

**REPUBLIC OF TURKEY
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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF PLANT PROTECTION
DOCTORAL THESIS**

**DETERMINATION OF THE GROWTH AND
HERBICIDE SENSITIVITY OF SOME INVASIVE
PLANTS UNDER DIFFERENT CARBON DIOXIDE,
TEMPERATURE AND NITROGEN CONDITIONS**

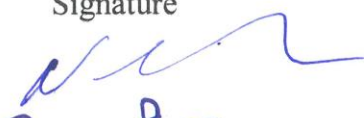

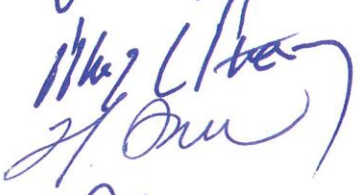

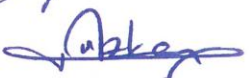
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I hereby declare that all information and results reported in this thesis have been obtained by my part as a result of truthful experiments and observations carried out by scientific methods, and that I have provided references appropriately and completely for the information which do not belong to my part within this study by virtue of scientific ethical codes.

12/02/2016

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ABSTRACT

DETERMINATION OF THE GROWTH AND HERBICIDE SENSITIVITY OF SOME INVASIVE PLANTS UNDER DIFFERENT CARBON DIOXIDE, TEMPERATURE AND NITROGEN CONDITIONS

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Global climate changes are supposed to impact the global ecosystems including those of plants. Invasive plants present a serious threat to plants, environment and, human and animal health. The global climate changes may impact the growth, invasion and management of invasive weed species. Hence, these studies were conducted to investigate the impact of recent climate changes on growth, control and nitrogen uptake of invasive species. In these two-years glasshouse experiments, we studied the effects of temperature, carbon dioxide (CO₂), nitrogen and herbicide application on biomass, growth, control and leaf tissue nitrogen concentration of four invasive weed species. High CO₂-concentration, high CO₂-concentration+high temperature improved the biomass and growth parameters of weeds including *Bromus tectorum* L., *Hordeum murinum* L., and *Lactuca serriola* L., while *Capsella bursa-pastoris* (L.) Medik. responded differently to climatic conditions compared with the other weeds. In general, high temperature had a negative or neutral effect on all the weed species in our studies. Nitrogen application had little effects on grasses while the broadleaved weeds mostly had a positive response to nitrogen application. Climatic conditions had no effect on activity of herbicide (glyphosate). This research work conclude that high-CO₂ concentration improved the growth of most of the invasive weeds in the experiment while elevated temperature mostly had negative or neutral effect on invasive weeds. Herbicide application provided equal and effective weed control under either of the CO₂ or temperature levels while N fertilization had improved very few growth parameters of invasive weeds under different climatic conditions.

Keywords: Global climate change, high CO₂-concentration, global warming, nitrogen, invasive species, growth, control.

ÖZET

BAZI İSTİLACI BİTKİLERİN FARKLI KARBONDİOKSİT, SICAKLIK VE AZOT KOŞULLARINDA GELİŞİMİ VE HERBİSİT DUYARLILIKLARININ BELİRLENMESİ

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Küresel iklim değişimlerinin bitkileri içeren küresel ekosistemleri etkilemesi beklenmektedir. İstilacı bitkiler; bitkiler, çevre, insan ve hayvan sağlığı için ciddi tehdit oluşturmaktadır. Küresel iklim değişiklikleri istilacı yabancı ot türlerinin büyümesini, çoğalmasını ve mücadelesini etkileyebilir. Bu nedenle, bu çalışmalar günümüz iklim değişimlerinin istilacı türlerin büyümesi, kontrolü ve nitrojen alınımındaki etkisinin araştırılması için yapılmıştır. İki yıllık bu sera denemelerinde, dört istilacı yabancı ot türlerinin biyokütle, büyüme, kontrol ve yaprak dokusu nitrojen konsantrasyonu üzerinde sıcaklığın, karbondioksit (CO₂), nitrojen ve herbisit uygulamalarının etkisini çalıştık. Yüksek CO₂ konsantrasyonu, yüksek CO₂ konsantrasyonu+yüksek sıcaklık *Bromus tectorum* L., *Hordeum murinum* L., ve *Lactuca serriola* L., yabancı otlarının büyüme ve biyokütle parametrelerini arttırırken, *Capsella bursa-pastoris* (L.) Medik. diğer yabancı otlarla karşılaştırıldığında iklim değişikliklerine farklı tepki vermiştir. Genel olarak, yüksek sıcaklığın çalışmalarımızdaki tüm yabancı ot türlerinde negatif veya nötr etkisi olmuştur. Dar yapraklı yabancı otların tersine, geniş yapraklı yabancı otlar çoğunlukla nitrojen uygulamasına pozitif tepki göstermiştir. İklim koşullarının herbisit (glyphosate) aktivitesi üzerinde bir etkisi olmamıştır. Bu araştırma çalışması, denemede yüksek CO₂ konsantrasyonunun, istilacı yabancı otların çoğunun büyümesini geliştirmesiyle sonuçlanmıştır. Herbisit uygulaması hem CO₂ hemde sıcaklık seviyelerinin altında, eşit ve etkili yabancı ot kontrolü sağlarken azotlu gübreleme, farklı iklim koşulları altında, istilacı yabancı otların az sayıda büyüme parametresini arttırmıştır.

Anahtar sözcükler: Küresel iklim değişiklikler, küresel ısınma, yüksek CO₂, azot, istilacı türler, büyüme, mücadele

FOREWORD

The global population is increasing at a quick pace while resources for food production are getting scarce day by day. In order to ensure food security, we will need to produce more food by utilizing the diminishing resources. In addition, we need to cope with all the challenges of current and future times in order to maintain a consistent supply of food and feed. Recent climate changes are among the major of these challenges. Importantly, the problem of climate change can aggravate the already existing challenges to food production. Weeds and invasive plant species can be a good example of such cases. Keeping in view the importance of such challenges, we conducted this research work in order to record the effect of a few salient climate changes on invasive weed species.

I am highly thankful to my supervisor Prof. Dr. M. Nedim DOĞAN who helped and supported me generously on all stages of my doctoral degree. I would also say huge thanks to Prof. Dr. Özhan BOZ who was always there for unconditional support whenever I needed that. Moreover, I am grateful to Assoc. Prof. Dr. Özkan EREN for his suggestions to choose my topic of research and conduct this work. I express bundle of thanks to Prof. Dr. Aydın Ünay for his support and help during my research and thesis write-up. It will be unjust if I do not mentioned my Turkish language teachers Okt. Hamza ÖZKAN, Okt. Nilay AKAY, Okt. Gökhan TÜRK, Okt. Nami ERDOĞAN who taught me Turkish language.

