The effects of carbon dioxide concentrations on some morphological and physiological characteristics of *ligustrum vulgare*

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Abstract

Carbon dioxide is one of the most important necessary factors for plant growth. In order to study morphological and physiological response of *ligustrum vulgare* to Co_2 enrichment, one-year-old seedlings of this species exposed to different carbon dioxide concentrations for two months. Treatments included 450 (control), 750, and 1100 ppm Co_2. The experiment was based on a completely randomized design with three replications. Then some morphological and physiological characteristics were measured. Results showed Co_2 concentrations of 750 and 1100 ppm increased all morphological traits (except collar diameter and root dry weight) and physiological characteristics such as proline, chlorophyll a and nitrogen. The highest amounts of proline, chlorophyll a and nitrogen were observed at concentration of 750 ppm. The amount of increase in proline was about 4-fold compared to the control. The study concludes that the increased carbon dioxide concentration in the future maybe improve the growth of this plant.

Keywords: carbon dioxide; *ligustrum vulgare*; proline; morphology; physiology

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Introduction

Over the last two centuries, accelerating rates of fossil fuel use and forest clearing by humans have led to increasing concentrations of carbon dioxide (CO_2) in the atmosphere (Houghton et al. 1996). From a pre-industrial-era concentration of 280 parts per million (ppm), atmospheric CO_2 rose to 364 ppm in 1997 (an increase of 30%), and it is likely to reach 560 ppm, double the pre-industrial concentration, within the next century. Because the atmosphere is well-mixed, this increase is fairly uniform over the entire surface of the planet. Plants, which need CO_2 to carry out photosynthesis are directly affected. Animals, which are not directly dependent on CO_2, are primarily affected by changes in the plants that they use for food or shelter (Dukes, 2000).

Without directly studying a plant species and the community in which it lives, it remains difficult to predict whether that species will benefit from elevated CO_2. It is usually possible to predict whether an individually-grown plant will increase its growth in response to CO_2 enrichment. Under these circumstances, most species that use

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the C_3 photosynthetic pathway respond favorably to increase in atmospheric CO_2. Species that use the C_4 and CAM pathways are less predictable; but in general the response is less vigorous than that of C_3 plants (Poorter 1993; Poorter et al., 1996). Most plants' growth is increased from 30 to 60% by doubling atmospheric carbon dioxide level (Taize and Ziger, 2003). Higher concentrations of carbon dioxide can have a positive effect on the efficiency of photosynthesis. Growth of forest trees or their re-growth also had a similar trend (Nasiri Mahallati et al., 2002). Increasing carbon dioxide that causes increase in photosynthesis and carbohydrate (Mott, 1990) can improve growth under stress conditions. In other words, the effects of enrichment by carbon dioxide can be influenced by environmental stresses (Chen et al., 1999). Rogers et al. (1999) reported that relative responses of the plant to increased carbon dioxide are much more intensified in environmental stresses and resource limitations.

Carbon dioxide enrichment in greenhouses can be used as a mechanism for reducing production time, improving quality and increasing plant vigor. Many studies have been conducted to examine the physiological response of the plants affected by CO_2 concentration during the past several decades. Changes in nitrogen, chlorophyll content, proline, and soluble sugar have been observed in many studies. The significant alteration on their levels is likely to cause marked effects on the entire metabolism of plant. In the present study, we investigated the morphological and physiological response of *ligustrum vulgare* to increase the concentration of carbon dioxide in greenhouse conditions.

Materials and Methods

Species under study

Ligustrum vulgare (wild privet, also sometimes known as common privet or European privet), is a species of Ligustrum native to central and southern Europe, north Africa and northwestern Iran. It is a semi-evergreen or deciduous shrub, growing to 3 m (rarely up to 5 m) tall. Plants from the warmer parts of the range show a stronger tendency to be fully evergreen; these have sometimes been treated as a separate variety *Ligustrum vulgare* var. italicum (Mill.) Vahl, but others do not regard it as distinct.

Methods

This study was conducted in spring 2015 in the greenhouse of the Faculty of Natural Resources, Yazd University. The experiment was done based on a completely randomized design with three replications. Treatments of the study were three concentrations of carbon dioxide, including 450, (control), 750, and 1100 ppm. Pots' soil contained crop soil, sand, manure, and leaf composts, with the ratio of 1: 1: 1: 1.

