. Irradiance, humidity and day length were the same as in the previous chamber experiment. Measurements made inside the plastic containers with data loggers (Onset HOBO, Onset Computer Corporation, Bourne, MA, USA) indicated that the beetles experienced air temperatures that were on average 1–2°C higher than the chamber air temperature. Recently, expanded soybean leaves from the ambient CO_2 and temperature plots at SoyFACE were harvested and the petioles were placed immediately in water-filled plastic vials prior to placement in the container as food for the mating pairs of beetles. New foliage was placed in the containers every three days. Beetle survivorship was recorded every three days and the data were analyzed using a Kaplan–Meier survivorship curve; to evaluate the null hypotheses that there was no difference among treatments, the survivorship curves were compared with a log rank test of equality (Origin Pro 8.5).

Effect of temperature and CO_2 on consumption

To examine the interactive effects of elevated CO_2 and temperature on the rate of leaf consumption by Japanese beetles, a factorial experiment was conducted at the SoyFACE research facility where soybeans were grown at two levels of CO_2 and temperature. Large plots (20 m diameter) of soybean (Pioneer 93B15) were grown under field conditions and exposed to elevated CO_2 and temperature at the SoyFACE research facility located south (40°02′ N, 88°14′ W) of the University of Illinois Urbana-Champaign, USA (SoyFACE, 2005). The FACE technology (Miglietta *et al*., 2001; Rogers *et al*., 2004; Prather *et al*., 2001) exposed vegetation to elevated CO_2 concentrations (550 μL/L) expected by 2050 (Prather *et al*., 2001) without physically enclosing vegetation. Pure CO_2 was injected above the plant canopy and upwind from tubing surrounding the experimental plots. Sensors within each plot monitored and controlled CO_2 concentration throughout the growing season. The experiment was a randomized block design with eight plots; in each block one plot was exposed to elevated CO_2 and one plot remained at the ambient conditions (~390 μL/L; $n = 4$). In 2009, an ambient air temperature and an elevated air temperature subplot (7 m^2) were added to each FACE plot, creating a split plot. In one subplot, surface temperature was maintained 3.5°C above the neighboring ambient-temperature plot with infrared heaters mounted on a frame above the plant canopy as in Kimball (2005) and Kimball *et al*. (2008). The experimental plots were in a 16 hectare field of continuous soybean that was planted and managed following standard agronomic practices for the midwestern US Plants were prereproductive for all experiments.

Eight undamaged plants were selected in each plot, where five Japanese beetles were enclosed in a mesh bag (1 mm × 4 mm mesh size) and placed on the first fully expanded trifoliate. Beetles were starved for 24 h prior to the experiment. Bagged foliage was photographed after 24 h of feeding and the amount of tissue removed was quantified with pixel counting software described previously. Immediately after the beetles were removed, the remaining leaf tissue was placed in liquid nitrogen and stored at −80°C for chemical analysis. This experiment was conducted in 2009 and repeated in 2011. Additional data collected in 2011 included the number of beetles that survived the 24 h time period and per capita feeding over that time period.

Plant metabolites

CystPI activity of soybean foliage was measured as in Zavala *et al*. (2008) to determine how elevated CO_2 and temperature act singly or in combination on plant defensive chemistry (Fig. 1). Leaf tissue (~200 mg per sample) was homogenized and suspended in 1.2 mL of buffer composed of 150 mmol/L NaCl, and 2 mmol/L ethylenediaminetetra-acetic acid (EDTA) adjusted to a pH of 7.2. Diluted papain (papaya proteinase) along with the sample were incubated for 30 min at 37°C, following which the chromogenic substrate p-Glu-Phe-Leu-pNA was prepared by suspension in 0.1 mol/L Sodium phosphate, 0.3 mol/L KCl, 0.1 mmol/L EDTA, and 3 mmol/L dithioerythreitol adjusted to a pH of 6.00, and added promptly. The samples were then run at 410 nm in a spectrophotometer for 15 min at 37°C. Total leaf protein was measured according to Bradford (1976) and the calculation of CystPI activity was normalized by differences in protein loading. Chemicals and reagents were obtained from Sigma (St. Louis, MO, USA).