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SYMBOLS AND ABBREVIATIONS INDEX

°C	Degree Celsius
a.i.	Active ingredient
C	Carbon
C.I.	Chlorophyll index
CH ₄	Methane
cm	Centimeter
cm ²	Centimeter square
CO ₂	Carbon dioxide
g	Gram
g a.i. ha ⁻¹	Gram active ingredient per hectare
g/l	Gram per liter
ha	Hectare
kg	Kilogram
L ha ⁻¹	Liter per hectare
m	Meter
ml	Milliliter
MSE	Mean square error
N	Nitrogen
N ₂ O	Nitrous oxide
No.	Number
ppm	Parts per million

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1. INTRODUCTION

Climate change has been among the most important challenges of recent times which may affect the natural ecosystems, agricultural productivity and food security (Howden et al. 2007; Solomon et al. 2009; Hanjra and Qureshi, 2010; Horlings and Marsden, 2011). The recent climate changes are not only affecting the crop plants and forests directly but they can also impact (negatively or positively) the pests of these crops (Olesen and Bindi, 2002; Rodenburg et al. 2011; Roos et al. 2011; Clements et al. 2014; Berthe et al. 2015). There are several components of these climate changes occurring worldwide (examples may include uneven rainfall, droughts, flooding, warming, and high concentrations for greenhouse gases). Humans are increasingly manipulating the existing resources in order to facilitate their self-existence. Burning of fossil fuels in automobiles, industries, and household has added enormous quantities of carbon dioxide (CO₂) and other gases to atmosphere. Steady rise in population was accompanied with changes in land use, burning of coal and other fossil fuels at a higher rate and environmental pollution due to anthropogenic activities. This has caused two distinct impacts on global climate i.e. rise in global temperature levels and increase in atmospheric CO₂-concentration. Pre-industrial CO₂ levels in the atmosphere were 280 parts per million (ppm), which nowadays have approached to 400 ppm (IPCC, 2007). The predictions of Intergovernmental Panel on Climate Change (IPCC) indicate that the CO₂-concentration in the atmosphere will approach to 700 ppm until the end of this century (IPCC, 2007). Similarly, IPCC predicts an approximate increase of 1.4–5.8 °C in the mean temperature of globe by the end of this century (IPCC, 2007). Accumulation of nitrogen (N) in high concentration in the environment is being considered as an important part of recent climate changes which can adversely impact the global ecosystems (Pardo et al. 2011). Nitrogen fertilization aimed at improving the crop productivity led to mass addition of this nutrient to our environment (Pardo et al. 2011; Driscoll et al. 2003).

The CO₂ from air is the source of raw material for food production by the plants. The addition of CO₂ to the environment as a result of human activity would have certain impacts on climate and plant species (Khatiwala et al. 2009; Moss et al. 2010). Importantly, the increasing atmospheric CO₂-concentration can favor the growth of C₃ plants (Woodward, 2002). The enzyme which catalyzes the fixation

of CO₂ in plants is named as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the activity of this enzyme is accelerated under elevated CO₂ levels in order to produce photosynthetic products i.e glucose (produced as a result of carboxylation of ribulose-1,5-bisphosphate) (Lorimer, 1981; Portis Jr, 1992).

In contrast, the C₄ plants express a variable response to high CO₂-concentrations, i.e. C₄ plants either respond positively or neutrally to the rising CO₂ levels in the atmosphere (Ziska, 2000; Morgan et al. 2001). An increase to 800-1000 μmol mol⁻¹ in the CO₂ levels can significantly increase the dry weight of C₃ plants (Kimball et al. 2002). The increased CO₂-concentration in the atmosphere can reduce the water conductance through stomata which leads to improved photosynthetic water productivity (Ruhil et al. 2014). Hence, the C₃ weeds are likely to be favored by increase in the atmospheric CO₂-concentration. Higher CO₂-concentration can improve the root growth, increases soil and microbial respiration, and modifies the root structure to absorb water and nutrients from the soil in higher quantities (Tingey et al. 2000; Zak et al. 2000). Increased CO₂-concentration also slows down the moisture loss from the soil; hence, the water is available in the soil for more time to support the weed growth (Fuhrer, 2003).

The evidences from the recent climatic data indicate that the global temperature is increasing steadily (IPCC, 2007). This increase in temperature would have important consequences on the earth's vegetation. The growth rates and phenological developments of plants are likely to be impacted by increased global temperature (Rustad et al. 2001). The plant phases like germination, tillering, and flowering can be seriously impacted by an increased atmospheric temperature (Mohammed and Tarpley, 2009). The global increase in temperature can result in a faster evapotranspiration that results in quick removal of moisture from the soil. Increasing soil temperature decreases the herbicide persistence in the soil (Bailey, 2004).

Besides the CO₂ and temperature, the excessive N concentration in terrestrial and aquatic ecosystems may be considered important among the climate changes of recent times. These N additions to environment result from anthropogenic activities such as N application to crops, wastes from industry and humans. Nitrogen fixation by legumes and precipitation are the other sources of N addition to environment. These N additions to environment cause serious ecological damages (Rouse et al. 1999; Brooks, 2003; Driscoll et al. 2003). Increased plant

invasions and damage to native vegetation can result from such N pollution (Brooks, 2003). For example, a study from China indicated that higher soil N levels caused by industry increased the invasion by *Spartina alterniflora* Loisel. (Zhao et al. 2015).

The plants interfering the human interests by causing negative effects such as impaired crop growth and reduced crop productivity, diminished aesthetic value of a landscape, and cause ill effects on human or animal health are called weeds (Zimdahl, 2013). Although, the weeds carry certain benefits in ecological perspectives, however, these are considered serious threat to human and animal health, and food security (Oerke, 2006; Ozaslan et al. 2016). The reasons are the quantitative and qualitative damages caused by weeds to crop plants (Kropff and Spitters, 1991; Oerke and Dehne, 2004; Oerke, 2006).

