LARVAL DEVELOPMENT AND MORTALITY OF THE PAINTED LADY BUTTERFLY, *VANESSA CARDUI* (LEPIDOPTERA: NYMPHALIDAE), ON FOLIAGE GROWN UNDER ELEVATED CARBON DIOXIDE

Bridget F. O'Neill[1], Arthur R. Zangerl[1], Clare L. Casteel[2], Jorge A. Zavala[3], Evan H. DeLucia[2,4], and May R. Berenbaum[1,4]

ABSTRACT

To determine the indirect effects of elevated CO_2 on larval Lepidoptera, we compared the growth and development of larvae of *Vanessa cardui* Linnaeus (Lepidoptera: Nymphalidae) on *Glycine max* Linnaeus (soybean) foliage grown under ambient or elevated levels of CO_2 from first instar to pupation. There was no significant difference in larval survivorship or the duration of larval development between the two treatments. The usual sexual difference in pupal size in *V. cardui* was lost when individuals were reared on a diet of *G. max* foliage grown under elevated CO_2 conditions. Despite the fact that increases in levels of atmospheric CO_2 are projected to increase the susceptibility of soybean foliage to coleopteran pests, impacts of such exposure may vary with herbivore taxon, making projections of future yield losses challenging.

Atmospheric carbon dioxide (CO_2) levels have risen from 280 ppm since the start of the Industrial Revolution to 384 ppm today, and levels are expected to double within the next 100 years (IPCC 2007). Several studies examining the effect of elevated CO_2 on foliage-feeding larval Lepidoptera have revealed that these insects generally experienced higher mortality and prolonged development on plants grown under elevated levels of CO_2 (Hättenschwiler and Schafellner 1999, Agrell et al. 2000, Wu et al. 2006, Knepp et al. 2007). The reduced levels of nitrogen and increased phenolic concentrations found in these leaves, contribute to increasing development time and decreasing survival (Lindroth et al. 1995, Traw et al. 1996). Prolonged development also contributes to increased mortality by increasing exposure to predators and parasitoids (Stiling et al. 1999, Chen et al. 2007).

In contrast with these studies, Karowe (2007) documented that larval *Colias philodice* Latreille (Lepidoptera: Pieridae) consuming foliage of two species in the Fabaceae (*Trifolium pratense* and *Melilotus alba*) grown under elevated CO_2 do not experience prolonged development. The nitrogen-fixing properties of plants in the Fabaceae family may counterbalance the expected protein depletion seen in larvae feeding on plants grown under elevated CO_2. Whether or not global atmospheric climate change similarly affects suitability of foliage of other legume species for lepidopterans, particularly economically

[1]University of Illinois, Urbana-Champaign, Department of Entomology, 320 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801 USA.
[2]University of Illinois, Urbana-Champaign, Department of Plant Biology, 265 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801 USA.
[3]Consejo Nacional de Investigaciones Cientificas y Technicas, Catedra de Bioquimica, Facultad de Agronomia, University of Buenos Aires, Avenida San Martin 4453, Buenos Aires, Argentina.
[4]University of Illinois, Urbana-Champaign, Institute of Genomic Biology, 1206 West Gregory Drive, Urbana, IL 61801 USA.

important commodity species, is important to determine for projecting future impacts of global atmospheric climate change on agriculture.

In this study, we examined the growth and survivorship of larvae of *Vanessa cardui* Linnaeus, the painted lady butterfly (Lepidoptera: Nymphalidae), on *Glycine max* Linnaeus (soybean) foliage grown under elevated and ambient levels of CO_2. *G. max* is an important crop species in the United States, particularly in Illinois, and there is great interest in how herbivores feeding on this plant will respond to future atmospheric change. *G. max* is a species in the Fabaceae family so lepidopteran species feeding upon it may not suffer the same nutritional deficiencies that have been seen in other lepidopterans feeding on non-legumes grown under elevated CO_2 levels. *V. cardui* is a native lepidopteran with populations prone to periodic outbreaks in soybean fields (Poston et al. 1977). We hypothesized that *V. cardui* will experience the same larval duration and mortality when reared on *G. max* grown under ambient and elevated levels of CO_2, due to the nitrogen-fixing properties of this plant species. We measured larval development time, mortality, and pupal weight to determine whether lepidopteran folivores of soybean are buffered against changes in their hostplant induced by growth under elevated CO_2.

