

IMPACT OF ELEVATED CARBON DIOXIDE AND INCREASED TEMPERATURE ON
JAPANESE BEETLE HERBIVORY

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Plant Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

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Abstract

To examine how the major elements of global change affect herbivory in agroecosystems, a multi-factorial experiment was conducted in which soybeans were grown at two levels of carbon dioxide and temperature, including those predicted for 2050, under otherwise normal field conditions. Japanese beetles (*Popilla japonica* Newman) were enclosed on foliage for 24 hours, after which the beetle survivorship, total and per capita leaf consumption, and leaf protease inhibitor activity was measured. The direct effect of temperature on beetle consumption and survivorship was also 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 CO₂, but there were no interactive effects of CO₂ 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 a 3.5° C increase in temperature will increase the optimal feeding window for the Japanese beetle by 290%. Elevated temperature and CO₂ 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, herbivory, FACE (free-air CO₂ enrichment), Glycine max, *Popillia japonica*, temperature dependence, Japanese Beetles

Acknowledgments

Foremost, it is impossible for me to overstate the gratitude that goes to my advisor Professor Evan Delucia for providing me with this rare opportunity. His patience, oversight, and advising style has allowed me to develop into a confident independent biologist.

I would also like to thank May Berenbaum, Jorge Zavala and Sardoy Pedro Miguel for valuable input on my experiment. Many thanks go to Carl Bernacchi for overseeing operation of the TFACE experiment, Andy Vonlocke for providing air temperature data at SoyFACE, Randy Nelson and Ed Johnson for providing soybean seeds for the growth chamber experiments, Bert Berla for providing thermal images of Japanese beetles and Lauren Segal, Allen Lawrence, Allen Yanahan and Mike Donovan for assistance with data collection in the field.

Additional thanks go to John Cheeseman, Debbie Black and Clare Casteel for helping me with the transition from undergraduate to graduate student, introducing me to research and showing me all the possibilities that come with being a biologist.

I am indebted to the many people who have morally and intellectually supported me through this time. This includes my friends Cesar Chialvo, Mike Zasadzien, and Scott Scharfenberg, in addition to the Delucia Lab group, Saber Mirismailli, Paul Nabity, Chris Black, Krista Anderson, Ben Duval, and Mike Masters.

Lastly I would like to thank my parents Richard and Marta Niziolek for their unconditional love and encouragement that gives me strength and focus. Their open-mindedness and style of upbringing has made it possible for me to succeed, and for that I am forever grateful.

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 U.S. Department of Energy (No. DE-FG02-04ER63489) and by

USDA grant 2002-02723.

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Introduction

Prior to the Industrial Revolution, the atmospheric carbon dioxide concentration was stable at $\sim 270 \mu\text{l l}^{-1}$ for over 1000 years (IPCC 2001). With the increase of anthropogenic CO_2 emissions, the global concentration of CO_2 has increased to $390 \mu\text{l l}^{-1}$ today, and is expected to nearly double from preindustrial levels by the end of the century (IPCC 2001, NOAA 2011). As a result of increasing CO_2 concentrations, the mean global temperature is expected to increase between $2\text{-}4^\circ \text{C}$, though 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 $\sim 3^\circ \text{C}$ (Hayhoe et al. 2010, Lin et al. 2010). In addition to directly affecting yields, elevated CO_2 and temperature may affect crop production by altering susceptibility to insect herbivores (Zvereva & Kozlov 2006, O'Neill et al. 2008). While the effect of elevated CO_2 on folivorous insects typically manifests itself indirectly by altering leaf chemical and physical properties affecting palatability, 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, Ainsworth et al. 2007). This in turn may result in compensatory feeding and increased levels of feeding (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 foliage was greater in large plots exposed to elevated CO₂ compared to ambient air, in otherwise normal environmental conditions (Hamilton et al. 2005, Dermody et al. 2008). Hamilton et al. (2005) discovered that Japanese beetles (*Popilla japonica* Newman) prefer soybean grown under elevated CO₂ and observed that Japanese beetles feeding on foliage grown under elevated CO₂ 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 which stimulate feeding by Japanese beetles (Ladd 1986), plants grown under elevated CO₂ are more poorly defended (Ainsworth et al. 2002, O'Neill et al. 2008). Cysteine proteinase inhibitor (CystPI) was down-regulated in soybean plants exposed to elevated CO₂ (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. It is known that the expression of defense genes in soybean seeds varies with temperature and pathogen infection (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 linearly with increasing temperature (Yang & Joern 1994); 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, while the optimum temperature for pupa 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 CO₂ and temperature, which are major factors in 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 CO₂ affects chemical defenses and palatability of this species (Fig. 1; Hamilton et al. 2005, O'Neill et al. 2008, Zavala et al. 2008, Casteel et al. 2008, Dermody et al. 2008, Zavala et al. 2009, Casteel 2010), by examining how CO₂ 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, USA). Measurements were made in the ambient CO_2 /temperature 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; images were collected during the peak feeding period of 1000 to 1500 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 to 40°C (day and night temperature were the same and the photoperiod was 17 hours). Irradiance during the day and humidity in each chamber were controlled at 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (PAR) and 60%, respectively. Japanese beetles were collected from the field using commercial pheromone/lure traps (Bag-a-Bug®, Spectrum Brand,