Exotic, alien or non-native plant species are the ones which are introduced (through any means) to environments other than their native regions (Lake and Leishman, 2004). The exotic species may get naturalized and expand their range in new areas after passing a lag period (Allendorf and Lundquist, 2003). The exotic species can exhibit a positive, negative or neutral effect on the introduced ecosystem or habitat (Colautti and MacIsaac, 2004). Over the time, these exotic species may attain the status of invasive species (i.e. if they start damaging the local vegetation, environment and, human and animal health). Colautti and MacIsaac (2004) described the invasive plant species as the alien or non-indigenous plants which get established or have colonized into new habitats, get widespread and threaten the native biodiversity. The damages caused by invasive species may include the altered composition of local vegetation, negative effects on human and animal health, and the local biodiversity, disturbance in natural cycles in the forests, disruption of ecosystem function, accelerated soil erosion, destruction of the aesthetic beauty, decreased crop yields and damages to environment (Pimentel et al. 2000; Pejchar and Mooney, 2009). Many researchers have argued that native plant species can also be considered as invasive if they expand their range and are causing environmental or economic damages or both of these (Simberloff and Rejmánek, 2011; Carey et al. 2012; Simberloff et al. 2012; Heger et al. 2013). Simberloff et al. (2012) discussed that native plant species are likely to become invasive although they have a lower potential of becoming invasive than that of nonnatives. Such plants will be called native invasive if they attain the properties as that of the invasive plant species.

Bromus tectorum is an annual plant which can grow in diverse habitats. This weed is among the worst invasive plants currently invading many parts of the world (Conn et al. 2010; Liu et al. 2013; Anonymous, 2015a). For example this weed has invaded large areas in Canada and America (Valliant et al., 2007; Bykova and Sage, 2012). The weed can disturb soil properties of invaded areas (Ogle et al. 2003). For example, the result of a study indicated that *B. tectorum* severely disturbs the soil N balance (Rimer and Evans, 2006; Concilio et al. 2015). *Capsella bursa-pastoris* is an annual broad-leaved weed and included in the important invasive weeds of the world (Anonymous, 2015b). This weed has been included as invasive in the Invasive Plants Atlas of United States (Anonymous, 2015c). *Hordeum murinum* is another important invasive weed (Anonymous, 2015d). Conn et al. (2010) reported *H. murinum* as an important invasive weed in Alaska State of USA. Anthropogenic activities can aid this weed to attain higher growth and seed production (HilleRisLambers et al. 2010). *Lactuca serriola* has also been reported as invasive weed (Anonymous. 2015e). D'Andrea et al. (2009) modeled the potential distribution of *L. serriola* under climate change scenarios. The weed was found to have the potential to spread in many of new areas, and warming (as consequence of climate change) had helped to provide new locations for its range expansion to new areas/habitats.

Although these weed species have been described as invasive in a global context, however, owing to their widely expanded range, these may be considered as native invasive weed plants in Turkey. Importantly, the previous literature does not describe the expected behavior of these weed species under the rising CO₂-concentrations or global warming. The response of *B. tectorum* to warming is an exception which has been previously reported by Zelikova et al. (2013) from USA. However, research work reported in this thesis comprises of response of *B. tectorum* to several factors other than warming.

Glyphosate is among the most important herbicides which have contributed tremendously to weed control in cropped and non-cropped lands. It can control most types of vegetation and possesses a mode of action that includes inhibition of aromatic amino acids synthesis (which are involved in protein synthesis) and production of other compounds and hormones. This inhibition is achieved through blockage of the activity of enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Schönbrunn et al. 2001). Glyphosate is particularly important for control of invasive plant species owing to its non-selective nature and broad-spectrum

activity. Examples from recent literature confirm the effectiveness of glyphosate against several invasive plant species under different environments (Schulz et al. 2012; Robertson et al. 2013; Adams et al. 2014). Herbicides application is the most widely used method for controlling the invasive plant species and glyphosate is the most widely used herbicide for suppressing invasive plant species (Kettenring and Adams, 2011). Recent studies indicate that the climate changes can impact the weeds and the activity of herbicides including glyphosate (or other herbicides) used for controlling weeds and invasive plants. The increased CO₂-concentration in the atmosphere may decrease the efficacy of herbicides (Ziska et al. 2004). For example, the *Elytrigia repens* (L.) Nevski was more tolerant to glyphosate when grown under high CO₂-concentration (Ziska and Teasdale, 2000). The weeds may take advantage of changed climate and utilize more CO₂ and nutrients to improve their growth. Hence, there might be difficulties in controlling the certain weeds by the normal control practices. The herbicide absorption, uptake and metabolism can also be impacted by temperature (Kumaratilake and Preston, 2005). The decreased evapo-transpiration under high CO₂-concentration may decrease the uptake of soil applied herbicides. Higher leaf starch concentration under enriched CO₂ might reduce the herbicide activity. On the other hand, the efficacy of some herbicides may be improved due to better herbicide uptake and translocation.

In the proposed research work, the response of invasive (or native invasive) weed species (*B. tectorum*, *C. bursa-pastoris*, *L. serriola*, *H. murinum*) to high CO₂-concentration, elevated temperature, nitrogen fertilization and herbicide application has been studied in their native range. Such information has not been reported in the previous literature. This research work will help to understand the growth behavior of these weed species under changing climatic factors such as increasing CO₂-concentration, global warming, and enhanced N fertilization to agricultural fields. Similarly, the current management practices (such as glyphosate application) in non-cropped areas may not be effective to control these invasive weeds under high CO₂-concentration and elevated temperature. Hence, this research work will help to understand the response of these invasive weed species to herbicide (glyphosate) application under changing climatic factors i.e. high CO₂-concentration and elevated temperature. The information produced from this research work regarding the growth behavior and control of these invasive species under changing climatic factors (including global warming and

rising atmospheric CO₂ levels) will help to formulate the management strategies for these weeds before they expand their range and cause environmental and economic losses.

Hence, studies were conducted to find the answer to following questions;

1. Either the growth of invasive weed plants is increased or not under high CO₂-concentration and elevated temperature?
2. How the N application affects the growth of invasive weeds under high CO₂-concentration and elevated temperature?
3. Either the glyphosate efficacy against invasive weeds remains the same or not under normal and high CO₂-concentration and elevated temperature conditions?

2. REVIEW OF LITERATURE

2.1. Climatic Changes

Recent climate changes are expected to severely impact all ecosystems of the globe. Increasing CO₂-concentration in atmosphere and rising temperature are most important among the recent climate changes. Currently, the CO₂-concentration in the atmosphere is nearly 400 ppm, which was about 280 ppm before start of intensive industrialization. The researchers have worked out that CO₂-concentration in the atmosphere will reach to 700 ppm by the end of current century (IPCC, 2007). In addition to increase in atmospheric CO₂-concentrations, the concentrations of other greenhouses gases such as CH₄, N₂O and chlorofluorocarbons are also steadily rising (IPCC, 2001; 2007). Subsequently, the global temperature is expected to be increased by 3-12 °C in response to accumulation of greenhouse gases in atmosphere. This will seriously impact the ecological sequences, for example, the intensity, distribution and timing of rainfall may be seriously altered by rising global temperature.

