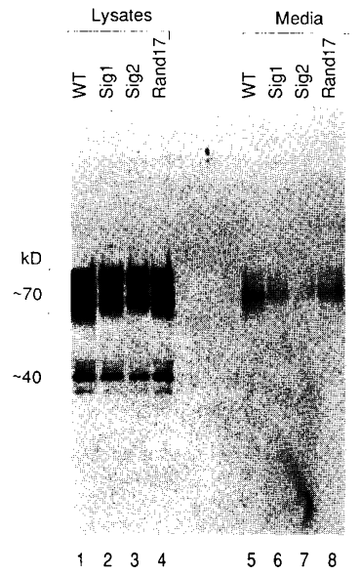


**Fig. 4.** Immunoprecipitation analysis of DAF proteins labeled metabolically with [$^3$H]ethanolamine. Transfected COS cells were incubated with [$^3$H]ethanolamine (200 μCi per 35-mm dish) for 16 hours. DAF was then immunoprecipitated from NP40 cell lysates (lanes 1 to 4) or culture media (lanes 5 to 8) that was collected at the end of the radiolabeling period.

membrane anchor. Similar conclusions have been reached regarding signal peptides for membrane translocation, whose function depends on their length and hydrophobicity rather than their specific sequence (*18*). It has been suggested that GPI anchor attachment requires a weakly hydrophobic domain since a single Asp → Val mutation in the COOH-terminal domain of Qa-2 converts this normally GPI-anchored protein into an integral membrane protein (*19*). Our data argue against this, since the hGH signal peptide contains a strongly hydrophobic core region. The length as well as the hydrophobicity of COOH-terminal domains appears to be important for GPI anchor attachment. Placental alkaline phosphatase (PLAP) synthesized with a hydrophobic COOH-terminal domain of 17 amino acids is anchored by a GPI anchor, whereas PLAP mutants that have 13 or fewer hydrophobic residues at the COOH-terminus are secreted (*14*). The hGH signal sequence contains a run of 13 hydrophobic amino acids that, in the context of the DAF COOH-terminus, appears to be sufficient to direct the attachment of a GPI anchor. Additional factors therefore may influence the precise minimal length requirement. DAF-Sig2 contains a COOH-terminal extension of five hydrophilic amino acids with an overall negative charge (Gln-Glu-Gly-Ser-Ala). This apparently does not affect processing and attachment of the GPI anchor.

Despite a wide degree of sequence diversity, signal peptides for membrane translocation are recognized by specific protein receptors (*20*). The finding that a secretion signal peptide can function in signaling GPI anchor attachment at the COOH-terminus of a protein suggests that the two processes may be related, mechanistically or evolutionarily. It is conceivable that the $NH_2$-terminal peptidase and the enzyme that cleaves the COOH-terminus of GPI-linked proteins have evolved from a common precursor. Whether the COOH-terminal signal for GPI attachment interacts with a protein receptor or membrane component, it appears that its overall conformation or character (hydrophobicity, length, secondary, or tertiary structure) rather than specific sequence is important for proper functioning.


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# The Effects of Enriched Carbon Dioxide Atmospheres on Plant–Insect Herbivore Interactions


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**Little is known about the effects of enriched $CO_2$ atmospheres, which may exist in the next century, on natural plant–insect herbivore interactions. Larvae of a specialist insect herbivore, *Junonia coenia* (Lepidoptera: Nymphalidae), were reared on one of its host plants, *Plantago lanceolata* (Plantaginaceae), grown in either current low (350 parts per million) or high (700 ppm) $CO_2$ environments. Those larvae raised on high-$CO_2$ foliage grew more slowly and experienced greater mortality, especially in early instars, than those raised on low-$CO_2$ foliage. Poor larval performance on high-$CO_2$ foliage was probably due to the reduced foliar water and nitrogen concentrations of those plants and not to changes in the concentration of the defensive compounds, iridoid glycosides. Adult pupal weight and female fecundity were not affected by the $CO_2$ environment of the host plant. These results indicate that interactions between plants and herbivorous insects will be modified under the predicted $CO_2$ conditions of the 21st century.**


BECAUSE OF FOSSIL FUEL CONSUMPTION and tropical deforestation, global atmospheric $CO_2$ concentrations are rising. The current atmospheric $CO_2$ level is 350 ppm, and this is expected to reach 700 ppm by the mid- to late 21st century (*1*). In addition to potentially altering global climate (*2*), it is expected that enriched $CO_2$ atmospheres will influence


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biotic interactions because of the critical role of $CO_2$ in photosynthesis. Both natural and agricultural crops respond to enriched $CO_2$ environments by increasing growth and water-use efficiency, especially those plants that possess the $C_3$ carbon-fixation pathway (*3*). In addition, under enriched $CO_2$ conditions, foliar nitrogen concentrations may decline (*4*), thereby reducing host plant quality for insect herbivores (*5*) and potentially inducing them to eat more (*4*). However, the effects of enriched $CO_2$ environments on natural plant–insect herbivore interactions over an entire insect generation are not well understood. We report that enriched $CO_2$ environments alter the dynamics between plantain, *Plantago lanceolata* (Plantaginaceae), and its specialist herbivore, the buckeye butterfly, *Junonia coenia* (Lepidoptera:Nymphalidae).