Madison WI, USA) and were starved for 24 hours 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 CO₂ conditions. Each Petri dish (128 total) was randomly assigned to one of the eight chambers (n = 16 per chamber). After 24 hours, 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 (ImageJ V1.40g by Wayne Rasband National Institutes of Health, USA). Statistical analysis and a linear fit were conducted using OriginPro 8.5.

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 dataloggers (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 CO₂ 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 feed 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 and a log rank test of equality (Origin Pro 8.5).

Effect of Temperature and CO₂ on Consumption

To examine the interactive effects of elevated CO₂ 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 CO₂ and temperature. Large plots (20-m diameter) of soybean (Pioneer 93B15) were grown under field conditions and exposed to elevated CO₂ 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 CO₂ concentrations (550 μl^{-1}) expected by 2050 (Prather et al. 2001) without physically enclosing vegetation. Pure CO₂ was injected above the plant canopy and upwind from tubing surrounding the experimental plots. Sensors within each plot monitored and controlled CO₂ concentration throughout the growing season. The experimental design was a randomized block with eight plots; in each block one plot was exposed to elevated CO₂ and one plot remained at the ambient conditions ($\sim 390 \mu\text{l l}^{-1}$; $n = 4$). In 2009 an ambient air temperature and an elevated air temperature subplot (7m²) 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 Infra red 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-ha field of continuous soybean that was planted and managed following standard agronomic practices for the midwestern US. Plants were pre-reproductive for all experiments.

Eight undamaged plants were selected in each plot, where five Japanese beetles were enclosed in a mesh bag (1x4 mm mesh size) and placed on the first fully expanded trifoliolate. Beetles were starved for 24 hours prior to the experiment. Bagged foliage was photographed after 24 hours 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-hour time period and per capita feeding.

Plant Metabolites

Cysteine proteinase inhibitor (CystPI) activity of soybean foliage was measured as in Zavala et al. (2008) to determine how elevated CO₂ 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 mM NaCl, and 2 mM EDTA adjusted to a pH of 7.2. Diluted papain along with the sample were incubated for 30 minutes at 37°C, following which the chromomeric substrate p-Glu-Phe-Leu-pNA was prepared by suspension in 0.1M Sodium phosphate, 0.3M KCl, 0.1Mm EDTA, and

3mM dithioerythritol adjusted to a pH of 6.00, and added promptly. The samples were then run at 410 nm in a spectrophotometer for 15 minutes 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.

Temperature Data

An empirical model was constructed to determine how an increase in air temperature of 3.5° C predicted for 2050 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 (Fig 4). Current air temperature during the time of peak damage by Japanese beetles (July – August) was obtained from a nearby weather station (Bondville, Illinois). Data analysis and graphing were carried out in Origin 8.5.

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 ($\pm 2^\circ\text{C}$) but high precision, ($\pm 0.5^\circ\text{C}$) 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°C to 29.4°C (Fig. 3; beetle temperature = $2.0 + 1.09 * \text{leaf temperature}$; $r^2=0.84$). Beetle temperature was approximately $3.5\text{-}4^\circ\text{C}$ higher than leaf surface in the aforementioned 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 hours increased linearly with increasing leaf temperature (tissue removed = $-8.17 + 0.6 * \text{air temperature}$; $r^2 = 0.96$) between 16°C and 37°C (Fig. 4), but declined precipitously above an air temperature of $\sim 37^\circ\text{C}$.

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 $\sim 38^\circ\text{C}$ 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 $\sim 32^\circ\text{C}$ in the containers (31°C chamber temperature; log rank $p = 2.09e^{-8}$, $df = 2$, Fig 5).

