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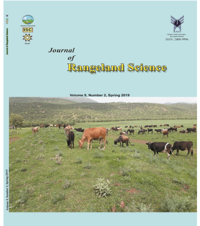

Research and Full Length Article:

Physiological Responses of *Pteropyrum aucheri* to Short-term Warming in Semi-arid Rangelands (Case Study: Kohpanj Region, Kerman Province, Iran)

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Abstract. Scientists predict climate changes and warmer conditions for the world in future. Predicting the warming effect on plant performance is one of the most important challenges in ecological sciences. In this study, the effects of short-term warming on physiological traits of *Pteropyrum aucheri* as one of the domain shrubs was assessed in riverside of semi-arid rangelands of Kohpanj region, Bardsir, Kerman province, Iran in 2014. Five 5×5 m^2 blocks were selected with the same elevation, slope and aspect. In each block, mature individuals of *Pteropyrum aucheri* were studied in 10 circular plots with 2m diameter during spring and summer. For simulating the warming phenomenon, Open-Top Chambers (OTCs) were placed on 5 plots and other plots were considered as control plots. OTC is applied in natural ecosystems and provides nearly the same growth condition for species with higher temperature. Results showed that the OTCs enhanced monthly average of air temperature (5cm above soil surface) as 1.5 ºC, surface soil temperature of 2 ºC and soil temperatures (5 cm below soil surface) as 1.7 ˚C in warming plots. Results showed that photosynthetic pigments (Chlorophyll a, b and total) were significantly reduced and carotenoid was significantly increased with short-term warming in warming plots ($p<0.05$). Water content, leaf area and dry matter production were none significantly declined with warming. In general, short-term warming affected photosynthesis performance of *Pteropyrum aucheri* but biomass was not considerably affected by warming.

Key words: Warming, Open-Top Chambers, Physiology, *Pteropyrum aucheri*

Introduction

In this century, global temperature has additional warming, ranging from 1.8ºC to 4ºC (IPCC, 2007). Climate scientists predict drier and warmer conditions for the world in future decades (IPCC, 2001). The impact of climate changes on plant performance is one of the most important challenges in ecology science as drier and warmer conditions may change the competitive relationship among shrubs in ecosystems (Llorens *et al.,* 2003). Temperature is the main factor in controlling the growth rate that can influence the growth and development in plants (Morison and Lawlor, 1999; Bayat *et al.,* 2016). Numerous experimental and observational studies have considered changes in vegetation communities in response to recent climate changes (Santamaria and VanVierssen, 1997; Root *et al.,* 2003). Shaver and Jonasson (1999) studied the response of ecosystems in the polar region to the increased temperature and pointed out that plant response to warming was low and occasionally climate changes did not significantly declined species diversity in high altitude. Although climate changes caused more carbon stock, previous studies indicated that raising primary production resulting in more carbon also depended on available nitrogen. Higher temperature can raise nitrogen with the increase of decaying rate of litters and nitrogen mineralization; consequently, it has a positive impact on primary production of plants in cold environments (Jonasson *et al.,* 1999).

Responses of plants to warming are influenced by environmental factors such as temperature, elevation, season, and species (Shaver *et al.,* 2000). Llorens *et al.* (2003) concluded that drought treatment significantly reduced the leaf photosynthetic rates of *Erica multiflora* and *Globularia alypum*. They suggested that drier conditions might decrease the annual productivity of these Mediterranean shrubs. A decrease of vegetation biomass along temperature gradient also was reported in European rangelands (Sardans *et al*., 2006). In summer, warming treatment might decrease photosynthetic performances. Conversely, in winter, warming might increase photosynthetic performances (Llorens *et al.,* 2003). Post and Pedersen (2008) assessed the effect of climate changes on plant community production and concluded that herbivore worms alleviated the positive effect of warming on plant community production. Bjerke *et al.* (2011) indicated a negative impact of warming on the net photosynthetic rate of the *Hylocomium splendens* whereas *Peltigera aphthosa* was unaffected by experimentally imposed winter warming. Damgaard *et al.* (2016) did not find any significant changes in the relative cover of different functional types for 28 years (1989-2012) and indicated that climate changes have not had a major impact on the plant community composition.