The changes in climate can affect the growth of some plants especially the ones having C₃ pathway of photosynthesis (Reddy et al. 2010). Invasive weeds can cause ecological and economic damages in the invaded areas. If the growth of invasive weeds is aided by recent climate changes, these weeds may offer tougher competition to crop plants. In this thesis chapter, we have reviewed: (1) Effect of recent climate changes (increasing CO₂ and temperature) on weeds' and other plants' growth and invasiveness, (2) High nitrogen concentration in the environment and plant invasion, and (3) The possible effects of climate change on weed management. The results of several studies indicated that climate change, particularly the rising CO₂ levels in the atmosphere increase growth and physiological activities of weeds.

2.2. Effect of Climate Change on Plant Growth and Invasion

2.2.1. Effect of High CO₂-Concentrations on Plants and Weeds

CO₂ is a raw material for food production in plants. The ambient CO₂ levels in the atmosphere may limit the photosynthetic activity of C₃ plants while increasing the CO₂ in air can stimulate the plant growth. Enhanced CO₂ supply will improve the photosynthetic activity which results in increased vegetative and reproductive

growth of plants. High CO₂-concentrations will also result in reduced stomatal opening, evapotranspiration, and photorespiration in C₃ plants. These events help to reduce moisture stress on plants. Some of recent studies have elaborated the impacts of high CO₂-concentration on weeds. Most of such studies have concluded that rising atmospheric CO₂-concentrations improve the growth, physiological activities and reproductive output of weeds.

Under the high CO₂-concentration, not only the growth rate and dry matter accumulation of weeds is increased, but also the losses caused to crops by weeds are increased (Ziska, 2000). For example, *Chenopodium album* L. when grown with soybean (*Glycine max* L.) under the high CO₂-concentration, the soybean yield reduction was increased from 28% to 39% compared with weed free treatment. Also, the dry weight of *Chenopodium album* was increased by 65% under high CO₂-concentration (Ziska, 2000). However, the C₄ weed *Amaranthus retroflexus* L. was not affected by high CO₂ levels with no increase in its dry weight (Ziska, 2000). In another study, Ziska and Teasdale (2000) provided the *Elytrigia repens* with two CO₂-concentrations; normal (~380 μmol mol⁻¹) and elevated (~720 μmol mol⁻¹); for determining its growth. The greenhouses for the experiment had a controlled environment for temperature, CO₂ and humidity. The growth, photosynthesis and biomass of the weed were increased at high CO₂-concentration compared with the normal concentration. Photosynthesis was significantly stimulated at higher CO₂ level.

Poorter and Navas (2003) reviewed the impact of increased CO₂ levels on vegetative growth and competitive performance of plants. They particularly considered the carbon economy parameters, vegetative biomass of isolated plants, and growth in competition. They concluded that at the high CO₂-concentration, the whole plant photosynthesis was increased which increased the leaf growth rate. However, the specific leaf area was decreased while relative growth rate was almost unaffected. Fast growing C₃ plants responded more to increased atmospheric CO₂ than the slow growing C₃ plants and C₄ plants as well.

Root and shoot biomass of invasive weed *Cirsium arvense* (L.) Scop. were increased under the high CO₂-concentration (Ziska et al. 2004). However, the growth of root was more pronounced at higher CO₂ levels. Similarly, Miri et al. (2012) determined the effect of ambient (350 ppm) and increased (700 ppm) CO₂ levels on root, leaf and shoot dry weight, chlorophyll contents and root-shoot ratio

of *Chenopodium album* and *Amaranthus palmeri* S.Watson. The vegetative growth was increased under the high CO₂ for both the weeds. However, the shoot attained a higher dry weight than the root; hence the root-shoot ratio was decreased. Also, the leaf weight and chlorophyll contents for weeds were improved when these were grown under high CO₂ levels.

Recently, Nord et al. (2015) have evaluated the effect of high CO₂-concentrations on the growth, mineral nutrition and physiology of a C₃ plant *Festuca arundinacea* Schreb. on different types of soils. The results indicated that high CO₂ did not affect the N concentrations in the *F. arundinacea*, however, high CO₂-concentration affected the phosphorus nutrition of this plant. Generally, the growth and photosynthesis activity of *F. arundinacea* was improved by high CO₂-concentrations.

Although significant work has been done to record the effect of increasing atmospheric CO₂-concentrations on growth of several weed and plant species, however, no work is on record regarding the effect of high CO₂-concentration on the species which we have worked on in our studies.

2.2.2. Effect of Elevated Temperature on Weeds and Plants

Plant ecosystems may respond variably to increasing global warming. Rustad et al. (2001) studied the effect of global warming on plant dry matter, N mineralization and soil respiration in a wide range of ecosystems and experimental sites. A warming of 0.3-6.0 °C for a period of 9 years or less was found to increase the plant dry matter accumulation by 19%, N mineralization by 46% and soil respiration by 20%.

The negative impacts of elevating atmospheric temperature on plants are on record. Fuhrer (2003) reviewed the effects of warming (elevated temperature) on crop plants, weeds and insect pests. The author discussed that the positive effects of high CO₂-concentrations in the atmosphere on the plants may be diminished by global warming. The warming favored more the C₄ weeds than C₃ weeds and decreased the yield of C₃ crop plants (Fuhrer, 2003). The results of study from Canada indicated that higher temperatures had a negative effect on the growth activities and physiology of *Brassica napus* L. (Qaderi et al. 2006). Higher

temperature decreased the leaf area and plant biomass, and disturbed the production of growth hormones as well (Qaderi et al. 2006).

Some studies also report that increasing atmospheric temperature may favor the growth of weeds. Satrapová et al. (2013) argued that weeds are probably favored by warmer climate. The growth and seed production of *A. retroflexus* was increased when it was grown under elevated temperature. A 45% increase in dry weight and 41% increase in seed production were noted due to increased temperature and precipitation. In another study, Zelikova et al. (2013) investigated the effects of climate change (different precipitation and temperatures) on growth and phenology of *Bromus tectorum*. Warming had decreased the biomass production of *B. tectorum* under water-limited environments, however, the seeds obtained from the control plots had a lower weight than the ones harvested from plots kept under warming.