In the field, no effect of feeding on foliage grown under elevated CO_2 and temperature, singly or in combination, was detected ($p > 0.05$, Fig 6A). The average leaf

tissue removal by five beetles caged on a single trifoliolate for 24 hours was $6.5 \pm 1.9 \text{ cm}^2$. However, feeding on foliage exposed to elevated temperature decreased insect survivorship (average number of beetles surviving the 24 hour 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-hour 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: $29.4\text{-}38.5^\circ \text{C}$), while in the heated plots the average daytime temperature was 37.1°C (range: $31.9\text{-}42.2^\circ \text{C}$; 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 2006-2010, 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. 9A). 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 9B). 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 CO₂ and the corresponding increase in temperature resulting from anthropogenic activity can alter herbivory rates in native and agricultural communities. Exposure of soybean to CO₂ 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 CO₂ (Fig. 5; O'Neill et al. 2008). However, when elevated temperature and CO₂ are applied simultaneously, the effect of elevated temperature on leaf damage appeared stronger than that of elevated CO₂, 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, 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 metabolisms, 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. 29-35° 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 35-37° 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-hour 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 CO₂ by changing aspects of leaf chemistry that affect leaf palatability (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 CO₂, and these reductions in transcript abundance may have contributed to observed reductions in CystPI activity (Zavala et al. 2008, Zavala et al. 2009). In contrast with these results no differences in CystPI activity with elevated CO₂ 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 hours of chewing damage to leaves, but the accumulation of the PI can take from 3-5 days to reach maximum levels (Zavala et al. 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.

Consistent with previous results (O'Neill et al. 2008), consumption of soybean leaves grown under elevated CO₂ significantly increased survival of Japanese beetles (Fig. 6). However, no difference in total consumption of foliage exposed to elevated or ambient CO₂ was detected. When given a choice, Japanese beetles prefer to feed on foliage exposed to elevated CO₂ (Hamilton et al. 2005) and their abundance is higher in elevated CO₂ 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 CO₂ on leaf consumption rates.

Global climate change is expected to affect plant-insect interactions, with potentially large economic costs associated with reduced crop yields (Scherer et al. 2000, Hamilton 2005). In this study, elevated temperature appeared to affect consumption rates by Japanese beetles more strongly than elevated CO₂, possibly because temperature acts both directly on insect metabolism and indirectly through changes in leaf palatability. 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 losses to the Illinois economy (Allen & Boote 2000, Bushermohle et al. 2000, USDA 2011,). Temperature stress is, however, a strong selective pressure and how expected increases in temperature will affect the fitness of insect populations in the future remains largely unknown (Bale 2002).

