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# Elevated CO2 Concentration Promotes Tomato Plant Growth but Impairs Spodoptera litura Performance

Papitchaya Teawkul, Wei-Ting Chen, Yuwatida Sripontan, Shaw-Yhi Hwang*

Department of Entomology, College of Agriculture and Natural Resources, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan

## Research Article

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***For Correspondence**

Department of Entomology, College of Agriculture and Natural Resources, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 402, Taiwan, Tel: +886 422840363; Fax: +886 4 22875024

Email: oleander@dragon.nchu.edu.tw

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**ABSTRACT**

Atmospheric $CO_2$ concentration increased throughout the last century and is expected to continue increasing in the future. Studies on the effects of elevated $CO_2$ concentration on insect herbivores have primarily focused on perennial trees, but relatively little is understood about the effects of elevated $CO_2$ concentration on crop plants and subsequently on their insect herbivores. This study assayed the effects of elevated $CO_2$ concentration on tomato plants growth and the tobacco caterpillar *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) fed on tomato plants. Our results demonstrated that the growth parameter and nutritional quality of tomato plants were altered by elevated $CO_2$ concentration; the tomato plants showed an increase in biomass and defense protein activities and reduced leaf nitrogen content and changes in secondary compound contents. Furthermore, S. *litura* larvae, which fed on tomato plants grown at elevated $CO_2$ concentration, had considerably decreased growth performance. In summary, elevated $CO_2$ concentration can strongly influence interactions between plants and insects and could have critical impacts on agro ecosystems.

## INTRODUCTION

Climate change is now widely recognized as a major environmental problem that affects biological and ecological process. Increased anthropogenic emissions caused primarily by the combustion of fossil fuels considerably elevate global $CO_2$ concentration[1]. The Intergovernmental Panel on Climate Change (IPCC) third assessment report stated that atmospheric $CO_2$ concentration has constantly and markedly increased from 280 ppm in 1750 to 367 ppm in 1990 and is expected to reach 550–990 ppm in 2100[2,3].

Increasing atmospheric $CO_2$ concentrations are expected to exert significant effects on plant physiology, chemistry, and morphology[4,5]. Studies have revealed that responses of plant growth at an elevated $CO_2$ concentration generally include an increase in the plant growth rate and a characteristic increase in the concentration of leaf carbohydrates and a decrease in the nitrogen content, thus causing a shift in the C:N balance[6,7]. Although the effects of elevated $CO_2$ concentration on plants are variable, plants growth often exhibit enhanced photosynthetic activity and increased productivity[8]. According with biomass produced of aspen oak trees and maple trees[9]. Studies have shown that elevated $CO_2$ concentration also influences the quality and quantity of plant secondary compounds[10-12]. Changes in nutrient availability alter trypsin inhibitor (TI) and polyphenol oxidase (PPO) activities in plants[13]. Previous studies also showed that phenolic contents in plants increase when the plants are grown at an elevated $CO_2$ concentration and this increase in phenolic content can have a negative effect on insects[14,15]. Phytochemical changes are major factors that determine the value of a plant as a food source to herbivorous insects and could have crucial implications for food security and natural ecosystems[16]. Phytophagous insects are primary consumers that can directly alter the nutritional properties of the host plant[13,16]. Variations in plant carbon and nitrogen content have been shown to have significant

effects on insect herbivores. The effects of elevated $CO_2$ concentration on insects have been widely studied, revealing various responses in insects[9]. Typically, the performance of phytophagous insects is negatively affected by the low nutritional quality of plant tissues at an elevated $CO_2$ concentration, and these insects consume more foliage to compensate for the nutritional quality[17,18]. A field experiment examined the effects of elevated $CO_2$ concentration on plant primary and secondary chemistry in a scrub-oak community as well as the consequences for herbivore performance, characterized by increased relative consumption rates and development time and decreased relative growth rate[19].