Plants of *ligustrum vulgare* (1-year-olds) grown in a greenhouse under the same conditions were transferred to plastic rooms of the size 3 × 2 × 2 m. Different treatments of carbon dioxide were individually designed for each room and carbon dioxide gas was injected using 50 kg capsules of carbon dioxide. Also, in order to control the amount of carbon dioxide precisely, Photo Acoustic Multi Gas Monitors 1312 were used in the rooms containing the gas. Average daily and night time temperature were 30 and 26° C, respectively which was identical for all treatments. The temperature at the plastics rooms was about 4° C more than the temperature compared to outside the rooms.

After treating for two months, growth characteristics including collar diameter (mm), height (cm), shoot and root wet weight (g), shoot and root dry weight (g), and wet and dry material biomass (g) and also some physiological characteristics including proline (Bates et al., 1973), soluble sugar (Kochert, 1987), chlorophyll (a, b, a + b) and carotenoid (Lichtenthaler, 1987), N, phosphorus, potassium (Ghazan Shahi, 1997), and leaf relative water content (Alidibe et al., 1990) were measured.

Data normality was investigated by Kolmogorov-Smirnov test; then one-way ANOVA, and Duncan multiple range test were performed with 5% level of significance using SPSS software version 13.0.

Results

Table 1
Analysis of variance for morphological traits in *ligustrum vulgare* under Co_2 enrichment

Traits	Sources	df	SS	MS	F	P	CV
Collar diameter	Co_2	2	12.18	6.09	1.75	0.25	**29.25**
Stem height	Co_2	2	1946.00	973.00	12.37	0.00	**12.61**
Number of leaves	Co_2	2	42588.66	21294.33	5.50	0.04	**22.25**
Shoot wet weight	Co_2	2	1522.20	761.10	25.90	0.00	**17.56**
Root wet weight	Co_2	2	1369.44	684.72	4.50	0.05	**24.13**
Wet weight biomass	Co_2	2	5639.66	2819.83	9.96	0.01	**28.61**
Shoot dry weight	Co_2	2	139.89	69.94	28.36	0.00	**17.82**
Root dry weight	Co_2	2	78.25	39.12	2.47	0.16	**39.71**
Dry matter biomass	Co_2	2	425.05	212.52	7.44	0.02	**36.82**

Table 2
Comparisons of effects of different concentrations of carbon dioxide on growth characteristics of *Ligustrum vulgare*

Characteristics	(450) Control	750	1100
Collar diameter (mm)	5.42 a	8.01 a	**5.69 a**
Stem height (cm)	52.66 b	69.66 b	**88.66 a**
Number of leaves	150.00 b	240.67 ab	**318.33 a**
Shoot wet weight (gr)	13.56 c	34.06 b	**44.93 a**
Root wet weight(gr)	14.17 b	25.57 ab	**44.10 a**
Wet weight biomass (gr)	27.73 b	59.63 ab	**89.03 a**
Shoot dry weight (gr)	3.79 c	9.20 b	**13.43 a**
Root dry weight (gr)	2.27 a	5.37 a	**9.47 a**
Dry matter biomass (gr)	6.06 b	14.57 ab	**22.90 a**

Analysis of variance for morphological traits showed CO_2 enrichment significantly affected the stem height, number of leaves, shoot wet weight, root wet weight, biomass wet weight, shoot dry weight, and dry matter biomass (Table 1). CO_2 concentration of 750 ppm significantly increased only the shoot wet weight and shoot dry weight. On the contrary, increasing CO_2 concentration to 1100 ppm significantly increased the stem height, number of leaves, shoot wet weight, root wet weight, biomass wet weight, shoot dry weight, and dry matter biomass compared to the control. CO_2 concentration of 1100 ppm significantly increased stem height, shoot wet weight and shoot dry weight of the plants compared to CO_2 concentration of 750 ppm (Table 2). The highest stem height (88.66 cm) was observed at CO_2 concentration of 1100 ppm. Number of leaves at CO_2 concentration of 1100 ppm increased more than two times compared to the control. Shoot dry weight and shoot wet weight performance of the plants were better than the other studied characteristics in the increased CO_2 concentration. Both characteristics increased at 750 and also at 1100 ppm equal to almost 3 and 4 times, respectively compared to control. The highest level of root wet weight was measured at CO_2 concentration of 1100 ppm which was three time more than the control. Dry matter biomass was also affected by increasing Co_2 and its level reached to 22.9 and 14.57 under 1100 and 750 ppm CO_2 concentration, respectively.

Analysis of variance for physiological traits showed CO_2 enrichment significantly affected the amounts of proline, chlorophyll a, and nitrogen (Table 3). The highest amount of proline, chlorophyll a, and nitrogen were observed at 750 ppm CO_2 concentration (Table 4). The amount of proline at 750 ppm CO_2 concentration was about 4-fold compared to the control. CO_2 enrichment did not affect the sugar, chlorophyll b, total chlorophyll, carotenoids, phosphorous, potassium, and relative water contents in the study.

