

Longevity and Fecundity of Japanese Beetle (*Popillia japonica*) on Foliage Grown Under Elevated Carbon Dioxide

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ABSTRACT Atmospheric levels of carbon dioxide (CO₂) have been increasing steadily over the last century. Plants grown under elevated CO₂ experience physiological changes that influence their suitability as food. Previous studies have found increased insect herbivory on plants grown under elevated CO₂. To determine effects of consuming foliage of soybean (*Glycine max*) grown under elevated CO₂ on adult survivorship and fecundity, Japanese beetles (*Popillia japonica* Newman) were fed for the duration of their adult lives leaves grown under elevated CO₂ (550 μmol/mol), under ambient atmosphere (370 μmol/mol), or grown under ambient atmosphere but supplemented with a solution of sugars. To determine effects of a diet of foliage grown under elevated ozone (O₃), another anthropogenic gaseous pollutant, beetles in the laboratory were fed soybean leaves grown under elevated CO₂, elevated O₃, or a combination of both elevated gases. Leaf tissue was also analyzed for longevity-enhancing antioxidants, because increases in dietary antioxidants can increase lifespan. Lifespan of Japanese beetles was prolonged by 8–25% when fed foliage developed under elevated CO₂, but consuming foliage that had taken up sugars to approximately the same level as foliage grown under elevated CO₂ had no effect on fecundity or longevity. Females consuming elevated CO₂ foliage laid approximately twice as many eggs as females fed foliage grown under ambient conditions. Consuming foliage grown under elevated O₃ had no effect on fecundity. No significant differences in total antioxidant content of foliage from ambient and elevated CO₂ conditions were detected. Although the precise mechanism is unclear, by altering components of leaf chemistry other than sugar content, elevated CO₂ may increase populations of Japanese beetles and their impact on crop productivity.

KEY WORDS elevated carbon dioxide, *Popillia japonica*, *Glycine max*, longevity, fecundity

Atmospheric levels of carbon dioxide (CO₂) have risen from 280 to 370 μmol/mol since the start of the Industrial Revolution (IPCC 2001). Current CO₂ levels are expected to double within the next 100 yr with increased burning of fossil fuels and clearing of forests that act as carbon sinks (Ehhalt et al. 2001). Elevated CO₂ stimulates photosynthesis and productivity in many plant species (Curtis and Wang 1998). Such changes would seem to increase future crop production, but effects on other species in these communities, such as insect herbivores, have not been extensively studied in a wide range of agroecosystems. Atmospheric levels of O₃, a gaseous pollutant, have been increasing as well (Ehhalt et al. 2001). Whereas O₃ decreases photosynthesis and plant productivity (Long and Naidu 2002), its plant-mediated effects on herbivorous insects have been less well studied than the effects of feeding on plants grown under elevated CO₂.

Early studies of the effects on insect herbivores of consuming plants grown under conditions of elevated CO₂ were conducted primarily on lepidopteran larvae. Lepidoptera are commonly used in field herbivory studies, because many species are ubiquitous and easy to obtain and rear through all life stages on a single host plant. Moreover, several species of Lepidoptera are serious pests of crops and forests, and there is much interest in how species in this order will perform on plants grown under future atmospheric conditions. Fajer et al. (1989) conducted a study with buckeye butterflies (*Junonia coenia*) on foliage of plantain (*Plantago lanceolata*) grown in chambers containing elevated CO₂ (700 μmol/mol). Caterpillars grew more slowly when fed foliage grown under elevated CO₂ and experienced higher larval mortality than caterpillars fed control foliage. Similar results were obtained in a study on cotton bollworm (*Helicoverpa armigera*) fed cotton bolls from plants grown under elevated CO₂ (750 μmol/mol, Chen et al. 2007). An increase in larval mortality also was observed when white marked tussock moth larvae (*Orgyia leucostigma*) were fed quaking aspen (*Populus tremuloides*) foliage grown under elevated CO₂ (696 μmol/mol, Agrell et al. 2000).