Open-Top Chambers (OTCs) were used to simulate warming in this research. Marion *et al.* (1997) designed OTCs to study the effect of increased temperature in ecosystems. OTC is placed in natural ecosystems and provides nearly the same growth conditions for species with higher temperature (Wan *et al.,* 2014). OTC can increase temperature (up to an average of 2.3°C) corresponding with the predicted global warming (Netten *et al.,* 2008). Suzuki and Kudo (2000) indicated that leaf nitrogen concentration and leaf mass per unit leaf area ($mg.cm^{-2}$) generally tended to decrease in the OTCs and fruit production was not influenced by the OTCs for all species. Zhao and Liu (2008) studied the effects of experimental warming, nitrogen fertilization and their combination on growth and photosynthetic performances of two coniferous species and pointed out that warming and fertilization significantly increased biomass accumulation and photosynthetic performances of species.

Hollister and Flaherty (2010) used OTCs to study the effect of experimental warming on vegetation biomass and their results showed that OTCs raised temperature 1-2 ºC and the response of species was different to warming while *Carex aquatilis* proportionally increased above-ground biomass and *Salix rotundifolia* increased the amount of above- and below-ground biomass in response to warming. Wahren *et al.* (2013) appraised the effects of experimental warming on vegetation composition and diversity using OTC to raise ambient growing-season temperatures by 1ºC.

Arid and semi-arid rangelands are sensitive ecosystems to climate changes (Maestre *et al.*, 2012). Shrub species are among main species in arid and semi-arid rangelands (Cutforth *et al.*, 1999). Strong responses of leaves to external conditions make these leaf traits appropriate proxies for a wide variety of stressors (Acevedo *et al.*, 2017). Relationships between plant abundance and temperature could be predicted by plant traits (Bellot *et al.*, 2004). Physiological characters of plant species are favorite functional indicators to response to the climate changes. Chlorophyll pigments are the sensitive organs encountered to environmental disturbs (Rampino *et al.*, 2006). Reducing the speed of photosynthesis reduces the activity of soil microbiology (Sardans *et al.*, 2006) and declines the available food of the root and if there is the water restriction, plant growth will be completely stunted (Chapin, 1980). Saeedi Goraghani *et al.* (2014) revealed that drought stresses significantly impacted on germination and growth indices of *Agropyron desertorum* in laboratory conditions. Akbari *et al.* (2016) studied the effect of drought stress on performance of two *Allium* ecotypes and reported that drought stress reduced leaf traits and has led to a decline in performance of *Allium*. Haeuser *et al.* (2017) found that plant differently reacted to heating, average colonization success of non-naturalized aliens was reduced by heating, but some species were not affected or performed even better with heating, particularly those with an annual life span and a high seed mass. Sastry *et al.* (2018) indicated that more productive species with higher photosynthetic rates may be more vulnerable to heat and drought stress, and more likely to be negatively affected by future increases in extreme climatic events. Prediction of shrubs response to warming is essential to sustainable rangeland management. *Pteropyrum aucheri* is one of the dominant shrub species in the riverside and seasonal waterways in the arid and semi-arid rangelands. The prediction of physiological reactions of this shrub to the increased temperature can help to select species in biological reclamation of riverside and hydrology managements. Therefore, in current research, the physiological response of *Pteropyrum aucheri* to short-term warming was studied in semi-arid rangelands of Kerman, Iran.

Materials and Methods

Study areas

The study was carried out in semi-arid rangelands of Kohpanj region, Bardsir, Kerman province, Iran with 14200ha located in 56 º 10´-56 º 51´ E longitudes and 29º 30´ -29º 59´ N latitudes. Study area is characterized by hot summers and cold winters. Spring precipitation occurs in April and May but the most precipitation comes as rain in autumn and winter. Annual mean precipitation is highly variable but it is usually less than 210 mm. Climate is semi-arid based on Domarten method. *Artemisia sieberi* was the dominant vegetation type. *Pteropyrum aucheri Jaub. & Spach is* the domain shrub in plain and riversides of the sampling sites.

