

# Response of beech (*Fagus sylvatica*) to elevated CO<sub>2</sub> and N: Influence on larval performance of the gypsy moth *Lymantria dispar* (Lep., Lymantriidae)

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**Abstract:** Two-year-old beech seedlings were kept from germination to bioassays with *Lymantria dispar* under the following conditions: ambient CO<sub>2</sub>/low N, elevated CO<sub>2</sub>/low N, ambient CO<sub>2</sub>/elevated N, and elevated CO<sub>2</sub>/elevated N. The effect of these growing conditions of the trees on the performance of the defoliator *L. dispar* was studied 2 years after initiating the tree cultivation. The developmental success of third-instar larvae of *L. dispar* was characterized by the weight gained, percentage of weight gain, relative growth rate (RGR), relative consumption rate (RCR), and efficiency of conversion of ingested food into body substance (ECI). Contrary to our expectations, additional N-fertilization did not increase and elevated CO<sub>2</sub> did not delay larval growth rate. However, the environmental treatments of the beech seedlings were found to affect the larval performance. Larvae consumed significantly higher amounts of foliage (RCR) on beech trees under controlled conditions (ambient CO<sub>2</sub> and low N) compared to those under elevated CO<sub>2</sub> and enhanced N. The opposite was true for ECI. The lowest efficacy to convert consumed food to body substance was observed under control conditions and the highest when the larvae were kept on beech trees grown under elevated CO<sub>2</sub> and additional N-fertilization. These opposite effects resulted in the weight gain-based parameters (absolute growth, percentage of growth, and RGR) of the gypsy moth larvae remaining unaffected. The results indicate that the gypsy moth larvae are able to change their ECI and RCR to obtain a specific growth rate. This is discussed as an adaptation to specific food qualities.

## 1 Introduction

The quality of leaves as food for phytophagous insects is affected by nutrients as well as by secondary metabolites (SCRIBER and SLANSKY, 1981; STRONG et al., 1984). Numerous studies have addressed the direct effects of CO<sub>2</sub> (LINDROTH et al., 1993, 1997; ROTH and LINDROTH, 1995; KINNEY et al., 1997; AGRELL et al., 2000) and N (BÜTTNER, 1956; OLDIGES, 1960; MATTSON, 1980; BRYANT et al., 1987; KYOTO et al., 1996; HÄTTENSCHWILER and SCHAFFELLNER, 1999) on tree physiology and on the synthesis of nutrients and secondary metabolites.

According to the CNB-theory, BAZAZZ et al. (1987) and TOUMI et al. (1988) hypothesized that elevated atmospheric CO<sub>2</sub> enhances the photosynthetic activity of plants. The surplus of assimilated carbon will be directed mainly to the metabolism of carbon-rich compounds, especially to the synthesis of secondary plant products. Accumulation of secondary metabolites is mainly responsible for a reduction in nutritional food quality which adversely affects the developmental success of herbivorous insects (SOO HOO and FRAENKEL, 1966). As a result of elevated CO<sub>2</sub>, the resistance of plants to pathogens and herbivorous insects would be expected to increase.

However, increased N-fertilization is expected to enhance plant growth by stimulating primary metabolism. In this case the concentration of proteins should increase and the plants should be more susceptible to

pathogens and herbivores. Fertilization with nitrogen increases the protein concentrations of leaves. Subsequently, concentrations of carbohydrates and carbon-based secondary plant metabolites in leaves decline (BRYANT et al., 1983). All these changes in the leaf chemistry might also affect the growth of leaf-feeding insects.

This paper aims to investigate the effects of elevated CO<sub>2</sub> and/or N on the performance of a defoliating insect. Our experimental system consisted of the tree species beech (*Fagus sylvatica*) and the phytophagous insect species, gypsy moth (*Lymantria dispar*). Because of the above-mentioned theories, the following three hypotheses were tested in bioassays: (1) trees grown under elevated atmospheric CO<sub>2</sub> will adversely affect larval performance; (2) additional N-fertilization of the trees will enhance growth rates of the larvae; and (3) the effects of elevated CO<sub>2</sub> and high N will compensate each other. Thus, trees grown under ambient CO<sub>2</sub>/low N or elevated CO<sub>2</sub>/elevated N should have the same impact on larval performance.