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In contrast to Lepidoptera, many coleopteran herbivores are foliage feeders only as adults. The impact of CO₂ on long-lived adults rarely has been assessed. A long-lived coleopteran of economic importance in Illinois is the Japanese beetle (Scarabaeidae, *Popillia japonica*). *P. japonica* first appeared in Illinois 50 years ago (USDA 2004) but was not common in central Illinois until the late-1990s (Gray and Steffey 1998). The adults of this species are leaf skeletonizers that feed on >300 species of plants (Potter and Held 2002). Adults emerge in early summer and live 4–6 weeks, with the females longer-lived than the males. Adults mate multiple times and females lay 4–6 eggs in each oviposition bout, leading to a lifetime total of 40–60 eggs (Fleming 1972). Adults and larvae are both considered to be economic pests, with adults feeding on crop and ornamental plant species and larvae feeding on turf and sod plant species.

Hamilton et al. (2005) found that foliage of soybean (*Glycine max*) grown under elevated CO₂ has increased sugar (glucose, sucrose, and fructose) content and that *P. japonica* preferentially feed on *G. max* foliage grown under elevated CO₂. This preference may be related to the increased sugar content, because sugars are strong phagostimulants for *P. japonica* (Ladd 1986). Hamilton et al. (2005) also found that *G. max* foliage grown under elevated O₃ had lower levels of glucose and fructose and lower levels of total herbivory.

We examined the effects of feeding on *G. max* foliage grown under elevated CO₂ or O₃ on longevity and fecundity of *P. japonica*. These life history traits are key factors in pest population growth and are useful in predicting future impacts of global atmospheric change on agriculture (Iversen and Harding 2007). Given the preference of *P. japonica* for soybean foliage grown under elevated CO₂ (Hamilton et al. 2005), we expected that consuming foliage grown under elevated CO₂ would improve beetle performance. Because of the adverse effects of elevated O₃ on soybean (Morgan et al. 2006), we also predicted that performance would decline when beetles fed on foliage grown under elevated O₃. We also evaluated the effect of increased dietary sugar content on beetles, independent of other effects of elevated CO₂ on *G. max* foliage.

Increases in fecundity have been linked in some species to the male diet (Cresoni-Pereira and Zucoloto 2006). To differentiate male and female contributions to fecundity, we recorded egg production of females consuming foliage grown under elevated CO₂ or ambient conditions after they were mated to males fed the same or alternate diet.

Dietary constituents, including some phytochemicals, with antioxidant properties are associated with enhanced longevity in several insect species (Orr and Sohal 1994). Previous studies on plants grown under elevated atmospheres have determined that antioxidant content increases in fruits (e.g., strawberries, *Fragaria* × *ananassa* Dutch; Wang et al. 2003) and foliage (e.g., European silver birch, *Betula pendula* Roth; Saleem et al. 2001). We measured total antioxi-

dants in foliage from plants grown under elevated CO₂ and under ambient conditions to determine whether changes in longevity are associated with changes in foliar antioxidant content.

Materials and Methods

Beetle Longevity and Fecundity. Virgin beetles were collected on emergence from the east side of Meadowbrook Park (Urbana, IL) during the last week of June and the first week of July 2003. Because all beetles emerged from the same area of the park, larvae fed on the same grass species and experienced the same weather conditions. Beetles were sexed by examining the tibial spurs on their first pair of legs (Fleming 1972). Beetles were divided into groups with one male and one female in each cage (0.95-liter plastic container with a no. 14 size mesh lid; height, 14 cm; diameter, 11.5 cm), containing a 3-cm-thick layer of fine sand for oviposition in a design similar to that used by Van Timmerman et al. (2000). Beetles were assigned randomly to each treatment and container, and females were measured so that female size was equal across treatments, to control for its effect on egg production (Saeki et al. 2005). Treatments were replicated 10 times. Beetles in each cage were fed soybean foliage from plants grown under elevated CO₂ (550 μmol/mol), elevated O₃ (1.2× ambient level), a combination of elevated CO₂ plus O₃, or ambient air (370 μmol/mol CO₂).