Temperature data

An empirical model was constructed to determine how an increase in air temperature of 3.5°C predicted for 2050

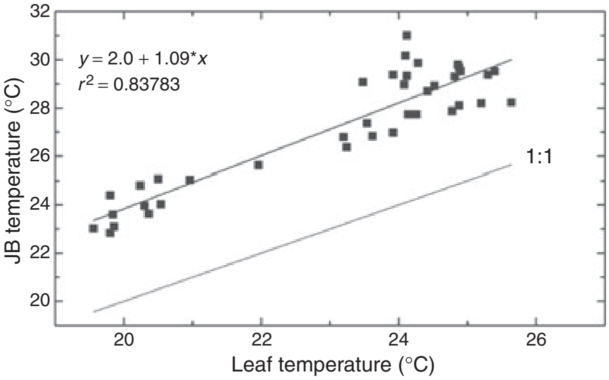

Fig. 3 Relationship between surface temperature of Japanese beetles and leaf surface temperature. Each point represents an independent measurement. Within the range of temperatures measured, beetle surface temperature was approximately 4°C above leaf temperature ($r^2 = 0.84$).

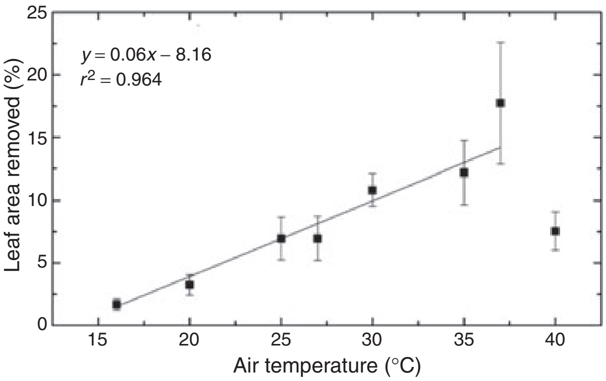

Fig. 4 The percentage of soybean leaf area removed by a single Japanese beetle after 24 h (±SE). The regression line is fitted to data from 16°C–37°C, and leaf area removed (%) as a linear function of temperatures under which Japanese beetles are most active ($r^2 = 0.97$). Each point is an average of eight independent measurements.

will affect the amount of soybean leaf tissue consumed by Japanese beetles. The model used the relationship between leaf consumption and air temperature (Fig. 2), and current and projected air temperatures to predict future feeding rates and duration. The temperature range, from now on known as the "optimal temperature range," used to determine when consumption would be highest in the field was based on observations by Fleming (1972), field data from Kreuger & Potter (2001) and a controlled laboratory experiment conducted during this research. Current air temperature during the time of peak damage by Japanese beetles (July–August) was obtained from a nearby weather station (Bondville, Illinois, USA). Data analysis and graphing were carried out in Origin 8.5.

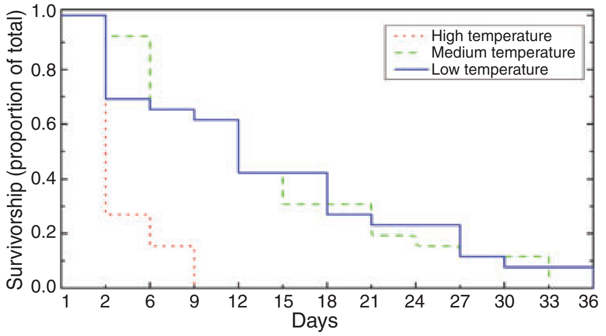

Fig. 5 Kaplan–Meier survivorship curves of adult Japanese beetles grown under three temperatures. Survivorship was significantly decreased at temperature of 38°C ± 0.14°C (dotted line; RH: 94.75% ± 0.92%), compared to 32.3°C ± 0.04°C (dashed line; RH: 89.6% ± 1.1%) or 27.43°C ± 0.45°C (solid line; RH: 89.8% ± 2.0%; $P < 0.001$). Test insects comprised equal number of males and females.

Results

To understand the thermal properties of Japanese beetles, thermal images taken in both sun and shade conditions were used to determine the relationship between leaf and body temperature. Thermal images (Fig. 2) have relatively low accuracy (± 2°C) but high precision, (± 0.5°C) so this method provides a robust comparison of beetle and leaf surface temperature within a single image (Fig. 2). Beetle temperature was highly correlated with leaf surface temperature in the range of 19.8–29.4°C (Fig. 3; beetle temperature = 2.0 + 1.09 × leaf temperature; $r^2 = 0.84$). Beetle temperature was approximately 3.5–4°C higher than leaf surface in this range.

