



## GROWTH OF JUVENILE *ARIANTA ARBUSTORUM* (LINNAEUS, 1758) FED ON WHITE CLOVER (*TRIFOLIUM REPENS* L.) GROWN UNDER AMBIENT OR ELEVATED ATMOSPHERIC CO<sub>2</sub>

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**ABSTRACT:** In many terrestrial ecosystems elevated atmospheric carbon dioxide (CO<sub>2</sub>) may reduce plant quality and change plant-herbivore interactions. The objective of this study was to evaluate the effects of elevated CO<sub>2</sub> (600 ppm vs. 350 ppm) on white clover (*Trifolium repens* L.) and to examine the feeding response and growth of juveniles of the helcid land snail *Arianta arbustorum* (L.). Leaves of *T. repens* grown at elevated atmospheric CO<sub>2</sub> had a lower nitrogen concentration and a lower specific leaf area than leaves grown under ambient CO<sub>2</sub> conditions. However, elevated CO<sub>2</sub> had no effect on leaf carbon concentration. Juvenile snails fed on either diet consumed about the same amount of leaf biomass. The lower leaf nitrogen concentration at elevated CO<sub>2</sub> resulted in a reduced nitrogen uptake. As a consequence, juvenile *A. arbustorum*, fed on *T. repens* grown under elevated CO<sub>2</sub>, tended to grow more slowly than snails fed on *T. repens* leaves produced under ambient CO<sub>2</sub>. This indicates that rising atmospheric CO<sub>2</sub> can indirectly affect the life cycle of terrestrial gastropods.

**KEY WORDS:** *Arianta arbustorum*, Pulmonata, Gastropoda, Helicidae, elevated carbon dioxide, herbivory

### INTRODUCTION

Natural and agricultural ecosystems are being exposed to a rapid increase in atmospheric CO<sub>2</sub> concentration. Furthermore, the accumulation of greenhouse gases is predicted to cause global temperature to rise from about 1 to 4°C over the next decades if emissions continue to increase as expected (IPCC 1996). The experimental and theoretical literature addressing the effects of rising atmospheric CO<sub>2</sub> on plant growth and community structure is growing rapidly (for reviews see BAZZAZ 1990, KAREIVA et al. 1993, POORTER 1993, KÖRNER 1995, KÖRNER & BAZZAZ 1996). Minor changes in nutrient content, plant chemistry, leaf toughness, or moisture could alter the interactions between plants and herbivores, either through feeding intensity or food preferences. An increased supply of carbon resulting from increased photosynthesis at elevated CO<sub>2</sub> has most often been found to increase the ratio of carbon to nitrogen in

plant tissues, but increased tissue concentrations of carbon-based secondary metabolites have also been found (AYRES 1993). There is increasing evidence that plant response to elevated atmospheric CO<sub>2</sub> is species-specific (HUNT et al. 1993, LEADLEY & KÖRNER 1996, LEADLEY & STÖCKLIN 1996). While economically important crops, forest trees, and representative C<sub>3</sub> and C<sub>4</sub> species as model systems have been repeatedly studied (see KÖRNER & BAZZAZ 1996), less attention has been focused on the potential changes in plant-herbivore interactions. Snail breeders are aware that snail growth might be affected by an increase in atmospheric CO<sub>2</sub> concentration, but detailed studies on the response of snails to food plants raised at elevated CO<sub>2</sub> are so far lacking. An exception is the study of LEDERGERBER et al. (1998) which showed changes in the feeding behaviour of juvenile *Helix pomatia* L. kept in model plant

communities grown either at ambient or elevated CO<sub>2</sub> concentrations.

In the present study we examine the feeding behaviour and growth of juveniles of the land snail *Arianta arbustorum* (L.) fed on white clover (*Trifolium repens* L.) raised under ambient or elevated CO<sub>2</sub> conditions. *A. arbustorum* is a simultaneously hermaphroditic land pulmonate snail (family Helicidae) common in moist habitats of north-western and central Europe (KERNEY & CAMERON 1979). Its shell growth is completed after 2–5 hibernations with the formation of a lip at the

edge of the aperture, which also indicates sexual maturity (BAUR & RABOUD 1988). The snails live on average 3–4 years as adults, but lifespans of up to 14 years have been recorded (BAUR & RABOUD 1988). Adult snails measure 16–22 mm in shell breadth. In the field, *A. arbustorum* deposits 1–4 clutches, each containing 20–80 eggs, per reproductive season. The eggs are laid in small holes in the soil or in decaying grass or moss (BAUR 1988). The nearly spherical eggs are approximately 3 mm in diameter (BAUR 1984). Hatching takes place 2–4 weeks after oviposition, depending on temperature.