Because it was determined previously that *P. japonica* are more numerous in elevated CO₂ plots and that the chemistry of soybean foliage differs between ambient and elevated CO₂ plots (Hamilton et al. 2005), our emphasis in this study was to evaluate variation in *P. japonica* performance when beetles were fed foliage grown under different conditions. Thus, the unit of replication for statistical analysis was individual beetles.

Beetles were fed foliage from SoyFACE, an open free air gas concentration enrichment system that exposes large field plots of soybean to elevated CO₂ and elevated O₃, singly or in combination (Long et al. 2004; <http://www.soyface.uiuc.edu>). The 32.4-ha field site has been in continuous crop production for 100 years (South Farms, University of Illinois, Savoy, IL; 40°03'21.3" N, 88°12'3.4" W). Soybeans ('Pioneer 93B15') had been growing for 36 days at the start of the experiment, 27 of them while elevated levels of CO₂ were being released and 15 of them while elevated levels of O₃ were being released. Average elevated CO₂ fumigation for the season was 553 μmol/mol; average elevated O₃ fumigation for the season was 63.3 nmol/mol. The concentration of CO₂ was measured with an infrared gas analyzer (model SBA-1; PP Systems, Hitchin, United Kingdom), and O₃ levels were measured with an O₃ analyzer (model 49C; Thermo Scientific Instruments, Franklin, MA). Foliage was cut from the top three trifoliates from each treatment, placed immediately in water-filled, rubber-capped vials, and transferred to the beetle cages. Foliage was changed every other day, and the cages were lightly

sprayed with water at this time to maintain moisture content of the sand and provide the beetles with supplemental water.

Cages were checked daily for adult beetle mortality. Oviposition was monitored weekly by sifting the sand for eggs and larvae with a 40-mesh screen. Male and female longevities were compared by Kaplan-Meier survival analysis in a stratified design with O₃ treatment as a factor and CO₂ as a stratified variable. Because no difference in survivorship distribution caused by O₃ fumigation was detected for either sex, the data were instead analyzed with CO₂ treatment as the factor variable and O₃ treatment as the strata variable, CO₂ being compared over pooled strata. Numbers of eggs in the different treatments were compared with the ambient control by a Mann-Whitney *U* test; this nonparametric test was selected because the variances were not homogeneous. All statistical tests were conducted with SPSS 9.0 statistical software (SPSS, Chicago, IL).

Eggs were collected from each treatment once a week, pooled, and weighed in groups of 10 to a precision of 0.0001 mg on an analytical balance (AE 100; Mettler-Toledo, Columbus, OH). Egg masses were normally distributed and did not require transformation before statistical analysis. Egg masses for each treatment were compared by two-way analysis of variance (ANOVA) with CO₂ and O₃ as main effects.

Effects of Sugar Supplementation on Beetle Longevity and Fecundity. In a separate experiment, beetles were fed soybean foliage grown under ambient or elevated CO₂, or ambient CO₂ but supplemented with sugar. Soybeans had been growing for 25 days at the start of the experiment, 18 of them while elevated levels of CO₂ were being released. Five males and five females were placed in each cage, and each treatment was replicated four times. A subset of leaves were separated for the sugar treatment, and their petioles were placed in an aqueous sugar solution containing 8.9 mg/g glucose, 1.0 mg/g fructose, and 4.3 mg/g sucrose (based on relative concentrations found in foliage grown under elevated CO₂; Hamilton et al. 2005). The petioles of leaves were inserted into rubber-capped vials containing the sugar solution then placed in the beetle cage and kept in solution until they were removed. Leaves in the other two treatments were placed in vials filled with distilled water. To confirm that the sugar content had been increased by this supplementation technique, glucose, fructose, and sucrose content for a separate collection of leaves was measured as in Rogers et al. (2004). Briefly, fresh leaves supplemented with sugars for 24 hours were weighed and ground in a glass bead beater, and sugar content was determined using an enzymatic assay.