*Plantago lanceolata*, a perennial herb with worldwide distribution, is one of the world's 12 most common weeds not associated with agriculture (*6*). It contains iridoid glycosides, carbon-based monoterpenoid compounds (*7*), which are deterrents to generalist insect herbivores (*8*). *Junonia coenia*, the buckeye butterfly, resides year-round in California, parts of Mexico, and the southeastern United States. It is a specialist on iridoid glycoside–containing plants, such as *P. lanceolata*, which it widely uses as a larval host plant (*9*).

To elucidate the effects of enriched $CO_2$ atmospheres on plant-insect interactions, we randomly assigned 100 2-week-old *P. lanceolata* seedlings from seeds collected in Cambridge, Massachusetts, to either low-$CO_2$ (350 ± 35 ppm) or high-$CO_2$ (700 ± 35 ppm) growth chambers (*10*). Plants were grown for 7 weeks before insect feeding began. Then, 100 newly hatched, first instar *J. coenia* larvae gathered from our laboratory-reared stock were randomly assigned to be fed either high $CO_2$– or low $CO_2$–grown *P. lanceolata* foliage. Insects were maintained individually in round plastic dishes (10 cm in diameter) with moistened paper towel strips taped to the lids to maintain humidity and plant turgor (*11*). Larvae were weighed every other day from hatching until pupation, with mortality and larval instar noted. Individuals that died early in the experiment were replaced with other first instar larvae and allowed to develop to pupation. Pupal weight was also measured as an estimate of fitness (*12*). Sex of the individual was recorded after adult emergence, and females were mated, fed daily with dilute honey water, and allowed to lay eggs on *P. lanceolata* leaves daily until they died (*13*).

Results of these experiments showed that the performance of these butterfly larvae was reduced when they were reared on high $CO_2$–grown *P. lanceolata*. Larvae fed high $CO_2$–grown foliage grew more slowly (*14*, Fig. 1, Table 1) and experienced nearly three times the mortality of individuals fed low $CO_2$–grown foliage (Table 1). Moreover, the development of early instars (first to third) took an average of three additional days on high $CO_2$–grown *Plantago* (Table 1). Delayed growth of the smaller and less vagile, early instar larvae may reduce the fitness of insects by increasing their exposure to parasitoids and predators (*15*) and may diminish their likelihood of completing development on the same host in climatically limited environments (*16*).

Male and female *J. coenia* larvae responded differently to high $CO_2$–grown *Plantago* foliage. Male larvae reared on high $CO_2$–grown *P. lanceolata* grew more slowly than males reared on low $CO_2$–grown plants, thereby increasing the time to pupation (entire larval period) (Table 1). However, female larval growth was not affected by the $CO_2$ environment of the host plant (Table 1). Despite differences in growth rate, final pupal weights attained by both male and female larvae were not significantly different between $CO_2$ treatments (Table 1), although females were significantly larger than males ($P<0.05$ by Student Newman-Keuls range test). Pupal weight of female lepidoptera is often correlated with their potential egg production (*12*). Because female pupal weight was not significantly affected by the $CO_2$ environment of their larval *Plantago* diet (Table 1), we were not surprised to find that fecundity (lifetime egg production per female) did not differ between $CO_2$ treatments (*13*) (Table 1). Thus, although feeding on high $CO_2$–grown *Plantago* significantly slowed larval growth, especially of males, it did not alter insect fitness as measured in terms of egg production.

Leaves of experimental plants were analyzed for tissue qualities known to influence insect herbivore performance: nitrogen, water, and defensive compounds (iridoid glycosides) (*5, 17*). These analyses showed that high $CO_2$–grown *P. lanceolata* was a poorer host for insect herbivores because of its significantly lower nitrogen and water concentrations. However, iridoid glycoside concentrations did not differ between $CO_2$ treatments (Table 1). Early instar larvae were apparently more susceptible to the decline in high $CO_2$–grown plant quality than late instar larvae: early instar larvae died or grew more slowly on high-$CO_2$ *Plantago*, whereas late instar larvae were not adversely affected by high $CO_2$–grown *Plantago*. In fact, larvae completed their final