## MATERIALS AND METHODS

Eggs were obtained from *A. arbustorum* collected in the embankment of a track 7 km SE of Basel (47°30'N, 7°38'E; altitude 380 m a.s.l.). The adult snails were kept singly in transparent plastic containers (14 x 10 x 5 cm) lined with moist soil. The containers were kept in an environmental chamber at 19±1°C and a light/dark cycle of 18:6 h for 1 month. Fresh lettuce was provided ad libitum as food. The containers were checked twice per week for eggs. The eggs were collected, cleaned and incubated in plastic dishes lined with moist paper towelling at 19°C. Newly-hatched snails were separated daily from remaining unhatched eggs to prevent egg cannibalism (BAUR 1992). The hatchlings were kept in families in transparent plastic containers (14 x 10 x 5 cm) lined with moist soil and powdered limestone, and fed on fresh lettuce. During the first two weeks the young snails were kept outdoors at a shaded place and thereafter at room temperature (20–22°C) and natural daylight.

Plants of *T. repens* were raised in climate controlled chambers with natural daylight and a 18/10°C temperature regime at 350 ppm (ambient) and 600 ppm (elevated) CO<sub>2</sub> atmosphere. Plants were obtained by clonal replication. They grew in a mixture of marl and calcareous grassland soil and were regularly watered to field capacity.

After weighing to the nearest 0.1 mg, 6-week-old *A. arbustorum* (shell breadth: 4–6 mm) were placed singly in petri dishes (6.5 cm in diameter) lined with moist soil. Each snail was fed either ambient-CO<sub>2</sub>-(350)-produced leaves ad libitum, or elevated-CO<sub>2</sub>-(600)-produced leaves. Two snails of similar weight (difference < 10%) of the same clutch (family) were randomly assigned to either of the two CO<sub>2</sub> treatments (15 juveniles per treatment). In this way genetic differences of snails between the treatments could be minimised. The experiment was run for 40 days. The food was replaced every 2–3 days. At the same time the leaf area eaten was recorded. For this purpose we photocopied each leaf before and after

Fig. 1. Five leaves of *T. repens* before (upper row) and after (lower row) grazing by juvenile *A. arbustorum*. Scale bar 1 cm



grazing and calculated the difference between the two leaf areas using a computer scanning program (Fig. 1).

For both treatments we used regression equations between leaf area and leaf dry weight to estimate the biomass consumed by snails. At the beginning of the experiment, specific leaf area (SLA = leaf area [in cm<sup>2</sup>] per dry weight of leaf biomass [in g]; N = 12 leaves), and the concentrations of carbon and nitro-

gen (N = 5 leaves) were determined for single leaves of *T. repens* from both treatments. At the end of the experiment, the snails were placed in containers with saturated air moisture for one night, after which they were re-weighed. This treatment was necessary to allow the snails to reach the same level of water saturation as they had at the beginning of the experiment.

## RESULTS

Single leaves of *T. repens* grown at elevated CO<sub>2</sub> did not differ in size from those grown at ambient CO<sub>2</sub> (elevated CO<sub>2</sub>: 0.91±0.05 cm<sup>2</sup> [mean ± S.E.]; ambient CO<sub>2</sub>: 1.01 ± 0.05 cm<sup>2</sup>; t = 1.56, df = 168, P = 0.12). However, the nitrogen concentration in the leaves was 16.6% lower at elevated CO<sub>2</sub> than in the leaves raised at ambient CO<sub>2</sub> (Table 1). In contrast, the leaf carbon concentration did not differ between the two treatments (Table 1). Specific leaf area (SLA) of *T. repens* raised at elevated CO<sub>2</sub> was 16.4% lower than in *T. repens* grown under ambient CO<sub>2</sub> (Table 1).

Juveniles of *A. arbustorum* fed on leaves produced at elevated CO<sub>2</sub> grazed a smaller leaf area than those

fed on plants raised at ambient CO<sub>2</sub> (Table 1). However, the amount of biomass consumed was similar in both groups. Due to the reduction in leaf nitrogen concentration at elevated CO<sub>2</sub>, the snails feeding on that diet received less nitrogen than the snails feeding on leaves produced at ambient CO<sub>2</sub>. As a consequence, the snails fed on leaves produced at elevated CO<sub>2</sub> tended to grow more slowly than those fed on leaves produced at ambient CO<sub>2</sub>. In both groups, the weight increase of individual snails was positively correlated with the amount of plant material consumed (ambient CO<sub>2</sub>: r = 0.89, N = 15, P < 0.0001; elevated CO<sub>2</sub>: r = 0.75, N = 15, P = 0.0009).