In conclusion, the effect of warming has been documented on many plant and weed species. However, the effect of warming on invasive weed species included in our studies is yet desired to be investigated. Although, previous research reports the effect of warming on *Bromus tectorum* Zelikova et al. (2013), however, it does not address the effects of N fertilization and herbicide application under high CO₂-concentration and elevated temperature, and interactive effects of CO₂-concentration and temperature, on this weed.

2.3. Effect of Climate Change (High CO₂-Concentrations and Global Warming) on Weed Invasion

Studies on invasive species are among the most important topics of recent decade (Rejmánek, 2000; DiTomaso, 2009). The modern science of biological invasion was founded in 1958 when C.S. Elton wrote a book named “The ecology of invasions by animals and plants” (Davis et al. 2001). This is a classical book describing species invasion hence called “Bible of invasion biology” (Simberloff, 2008).

The propagation materials of plant species have been transferred from one to other places by humans both intentionally and unintentionally (Bazzaz, 1986). Even the seeds of staple foods like wheat, rice and maize were transferred to all across the globe from their native ranges. In the recent past, humans have achieved

remarkable success in improving their means of transport. Increase in means of transport has increased the chances of transfer of living species from one part of earth to others (Vitousek et al. 1997).

The species which get a chance of introduction into new environments or ecosystems (other than their native environment) through any means are called as exotic or alien species (Lake and Leishman, 2004). For example, the weed species whose seeds are transferred to the geographical regions where these were not present originally, these will be regarded as 'alien' or 'exotic' weeds. These exotic species first pass through a lag period, get naturalized to new environments, and then expand their distribution range. Nowadays, alien or exotic species can be easily observed everywhere in the world (Allendorf and Lundquist, 2003).

Invasive species are an advanced form of alien or exotic species. As the exotic species are introduced to new environments, these may have a positive, negative or neutral influence on the introduced ecosystem or habitat. If an exotic species negatively impacts its new environment, or the organisms which are native to that environment, this will be called as an invasive species (Colautti and MacIsaac, 2004). More precisely, we can define invasive species as the exotic species which cause potential damages to human and animal health, negatively impact the local biodiversity, disrupt ecosystem function, destroy the aesthetic beauty and cause damages to environment (Pimentel et al. 2000; Pejchar and Mooney, 2009). By altering the composition of local vegetation, the invasive plants can accelerate the soil erosion, disturb natural cycles in the forests, pollute water bodies, infest field crops, and decrease crop yields. Many of the invasive plants have damaging effects on human health.

Recent climate changes can impact the plant invasion. Many studies have reported that increasing CO₂-concentrations in the atmosphere and global warming can aid the non-native species to naturalize and spread in the new environments. Changing climate can particularly impact the invasive species in arid areas. The growth of a shrub was increased to almost double when the atmosphere was enriched with free CO₂ in a desert area (Smith et al. 2000). The authors concluded that invasive species will be more successful under changing climate. This success of invasive species is supposed to intensively disturb the native vegetation (Smith et al. 2000).

Prunus laurocerasus L. is an exotic plant species found in temperate forests of Switzerland. Hättenschwiler and Körner (2003) investigated the effect of high CO₂-concentrations on the adaptation of *P. laurocerasus* in this forest by growing its plants in normal and high CO₂-concentrations. The high CO₂-concentration increased the growth of *P. laurocerasus* by more than 50%. The authors concluded that increasing CO₂-concentrations in the atmosphere will support the spread of *P. laurocerasus* in temperate forest of Switzerland. Bradley et al. (2010) argued that the role of climate change in promoting plant invasion is complex; the high CO₂-concentrations promote the plant invasions while warming and changed rainfall patterns affect plant invasions sometimes positively and other times negatively.

Invasive plant species will be more successful under increasing atmospheric CO₂-concentrations (Manea and Leishman, 2011). Ziska et al. (2011) reviewed the relationship between recent climate changes and weed invasiveness. The authors argued that climatic changes such as increasing CO₂-concentrations and global warming support the plant invasion. Increasing CO₂-concentrations can aid invasive weeds in becoming more noxious in crop fields causing a higher decrease in crop yields and increase in weed management costs. Global warming can help the invasive plant species to get establish and expand in cool and hilly areas.

Similarly, elevated temperature can also aid the invasiveness of plant species. For example, Chuine et al. (2012) reported that elevated temperature (1.5-3 °C) could support the invasion of *Setaria parviflora* (Poir.) Kerguelen, which is a non-native C₄ species in Mediterranean Basin. Compared to the native species, the elevated temperature only improved the growth, dry matter and reproduction capacity of *S. parviflora*. The authors suggested that future climate warming will support the growth and invasiveness of *S. parviflora*.

2.4. Nitrogen Fertilization and Plant Invasion

Addition of N to natural environments may promote the plant invasion. Berendse et al. (2001) had studied the effect of high CO₂ and N deposition on the growth of *Sphagnum* bogs. The results showed that high CO₂ did not affect the growth and biomass production of bogs while higher N concentrations had a negative effect on growth. Higher N concentrations had instead favored the growth of *Polytrichum strictum* Menzies ex Brid. and other higher plants which resulted in the shift in plant species composition.

Brooks (2003) argued that lower soil N contents of the deserts may be a possible reason for least plant invasions in the deserts. The author conducted a study in the Mojave Desert of California, USA; where N was added artificially to desert soils to check the response of exotic and local plants to this N application. Desert soil enrichment with N was found to have a positive effect on exotic species and a negative effect on local vegetation. The biomass and density of exotic plants such as *Schismus barbatus* (L.) Thell., *Schismus arabicus* Nees, *Bromus madritensis* L., and *Erodium cicutarium* (L.) L'Hér., were increased with N additions while a reverse effect was noted on local vegetation. The author concluded that N deposition in new sites can support the invasive species to dominate in new environments which will ultimately result in suppression of local vegetation and results in other serious ecological consequences.

A recent study suggests that the high CO₂-concentrations and N pollution in the environment increase the invasion of *Phragmites australis* (Cav.) Trin. ex Steud. The authors argued that this accelerated invasion can be controlled if the N is not abundant in the environment (Mozdzer and Megonigal, 2012).

Some studies have elaborated that N accumulation in the soil helps the establishment of invasive species by aiding them in attaining higher biomass. Bajpai and Inderjit (2013) studied the relationship between plant invasion and nitrogen availability. Higher plant growth and biomass accumulation was noted for invasive species *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. when the soil was rich in N. The soils of invaded and non-invaded sites by *A. adenophora* were compared, and higher soil N content (attained through litter deposition) was noted in the invaded soils. Also, microbes had played an important role in this N availability to invading species. The authors suggested that soil N contents strongly facilitate the invasion.