Cages were checked daily for mortality for 41 d. Median age at death per cage was calculated for each sex and analyzed by two-way ANOVA with sex and treatment as main effects. Differences between treatments were analyzed by Dunnett post hoc tests. Oviposition was monitored weekly as described previously. Total egg production was analyzed by a one-way ANOVA with treatment (ambient, elevated CO₂, and

sugar supplementation) as the main effect. Differences between treatments were analyzed by Tukey post hoc tests.

Effects of Male Versus Female Diet. This experiment was designed to partition male and female contributions to egg fecundity as a function of diet independent of longevity effects. Another cohort of beetles was divided into groups, with five males and five females, only five males, or only five females in each cage. Treatments were replicated four times. Beetles in each of the treatments were fed soybean foliage grown under either elevated or ambient CO₂. Five male beetles from a cage provisioned with leaves grown under elevated CO₂ were introduced to a mating chamber containing five female beetles from a cage provisioned with control leaves for 2 hours every day as long as beetles were alive. Five male beetles from a cage provisioned with control leaves were introduced to a mating chamber with five female beetles fed leaves grown under elevated CO₂ and left for 2 hours every day as long as beetles were alive. Cages with five males and five females together fed leaves grown under either elevated CO₂ or ambient conditions were kept as controls.

Oviposition was monitored as described earlier. Because egg number in this experiment was correlated with the number of days that male beetles were alive (total beetle days, $r = 0.510$, $P = 0.043$) but not with the number of days that female beetles were alive ($r = 0.07$, $P = 0.796$), we used male beetle days as a covariate in a two-way analysis of covariance (ANCOVA) to separately account for this source of variation (assumption of homogeneity of slopes was satisfied). Foliage diet of males and foliage diet of females were main effects in the model. Number of beetle days was calculated as the sum of days the male beetles were alive.

Antioxidant Analysis. Discs were collected from the most recent fully expanded leaf from three plants in the four replicate SoyFACE plots for ambient and elevated CO₂ in August 2003 and July 2004, flash-frozen in liquid nitrogen, and stored at -80°C . Antioxidants were measured following Lee et al. (2004). Leaf discs collected from the three plants in each ring were analyzed separately from each other. Leaf material (0.04 ± 0.01 g) was mixed with 583 μl acetonitrile, 167 μl hydrochloric acid, and 250 μl deionized water for a final volume of 1 ml. Samples were vortexed for 1 min, shaken for 2 hours at room temperature, centrifuged for 30 minutes at 4500 rpm, and dried overnight in a vacuum evaporator (RC 10.22; Jouan, Winchester, VT). Dried samples were dissolved in 1 ml methanol, with 0.25 ml of sample added to 3.75 ml of DPPH (2,2-diphenyl-1-picryl-hydrazyl, a stable free radical) solution. Equal volumes of 0.5, 0.25, 0.1, 0.075, and 0.05 mM BHT (butylated hydroxytoluene, a radical scavenging compound) were added to 3.75 ml DPPH solution for use as standards. Absorbance was measured in a spectrophotometer at 517 nm (Lamba 3B UV/Vis; Perkin Elmer, Waltham, MA) after a 30-min incubation at 21°C. Antioxidant amounts for foliage from each FACE plot ($n = 4$) were