Table 1. Comparison of *T. repens* leaves raised at two CO<sub>2</sub> concentrations and the response of juvenile *A. arbustorum* fed on either of the two diets for 40 days. Data are means ±SE, with sample size in parentheses. P-values result from unpaired t-tests. Percentage values were arcsine-transformed for statistical analysis

Variable	Ambient CO <sub>2</sub> (350 ppm)	Elevated CO <sub>2</sub> (600 ppm)	t	P
nitrogen concentration of <i>T. repens</i> (% dry matter)	3.56±0.20 (5)	2.97±0.14 (5)	2.47	0.039
carbon concentration of <i>T. repens</i> (% dry matter)	43.95±0.54 (5)	44.75±0.22 (5)	1.39	0.202
specific leaf area (SLA; cm <sup>2</sup> g <sup>-1</sup> )	274±16 (12)	229±12 (12)	2.27	0.033
leaf area grazed (cm <sup>2</sup> )	6.94±0.54 (15)	5.00±0.41 (15)	2.84	0.008
biomass (dry weight) consumed (mg per snail)	27.03±2.17 (15)	23.94±2.07 (15)	1.03	0.311
nitrogen consumed (mg per snail)	0.96±0.08 (15)	0.71±0.06 (15)	2.56	0.016
relative weight increase of snails (%)	137.0±13.0 (15)	112.9±13.5 (15)	1.45	0.158

## DISCUSSION

The present study showed that a reduction in leaf nitrogen concentration at high CO<sub>2</sub> can lead to a slower growth, thus confirming results of studies on insects (for reviews see LINCOLN et al. 1993, WATT et al. 1995). Measurements of juvenile growth should provide reliable estimates of net food quality. Faster

growth rates are likely to reflect more favourable cost-benefit ratios of different food types to an animal. Plant tissue quality, particularly nitrogen concentration and digestibility, are the main factors influencing gastropod growth and reproduction (ROLLO & HAWRYLUK 1988, ROLLO & SHIBATA 1991). Gastro-

Pods commonly supplement their nitrogen-poor diets with faeces, carrion and (rarely) living animals (FRÖMMING 1954). Leaves of plants grown at elevated CO<sub>2</sub> concentrations often have lower nitrogen concentrations than plants grown at ambient CO<sub>2</sub> (BAZZAZ 1990, KÖRNER & MIGLIETTA 1994). Our data seem to confirm this general pattern. The decrease in plant nitrogen concentration may also result in consumption of greater amounts of plant material grown under elevated CO<sub>2</sub> conditions (LEDERGERBER et al. 1998, LINCOLN et al. 1993, WATT et al. 1995). In the present study, however, juvenile snails consumed smaller areas of *T. repens* leaves grown under elevated CO<sub>2</sub> conditions. Due to the lower specific leaf area of *T. repens* at elevated CO<sub>2</sub>, the snails from both groups consumed a similar amount of biomass. Thus, the snails did not compensate for the lower nitrogen concentration in *T. repens* raised at elevated CO<sub>2</sub> by eating larger leaf areas. It is possible that the amount of food that can pass through the digestive tract is limited in juvenile *A. arbustorum*. This hypothesis needs testing.

*T. repens* and other legume species produce cyanogenic glucosides as a deterrent to herbivory

(JONES 1973). However, there is substantial variability in the quantity of cyanogenic glucosides produced by *T. repens* and in the tolerance of gastropods to these compounds (SOUTH 1992). In the present study juvenile *A. arbustorum* appeared to be quite tolerant to this plant defence compound.

In the long term, plant species may respond to environmental changes such as elevated atmospheric CO<sub>2</sub> by altering the structure and composition of tissue, shifting in abundance and distribution, by going extinct, or by evolving. Predicting the kind of response is difficult. There are almost no species for which we know enough relevant ecology, physiology and genetics to predict their evolutionary response to environmental changes. There is even more uncertainty with respect to possible changes in plant-herbivore interactions and in the responses of herbivores to environmental change. Our results indicate a negative effect of rising atmospheric CO<sub>2</sub> on snail growth which could change the life cycle.

## ACKNOWLEDGEMENTS

We thank C. KÖRNER for allowing us to use climate chambers and J. ARNONE for comments on the manuscript. Financial support was received from the Swiss

National Science Foundation (Priority Programme Environment, IP Biodiversity, grant 5001-44620).

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received: June 10th, 1999

accepted: September 27th, 1999