MATERIALS AND METHODS

Soybeans were grown under ambient (384 ppm CO_2) and elevated CO_2 (550 ppm) atmospheres at the Soy Free Air gas Concentration Enrichment (FACE) site on the UIUC campus. SoyFACE is an open system that exposes large field plots of soybean to elevated CO_2 and elevated O_3, individually or in combination. The 32.4 ha field site has been in continuous crop production for 100 years (South Farms, University of Illinois, 40° 03'21.3" N 88° 12'3.4" W, Savoy, Illinois, USA). The cultivar planted at this site is the Pioneer 93B15 cultivar. The concentration of CO_2 was measured with an infrared gas analyzer (Model SBA-1, PP Systems, Hitchin, UK). Treatment plots are 100 m from any other plot, have a diameter of 20 m, and cover 350 m^2. There are a total of 16 plots with treatments replicated four times in a randomized block design (Long et al. 2004). *V. cardui* eggs were purchased from Carolina Biological Supply Company (Burlington, North Carolina, USA).

Ten plants were chosen in each atmospheric treatment plot for a total of 40 plants per treatment. Six to 12 newly hatched larvae were placed on one leaf on each plant and the leaves were bagged with fine mesh to prevent movement off the plant on 14 July 2006. The soybeans had been growing for 50 days at this point in the growing season, 44 of them under elevated levels of CO_2. The larvae were spaced evenly across the leaf to prevent competition for leaf material, although crowding has been shown to have no effect on *V. cardui* larval duration or mortality (Poston et al. 1978). The total number of larvae under the ambient treatment was 395 on 40 plants; the total number of larvae under the elevated CO_2 treatment was 325 on 40 plants. Bags were checked every other day; surviving larvae were identified to instar, counted, and moved to a new leaf if insufficient leaf material remained to maintain development. High mortality during the first instar led to much lower larval densities during the subsequent instars, around one or two larvae per bag. After larvae pupated, the pupae were moved from the field to a greenhouse on the UIUC campus. Pupae were weighed, sexed and placed in a large mesh flight cage (1.219 m. long, 76.2 cm. wide, and 1.022 m. tall).

Larval durations for each stadium were analyzed by linear mixed models, with stadium length as the dependent variable, CO_2 level as a fixed factor, and plot as a random factor in each model (R Development Core Team 2009; package nlme Pinheiro et al. 2009). Larval mortality for each stadium was analyzed by Kaplan-Meier survival analysis, with length of instar duration in days as the time, survival as the status, and treatment as the factor with the values given as log rank (SPSS 2007). Differences in pupal mass were evaluated by two-way analysis of variance, with treatment and sex as the independent variables (SPSS 2007). Power analyses for each of the different statistical tests were conducted at the $P = 0.05$ level (PASS 2008).

RESULTS

There were no significant differences in duration for any instar (first instar $P = 0.13$; second instar $P = 0.27$; third instar $P = 0.88$; fourth instar $P = 0.30$; fifth instar $P = 0.52$; total duration $P = 0.55$; df = 6 for all analyses) (Table 1). Statistical power of the linear mixed models varied greatly for the different instars (first instar power = 0.88; second instar power = 0.81; third instar power = 0.07; fourth instar power = 0.41; fifth instar power = 0.24; total larval period power = 0.99). There were also no significant differences in mortality for any instar (first instar $P = 0.43$; second instar $P = 0.52$; third instar $P = 0.11$; fourth instar $P = 0.07$; fifth instar $P = 0.21$; total duration $P = 0.24$; df = 1 for all analyses) (Table 2; Fig. 1). Statistical power of the log rank tests also varied greatly for the different instars (first instar power = 0.69; second instar power = 0.86; third instar power = 0.27; fourth instar power = 0.17; fifth instar power = 0.73; total larval mortality power = 0.05).

Table 1. Mean instar duration of *Vanessa cardui* larvae consuming soybean foliage grown under ambient and elevated CO_2 conditions.