In conclusion, N accumulation in the environment can impact the invasiveness of many weeds and plant species. Nevertheless, no study reports the response of invasive weed species included in our experiments to N fertilization under high CO₂-concentration and elevated temperature.

2.5. Effect of Climate Change on Weed Management

Some records also show the efficacy of various herbicides under the varying CO₂ and temperature levels. Ziska and Teasdale (2000) grew the *Elytrigia repens* at two CO₂ levels, normal (~380 μmol mol⁻¹) and elevated (~720 μmol mol⁻¹). At higher CO₂ level, efficacy of glyphosate (applied at 2.24 kg ai ha⁻¹) to control *Elytrigia repens* was decreased.

Bailey (2004) studied the persistence and weed control efficacy of herbicide isoproturon in the soil in response to changing climate. Over a period of 22 years, the soil persistence and efficacy of this herbicide against weeds was reduced by one-fourth. It implies that quantity of this herbicide which was sufficient to control 100% of weeds in year 1980; controlled the same weeds by 75% in 2001. In other words, it can be implied that the herbicide which was effective in soil against weeds for 120 days during 1980, was effective only for 90 days in 2001. The author concluded that this decrease in herbicide efficacy was attributed to increasing environmental temperature.

In a study from USA, *Cirsium arvense* plants were studied for their growth, dry matter and response to glyphosate application under normal and high CO₂-concentrations (Ziska et al. 2004). The herbicide was applied at 2240 g a.i. ha⁻¹ under field conditions in a two years study. The growth of this weed was significantly stimulated by high CO₂-concentrations. The plants grown under high CO₂-concentration had higher tolerance for glyphosate. This increased tolerance was probably the result of dilution and not the reduced uptake of herbicide (Ziska et al. 2004).

A study from Australia indicated the positive effect of warming in improving herbicide efficacy. Kumaratilake and Preston (2005) studied the effect of different temperatures on the efficacy of glufosinate against *Raphanus raphanistrum* L. In contrast to lower temperatures (5/10 and 15/20 °C), the glufosinate had a higher activity against *R. raphanistrum* at higher temperature (20/25 °C). The researchers further studied the reasons for improved glufosinate efficacy against the weed. The absorption of glufosinate was same at the different temperatures; however, translocation was significantly improved at the higher temperature.

Ziska and Goins (2006) studied the effects of high CO₂-concentration on soybean crop, its weeds and efficacy of glyphosate herbicide in a two years field experiment. The growth of soybean plants was positively affected by high CO₂-concentration. In the first year of study, the weed flora comprised of only C₄ species, hence, the weeds did not get a positive affect from high CO₂-concentrations. However, in the second year of study, the weeds were a mixture of C₃ and C₄ weeds, the growth of weeds was improved by high CO₂ and subsequently the efficacy of glyphosate was disturbed against weeds.

It is obvious that the response of many weeds and invasive plant species has been investigated under high CO₂-concentration and elevated temperature, however, no study reports the response of weeds included in our studies to herbicide application under simulated climatic conditions i.e. high CO₂-concentration and elevated temperature.

3. MATERIALS AND METHODS

3.1. Study Site

Experiments were conducted in the glasshouse of the Department of Plant Protection, Faculty of Agriculture, Adnan Menderes University, Aydin (37.75°N, 27.75°E), Turkey during winter season of 2013-2014 and repeated in 2014-2015.

3.2. Determination of Test Plant Species for Experiments (Screening Studies)

An initial screening experiment was conducted to evaluate the response of invasive weed species to high CO₂-concentration in order to select the weed species which will be used as material in further studies. With this aim, seeds of 33 invasive weed species were donated by Dr. Özkan Eren (Associate Profess, Biology Department, Adnan Menderes University Aydin, Turkey) who had collected these seeds for use in other experiments. The weeds were tested for their germination percentage by sowing 50 seeds of each weed in three replications. These seeds were sown in plastic trays filled with soil. Eleven invasive weed species which had a higher germination percentage were selected for initial experiment (Table 3.1). The seedlings of these 11 weeds were transplanted to 2 kg plastic pots after emergence in order to evaluate the reponse of these invasive weeds to different CO₂-concentrations (ambient and elevated). Each pot contained four plants from either of the invasive plants.

Table 3.1. List of weed species in the screening experiment

No.	Weeds	No.	Weeds
1.	<i>Avena barbata</i>	7.	<i>Lactuca serriola</i>
2.	<i>Bromus tectorum</i>	8.	<i>Lolium multiflorum</i>
3.	<i>Capsella bursa-pastoris</i>	9.	<i>Medicago sativa</i>
4.	<i>Carduus nutans</i>	10.	<i>Poa bulbosa</i>
5.	<i>Cirsium vulgare</i>	11.	<i>Potentilla recta</i>
6.	<i>Hordeum murinum</i>		

These invasive plants were exposed to either of the normal (400 ppm) or elevated (800 ppm) carbon dioxide levels in the two compartments (5×5 m) of a

glasshouse. The experiment was conducted under completely randomized design with four replications.

The data on fresh weight (g), dry weight (g) and plant height (cm) were recorded four times during the weeds' life span i.e. on 3, 5, 7 and 9 weeks after sowing (WAS). The plants were harvested and immediately weighed on an electric balance to note fresh weight. The same plants for the respective treatments were then put in paper bags and dried in an oven (Memmert Schutzzart DINEN 60529-IP20) at 70 °C until the constant weight. Afterwards, the dry weight for each treatment was recorded using an electric balance. The plant height (cm) was recorded with the help of a transparent meter rod from ground level to the tip of top leaf. Chlorophyll index was recorded at 5, 7 and 9 WAS using PlantPen NDVI 300 (Photon Systems Instruments, Czech Republic).

The standard errors were calculated for the collected data using Microsoft Excel Program. The data were drawn into line illustrations using Microsoft Excel Program. Standard errors were inserted in the line illustrations to express the difference among treatments.

3.3. Response of Invasive Weeds to High CO₂-Concentration, Elevated Temperature, N Fertilization and Herbicide Application

The following four weed species were tested for their response to high CO₂-concentration, elevated temperature, N fertilization and herbicide application.