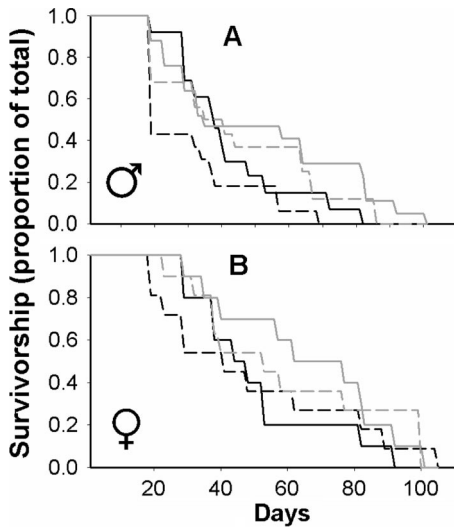


Fig. 1. Survivorship of male (A) and female (B) beetles fed soybean leaves grown under elevated levels of atmospheric CO_2 or O_3 . Male survivorship differed significantly when fed foliage from plants grown under elevated CO_2 compared with ambient CO_2 (Kaplan-Meier survival analysis, log rank = 4.74, df = 1; A). Female survivorship was not significantly affected by the growth environment of foliage (log rank = 1.77, df = 1; B). The starting number of beetles was 10 in each case. Solid black lines represent beetles fed leaves grown under ambient conditions, solid gray lines represent beetles fed leaves grown under elevated CO_2 , dashed black lines represent beetles fed leaves grown under elevated O_3 , and dashed gray lines represent beetles fed leaves grown under combined elevated CO_2 and O_3 .

converted to BHT equivalents and a one-way ANOVA was conducted with treatment as the main effect.

Results

Beetle Longevity and Fecundity. Feeding on foliage from plants grown under elevated O_3 affected neither male nor female survivorship distributions (male log rank = 2.25, df = 1; female log rank = 0.42, df = 1). Male beetles fed foliage from plants grown under elevated CO_2 lived significantly longer than control males (Fig. 1A). Female survivorship was not significantly affected by the growth environment of foliage (Fig. 1B). Female beetles fed foliage from plants grown under elevated CO_2 laid significantly more eggs than females fed foliage grown under ambient conditions (mean egg number CO_2 = 26.9 ± 4.15 SE, mean egg number ambient = 9.7 ± 3.34 SE; Mann-Whitney U, $P = 0.011$). In comparison with beetles from the ambient treatment, there was no significant difference in fecundity of beetles fed foliage grown under elevated O_3 (mean, 6.8 ± 2.6 ; $P = 0.512$) or foliage grown under elevated CO_2 plus O_3 (mean, 13.1 ± 3.9 ; $P = 0.387$).

Female beetles that consumed leaves grown under elevated CO_2 laid significantly lighter eggs (mean egg mass: 1.1 ± 0.6 μg) than females that consumed leaves grown under ambient air (mean egg mass: 1.6 ± 0.5 μg ;

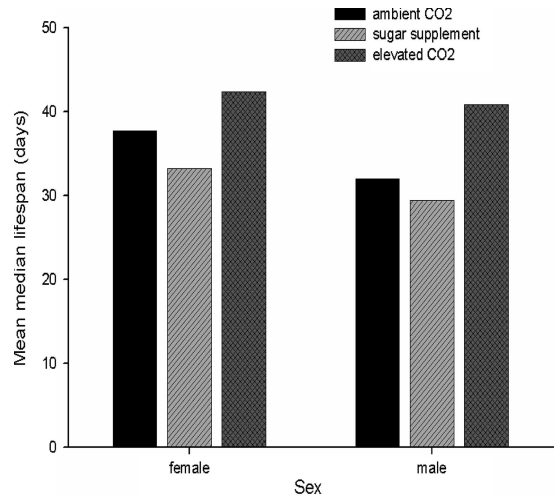


Fig. 2. Mean median survivorship per cage of female (left) and male (right) beetles fed soybean leaves grown under different atmospheric environments or supplemented with sugar. A significant treatment effect was found from two-way ANOVA ($F = 10.93$; df = 2,18; $P = 0.001$). A nearly significant effect of sex was detected ($F = 4.108$; df = 1,18; $P = 0.058$), but there was no interaction between sex and treatment ($F = 0.452$; df = 2,18; $P = 0.644$). A Dunnett test comparing elevated CO_2 to ambient (control) was significant ($P = 0.0140$). The Dunnett test comparing sugar supplementation to control was not significant ($P = 0.220$). There were 31 females and 32 males fed leaves grown under ambient conditions, 32 females and 23 males fed leaves grown under elevated CO_2 conditions, and 25 females and 14 males fed leaves grown under ambient conditions and supplemented with additional sugars.