Research Methods

Five5×5 m^2 blocks were selected with the same elevation, slope and direction. In each block, mature individuals of *Pteropyrum aucheri* were chosen in 10 circular plots with 2m diameter. For simulating the warming phenomenon, Open-Top Chambers (OTCs) were placed on 5 plots and other plots were considered as control plots. OTC was used to investigate the short-term effect of temperature on the early growth and physiology of *Pteropyrum aucheri*. OTC has cone-shaped transparent structures, an open top to allow direct solar irradiation into the chamber. The inwardly inclined sides trap part of incoming heat like a greenhouse. OTCs were used with 200cm diameter and 100 cm height. We monitored soil (5 cm under top soil), near-surface and air (5cm above soil) temperature in control and warmed plots monthly from April to August in 2014. Plant traits included leaf area, relative water content, total chlorophyll, chlorophyll a and b, carotenoid and production.

Leaf area and production

In each plot, smaller 20×20 cm^2 quadrates were plotted and annual production was harvested, dried and weighted. Also, 10 leaves were randomly sampled and their images were drawn on the graph and their area on scale mm^2 was measured.

Relative water content (RWC)

In each plot, 10 leaves were sampled by main branches to measure the relative water content at about 10 am. Leaves were separately put in plastic containers and then, samples were quickly transferred to the laboratory inside the ice flask to prevent from water loss. Then, leaves were immersed in the distilled water for 24 hours at room temperature without light; the samples were quickly and accurately dried and wiped to calculate the turgid weight, and were placed in an oven for 48 hours at 70 °C to measure dry weight. The relative water content was calculated according to the following equation (equation 1) (Mahmood *et al.,* 2003):

RWC (%) = [(W-DW) / (TW-DW)] x 100 (1)

Where,

W = Sample fresh weight

TW = Sample turgid weight

DW = Sample dry weight

Photosynthetic pigments

Photosynthetic pigments including total chlorophyll, chlorophyll a, b and carotenoids (carotenoid and xanthophyll) were estimated using Lichtenthaler (1987) method. 0.2 g of the leaves was rubbed with 15 ml of acetone and dissolved it. 3 ml of samples were poured into cuvette, and the absorbance of the solution was read by the spectrophotometer (PD-303 model) at Agriculture Education Center in Bardsir. For this purpose, the spectrophotometer was first calibrated and the absorbance of the solution was calculated at 646.8, 66.25, and 470 nm wavelengths, and the concentration of pigments using the following equations (equations 2-5):

Chlorophyll a = 12.25 A663.2 – 2.79 A 646.8 (2)

Chlorophyll b = 21.21 A646.8 – 5.1 A663.2 (3)

Total chlorophyll = 7.15 A663.2 – 18.71 A646.8 (4)

Carotenoid= (1000A470-1.8chla-85.02chlb)/198 (5)

Where A is the absorbance wave lengths by the spectrophotometer

SPSS 20 software was used for data analysis. The mean of each physiological characteristic of *Pteropyrum aucheri* was compared in the warming and control plots using independent t-test.

Results

Temperature comparison of OTCs and control plots revealed that monthly maximum difference of soil surface temperature was 2°C in June (Table 1).

Monthly maximum difference of soil temperature, measured at 5 cm depth, was 1.7ºC in June (Table 1). Maximum difference of air temperature, measured at 5 cm above soil, was 1.5°C higher in the OTC plots in July (Table 1).

The increase in temperature significantly affected carotenoid, total chlorophyll and chlorophyll a, b ($p<0.05$) and no significant effect on the relative content of water, leaf area and production (Table 2). The average contents of chlorophyll a were estimated as 5.34±0.7 and 5.01±0.6µg/g in control and warming plots, respectively (Table 2). The average of chlorophyll b was 4.90 ± 0.8 and 4.14± 0.8µg/g in control and warming plots, respectively (Table 2).

The mean total chlorophyll was 13.30±8.5 and 7.38±7µg/g in the control and warming plots, respectively (Table 2). The average of carotenoid in the control and warming plots was estimated 0.30±0.18 and 0.45±0.2 µg/g, respectively (Table 2). The average of relative water content was 56.89± 40 and 51.02±38% in control and warming plots (Table 2). The average leaf area in the control and warming plots was 18.41±9.2 and 16.23±7.2 mm^2, respectively. The average of production in the control and warming plots was 945±543 and 871±539 g/m^2 (Table 2).