Figures

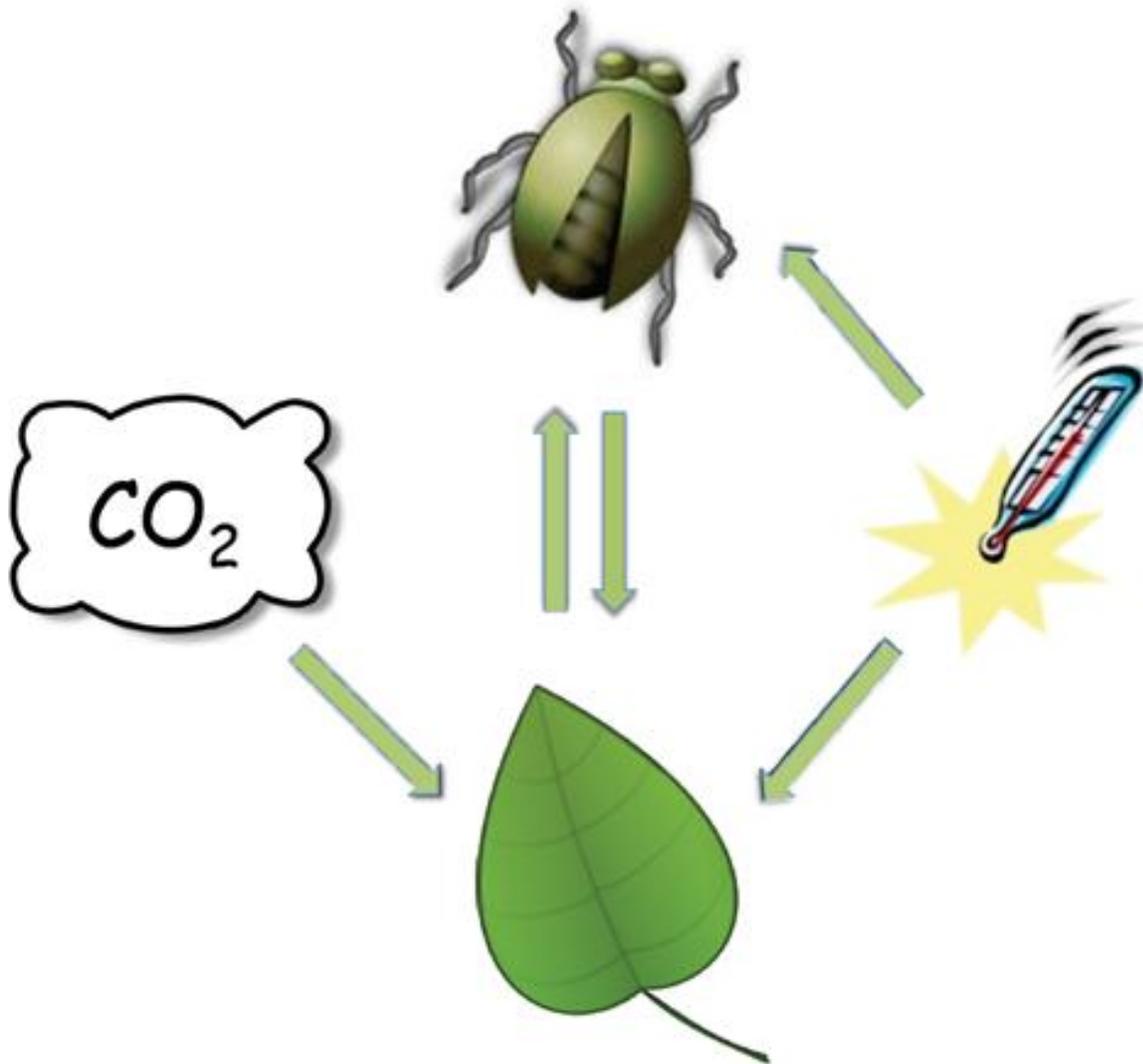


Figure 1: A conceptual model illustrating the interactions between predicted increases in CO₂ and temperature on leaf consumption of soybean by Japanese beetles. At concentrations predicted for 2050, CO₂ does not directly affect Japanese beetles. However, elevated CO₂ alters plant chemistry that in turn increases palatability of foliage, Japanese beetle lifespan, and fecundity. Elevated temperature also can affect leaf attributes that affect nutritional suitability and additionally, directly affects insect metabolism.