Numerous studies have focused on the effects of elevated $CO_2$ concentration on insect herbivores, particularly those found on deciduous tree species[9,20-22], and numerous studies have been conducted in temperate regions[23-26]. However, relatively little is known about the effects of elevated $CO_2$ concentration on subtropical crop plants and subsequently on their insect herbivores. The objective of this study was to investigate the effects of elevated $CO_2$ concentration on plant performance and photochemistry and on the performance of subtropical insects.

## MATERIALS AND METHODS

### Plants

The present study was conducted under controlled greenhouse conditions (temperature 26 ± 2°C, 16L:8D photoperiod) from the fall of 2013 to January 2014. Tomato (Lycopersicon esculentum Mill. Var. cerasiforme (Dunal) Alef.) seeds were bought from a local seed company (Known-You Seed Co., Ltd., Kaohsiung, Taiwan) and soaked in warm water (50°C) for 20 min for surface sterilization. The seeds were then sown in 104-well pot trays (22 cm × 14.5 cm) with commercial potting soil (Known-You Seed Co., Ltd.). These pot trays were then placed in a greenhouse chamber at ambient or elevated $CO_2$ concentration. Three weeks after seed germination, the seedlings were transplanted into plastic pots (diameter, 12 cm; height, 10.5 cm) with field soil. A commercial synthetic fertilizer (50 mL/pot, concentration 0.5 g/1000 mL, N:P:K=20:20:20, Hyponex) was added weekly to each plant, and the plants were watered daily. In this experiment, two $CO_2$ concentrations were maintained in greenhouse chambers: (i) ambient $CO_2$ (450 ± 10 ppm, corresponding to the background atmosphere entering the greenhouse chamber) or (ii) elevated $CO_2$ (900 ± 5 ppm, double the ambient $CO_2$ concentration). $CO_2$ concentrations were monitored inside each chamber to check the $CO_2$ concentrations, and the data log was checked every other week.

Three weeks after transplantation, the tomato plants were used for bioassays. For plant growth performance analysis, 30 plants were randomly selected from each chamber and harvested. These plants were cut from the hypocotyls and recorded for the fresh weight. In addition, leaf areas were measured during sampling. All leaves from each sampled plant were removed using scissors and the leaf area was measured using the portable area meter (Model LI-3000A, LI-COR, Lincoln, NE, USA). Subsequently, the above-ground tissue of each sampled plant was placed in a paper bag and oven-dried at 45°C for 2 weeks. The dry weight of each sample was measured.

### Insects

In this study, *Spodoptera litura* (Fabricius) larvae were used for the insect feeding trial. The larvae were collected from a field in Taichung, Taiwan and were maintained at the insect–plant interaction laboratory of National Chung Hsing University. S. *litura* eggs were placed in plastic cups (10-cm width × 5.5-cm length) and were covered by tissue papers upon hatching. In the laboratory, all larvae were fed with an artificial diet[27]. The larvae were transferred into 30 holes tray individually before the fifth instar and reared until pupation. Pupae were sterilized using 1.31% bleach for 15 min and rinsed using water. The pupae were placed in plastic cups until eclosion. Adults were transferred into a cylinder (14-cm width × 21.5-cm length) and fed with a honey solution modified from Yadav et al.[27] until they laid eggs. During the bioassays, the insects were reared under controlled conditions in a growth chamber (temperature 25 ± 1°C, 12L: 12D photoperiod).