## 2 Materials and methods

### 2.1 Beech seedlings

Groups of 12 beech saplings were grown in one container of size 60 × 40 × 40 cm. The trees had been kept from

germination to bioassay continuously in glasshouse compartments under an ambient (350 ppm) or a standardized elevated  $\text{CO}_2$  atmosphere (700 ppm). Plants were sufficiently watered throughout the study and fertilized with a standardized Hoagland solution (HOAGLAND and ARNON, 1950) ( $4 \text{ l/m}^2$  soil surface of the container) which was applied once every 2 weeks. In experiments with enhanced nitrogen,  $\text{NH}_4\text{NO}_3$  was added to the nutrient solution to achieve a final amount of  $5 \text{ g N/m}^2$  surface. The following four experimental  $\text{CO}_2/\text{N}$ -regimes were run: (1) ambient  $\text{CO}_2$ /low N; (2) elevated  $\text{CO}_2$ /low N; (3) ambient  $\text{CO}_2$ /elevated N; and (4) elevated  $\text{CO}_2$ /elevated N.

## 2.2 Insects

*L. dispar* larvae were reared from egg masses provided by the USDA/APHIS Otis Method Development Center, Cape Cod, MA, USA. The insects were fed on a high wheatgerm diet (BELL et al., 1981) and raised in growth chambers at  $24^\circ\text{C}$ , 70% RH, photoperiod 16 h light: 8 h dark.

## 2.3 Bioassays

Gypsy moth larvae were reared in the same compartments and conditions as the beech saplings. Prior to the initiation of the bioassays, transparent plastic cages ( $10.5 \times 5 \times 8 \text{ cm}$ ) were attached to twigs so that 3–4 leaves were enclosed inside each cage. Ventilation of the cages was ensured by windows that were cut in two of the side walls and covered with fine nylon mesh gauze. Since the caged leaves were provided as larval food, it was necessary to calculate their dry matter by non-destructive methods. Therefore, their maximum length and width were measured and the dry mass was estimated by a regression model which was developed and validated before commencing the bioassays. The regression equation was  $y = 2037 \times 10^{-2} \times l \times w$ , where  $y$  = dry mass of the leaf,  $l$  = maximum length and  $w$  = maximum width of the fresh leaf. The model fits with  $r^2 = 0.92$ .

Freshly moulted third-instar larvae were weighed and then transferred individually into a cage. The larvae were checked daily and, in case of future food shortage, caged on new twigs which were prepared as described above. The bioassays were completed after 312 h. The remaining leaf material on the enclosed twigs and in the bottom of the cages was collected, dried ( $70^\circ\text{C}$ , 24 h) and then weighed. The larvae were weighed to record their growth during the 312 h of the experiment. In each  $\text{CO}_2/\text{N}$ -regime 16 replicates were performed.

## 2.4 Calculations

The differences between the calculated dry masses before and after the experiment were used as a measure of the amount of food eaten. The following growth indices were calculated according to WALDBAUER (1968): relative consumption rate [ $\text{RCR} = \text{dry weight of food consumed}/(\text{days of period} \times \text{mean weight of larva during the period})$ ]; relative growth rate [ $\text{RGR} = \text{weight gain of larva during period}/(\text{days of period} \times \text{mean weight of larva during the period})$ ]; and efficiency of conversion of ingested food [ $\text{ECI} = 100 \times \text{biomass gained}/\text{food consumed}$ ].

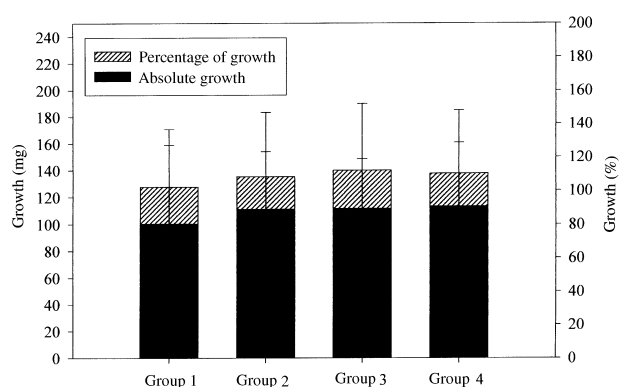
Statistical processing of the results was carried out with the computing program SPSS for Windows 9.0 (SPSS Inc., Chicago, IL, USA). The Gaussian distribution of all data was tested using the test of Lilliefars. The equality of variances of two means was tested using the Levene test. Significant differences between means of normally distributed data were tested using the ANOVA and LSD test, those with

unequal variances were tested using the Dunnett  $C$ -test. Significant differences between two means of the groups were tested using Student's  $t$ -test or the Mann-Whitney  $U$ -test, depending on the equality of variances. Significance was defined with  $P < 0.05$ .