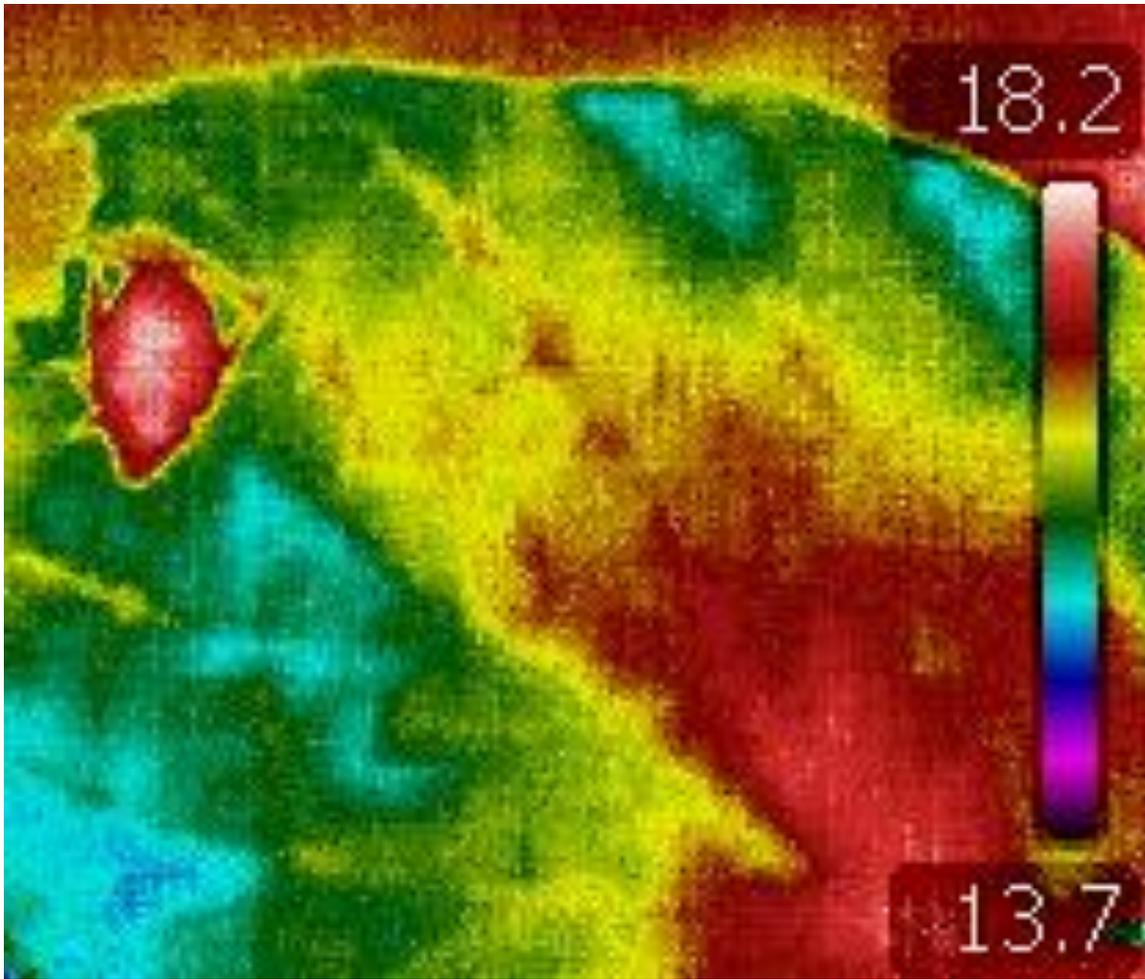


Figure 2: A representative false-color image of surface temperature of a Japanese beetle feeding on a soybean leaf. Images were taken with a thermal camera (Flir System ThermalCAM T400, Boston, MA). The Japanese beetle (upper left) is located on a cut soybean leaf.

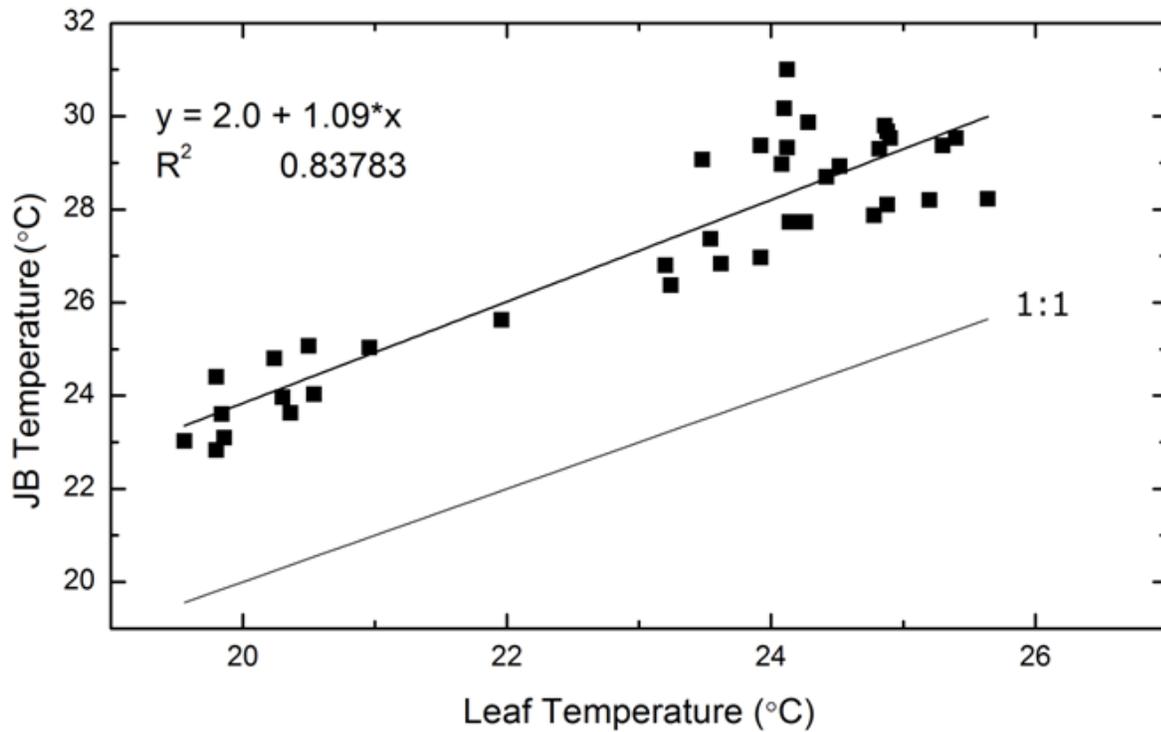


Figure 3: Relationship between surface temperature of Japanese beetles and leaf surface temperature. Each point represent an independent measurement. Within the range of temperatures measured, beetle surface temperature was approximately 4 C ° above leaf temperature ($r^2 = 0.84$).