Instar	Mean ambient Duration (days)	Mean CO_2 Duration (days)	*t* value	*P*
First	2.59 ± 1.50	2.33 ± 1.34	-1.76	0.13
Second	2.74 ± 1.04	3.38 ± 1.28	1.20	0.27
Third	4.62 ± 1.57	4.50 ± 1.45	-0.16	0.88
Fourth	2.74 ± 1.11	2.24 ± 0.88	-1.12	0.30
Fifth	4.93 ± 1.57	5.23 ± 1.51	0.68	0.52
Total Larval Duration	4.64 ± 5.85	4.17 ± 5.62	-0.63	0.55

Table 2. Larval mortality for each *Vanessa cardui* instar consuming soybean foliage grown under ambient and elevated CO_2 conditions.

Instar	Ambient Larvae Entering Stage	Ambient Mortality	CO_2 Larvae Entering Stage	CO_2 Mortality	Kaplan Meier log rank value	*P*
First	395	81.6% ± 6.8%	325	87% ± 8%	0.62	0.43
Second	78	31.8% ± 29.6%	63	12.4% ± 10.9%	0.41	0.52
Third	71	26.7% ± 15.7%	44	36% ± 10.3%	2.54	0.11
Fourth	52	3.2% ± 3.7%	41	5.9% ± 6.8%	3.27	0.07
Fifth	49	46.2% ± 38%	38	22.1% ± 19.4%	1.58	0.21
Entire Larval Period	395	94.2% ± 0.04%	325	94.2% ± 0.04%	1.40	0.24

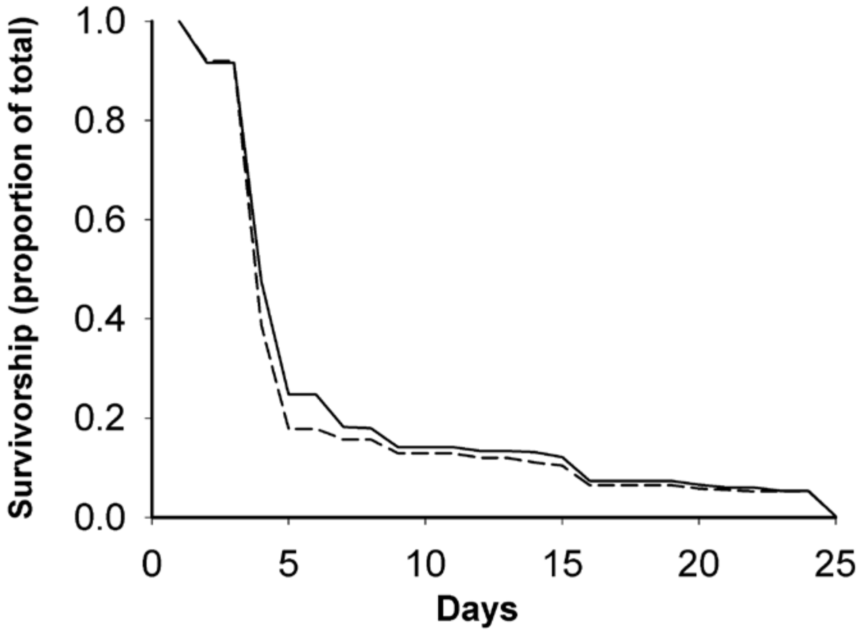

Figure 1. Larval survivorship of *Vanessa cardui* consuming soybean foliage grown under ambient and elevated CO_2. Solid black lines = larvae reared on plants grown under ambient levels of CO_2 (starting N = 395), dashed black lines = larvae reared on plants grown under elevated levels of CO_2 (starting N = 325).

There was no overall effect of atmospheric treatment on fresh pupal mass ($F = 1.24$; df = 1; $P = 0.27$). There was a significant difference in pupal mass between the sexes ($F = 4.30$; df = 1; $P = 0.05$), but this difference was driven by a significant interaction between treatment and sex ($F = 4.78$; df = 1; $P = 0.04$) (Fig. 2) such that a sexual dimorphism occurred at ambient CO_2 but not at elevated CO_2. Statistical power of the ANOVA was low (power = 0.52).