CO_2 main effect ANOVA; df = 1,13; $F = 7.126$; $P = 0.019$). Ozone treatment had no overall effect on egg mass (mean, 1.5 ± 0.7 μg ; O_3 main effect ANOVA; df = 1,13; $F = 1.394$; $P = 0.259$). There also was no significant interaction effect of CO_2 and O_3 treatment on mean egg mass (mean, 1.3 ± 0.4 μg $\text{CO}_2 \times \text{O}_3$ interaction from ANOVA; df = 1,13; $F = 1.256$; $P = 0.283$).

Effects of Sugar Supplementation on Beetle Longevity and Fecundity. Sugar supplementation increased sugar content of foliage grown under ambient air from sucrose, fructose, and glucose concentrations of (means \pm SE) 1.47 ± 0.46 , 0.74 ± 0.18 , and 0.53 ± 0.18 nmol/mg leaf fresh weight, respectively, to 14 ± 10.67 , 4.4 ± 0.68 , and 39.8 ± 5.78 nmol/mg leaf fresh weight, respectively. Although the supplementation effectively increased sugar concentrations, the levels were higher than those typically observed in plants exposed to elevated CO_2 under field conditions (Rogers et al. 2004, Hamilton et al. 2005). In the two-way analysis of survivorship, only treatment had a significant effect on median survivorship (Fig. 2). Because there was no sex or sex \times treatment interaction, both sexes responded similarly. Post hoc comparisons to the ambient treatment showed that beetles lived longer only if fed leaves grown elevated CO_2 ; sugar supplementation did not enhance longevity.

Females fed foliage grown at elevated CO₂ produced significantly more eggs than females fed foliage grown under ambient air and more than females fed elevated levels of sugar (mean egg number ambient = 82.8 ± 6.0, mean egg number elevated CO₂ = 148.5 ± 11.2, mean egg number sugar supplemented = 98.8 ± 20.0, one-way ANOVA; df = 2,9; *F* = 13.409; *P* = 0.049; Tukey post hoc tests: CO₂ versus ambient, *P* = 0.002; CO₂ versus sugar, *P* = 0.011); there was no significant difference in egg production between females fed elevated levels of sugars and females fed leaves from ambient air (Tukey post hoc test, *P* = 0.478).

Effects of Male Versus Female Diet. After adjustment for male survivorship in a two-way ANCOVA, only a female effect of diet on total egg production was significant (ANCOVA main effect of female diet, df = 1,11; *P* = 0.013). On average, females in cages fed leaves grown under elevated CO₂ produced 140 ± 9.4 eggs compared with 100 ± 9.4 eggs in cages with females feeding on leaves grown under ambient CO₂. Neither male diet nor the interaction between male and female diet had a significant effect on egg production (male diet, df = 1,11; *P* = 0.362; interaction, df = 1,11; *P* = 0.430). Thus, apart from the effect that feeding on foliage grown under elevated CO₂ had in enhancing egg production by increasing longevity of both males and females, feeding on elevated CO₂ foliage also contributed to egg production through additional mechanisms in females. The relatively restricted time for mating in cages between males and females fed different diets did not seem to be an issue in this experiment; had egg production been impaired in these matings, a significant interaction effect would have resulted.

Antioxidant Analysis. There was no significant difference in antioxidant content expressed as BHT equivalents between leaves grown under elevated CO₂ and those grown under ambient conditions (mean BHT equivalent: ambient = 0.62 ± 0.03; mean BHT equivalent: CO₂ = 0.63 ± 0.03; ANOVA, df = 1, 22; *F* = 0.314; *P* = 0.581).

Discussion

Fecundity of *P. japonica* fed on leaves grown under elevated CO₂ consistently was greater than fecundity of beetles fed leaves grown under current levels of CO₂. The indirect effects of elevated CO₂ on beetle fecundity were manifested in two ways. First, because both male and female beetles lived longer, there were more opportunities to mate and more time to lay eggs. This phenomenon has been observed previously in female *P. japonica* by Ladd (1987), who found that increased longevity caused by feeding on certain host plant species led to an increase in egg production. Second, there was an additional positive effect that is not related to longevity, because the ANCOVA showed a significant female effect of feeding on foliage grown under elevated CO₂ after removal of variation in longevity. Thus, increased longevity and fecundity of *P. japonica* may contribute to greater damage by this

herbivore as the concentration of CO₂ in the atmosphere continues to increase.