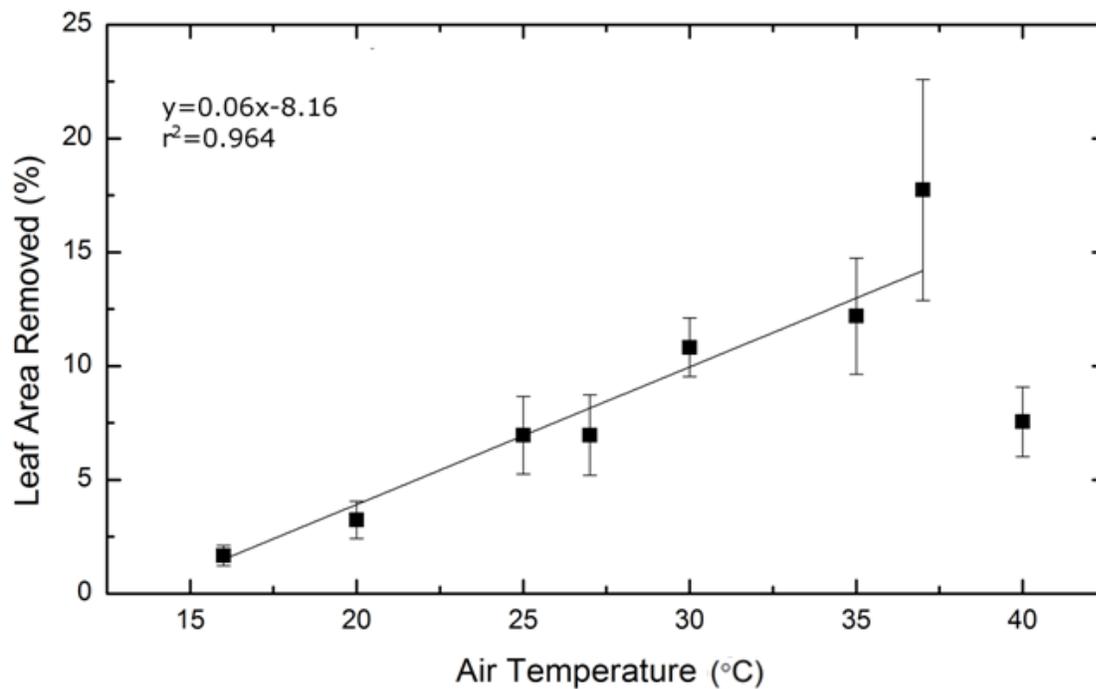


Figure 4: The percentage of soybean leaf area removed by a single Japanese beetle after 24 hours (\pm SE). The regression line is fitted to data from 16-37° C, and leaf area removed (%) as a linear function of temperatures under which Japanese beetles are most active ($r^2 = 0.97$). Each point is an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