DISCUSSION

Vanessa cardui larval development time and mortality did not differ for insects raised on foliage grown under ambient or elevated levels of CO_2. This lack of a significant increase in larval development time on soybean foliage grown under elevated CO_2, and the lack of a significant difference in larval mortality between the two treatments were unexpected when compared with previous studies on larval Lepidoptera reared under elevated CO_2 conditions (Wu et al. 2007), but followed our hypothesis. There are several possible explanations for these findings. One is that the nitrogen content of the foliage of soybeans grown under elevated CO_2 is higher than the nitrogen content in foliage of other plant species grown under elevated CO_2 (Ainsworth et al. 2007, Zhang et al. 2007). Nitrogen is extremely important for growing insects, including larval Lepidoptera (Goverde et al. 2008, Nerg et al. 2008). This would also help to explain the Lepidoptera feeding results seen by Karowe (2007) when larvae were given legumes grown under elevated levels of CO_2. The second factor may be the fact that growth under elevated CO_2 reduces the production of defense

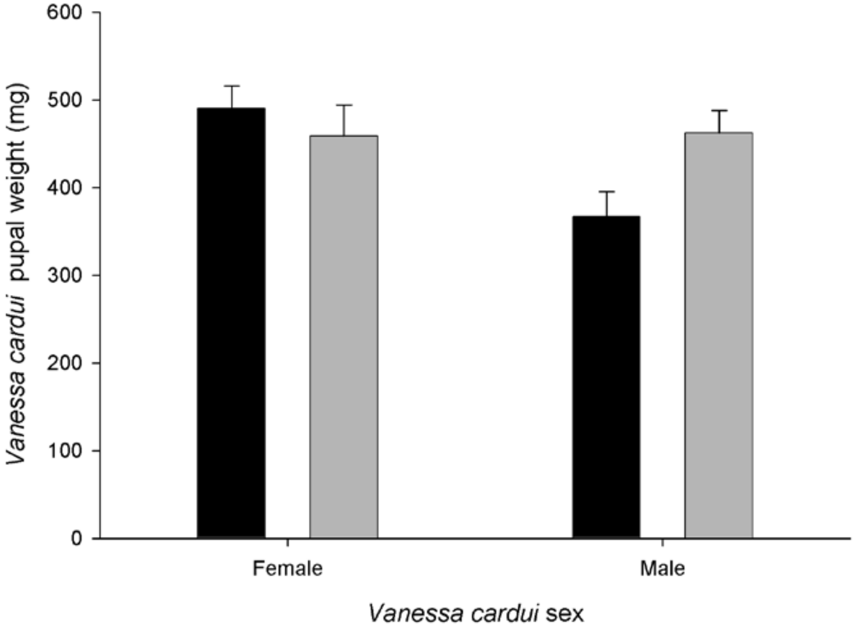

Figure 2. Interaction of treatment and sex on *Vanessa cardui* female (left) and male (right) pupal fresh weights. Female pupal fresh weights were not significantly different between pupae of larvae fed on *G. max* grown under ambient or under elevated levels of CO_2 (ANOVA, Tukey HSD, $P = 0.890$). Weights were not significantly different between male pupae fed on *G. max* grown under ambient or elevated levels of CO_2 (ANOVA, Tukey HSD, $P = 0.080$). Pupal fresh weights were significantly different between females and males fed on *G. max* grown under ambient levels of CO_2 (ANOVA, Tukey HSD, $P = 0.015$). Pupal fresh weights were not significantly different between females and males fed on *G. max* grown under elevated levels of CO_2 (ANOVA, Tukey HSD, P = 0.191). Numbers of larvae surviving to pupation were 20 on plants grown under ambient levels of CO_2; and 17 on plants grown under elevated CO_2. Pupal fresh weights were measured in milligrams. Black bars indicate pupae reared on *G. max* grown under ambient levels of CO_2, grey bars indicate pupae reared on *G. max* grown under elevated levels of CO_2.

compounds in soybeans (Zavala et al. 2008). Most defenses in other plant species (particularly phenolics) increase under elevated CO_2 conditions (Reddy et al. 2004, Mattson et al. 2005).