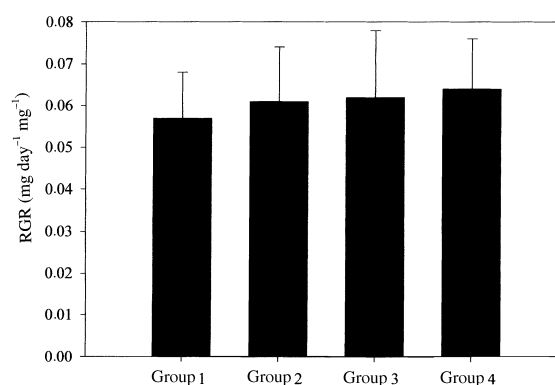
## 3 Results

### 3.1 Larval weight gain

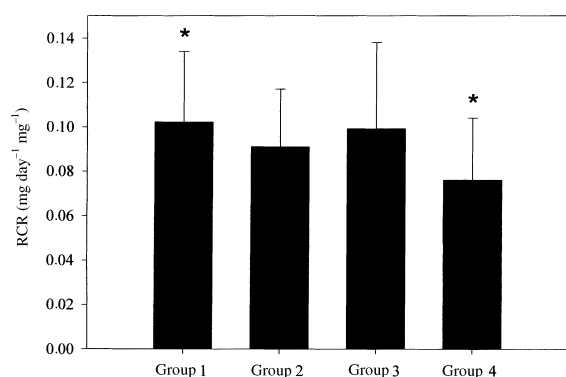
Figure 1 shows the weight gained (mg) of the larvae during the investigation period of 312 h. Larvae fed on beech saplings treated with N of the pure Hoagland solution and ambient  $\text{CO}_2$  showed the lowest weight gain, but the differences between the four groups are not significant according to the ANOVA. The same is true for the percentages of total growth of the larvae (fig. 1). With regard to the relative growth rates, no significant effects of the  $\text{CO}_2$  and N treatment of the beech saplings were observed (fig. 2). Thus, the four combinations of  $\text{CO}_2$  and N treatments had no influence on the growth of the gypsy moth larvae.



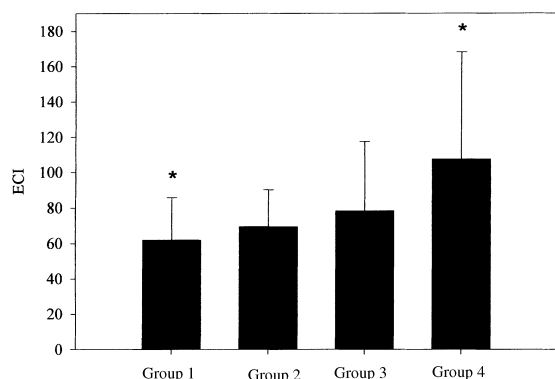
**Fig. 1.** Percentage and absolute weight gained of gypsy moth larvae after feeding on  $\text{CO}_2$ - and N-treated beech saplings. Group 1, ambient  $\text{CO}_2$ -low N; Group 2, elevated  $\text{CO}_2$ -low N; Group 3, ambient  $\text{CO}_2$ -elevated N; Group 4, elevated  $\text{CO}_2$ -elevated N



**Fig. 2.** Relative growth rates (RGR) of gypsy moth larvae after feeding on  $\text{CO}_2$ - and N-treated beech saplings. Groups as indicated in fig. 1



**Fig. 3.** Relative consumption rate (RCR) of gypsy moth larvae after feeding on CO<sub>2</sub>- and N-treated beech saplings. Groups as indicated in fig. 1. Asterisks indicate significantly different means on the  $P < 0.05$  level according to ANOVA and LSD



**Fig. 4.** Efficiency of conversion of ingested food to body substance (ECI) of gypsy moth larvae after feeding on CO<sub>2</sub>- and N-treated beech saplings. Groups as indicated in fig. 1. Asterisks indicate significantly different means on the  $P < 0.05$  level according to ANOVA and LSD

### 3.2 Larval RCR and ECI

Between the four groups, there was no significant difference in the total amount of material eaten. However, the relative consumption rate was significantly higher when the larvae fed on beech saplings were treated with low N and ambient CO<sub>2</sub> compared to those with elevated N and CO<sub>2</sub> (fig. 3). The opposite physiological stage was observed with regard to the efficiency of conversion of the ingested food to body substance (ECI- value). Larvae kept on saplings fertilized exclusively with Hoagland solution and grown under ambient CO<sub>2</sub> transferred the eaten foliage less efficiently to the body substance than those on trees under elevated N and CO<sub>2</sub> (fig. 4).

## 4 Discussion

Based on general biological observations, it would be expected that increasing the concentration of nitrogen in leaves would enhance the developmental success of herbivorous insects (MATTSON, 1980; SCRIBER and

SLANSKY, 1981; WHITE, 1984, STRONG et al., 1984). However, as reported in the review by KYOTO et al. (1996), phytophagous forest insects do not respond to plant-derived nitrogen in a regular manner. Foliage of nitrogen-fertilized quaking aspen-fed larvae of the large aspen tortrix (*Choristoneura conflictana*) increases the weights of pupae (BRYANT et al., 1987). On the contrary, larvae of the nun moth, *Lymantria monacha*, fed needles of pines on poor, sandy soils but fertilized with nitrogen suffered higher mortality compared to those fed needles from untreated control trees (BÜTTNER, 1956). This observation reflects the low preference of *L. monacha* for these treated pines (OLDIGES, 1960). Pine needles from N-fertilized stands caused lower cocoon weights in *Neodiprion sertifer* compared to corresponding controls (LARSSON and TENOW, 1984). In the present study sole N-fertilization of the beech saplings does not significantly affect any of the measured developmental parameters of gypsy moth larvae.