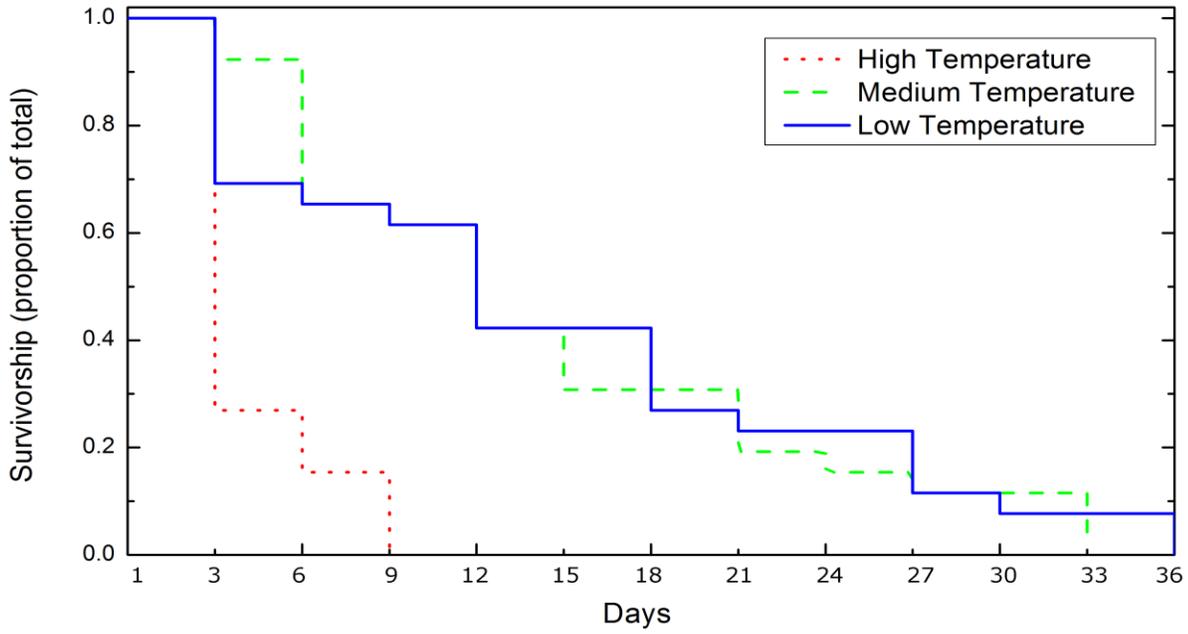


Figure 5: Kaplan-Meier survivorship curves of adult Japanese beetles grown under three temperatures. Survivorship was significantly decreased at temperature of $38 \pm 0.14^{\circ} \text{C}$ (dotted line ; RH: $94.75 \pm 0.92\%$), compared to $32.3 \pm 0.04^{\circ} \text{C}$ (dashed line; RH: $89.6 \pm 1.1\%$) or $27.43 \pm 0.45^{\circ} \text{C}$ (solid line; RH: $89.8 \pm 2.0\%$; $p < 0.001$). Test insects comprised equal number of male and females.

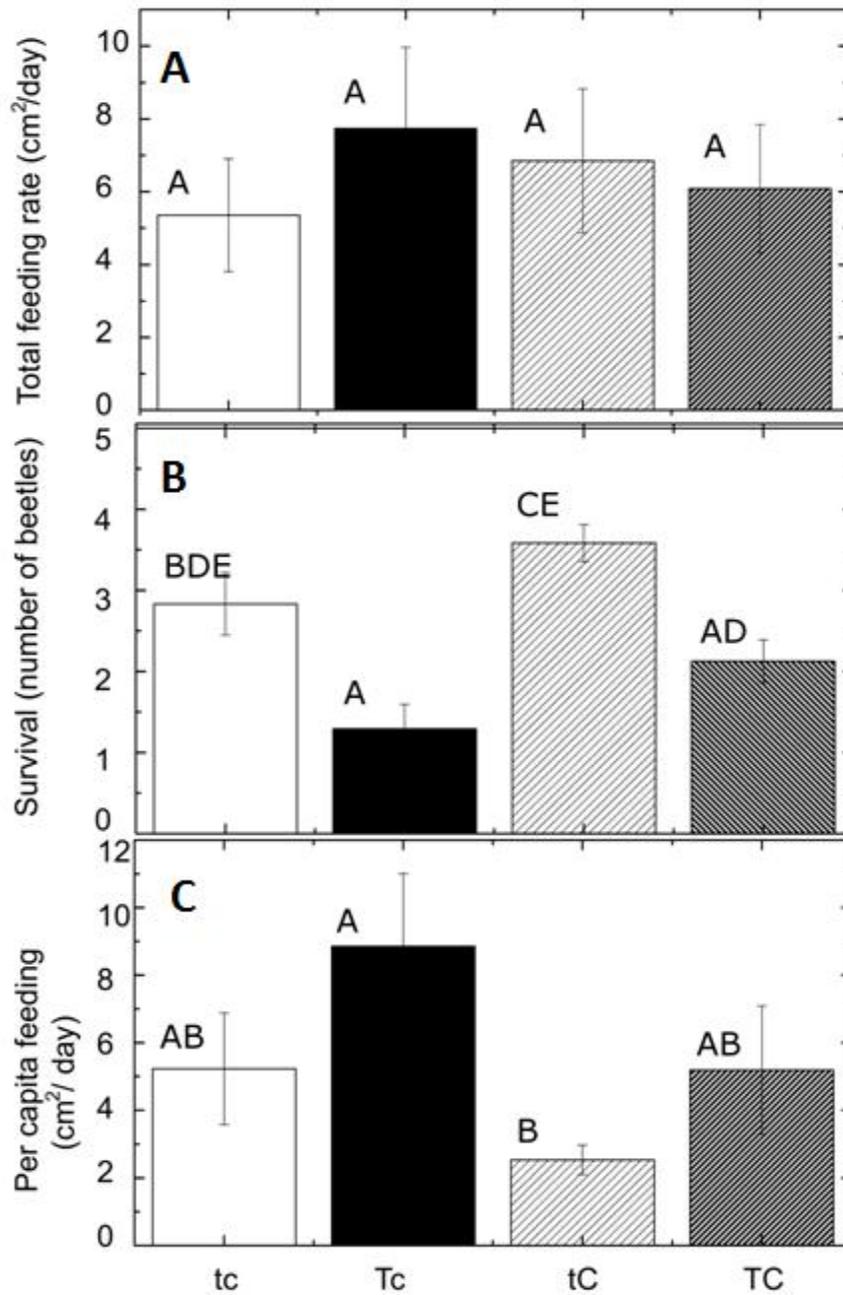


Figure 6: (A) Total consumption rate (cm²/day); (B) survivorship; and (C) per capita consumption (cm²/day) in the field after 5 beetles were bagged on the first fully expanded trifoliolate for 24 hours. Means with different letters are significantly different (Tukey LSM, $p < 0.05$)