Table 1. Mean soil surface, soil below and air temperature in control and warming plots

Months	Soil surface temperature°C		Soil below temperature°C		Air temperature°C	
	Warming	**Control**	**warming**	**control**	**warming**	**control**
April	25±7.9	24±5.8	22±7.3	21±7.5	21±4.9	20±3.6
May	28±4.4	27±3.5	26.5±5.7	25.5±3.7	27±2.3	27±2.7
June	40±2	38±2.8	38.7±2	37±1.5	37±5.6	36±6.7
July	40±3.3	39±3.4	39±3.1	38±3.5	38.5±2.8	37±2.5
August	44±1	43±1.2	43±2.4	42±2.2	41±2.43	40±3.3
Mean	35.4±8.3	34.2±8.2	33.84±8.9	32.7±9	32±8.2	32.9±8.5

Table 2. Means compartion of leaf traite of *Pteropyrum aucheri in* control and warming plots

Leaf Traits	Mean		t
	Control Plots	**Warming Plots**	
Chlorophyll a (µg / g)	5.34±0.70	5.01±0.6	2.51*
Chlorophyll b (µg / g)	4.90 ± 0.8	4.14± 0.8	2.34*
Total Chlorophyll (µg / g)	13.30±8.5	7.38±7.0	8.65**
Carotenoid (µg / g)	0.30±0.18	0.45±0.2	-3.27*
Relative Water Content (%)	56.89± 40	51.02±38	1.98^{NS}
Leaf Area (mm^2)	18.41±9.2	16.23±7.2	1.11^{NS}
Production (gr/m^2)	945±543	871±539	1.65^{NS}

** and *statistical significance at the 1% and 5% level respectively, NS=non-significant

Discussion and Conclusion

OTCs increased temperature of the air, soil and soil surface from 1 to 2 ºC. Zhao and Liu (2008) also concluded that OTCs increased the average air temperature and soil temperature as 1.2 ºC. Netten *et al.* (2008) also concluded that OTCs increased the average temperature to 2.3ºC. In general, results indicated that OTC was a usable instrument to stimulate the warming in semiarid rangelands. OTC can be used as an inexpensive artificial warming device as compared to active warming devices such as active greenhouses and heating elements that are more expensive to construct and to maintain (energy and personnel) (Liboriussen *et al.*, 2005). Since OTCs are easy and feasible to construct and can withstand extreme weather conditions, they are suitable for use in remote and harsh natural ecosystems. They are also especially suitable for long-term researches; the largest OTC can provide temperature increases that correspond with predicted climatic warming (Netten *et al.*, 2008). Main limitations are that the temperature increase cannot be controlled and OTCs obviously protect ecosystems from wind. In terrestrial systems, this can be an important disadvantage (Marion *et al.*, 1997).

Chlorophyll Pigments

Chlorophylls are essential molecules that are responsible for receiving solar energy in photosynthetic systems (Tanaka and Tanaka, 2006). The investigation of chlorophylls needs to survey the effect of raising temperature on photosynthetic performance (Bellot *et al.*, 2004). The results of this study indicated a significant decrease in chlorophyll a, b and total due to raising temperature. Also, the results of previous studies have shown that drought stress reduced the amount of chlorophyll in *Camellia sinensis* (Xu *et al.*, 2012). Reducing the amount of photosynthetic pigments under stress conditions can be attributed mainly to the destruction of the chloroplast structure (Neocleous and Nasilakakis, 2007). El-Tayeb (2005) pointed out that reduction of photosynthetic pigments in stress conditions may be due to instability complex protein and chlorophyll degradation by enhancing the activity of the chlorophyllase enzyme. Results revealed that the amount of carotenoids increased with increasing temperature. In chloroplasts, carotenoids act as an auxiliary pigment that induces the synthesis of carotenoids under stress conditions because of their protective role in photosynthesis (Lei *et al.*, 2007). The study on *Plantago coronopus* showed that salinity stress increased the amount of carotenoid in the plant (Koyro, 2006).

Relative water content

Relative water content is one of the physiological parameters of plant response to enviromental stresses (Colom and Vazzana, 2003). Increasing temperature has a significant relationship with the rate of water shortage due to evapotranspiration (Kirschbaum, 2000). Reducing water potential prevents from cell division, reduces pure photosynthesis, and protein synthesis and changes the hormonal balance of the major tissue (Ma *et al.*, 2006). The results of this study showed that increasing temperature had no significant effect on relative water content in leaves. Similarly, former results showed that the relative water content in drought stress has been declined in many plants (Lei *et al.*, 2007). Martinez *et al.* (2007) and Ghanbari *et al.* (2013) also indicated that no difference was observed in the amount of relative water content of plants under stress and control conditions.