To assess the impact of CO<sub>2</sub>-mediated alteration of foliage on herbivores, it is usual to conduct short-term studies on growth rates, consumption rates and food processing (LINDROTH, 1996) as we did. Lack of sufficient CO<sub>2</sub>-treated plant material normally prevents large-scale bioassays. The scarce information available to date shows a slight general trend in the insect's response to high-CO<sub>2</sub> foliage which depends on the insect species and especially on the corresponding host tree species (LINDROTH, 1996). The most pronounced impact of CO<sub>2</sub>-altered leaves is observed with pioneer tree species, such as quaking aspen (*Populus tremuloides*), causing a significant decrease in the growth rate of the forest tent caterpillar (*Malacosoma disstria*) and gypsy moth *L. dispar*. Foliage from slower growing tree species affects the weight gain of herbivores inconsistently. Whereas high-CO<sub>2</sub> leaves from red oak (*Quercus rubra*) stimulate the growth rate of *L. dispar*, those from a typical climax tree species sugar maple (*Acer saccharum*) remained without any effect (LINDROTH, 1996). The latter result was confirmed in our study in which foliage from CO<sub>2</sub>-treated beech seedlings had no effect on the growth of *L. dispar*. Understanding the tree species investigated by the LINDROTH group as an ecological sequence from the pioneer species quaking aspen to the climax species sugar maple, the present study, including the Central European climax species beech, agrees with the above order where beech corresponds to sugar maple.

The treatment of the beech saplings altered the nutritional quality in an unexpected manner. Whereas the enhancement of a single factor, CO<sub>2</sub> or N, caused only less pronounced effects on the larval development, the combination of both revealed significant impacts. Gypsy moth larvae which fed on beech saplings treated with elevated N and CO<sub>2</sub> had a lower relative consumption rate (RCR) but higher efficiency of conversion of ingested food to body substance (ECI) than the untreated group, with no difference in their growth. This means they ingested less but converted a higher percentages of the ingested food to their body substances, obtaining

the same weight as the larvae in the other groups. A lower feeding activity of phytophagous insects on N-treated trees was also found by BÜTTNER (1956), whereas LINDROTH et al. (1993) reported a higher activity of *L. dispar* larvae fed on CO<sub>2</sub>-treated aspen. However, feeding activities might reflect the quality of the food. Larvae on food of poor quality or additional toxic character might feed less than those on rich food.

Phenolic compounds induced by elevated CO<sub>2</sub> lower the nutritional quality of leaves and result in a decrease in the efficiency of digesting nutrients (SOO HOO and FRAENKEL, 1966). The results presented show that, within a certain range, *L. dispar* larvae are able to compensate for alterations of the nutritional quality by ingesting less food but increasing the efficiency to convert it to body substance. On the one hand, this mechanism prevents the larvae from incorporating too many harmful substances (phenolic compounds) but keeps them at the normal growth rate. JAGANADH and NAIR (1996) obtained similar results in *Spodoptera mauritia* and RAPS and VIDAL (1998) in the diamond-back moth *Plutella xylostella*; both species were able to compensate for food quality by altering food utilization values in order to maintain a specific growth rate. On the other hand, an elevation of nitrogen and CO<sub>2</sub> might have increased the quality of the beech leaves by increasing the amount of nutrients and/or carbohydrates (BRYANT et al., 1983). Thus, the larvae did not need to consume as much leaf material as the others to maintain the 'normal' growth rate. SHEPPARD and FRIEDMAN (1992) found that an increase in food quality resulted in an increased ECI of gypsy moth larvae, whereas the RGR remained the same, which corroborates the investigations in the present study. When gypsy moth larvae do not change their ECI to compensate for specific food qualities they are not able to regulate their growth and thus their weight gained decreased after feeding on decreased food qualities (STOCKHOFF, 1992). This regulation through the ECI and RCR enables the gypsy moth to compensate for the lack of growth due to infections from pathogens or parasites, such as a parasitic protozoa (*Vairimorpha* spec., Microsporidia, Burenellidae) (HENN and SOLTER, 2000). It seems that a change in the ECI and RCR might indeed be a mechanism by which the gypsy moth larvae is able to react to specific food qualities or difficult developmental conditions and to regulate its growth to a specific level.

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