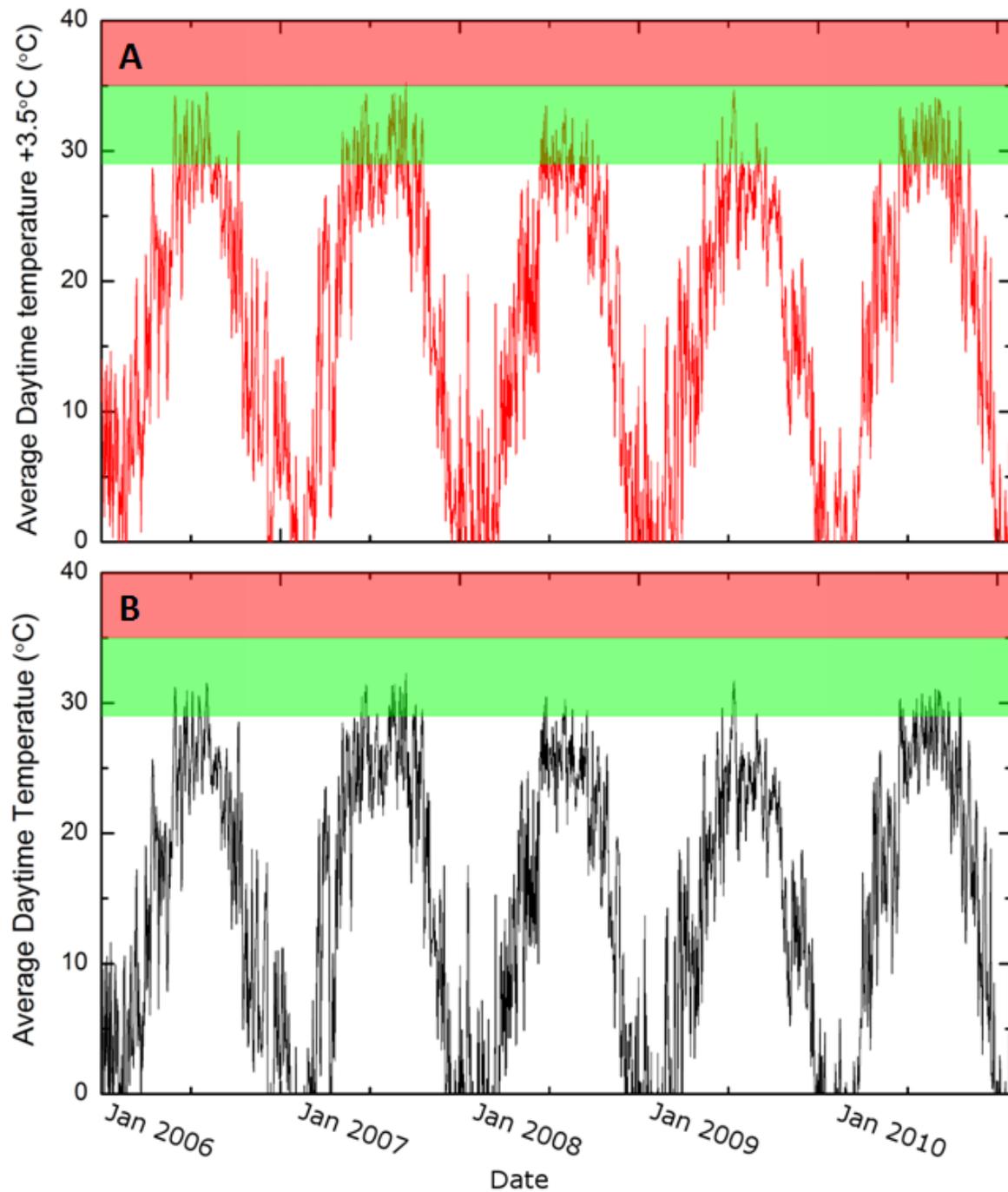


Figure 7: (A) Observed daytime temperature (0800-1800hr) obtained from the Surfrad Solar Research site located in Bondville, Illinois for the years 2006 through 2010. (B) Observed daytime temperature increased by 3.5°C to represent temperatures expected by

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.

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