Table 3
Analysis of variance for physiological traits in *ligustrum vulgare* under Co_2 enrichment

Traits	Sources	df	SS	MS	F	P	CV
Proline	Co_2	2	0.00	0.00	38.38	0.00	**24.93**
Sugar	Co_2	2	2.28	1.14	4.20	0.07	**0.62**
Chlorophyll a	Co_2	2	8.82	4.41	10.48	0.01	**2.1**
Chlorophyll b	Co_2	2	290.22	145.11	1.14	0.38	**64.95**
Total chlorophyll	Co_2	2	569.07	284.53	1.67	0.26	**29.53**
Carotenoids	Co_2	2	15.51	7.75	0.60	0.57	**52.60**
Nitrogen	Co_2	2	5.49	2.74	29.34	0.00	**11.05**
Phosphorus	Co_2	2	0.00	0.00	2.03	0.21	**30.58**
Potassium	Co_2	2	15292941.63	7646470.81	1.56	0.08	**11.54**
Leaf relative water content	Co_2	2	359.99	179.99	1.71	0.25	**14.13**

Table 4
Comparisons of the effects of different concentrations of carbon dioxide on the physiological characteristics of *Ligustrum vulgare*

Characteristics	**Co_2 concentration (ppm)**		
	1100	750	(450)Control
Proline (mg g^{-1} fw)	0.002 b	0.008 a	0.002 b
Sugar (mg g-1 wd)	0.083 ab	0.08 a	0.08 a
Chlorophyll a (mg g-1 fw)	30.16 b	32.24 a	30.11 b
Chlorophyll b (mg g-1 fw)	17.24 a	10.45 a	24.36 a
Total Chlorophyll (mg g-1 fw)	47.42 a	33.21 a	51.85 a
Carotenoids (mg g-1 fw)	5.35 a	5.20 a	2.49 a
Nitrogen (%)	2.03 b	3.85 a	2.42 b
Phosphorus (mg/kg)	0.07 a	0.05 a	0.04 a
Potassium (mg/kg)	10.83 a	8.62 a	**9.31 a**
Leaf relative water content (%)	65.30 a	80.72 a	**71.66 a**

Discussion

Results showed that CO_2 enrichment positively affected morphological traits of the plants under study. Increased morphological traits maybe relate to increasing chlorophyll a and then the photosynthesis products such as carbohydrates. But contrary to expectations, the findings showed that CO_2 enrichment did not significantly increase the amount of soluble sugars in *ligustrum vulgare*. Moreover, contrary to our findings some researchers have reported an increase in soluble sugar contents. For example, Ainaworth et al. (2004) reported that increasing carbon dioxide in *Glycine max* has significantly increased soluble carbohydrates content up to 20% and has insoluble carbohydrates content up to 58%. It is possible that significant amount of soluble sugars produced by photosynthesis in our experiment were temporarily used for the growth of these traits in *ligustrum vulgare*.

Results of this study showed that increasing the concentration of carbon dioxide to 750 ppm caused an increase in the nitrogen content of the plants. At this concentration, a significant increase was also observed in proline amino acid. Possibly nitrogen through nitrogen metabolic recovery cycle has caused an increase in amino acid and a likely increase in the proline content of the plant. Carbon dioxide at the concentration of 1100 ppm had no effect on the nitrogen content of the studied plant. Reducing the amount of nitrogen from 750 to 1100 ppm can be attributed to the balance between nitrogen and carbon. Reducing the amount of nitrogen at 1100 ppm has caused a reduction in the amount of proline and possibly protein synthesis. The balance between the two can play an important role in reproductive and growth steps of the plant. When nitrogen enters molecules of amino acids and fix there, some of the metabolic materials of tri-carboxylic acid cycle (TCA cycle) are used, but

TCA cycle action continuity requires refilling the mentioned metabolic materials used. Supply and refill of these metabolic materials led to the consumption of hydrocarbons and its derivatives. These compounds provide biosynthesis of amino acids with carbon skeletons, thus inorganic nitrogen causes reduction in carbohydrates' reserves in the plant (Noggle and Fritz, 1983). Increased carbon dioxide in common clover causes increase in total nitrogen percentage and biological nitrogen fixation (Zanetti et al., 1996). On the contrary, the results of some studies showed that by increasing carbon dioxide, nitrogen concentration reduced in grapes (Moutinho-Pereira et al., 2009). Similarly, studies showed that by increasing carbon dioxide in rice, the concentration of nitrogen in the cluster reduced to 7% (Zhang et al., 2013).

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