To determine the effect of air temperature on consumption rate, Japanese beetles were allowed to feed on soybean at eight different temperatures in controlled environment chambers. The percentage of leaf tissue removed after 24 h increased linearly with increasing leaf temperature (tissue removed = −8.17 + 0.6 × air temperature; $r^2 = 0.96$) between 16°C and 37°C (Fig. 4), but declined precipitously above an air temperature of ~37°C.

Survivorship of Japanese beetles also declined sharply at high temperatures. At an air temperature of 36°C in the controlled environmental chamber, which corresponded to an air temperature of ~38°C in the immediate vicinity of beetles inside the plastic containers, longevity was approximately nine days but increased to 33 days or longer at temperatures below ~32°C in the containers (31°C chamber temperature; log rank $P = 2.09\text{e}^{-8}$, df = 2, Fig. 5).

In the field, no effect of feeding on foliage grown under elevated CO_2 and temperature, singly or in combination,

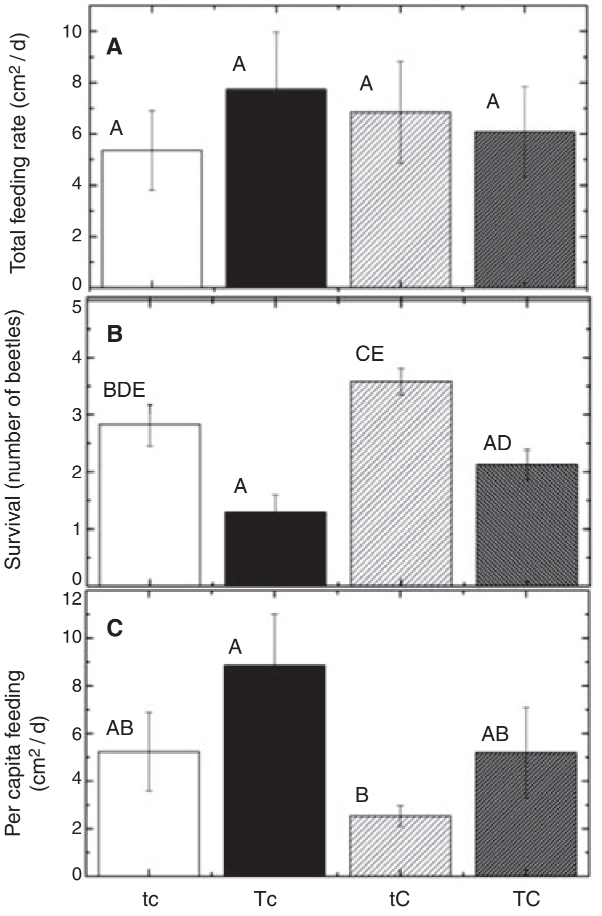

Fig. 6 (A) Total consumption rate (cm^2/d); (B) survivorship; and (C) per capita consumption (cm^2/d) in the field after five beetles were bagged on the first fully expanded trifoliate for 24 h. Plants were grown in the SoyFACE experiment at ambient temperature and ambient CO_2 concentration (~390 μL/L; tc), elevated temperature (plus 3.5°C) and ambient CO_2 concentration (Tc), ambient temperature and elevated CO_2 concentration (tC), or elevated temperature and CO_2 concentration (TC). Means with different letters are significantly different (Tukey LSM, $P < 0.05$)