### Short-Term Feeding Trial

For the insect feeding trial, the second instar larvae of S. *litura* were individually transferred to petri dishes (5-cm diameter × 1-cm height) before molting to the third instar. After starving for 12 h, third instar larvae were weighed. Larvae with a similar size were used in the bioassays. Overall, 90 larvae were selected. Among these larvae, 30 larvae were initially frozen at -20°C for 24 h and oven-dried at 45°C for 48 h to count the larval water content to later calculate the initial dry weight of the larvae used for the bioassays. Then, 30 plants from both elevated $CO_2$ and ambient $CO_2$ concentration chambers were freshly cut at the sixth leaves (counted from above the hypocotyls). Each leaf was placed in a microtube (2 mL; Scientific Specialties Inc., Lodi, CA, USA) and filled with water and covered with parafilm for retaining the moisture of the leaf. The remaining 60 larvae were used for the insect feeding performance trial, and each larva was placed into a petri dish (9-cm diameter × 1.5-cm height) containing fresh foliage from respective $CO_2$ chambers. The feeding trial was conducted for 46 h in the growth chamber (25 ± 1°C, 12L: 12D photoperiod), and all of the larvae were then immediately separated. The larvae were frozen at -20°C for 24 h and were oven-dried at 45°C for 2 weeks to obtain the final dry weight. The growth parameters were calculated following the methods of Waldbauer[28]: Relative growth rate (RGR)=[(Final dry weight of insect-Initial dry weight of insect)/Initial dry weight of insect]/Duration.

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## CHEMICAL ANALYSES

Foliage of tested plants was collected for chemical analysis during the bioassays. Foliar nutritional and defense compounds were analyzed from the foliage. Fifteen plants were used for each treatment. The sixth leaf from each plant was collected for analyzing defense protein, trypsin inhibitor (TI), and polyphenol oxidase (PPO) activities. In addition, the remaining foliage was collected for determining foliar nitrogen, total nonstructural carbohydrate (TNC), and total phenolic contents.

The TI assay was performed according to the method of Rodriguez-Saona et al.[29]. Leaf tissues were ground in liquid nitrogen and homogenized in a Tris–HCl buffer (50 mM, pH 7.8), and the supernatant of the leaf extract was centrifuged at 12,000 rpm at 4°C for 20 min. For the TI assay, three sets (sample, blank, and standard) were prepared. The standard set was prepared with double-distilled water (DDW), trypsin solution (0.8 mg/mL, 0.25 mM HCl) (trypsin; Sigma), and heated 2% casein solution (100°C and 15 min, 10 mM pH 7.6 phosphate buffer) and was incubated at 37°C for 20 min. The sample set was prepared with DDW, clarified leaf extract, and heated 2% casein solution and was incubated at 37°C for 20 min. After adding the trypsin solution, the sample set was incubated again at 37°C for 20 min. The blank set was prepared with DDW, clarified leaf extract, and heated 2% casein solution and was incubated at 37°C for 20 min. Additional DDW was added and the blank set was incubated again at 37°C. The reactions were stopped with a trichloroacetic acid solution (TCA, 10% w/v $H_2O$). Enzyme activity was calculated using the following equation: [(OD280 of standard + OD280 of blank - OD280 of sample)/OD280 of standard]×100%. PPO activity was determined using extract of the sixth leaf[30-33]. Leaf samples were ground in liquid nitrogen and homogenized in a grinding buffer (pH 7.0 K-P containing 7% (w/v) polyvinylpolypyrrolidone). A 10% solution of triton X-100 was added to the homogenate, which was centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant was used to determine PPO activity. Bovine serum albumin was used as a standard[34]. PPO activities were reported as ΔOD470 min−1 mg fresh weight−1[35]. Nitrogen content was determined using a micro-Nesslerization technique[36]. The foliage was freeze-dried, frozen in liquid nitrogen, and subsequently ground to powder, and then subjected to acid-digestion[37]. Glycine p-toluenesulfonate (5.665%) was used as a nitrogen standard. For the TNC content analysis, starch was first extracted and enzymatically converted to glucose. A glucose–peroxidase system was used with 4-aminophenazone as the oxygen acceptor[38]. Total phenolic contents were determined using the Folin–Ciocalteu method[39]. Phenolics were extracted with $CH_3OH$, and the phenolic content in the extract was then determined using a spectrophotometer with gallic acid as a standard. The mean and standard error (SE) for all chemical contents were calculated.

## STATISTICAL ANALYSES

The mean and SE was calculated for plant growth (fresh weight, dry weight, and leaf area), insect performance (growth rate), and plant chemical components (PPO, TI, total nitrogen, TNC, and total phenolic content). The effects of $CO_2$ concentrations on larval performance and their host plant were analyzed using the t-test, SAS for Windows version 9. Inc., Cary, NC, USA was used to analyze the differences between the mean values of each treatment.