Previous studies on chewing insects under atmospheric change found that fecundity decreases more often than it increases. Fecundity of cotton bollworms (*Helicoverpa armigera* Hubner) feeding on spring wheat grown under elevated CO₂ (750 μmol/mol) was significantly lower, with successive generations laying fewer eggs than the original populations (Wu et al. 2006). Fecundity of alpine grasshoppers (*Miramella alpina*) that fed on several species of dwarf shrubs grown under elevated CO₂ (550 μmol/mol) was lower than those fed foliage grown in ambient air (Asshoff and Hattenschwiler 2005); nutritional deficiencies experienced by the nymphs led to reduced adult biomass and subsequently reduced egg mass.

In contrast to cotton bollworms and grasshoppers, the fecundity of aphids increase when feeding on foliage grown in elevated CO₂ (Chen et al. 2004, 550 and 750 μmol/mol; Xing et al. 2003, 550 and 700 μmol/mol). The authors of both studies hypothesized that increases in plant sugars and other soluble carbohydrate caused this increase and indeed found that higher fecundity was correlated with greater carbohydrate levels.

Although the increase in leaf sugars in soybean (Ainsworth et al. 2002, Rogers et al. 2004, Hamilton et al. 2005) is a potent feeding stimulus for *P. japonica* (Ladd 1986), it did not explain increased fecundity. Elevated CO₂ alters many other facets of leaf chemistry that potentially affect longevity and fecundity (Peñuelas and Estiarte 1998, Zvereva and Kozlov 2006). Antioxidants, for example, promote longevity (Harman 1956, Orr and Sohal 1994), and increases in antioxidant levels have been detected in plants exposed to elevated CO₂ and elevated O₃ (Saleem et al. 2001, Wang et al. 2003). However, no increase in antioxidant content was detected for soybean in this study or in a multiyear study using the same cultivar (K. Gillespie, unpublished data). Reductions in insect growth and fitness are associated with low levels of leaf nitrogen (Mattson 1980) and the leaf nitrogen concentration of soybean, although generally high because of its ability to reduce atmospheric nitrogen, is slightly lower (8–12%) when grown under elevated CO₂ (O. Dermody, unpublished data). However, small reductions in leaf nitrogen would contribute to lower fecundity rather than the increase in fecundity observed in this study for beetles that consumed foliage developed under elevated CO₂.

Recently, Zavala et al. (unpublished data) reported a substantial reduction in the production of cysteine proteinase inhibitors, specific deterrents to beetles, in soybeans grown under elevated CO₂. Soybean has one constitutive and two inducible cysteine proteinase inhibitor genes (Botella et al. 1996, Zhao et al. 1996). By interfering with the function of proteinases in the gut, cysteine proteinase inhibitors in plant tissues decrease growth and development of insects (Fabrick et al. 2002, Kim and Mullin 2003). Reductions in cysteine proteinase inhibitor activity in leaves grown under

elevated CO₂ may have contributed to greater fecundity of *P. japonica*.

The increase in fecundity of *P. japonica* fed high-CO₂ foliage may not necessarily contribute directly to greater population size and herbivore damage, because increased egg production by *P. japonica* in elevated CO₂ seems to have come at the cost of reduced egg weight, potentially leading to decreased juvenile survivorship. This potential reduction is unlikely to be large, however, because the product of mean egg production and mean egg mass of females fed leaves grown under elevated CO₂ is still double that of females fed leaves grown under ambient conditions (29.6 versus 15.5 μg).

Popillia japonica is a voracious feeder, and current control efforts are estimated to cost more than \$460 million a year (USDA 2004). The future population increase suggested by these studies could enhance the pest status of this species and the cost of control. The increase in *P. japonica* longevity and fecundity, coupled with a marked behavioral preference for soybeans grown at elevated CO₂ (Hamilton et al. 2005), suggests that defoliation of soybeans by *P. japonica* may in the future become more extensive as a consequence of global atmospheric change.

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