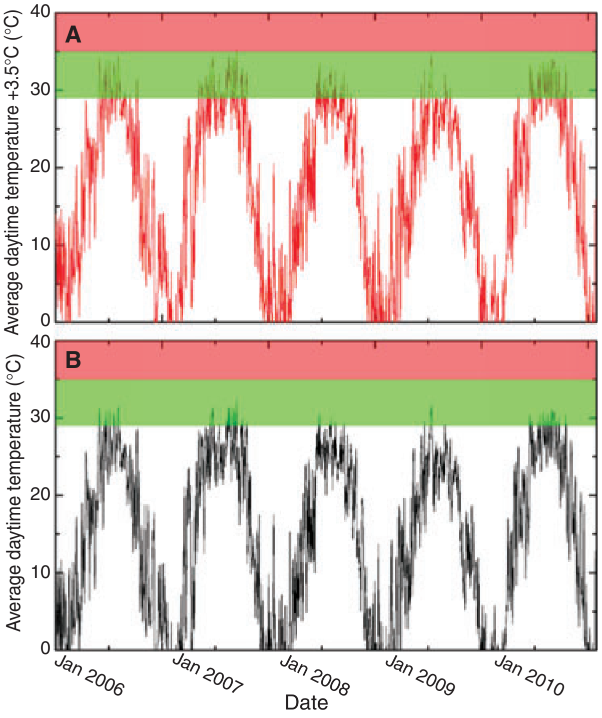

Fig. 7 (A) Observed daytime temperature (0800–1800 h) obtained from the Surfrad Solar Research site located in Bondville, Illinois for the years 2006–2010. (B) Observed daytime temperature increased by 3.5°C to represent temperatures expected by 2050. Duration of Japanese beetle activity increases with temperature up to the green band. The green band represents temperatures at which Japanese beetle activity is highest. Japanese beetle activity decreases within the red region.

was detected ($P > 0.05$, Fig. 6A). The average leaf tissue removal by five beetles caged on a single trifoliate for 24 h was 6.5 ± 1.9 cm^2. However, feeding on foliage exposed to elevated temperature decreased insect survivorship (average number of beetles surviving the 24 h period for ambient and elevated temperature was 3.21 ± 0.31 and 1.71 ± 0.28, respectively; $P < 0.01$, Fig. 6B), while feeding on foliage under elevated CO_2 increased survivorship (average number of beetles surviving the 24 h period for ambient and elevated CO_2 was 2.06 ± 0.34 and 2.86 ± 0.25, respectively; $P = 0.012$, Fig. 6B). There was a trend of higher per capita consumption under elevated temperature ($P = 0.067$, Fig. 6C) and lower consumption under elevated CO_2 ($P = 0.064$, Fig. 6C). Average daytime temperature in the control plots for the duration of the experiment was 33.6°C (range: 29.4–38.5°C), while in the heated plots the average daytime temperature was 37.1°C (range: 31.9–42.2°C; D. Drag unpublished data).

The average CystPI activity across treatments in 2009 and 2011 were 1.57 ± 0.51 nmol PI/mg protein and 1.41 ± 0.40 nmol PI/mg protein, respectively. Similarly, the average protein for 2009 and 2011 across treatments was 0.066 ± 0.014 mg protein/g frozen tissue and 0.072 ± 0.015 mg protein/g frozen tissue, respectively. No significant differences were detected across treatments for CystPI or protein concentration in either year.

A simple empirical model predicting the effect of elevated temperature on the consumption of soybean leaf tissue was constructed by combining measurements of the temperature dependence of leaf consumption (Fig. 4) and historical air temperature data. Based on temperature data collected from 2006 to 2010, 97.2% of this time was below the optimal temperature range (Fig. 4; Fleming, 1972, Kreuger & Potter, 2001), and 2.8% of the days fall within the optimal temperature range (Fig. 7A). A 3.5°C increase in temperature is expected by 2050; this change in temperature increases from 2.8% to 8.2% the yearly time Japanese beetles will spend during the temperature range conducive to high consumption. Under these circumstances only 0.35% of the time would fall above the ideal temperature range (Fig. 7B). Ideal temperatures occurred between June and late August, which corresponds to the Japanese beetle season at present in central Illinois. These results suggest that the 3.5°C increase in air temperature predicted for 2050 will increase the duration of feeding by Japanese beetles nearly threefold.

Discussion

Elevated atmospheric CO_2 and the corresponding increase in temperature resulting from anthropogenic activity can alter herbivory rates in native and agricultural communities. Exposure of soybean to CO_2 levels, expected by 2050, greatly increases damage to foliage by chewing insects, particularly the invasive Japanese beetle (Coviella & Trumble, 1998; Hamilton *et al*., 2005; Dermody *et al*., 2008). The survivorship of beetles is increased when they consume foliage grown under elevated CO_2 (Fig. 5) (O'Neill *et al*., 2008). However, when elevated temperature and CO_2 are applied simultaneously, the effect of elevated temperature on leaf damage appeared stronger than that of elevated CO_2, possibly because of its direct effect on insect metabolism (Fig. 1); this response may mean soybean will experience greater damage to soybean foliage as the climate warms. The results of this study show that the expected increase in temperature will ultimately result in increased time spent feeding by Japanese beetles, along with an increase in the rate of herbivory, and no decrease in overall Japanese beetle survivorship, absent any other changes in beetle behavior and physiology.