## RESULTS

In this study, the effects of elevated $CO_2$ on tomato plant growth (foliar fresh weight, dry weight, and leaf area) was analyzed, and the results indicated that foliage fresh and dry weights of elevated $CO_2$ concentration-treated plants were higher than those of ambient $CO_2$ concentration-treated plants **(Figure 1A, B).** In addition, the leaf area was larger for elevated $CO_2$-treated plants **(Figure 1C).**

Phytochemistry results revealed that foliar nitrogen content decreased markedly with elevated $CO_2$ concentration compare with ambient $CO_2$ **(Figure 2A).** By contrast, TNC content was not significantly affected by $CO_2$ concentration **(Figure 2B).**

Results of plant defense protein analysis showed that TI activity was significant higher at an elevated $CO_2$ concentration **(Figure 3B).** However, PPO activity and total phenolic contents were not significantly at an elevated $CO_2$ concentration **(Figure 3A, C).**

Results of the insect performance assay showed that the performances of S. *litura* larvae were significantly different between the $CO_2$ treatments. The growth rate was much lower in larvae fed on elevated $CO_2$ concentration-treated foliage than in larvae fed on ambient $CO_2$ concentration-treated foliage **(Figure 4).**

## DISCUSSION

Enhanced plant growth performance at an elevated $CO_2$ concentration has been recorded for various crop plants, such as peanut[11], tobacco[40], and rice[41]. Our study results demonstrated that tomato plant growth was affected by $CO_2$ concentration. Tomato plants grew faster at an elevated $CO_2$ concentration; thus, both the above-ground weight and leaf area increased at an elevated $CO_2$ concentration. In addition to our study, Ferris et al.[42] showed that leaf areas are sensitive to $CO_2$ concentration, suggesting that the effects of $CO_2$ concentration on leaf area are necessary for driving enhanced biomass production. Increased photosynthesis efficiency at an elevated $CO_2$ concentration may be responsible for the enhanced biomass production. Ainsworth and Rogers[43] showed that elevated $CO_2$ concentration could enhance photosynthesis efficiency and reduce stomatal conductance of plants.

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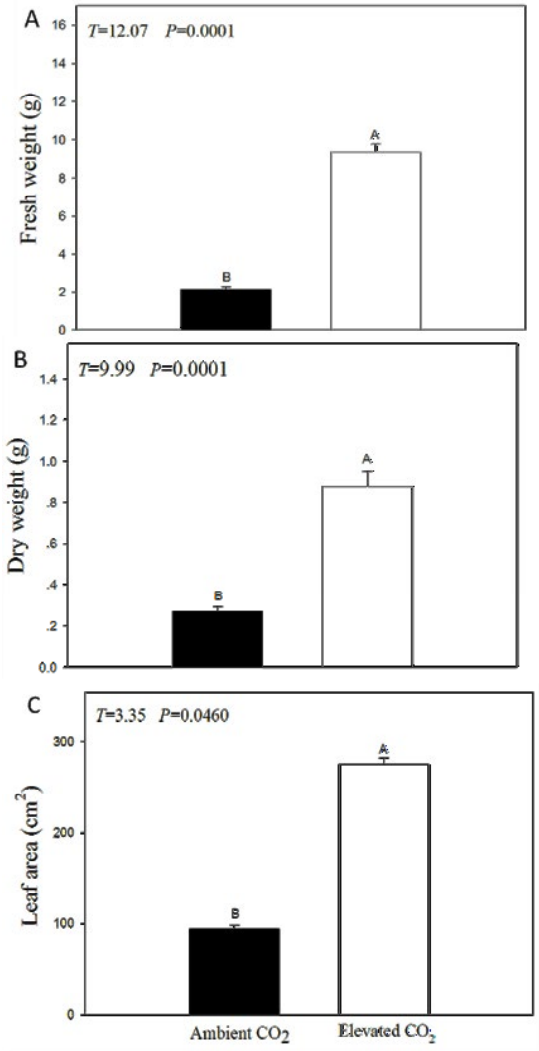


**Figure 1.** Growth performance of tomato plants grown under ambient (450 ppm) and elevated (900 ppm) $CO_2$ conditions (A) Fresh weight, (B) dry weight, (C) leaf area. Mean ± SE (n = 15). Bar with different letters mean significant differences between treatments (Tukey's test, $P < 0.05$).