Although very few larvae survived to pupation (Fig. 1), the results of the analysis of pupal masses suggest that elevated CO_2 may have influenced pupal mass. There was no overall difference in pupal weight between the two treatments (Fig. 2), which was again unexpected in view of previous works by Wu et al. (2007) in which pupal masses of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) fed cotton bolls grown under elevated CO_2 were reduced, but as with larval duration and mortality followed our hypothesis. The difference in pupal weight between males and females raised on foliage grown under ambient levels of CO_2 is expected, as female Lepidoptera are normally heavier than males (Poston et al. 1977). The lack of difference in pupal weight recorded for females and males raised on foliage grown under elevated CO_2 was due to an increase in pupal mass of males under those conditions. *Popillia japonica* Newman

(Japanese beetle) males live significantly longer when fed a diet of *G. max* foliage grown under elevated CO_2 levels than males fed a diet of foliage grown under ambient CO_2 levels (O'Neill et al. 2008). *V. cardui* males with larger pupal masses would have more nutritional reserves as adults to fuel an increased lifespan (Murúa et al. 2008), and these larger males would be able to maintain a larger territory (Enrique et al. 2008) or be able to win more fights for mating opportunities (Brown and Alcock 1991). It should be noted that all individuals came from a laboratory strain that was not fed soybean foliage, and they may not display the same survivorship or feeding behavior of wild-type individuals (Hernandez et al. 2009).

Statistical power of all analyses was fairly low. While an *a priori* power analysis suggested that our initial sample sizes would be adequate to resolve significant differences, the high mortality seen in the first instar reduced the sample sizes to such a degree that statistical power was lost. If this experiment were to be repeated initial sample sizes would have to be increased by several hundred individuals, and space at this shared research site would not be adequate.

The lack of difference in *V. cardui* larval development under conditions of global atmospheric change suggests that defoliation of soybeans by *V. cardui* may not change in the future as greatly as anticipated by previous studies on non-legumes. This work demonstrates that plant-insect interactions are idiosyncratic with respect to impacts of global climate change and future projections must be based on appropriate studies.

ACKNOWLEDGMENTS

This research was supported in part by grants from the U.S. Department of Energy (No. DE-FG02-04ER63489) and the National Research Initiative of USDA Cooperative State Research, Education and Extension Service (2002–02723). The SoyFACE experiment is supported by the Illinois Council for Food and Agricultural Research (C-FAR), Archer Daniels Midland Co., and USDA-ARS.

LITERATURE CITED

Agrell, J., E. P. McDonald, and R. E. Lindroth RE. 2000. Effects of CO_2 and light on tree phytochemistry and insect performance. Oikos. 88: 259-272.

Ainsworth, E. A., A. Rogers, A. D. B. Leakey, L. E. Heady, Y. Gibon, M. Stitt, and U. Schurr. 2007. Does elevated atmospheric [CO_2] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? J. Exp. Bot. 58: 579-591.

Brown, W. D., and J. Alcock. 1991. Hilltopping by the red admiral butterfly mate searching alongside corridors. Journal of Research on the Lepidoptera. 29: 1-10.

Chen, F.J., G. Wu, M. N. Parajulee, and F. Ge. 2007. Impact of elevated CO_2 on the third trophic level: a predator *Harmonia axyridis* and a parasitoid *Aphidius picipes*. Biocontrol Sci. Tech. 17: 313-324.

Enrique, P., C. Peixoto, and W. W. Benson. 2008. Body mass and not wing length predicts territorial success in a tropical Satyrine butterfly. Ethology. 114: 1069-1077.

Goverde, M., A. Bazin, M. Kery, J. A. Shykoff, and A. Erhardt. 2008. Positive effects of cyanogenic glycosides in food plants on larval development of the common blue butterfly. Oecologia. 157: 409-418.

Hättenschwiler, S., and C. Schafellner. 1999. Opposing effects of elevated CO_2 and N deposition on *Lymantria monacha* larvae feeding on spruce trees. Oecologia. 118: 210-217.

Hernandez, E., J. Toledo, T. Artiaga-Lopez, and S. Flores. 2009. Demographic changes in *Anastrepha obliqua* (Diptera: Tephritidae) throughout the laboratory colonization process. J. Econ. Entomol.. 102: 542-551.

(IPCC) Intergovernmental Panel on Climate Change. 2007. Fourth Assessment Report. Climate Change 2007, Cambridge University Press, Cambridge, UK.

Karowe, D. N. 2007. Are legume-feeding herbivores buffered against direct effects of elevated carbon dioxide on host plants? A test with the sulfur butterfly, *Colias philodice*. Global Change Biology. 13: 2045-2051.

Knepp, R. G., J. G. Hamilton, A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2007. Foliage of oaks grown under elevated CO_2 reduces performance of *Antheraea polyphemus* (Lepidoptera: Saturniidae). Environ. Entomol. 36: 609-617.