Leaf area and production

The results showed that the increasing temperature had no significant effect on average leaf area and the production. The Reducing annual production under warmer conditions can be due to the slow-down in photosynthesis (Llorens *et al.*, 2003). The decrease in leaf area can also affect the amount of annual production (Pereira, 1995). The main reason for the decrease in plant production in arid and semi-arid areas under increasing temperature may be the increase under the effect of the stress of water shortages in these areas (Llorens *et al.*, 2003). Kalantar Ahmadi *et al.* (2014) pointed out that the leaf anatomy has also changed and the leaves were smaller and thicker following drought stress.

Warming affects plant growth by changing the potential of plant photosynthesis (Penuelas and Llusia, 2002). Increased temperature directly affects photosynthesis potential and growth rate of species and indirectly physiological process, water content and changes of growth season (Llorens *et al.*, 2003; Klein *et al.*, 2005). Plant species react to stress conditions with decreased photosynthesis rate and subsequently decreased production (Yang *et al.*, 2007). Results of this study showed short-term warming decreasingly affected photosynthesis performance of *Pteropyrum aucheri* but biomass was not considerably declined by warming.

Intensity and duration of stress are effective factors in species reactions to stresses (Barnabas *et al.,* 2008). Acevedo *et al.* (2017) found that lignin, cellulose, leaf water content and leaf area are the leaf traits significantly reacting to long-term stress so that using long-term warming and bigger OTC can be applied to insure *Pteropyrum aucheri* performance to climate changes. This research that considered prediction of plant reaction to warming using experimental methods can provide good information for choosing proper species in future plans of rangeland reclamation and development. Grazing management also needs to attend species endurance with raising temperature.

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عکس العمل فیزیولوژیکی گونه پرند (*Pteropyrum aucheri*) به افزایش دمای کوتاه مدت در مراتع نیمه خشک (مطالعه موردی: ناحیه کوه پنج، استان کرمان، ایران)

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چکیده. اقلیم شناسان برای جهان افزایش دما را پیش بینی می‌کنند. پیش‌بینی تاثیر افزایش دما بر عملکرد گیاهان یکی از مهمترین چالش‌های علوم اکولوژی است. در این مطالعه تاثیر افزایش دما بر مشخصات فیزیولوژیکی گونه پرند (*Pteropyrum aucheri*) یکی از گونه‌های غالب در بستر رودخانه مراتع نیمه خشک استان کرمان در سال ۱۳۹۳مطالعه شد. ۵ قطعه ۵×۵ متر مربع با ارتفاع، شیب و جهت یکسان انتخاب شدند. در هر قطعه، پایه‌های بالغ گونه پرند در ۱۰ پلات دایره‌ای با قطر ۲متر در بهار و تابستان بررسی شدند. به منظور شبیه‌سازی افزایش دما، اتاقک‌های سر باز (OTCs) بر روی ۵ پلات قرار گرفتند و پلات‌های دیگر به عنوان پلات کنترل لحاظ شدند. از آنجا که OTC در محیط طبیعی قرار می‌گیرد، برای گونه‌ها تقریبا شرایط رشد یکسان با دمای بیشتر را فراهم می‌کند. OTCs میانگین ماهانه دما هوا (۵ سانتیمتر بالای سطح خاک) را ۱/۵درجه سانتیگراد، سطح خاک را ۲ درجه سانتیگراد و خاک (۵ سانتیمتر زیر سطح خاک) را ۱/۷ درجه سانتیگراد در پلات‌های تیمار افزایش دادند. نتایج نشان داد افزایش دما در پلات‌های تیمار به طور معنی‌داری رنگیزه‌های فتوسنتزی (کلرفیل a، b و کل) را کاهش و کارتینوئید را افزایش داده بود ($p<۰/۰۵$). اما کاهش محتوای نسبی آب، شاخص سطح برگ و تولید تحت تاثیر افزایش دما معنی‌داری نبودند ($p<۰/۰۵$). اگر چه افزایش دمای کوتاه مدت عملکرد فتوسنتز گونه پرند را تحت تاثیر قرار داد، اما تاثیر مشهودی بر بیوماس گونه پرند نداشت.

کلمات کلیدی: افزایش دما، اتاقک‌های سرباز، فیزیولوژی، پرند