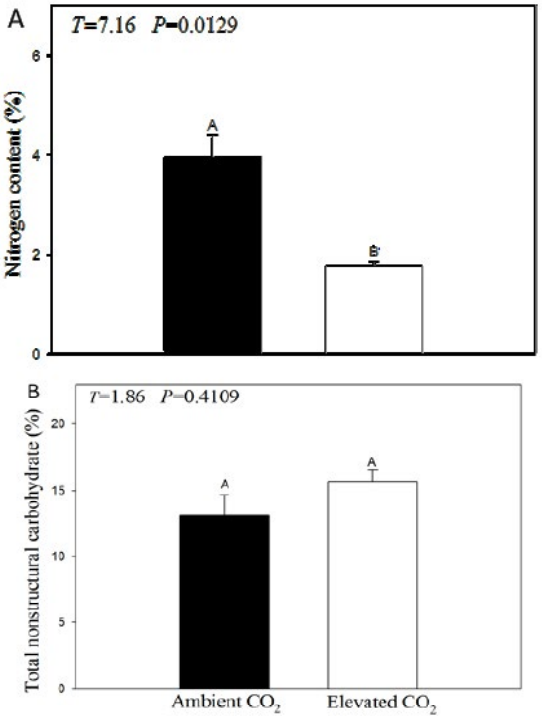


**Figure 2.** Chemical composition of tomato foliages grown under ambient (450 ppm) and elevated (900 ppm) CO2 conditions. (A) Nitrogen content, (B) total nonstructural carbohydrate. Mean ± SE (n = 15). Bar with different letters mean significant differences between treatments (Tukey's test, P < 0.05).

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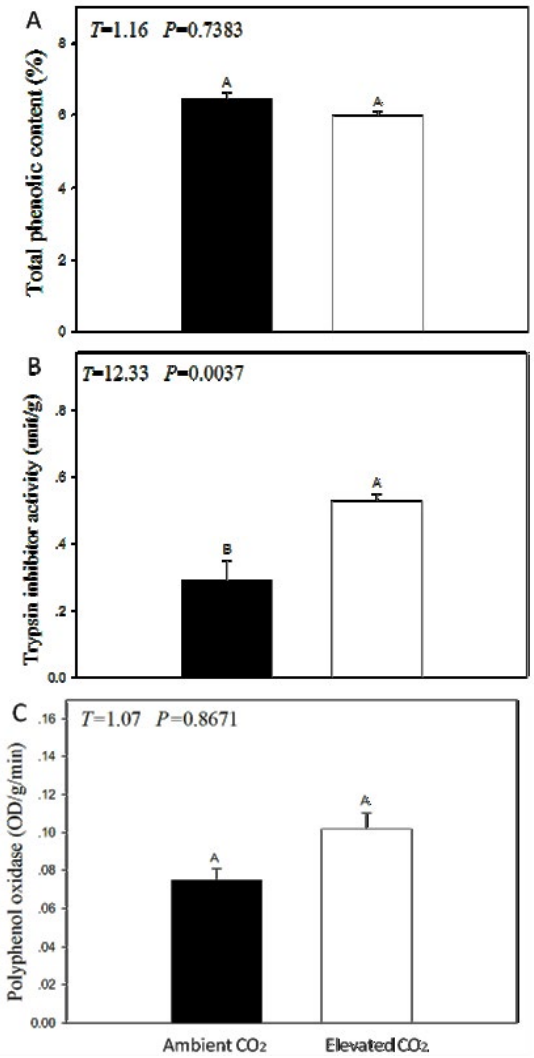


**Figure 3.** Chemical composition of tomato foliages grown under ambient (450 ppm) and elevated (900 ppm) $CO_2$ conditions. (A)Total phenolic content (B) Trypsin inhibitor activity, (C) Polyphynol oxidase activity. Mean ± SE (n = 15). Bar with different letters mean significant differences between treatments (Tukey's test, $P < 0.05$).