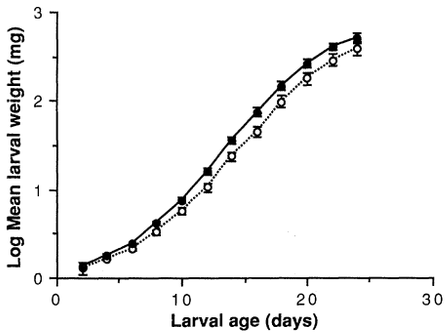


**Fig. 1.** Growth of *Junonia coenia* on either low $CO_2$–(●) or high $CO_2$–(○) grown *Plantago lanceolata* (error bar = 1 SE). Initially, $n = 50$ larvae for both treatments; at day 24, $n = 35$ for low-$CO_2$ treatment (15 individuals pupated, all replacement larvae survived); $n = 40$ for high-$CO_2$ treatment (7 individuals pupated, 3 replacement larvae died).

**Table 1.** Effects of enriched $CO_2$ environments on *Junonia coenia* larvae reared on *Plantago lanceolata*. All statistical comparisons were performed with *t* tests except that for mortality, which was compared by means of a *G* test. Data are presented as $\bar{X}$ ± SD, with sample sizes shown in parentheses; NS, not significant.

| Characteristic | Low $CO_2$ (350 ppm) | High $CO_2$ (700 ppm) | *P* |
|---|---|---|---|
| | *Insect characteristics* | | |
| Mortality (%) | 7.4 (54) | 20.7 (58) | <0.05 |
| Development time (days) | | | |
| Instars 1, 2, 3 | 14.8 ± 3.1 (50) | 17.3 ± 3.6 (47) | <0.001 |
| Instars 4, 5 | 10.4 ± 1.8 (50) | 9.2 ± 1.6 (47) | <0.001 |
| Time to pupation (days) | | | |
| Male | 24.3 ± 3.8 (24) | 26.7 ± 3.1 (23) | <0.05 |
| Female | 26.5 ± 2.9 (23) | 27.4 ± 4.7 (21) | NS |
| Pupal weight (mg) | | | |
| Male | 362.2 ± 38.8 (24) | 384.4 ± 37.8 (23) | NS |
| Female | 427.3 ± 48.2 (23) | 415.2 ± 58.1 (21) | NS |
| Fecundity (eggs per female) | 634.9 ± 257.4 (14) | 512.7 ± 175.7 (11) | NS |
| | *Plant characteristics* | | |
| Leaf nitrogen (%) | 1.31 ± 0.27 (20) | 1.00 ± 0.21 (20) | <0.001 |
| Leaf water (%) | 79.53 ± 1.9 (20) | 76.58 ± 2.4 (20) | <0.001 |
| Leaf iridoids (%) | 3.34 ± 0.98 (15) | 2.68 ± 0.83 (15) | NS |

two instars faster on high $CO_2$–grown *Plantago* than on low $CO_2$–grown *Plantago* (Table 1). Unlike early instar larvae, late instar *J. coenia* larvae can compensate for poorer host quality induced by enriched $CO_2$ environments by consuming more foliage (*18*).

Because reduction in foliar nitrogen is a predominant plant response to enriched $CO_2$ environments (*4*), we suggest that in enriched $CO_2$ environments interactions between plants and their insect herbivores will be modified. In the enriched $CO_2$ atmospheres expected to occur in the future, foliage-feeding herbivorous insects will have to confront poorer quality host plants, which may induce both lengthened larval periods and greater mortality. Plants that grow better in enriched $CO_2$ environments (*3*) might then experience a reduced herbivore load, thereby further increasing their yield. However, in contrast to this phytocentric scenario, those insect herbivores that do survive will consume significantly more foliage than their low $CO_2$–reared ancestors, potentially compensating for their reduced numbers and negating the plants' increased growth. The ramifications of these changes caused by enriched $CO_2$ atmospheres for other natural plant–insect herbivore systems, and potentially at higher trophic levels, warrants further attention if we hope to meet the challenges presented by our future biosphere.

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10. Plants were maintained in growth chambers with 14-hour, 27°C day:10-hour, 25°C night, at 60% relative humidity. Light provided by incandescent and fluorescent bulbs was at approximately 500 $\mu$mol $m^{-2}$ $s^{-1}$. Plants were watered daily and individually fertilized every other week for 6 weeks, and then weekly for 4 weeks with 0.1 g of Peters 15:15:15 NPK water-soluble fertilizer in 100 ml of water. Two growth chambers were used per $CO_2$ treatment, and plants in each treatment were rotated both within and between chambers weekly to minimize chamber and pseudo-replication effects.
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***"Defying the law of gravity, Sarge."***