1. *Bromus tectorum* (C₃)
2. *Capsella bursa-pastoris* (C₃)
3. *Hordeum murinum* (C₃)
4. *Lactuca serriola* (C₃)

3.4. Seed Collection

Populations of four invasive plants were collected from a vast range of land area (Table 3.2 and 3.3). The geographical location of each seed population was recorded using geographical positioning system (GPS; Magellan EXPLORIST 710 EL GPS Outdoor Elektronik). The least distance between the seed collection

location of each population of weed species was kept more than 5 km. The collected seed populations were tagged with location name and GPS coordinate, and then shifted to lab. Seed localities represented diverse environments such as canal banks, top of mountains, plain fields, crop and vegetable fields, roadsides, and fruit gardens. The seeds were collected from an altitude of 16.7 to 1686.3 m and 22.0 to 951.0 m in the first and second year of study, respectively (Table 3.2). The seeds of all collected populations were dried in shade for 7 to 10 days, separated from chaff and mixed thoroughly. The collected populations from a variety of location were pooled to form a composite sample for each weed. The composite samples of populations were then stored at room temperature (25 °C) and used when the studies were conducted.

Table 3.2. Localities of target plants in the first year of experiment 2013-2014

No.	City/town	Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	Weed*
1.	Denizli	Demirli	37.79	28.81	1142.4	1, 2
2.	Denizli	Babadağ	37.80	28.84	778.6	1, 2
3.	Denizli	Serinhisar 1	37.60	29.27	1031.5	1, 2
4.	İzmir/Ödemiş	Hamam Köy	38.01	27.99	726.9	1, 4
5.	İzmir/Ödemiş	Küre Geçidi	38.05	27.99	919.8	1, 2
6.	İzmir/Ödemiş	Çamlıca köyü 1	38.09	27.95	326.0	1, 2, 3, 4
7.	Denizli	Serinhisar 2	37.57	29.28	924.9	1, 2
8.	Denizli	Honaz Dağı Milli Parkı	37.67	29.22	863.1	1, 2, 3
9.	Denizli	Honaz 1	37.68	29.26	1686.3	1, 2
10.	İzmir/Ödemiş	Çamlıca köyü 2	38.11	27.96	324.8	1, 2, 3, 4
11.	Denizli	Akkent Köyü Yolu	38.14	29.38	806.3	1, 2
12.	Aydın	ADÜ, Ziraat Fakültesi	37.74	27.74	284.1	1, 2, 4
13.	Aydın	Ovaeymir	37.78	27.83	26.7	2,
14.	Aydın	Çaybaşı	37.76	27.83	26.7	1, 2
15.	Aydın	Boydere	37.74	27.79	40.7	1, 2, 3
16.	Aydın	Koçarlı	37.75	27.75	109.4	1, 3
17.	Aydın	Yeni Köy	37.76	27.60	117.0	1, 2, 3

Table 3.2. Contiues

18.	Aydın	Karadut Köyü	37.72	27.55	16.7	1, 3
19.	Aydın/Söke	Söke Girişi	37.74	27.42	20.4	1, 3
20.	Aydın/Söke	Sazlı Köy	37.76	27.43	23.3	1, 2, 3
21.	Aydın	7 Eylül Mah.	37.83	27.84	49.0	1, 2, 3
22.	Aydın	Tekke Köyü girişi	37.77	27.64	50.0	3,
23.	Aydın/Söke	Söke Arıtma Tesisi	37.72	27.55	16.7	3
24.	Aydın/Nazilli	Direcik köyü	37.84	28.28	58.1	1, 2, 3
25.	Aydın/Nazilli	Bozdoğan yolu	37.80	28.31	83.3	1, 2, 3

*1: *L. serriola*, 2: *H. murinum*, 3: *B. tectorum*; 4: *C. bursa-pastoris*

Table 3.3. Localities of target plants in the second year of experiment 2014-2015

No.	City/Town	Locality	Latitude (°N)	Longitude (°E)	Altitude (m)	Weed*
1.	Aydın	ADÜ, Ziraat Fakültesi	37.74	27.74	284.1	1, 2, 4
2.	Aydın	Yenipazar	37.89	28.18	91.5	2, 4
3.	Aydın	Karacasu	37.73	28.64	373.1	2, 3
4.	Aydın /Karacasu	Afrodisias	37.71	28.74	546.3	2, 3
5.	Aydın /Karacasu	Afrodisias	37.72	28.78	951.0	2, 3
6.	Aydın /Karacasu	Ataeymir	37.70	28.78	628.7	2, 3
7.	Aydın /Karacasu	Yazır	37.70	28.71	501.4	3, 4
8.	Aydın /Karacasu	Bingeç köyü	37.63	28.65	848.1	2, 3
9.	Aydın /Karacasu	Bingeç köyü	37.62	28.61	843.9	2, 3
10.	Aydın /Karacasu	Yaykın	37.61	28.58	799.0	2, 3
11.	Aydın /Karacasu	Yaykın	37.60	28.68	917.5	2, 3
12.	Aydın /Bozdoğan	Kemer barajı	37.58	28.52	315.9	3, 4
13.	Aydın	Koçarlı	37.77	27.70	22.0	1
14.	Aydın	Koçarlı-İncirlova	37.81	27.71	22.2	1, 4
15.	Aydın	Germencik	37.87	27.50	43.4	1, 4

Table 3.3. Contiues

16.	Aydın	Serçeköy	37.85	27.93	47.9	1
17.	Aydın	Koçarlı	37.76	27.73	112.2	1, 4
18.	Aydın /Koçarlı	Çakmar	37.75	27.75	109.4	1, 4
19.	Aydın /Koçarlı	Zeytinköyü	37.74	27.79	40.7	1, 4
20.	Aydın	Işıklı Veterner Fak.	37.83	27.79	36.7	1, 4
21.	Aydın	7 Eylül Mah.	37.83	27.84	49.0	1

*1: *L. serriola*, 2: *H. murinum*, 3: *B. tectorum*; 4: *C. bursa-pastoris*

3.5. Experimental Conditions

The experiments were established in a closed glasshouse having four separate compartments. Each of these compartments had an area of 5×5 m (Fig. 3.1a). Both the temperature and CO₂ inside each of the chambers were adjusted through automatic control system. Each glasshouse compartment had wiring of 4 mm diameter perforated plastic pipes. These plastic pipes were connected to CO₂ cylinders placed outside the glasshouse chambers (Fig. 3.1b). CO₂ sensors were used to check the CO₂-concentration in the chambers with high CO₂ levels treatments. This device was adjusted to sense the CO₂-concentration in the growth rooms every minute and supply CO₂ as soon as it was decreased below the desired levels (Fig. 3.1c). Locally manufactured air-conditioning systems were used to maintain the desired day/night temperature levels for the respective treatments.