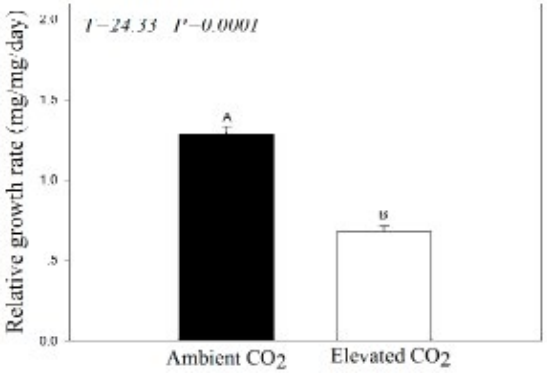


**Figure 4.** Relative growth rate of *Spodoptera litura* larvae fed with tomato foliages grown under ambient (450 ppm) and elevated (900 ppm) $CO_2$ conditions. Mean ± SE (n = 30). Bar with different letters mean significant differences between treatments (Tukey's test, $P < 0.05$).

In addition to changes in plant growth performance, our results showed that elevated $CO_2$ concentration caused changes in the nutritional quality of plants. The changes in primary metabolite contents, decreased nitrogen contents and increased carbon contents, substantially increased in the leaf C:N ratio[11]. The past studies indicating that the additional photosynthesis products formed starch and sugars due to the limited nitrogen supply[44,45]. Although in this study the result of TNC analysis showed no significant difference between ambient and elevated $CO_2$ conditions. After plant chemical contents changed, it could have a significant impact on herbivores[19], and nitrogen content is likely the most essential component for herbivore performance. Therefore, the increase in the foliar C:N ratio at an elevated $CO_2$ concentration could limit nutrient availability for insects[46]. Regarding secondary metabolite phenolic compounds, our results showed that foliar phenolic content no difference between ambient and elevated $CO_2$ concentration; however, Penuelas et al.[47] showed various results for different plant species. Thus, the

effects of elevated $CO_2$ concentration on foliar phenolic content are not consistent; additional studies are required to elucidate these effects. Regarding plant defense proteins, we found very different results, TI activities increased at an elevated $CO_2$ concentration, but PPO activity was not significantly different. Studies have shown that TI and PPO could be induced to protect plants from various stress conditions[48,49].

Foliar chemistry changes with elevated $CO_2$ concentration can affect the performance and food utilization of herbivorous insects. Nitrogen deficiency might be associated with plant growth limitation and a concomitant increase in the level of foliar secondary metabolism[50]. Nitrogen content is often considered the most important resource for phytophagous insects; therefore, reduced nitrogen content might affect insect growth performance. Our study demonstrated that, the relative growth rate was poor when the larvae fed on elevated $CO_2$-treated foliage. A study showed that caterpillars fed on nutrient-poor foliage would compensate for nutrient requirements by consuming more foliage[12]. Similarly, other studies have shown decreased digestibility and growth rates and increased consumption rates and mortality for insect herbivores fed on elevated $CO_2$-treated foliage[11,51-53]. In conclusion, our study demonstrated that crop plants at an elevated $CO_2$ concentration responded with foliar changes, and these phytochemical changes could have potential effects on subsequent insect herbivore growth performance.

The effects of elevated $CO_2$ concentration have been reported in several studies, and most of these studies have focused on temperate forest tree–insect interactions[7,54-58]. However, little is known about the responses of crop plant–insect interactions on elevated $CO_2$ concentration in the subtropical environment. Our study provides evidence on how the global environmental change might affect crop plant–insect interactions, and this evidence could be extremely helpful for managing agroecosystems in the future.

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