Up to a critical threshold of approximately 37°C, increasing air temperature stimulated leaf consumption by Japanese beetles; but at higher temperatures, consumption rates and survivorship dropped precipitously (Figs. 4 and 5). Consumption rate increased 1% with every degree between 16°C and 37°C. Physiological thermoregulation in most insects is imperfect and increasing temperature stimulates metabolism, necessitating higher rates of food consumption (Calderwood, 1961; Schults *et al*., 1991; Yang & Joern, 1994; Clarke & Fraser, 2004; Dingha *et al*., 2009). The temperature at which maximum leaf consumption occurred in this study (37°C) is slightly higher than the daytime temperatures in the field when flight activity and consumption rates are greatest (e.g., 29°C–35°C; Kreuger & Potter, 2001). Results from this study and those of Kreuger and Potter (2001) suggest that foliar damage by Japanese beetles will be greatest in the range of 35°C–37°C, declining at higher temperatures.

The decrease in leaf consumption at >37°C observed in this study was caused by increased mortality. In this laboratory experiment, where beetles were not allowed to seek shade and thus behaviorally thermoregulate, beetles survived for only 9 days at ~38°C, compared to >33 days below ~32°C (Fig. 5). The adult lifespan of a Japanese beetle in the field is from 30–45 days (Potter & Held, 2002). Similarly, field experiments show, at air temperatures exceeding 37°C, survivorship over a 24 h period was significantly lower compared to survivorship at an average temperature of 33.6°C (Fig. 6B). While the proximate cause of beetle mortality was not examined in this study, dehydration may have played a role. At high temperatures, the evapotranspiration rate may exceed water intake by Japanese beetles, which occurs through foliage consumption (Fleming, 1976; May, 1979).

Because of their capacity to behaviorally thermoregulate, increased mortality of Japanese beetles at temperatures above 37°C under field conditions will not be as dramatic as in the experiments reported here where beetles were confined to individual leaves. Above an air temperature of 35°C, Japanese beetles seek shade or burrow into the ground (Hawley & Metzger, 1940; Kreuger & Potter, 2001), thereby minimizing temperature-induced mortality. Whether beetles die at >37°C or burrow, the consequences of high temperature for immediate rates of herbivory would be the same – beetles are not feeding and a reduction of chewing damage to foliage would be expected at these high temperatures.

Because of their capacity to physiologically thermoregulate, it is difficult to assign a precise air temperature at which leaf consumption by Japanese beetles would decline. The surface temperature of Japanese beetles was approximately 4°C higher than the surrounding leaf temperature (Fig. 3). The difference between beetle surface temperature and air temperature should, however, be somewhat less than 4°C as the surface temperature of actively transpiring soybean leaves is typically somewhat lower than air temperature (Reicosky *et al*., 1985). Kreuger and Potter (2001) observed that the thoracic temperature of Japanese beetles was 0.1–2.3°C higher than air temperature under shade conditions and 3.1–4.1°C higher while located in the sun. This elevation in beetle temperature is accomplished primarily by basking and metabolic thermoregulation (May, 1979).

In addition to directly affecting consumption rates by modulating insect metabolism, variation in air temperature may modulate herbivory indirectly, operating like elevated CO_2 by changing aspects of leaf chemistry that affect leaf suitability (Fig. 1). Previously, Casteel *et al.* (2008) demonstrated that genes coding for jasmonic acid, an important signaling hormone (Howe, 2001), and CystPI were suppressed in soybean plants grown under elevated CO_2, and these reductions in transcript abundance may have contributed to observed reductions in CystPI activity (Zavala *et al.*, 2008, 2009). In contrast with these results, no differences in CystPI activity with elevated CO_2 or temperature were detected in this study. Sampling times relative to the induction response of CystPI may explain this apparent inconsistency. mRNA coding for CystPI expression is induced within 12 h of chewing damage to leaves, but the accumulation of the PI can take from 3 to 5 days to reach maximum levels (Zavala & Baldwin, 2004; Horn *et al.*, 2005). It is possible that foliage collected 0–3 days after herbivory had not yet achieved detectable differences in CystPI activity.