Lindroth, R. L., G. E. Arteel, and K. K. Kinney. 1995. Responses of three saturniid species to paper birch grown under enriched CO_2 atmospheres. Funct. Ecol.. 9: 306-311.

Long, S. P., E. A. Ainsworth, A. Rogers, and D. R. Ort. 2004. Rising atmospheric carbon dioxide: Plants FACE the future. Ann. Rev. Plant Biol. 55: 591-628.

Mattson, W. J., R. Julkunen-Tiitto, and D. A. Herms. 2005. CO_2 enrichment and partitioning to phenolics: do plant responses accord better with the protein competition or the growth differentiation balance models? Oikos. 111: 337-347.

Murúa, M.G., M. T. Vera, S. Abraham, M. L. Juaréz, S. Prieto, G. P. Head, and E. Willink. 2008. Fitness and mating compatibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) populations from different host plant species and regions in Argentina. Ann. Entomol. Soc. Am. 101: 639-649.

Nerg, A.-M., A. Kasurinen, T. Holopainen, R. Julkunen-Tiitto, S. Neuvonen, and J. K. Holopainen. 2008. The significance of ectomycorrhizas in chemical quality of silver birch foliage and above-ground insect herbivore performance. J. Chem. Ecol. 34: 1322-1330.

O'Neill, B.F., A. R. Zangerl, E. H. DeLucia, and M. R. Berenbaum. 2008. Longevity and fecundity of the Japanese beetle, *Popillia japonica* (Newman), on foliage grown under elevated carbon dioxide. Environ. Entomol. 37: 601-607.

(PASS) Power Analysis and Sample Size. 2008. Power Analysis and Sample Size Software Program. Kaysville, UT:NCSS.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and the R Development Core Team. 2009. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-92.

Poston, F. L., R. B. Hammond, and L. P. Pedigo. 1977. Growth and development of the painted lady on soybeans (Lepidoptera: Nymphalidae). J. Kansas Entomol. Soc. 50: 31-36.

Poston, F. L, L. P. Pedigo, and R. B. Hammond. 1978. A leaf consumption model for the painted lady on soybeans. J. Kansas Entomol. Soc. 51: 191-197.

R Development Core Team. 2009. R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Reddy, G. V. P., P. Tossavainen, A.-M. Nerg, and J. K. Holopainen. 2004. Elevated atmospheric CO_2 affects the chemical quality of *Brassica* plants and the growth rate of the specialist, *Plutella xylostella*, but not the generalist, *Spodoptera littoralis*. J. Agric. Food Chem. 52: 4185-4191.

SPSS. 2007. SPSS for Windows, Rel. 16.0.1. Chicago, IL: SPSS, Inc.

Stiling, P., A. M. Rossi, B. Hungate, P. Dijkstra, C. R. Hinkle, W. M. Knott, and B. Drake. 1999. Decreased leaf-miner abundance in elevated CO_2: reduced leaf quality and increased parasitoid attack. Ecological Applications. 9: 240-244.

Traw, M. B., R. L. Lindroth, and F. A. Bazzaz. 1996. Decline in gypsy moth (*Lymantria dispar*) performance in an elevated CO_2 atmosphere depends upon host plant species. Oecologia. 108: 113-120.

Wu, G., F.-J. Chen, and R. Ge. 2006. Response of multiple generations of cotton bollworm *Helicoverpa armigera* Hübner, feeding on spring wheat, to elevated CO_2. J. Appl. Entomol. 130: 2-9.

Wu, G., F.-J. Chen, Y.-C. Sun, and F. Ge. 2007. Response of successive three generations of cotton bollworm, *Helicoverpa armigera* (Hübner), fed on cotton bolls under elevated CO_2. J. Environ. Sci. 19: 1318-1325.

Zavala, J. A., Casteel, C. L., E. H. DeLucia, and M. R. Berenbaum. 2008. Anthropogenic increase in carbon dioxide compromises plant defense against invasive insects. Proceedings of the National Academy of Sciences of the United States of America. 105: 5129-5133.

Zhang, Y., B. Duan, Y. Qiao, K. Wang, H. Korpelainen, and C. Li. 2007. Leaf photosynthesis of *Betula albosinensis* seedlings as affected by elevated CO_2 and planting density. Forest Ecology and Management. 255: 1937-1944.