While no changes in CystPI were detected in this study, other aspects of leaf chemistry may have contributed to observed patterns of herbivory under elevated CO_2 or temperature. In particular, changes in the antioxidant composition of foliage may have enhanced longevity of Japanese beetle under elevated CO_2. Flavonoids and isoflavonoids contribute to plant defense against oxidative stress (Dakora & Phillips, 1996) and these compounds can be induced by wounding (Hagerman & Butler, 1991). However, individual flavonoids with prooxidant properties can be detrimental to insect growth (Ahmad & Pardini, 1990), so the relative proportions of different flavonoids and isoflavonoids in the diet are critical in determining whether their integrated effect is positive or negative (Johnson & Felton, 2001). O'Neill *et al.* (2010) documented the presence of the isoflavones genistein and daidzein and the flavonols quercetin and kaempferol in soybean foliage, and the concentrations of quercetin glycosides were considerably higher in plants grown under elevated CO_2. The increase in quercetin glycosides may have enhanced the nutritional quality of leaves promoting longevity under elevated CO_2 (Fig. 6). Antioxidant capacity in soybean is modulated by exposure to ozone (Gillespie *et al.*, 2011), but how it is affected by the interaction with other environmental stresses is not well understood.

Consistent with previous results (O'Neill *et al.*, 2008), consumption of soybean leaves grown under elevated CO_2 significantly increased survival of Japanese beetles (Fig. 6). However, no difference in total consumption of foliage exposed to elevated or ambient CO_2 was detected. When given a choice, Japanese beetles prefer to feed on foliage exposed to elevated CO_2 (Hamilton *et al.*, 2005) and their abundance is higher in elevated CO_2 plots at SoyFACE (Hamilton *et al.*, 2005; Dermody *et al.*, 2008). Daytime air temperatures were high during this experiment (29.4–38.5°C) and in the range where temperature inhibits feeding by Japanese beetles. These high temperatures may have compromised the ability to detect an effect of CO_2 on leaf consumption rates.

Global climate change is expected to affect plant-insect interactions, with potentially large economic costs associated with reduced crop yields (Scherm *et al.*, 2000; Hamilton *et al.*, 2005). In this study, elevated temperature appeared to affect consumption rates by Japanese beetles more strongly than elevated CO_2, possibly because temperature acts both directly on insect metabolism and indirectly through changes in leaf suitability. Results from this study suggest that the direct effect of the expected 3.5°C increase in temperature in the central United States by 2050 will increase the amount of time that the temperature is ideal for Japanese beetles consumption by 290%. Japanese beetles feed on a large number of different plant species (Fleming, 1972; Potter & Held, 2002), and an increase of feeding time of this magnitude, in addition to an increase in rate of consumption, may result in substantial damage to valuable ornamental plants as well as affecting soybean yields. As little as 20% leaf damage directly before seed fill will decrease yield by 15%, resulting in considerable economic loss (Allen & Boote, 2000; USDA, 2011). Temperature stress is, however, a strong selective pressure and how expected increases in temperature will affect the evolution and adaptation of insect populations in the future remains largely unknown (Bale *et al.*, 2002).

Acknowledgments

The authors thank Jorge Zavala and Clare Casteel for guidance with this research. We also thank Carl Bernacchi for overseeing operation of the TFACE experiment; Andy VanLoocke for providing air temperature data at SoyFACE; Randy Nelson and Ed Johnson for providing soybean seeds for the growth chamber experiments; and Lauren Segal, Allen Lawrance, Alan Yanahan, and Mike Donovan for assistance with data collection in the field. The authors gratefully acknowledge Bert Berla for providing thermal images of Japanese beetles. The SoyFACE experiment is supported by the Illinois Council for Food and Agricultural Research (C-FAR), Archer Daniels Midland Co., and USDA-ARS. This research was supported in part by grants from the US Department of Energy (No. DE-FG02–04ER63489) and by USDA grant 2002–02723.

Disclosure

The authors have not potential conflicts of interests to disclose.

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Accepted February 20, 2012