These climatic conditions established in the four chambers were as following;

- 1) Control or ambient environment (CO₂=400-450 ppm; temperature 20/10 °C day/night)
- 2) Elevated temperature (CO₂=400-450 ppm; temperature 25/15 °C day/night)
- 3) High CO₂+elevated temperature (CO₂=800-900 ppm; temperature 25/15 °C day/night)
- 4) High CO₂ (CO₂=800-900 ppm; temperature 20/10 °C day/night).

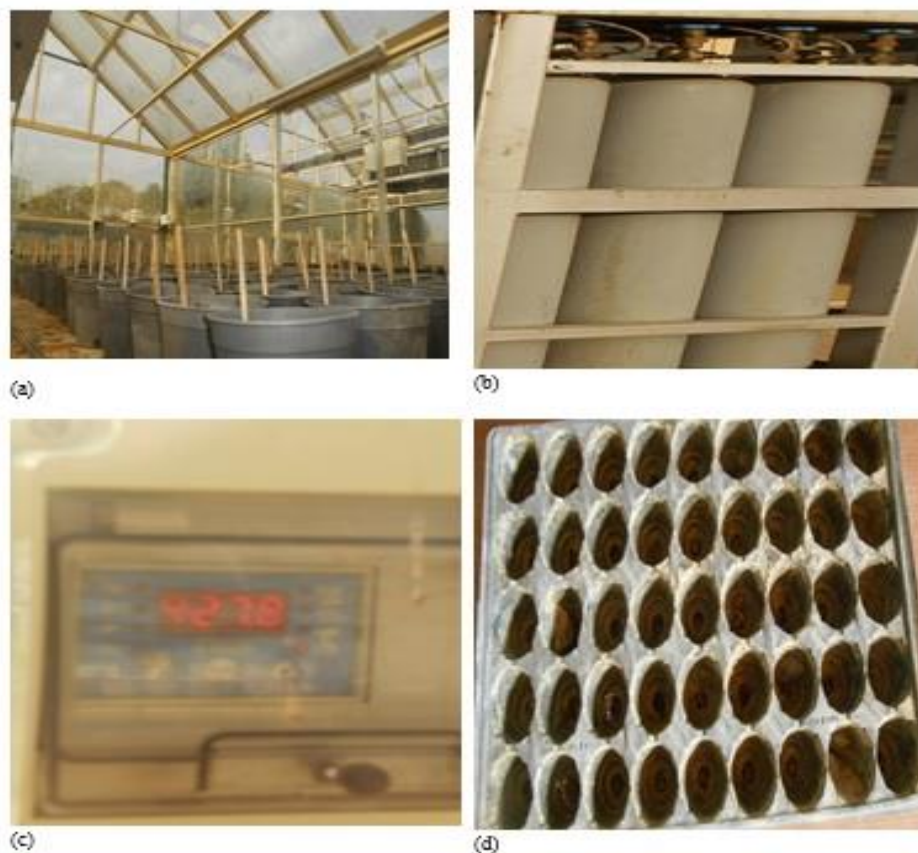


Fig. 3.1. (a) Glasshouse compartment, (b) CO₂ cylinders, (c) CO₂-concentration reader, (d) Plastic tray for growing seedlings.

3.6. Potting Medium and Experimental Design

In each year, the experiments were conducted using completely randomized design (CRD) with five replications. The seeds of each weed species were taken from the composite samples (as explained in section ‘3.4. Seed collection’) and sown in trays (20×10 cm). Each tray had 45 holes; each hole was 5 cm deep and 5 cm wide (Fig. 3.1d). The trays were filled with well-pulverized potting mix, while one seed per hole was sown on 12 November during each year of study (i.e. 2013 and 2014) to obtain seedlings. The trays were then kept in a moistened place so that the seeds can obtain moisture through seepage. The trays were monitored closely to observe the germination of invasive weeds. The 22 days old seedlings of each weed species were transplanted to 3 kg plastic pots. The plastic pots had a width and depth of 20 cm and 15 cm, respectively. These plastic pots were filled with a fine

soil. The properties of soil used in the experiment have been presented in the Table 3.4. The plastic pots filled with soil were arranged in each of the four compartments of glasshouse.

Table 3.4. A physico-chemical analysis of soil used in the experiments in 2013-2014 and 2014-2015

No.	Attribute	Concentration	
		2013-2014	2014-2015
1.	Sand (%)	74.92	67.57
2.	Silt (%)	22.47	26.72
3.	Clay (%)	2.61	5.71
4.	Texture	Loamy sand	Sandy loam
5.	Total salts (%)	0.0197	0.0204
6.	pH	7.50	7.62
7.	Organic matter (%)	3.47	3.11
8.	Phosphorus (ppm)	126	96
9.	Potassium (ppm)	239	283
10.	Calcium (ppm)	2326	3035
11.	Sodium (ppm)	53	86
12.	Iron (ppm)	22.16	20.46
13.	Zinc (ppm)	3.90	3.48
14.	Manganese (ppm)	15.12	19.76
15.	Copper (ppm)	11.74	12.22
16.	Boron (ppm)	1.99	1.83

The pots were irrigated and seedlings were transplanted on 05 December 2013 and 2014 for the first and second year of experiment, respectively. The glasshouse compartments were maintained with the climatic conditions as mentioned in the section “3.5 Experimental conditions”. The weed species were carefully monitored and irrigated when required.

3.7. Effect of Climatic Conditions and Nitrogen (N) Application on Growth and Leaf Tissue N of Invasive Weeds

In this experiment, the response of invasive weed species was determined to different levels of N in relation to different climatic conditions. The climatic

conditions have been explained in section ‘3.5. Experimental conditions’. These doses of N were:

- 1) 0 kg N ha⁻¹ (control)
- 2) 60 kg N ha⁻¹, and
- 3) 120 kg N ha⁻¹.

The arrangement of treatments for this experiment has been presented in Fig. 3.2. Nitrogen doses in the experiment were applied in two equal splits. In each year, first split of N was applied on 10 December and second on 01 January. The N in the form of urea (46% N) was dissolved in water and delivered to plants as solution.

3 = High CO₂ + elevated temperature				2 = Elevated temperature		
CO ₂ = 800-900 ppm Temperature = 25/15 °C (day/night)				CO ₂ = 400-450 ppm Temperature = 25/15 °C (day/night)		
	N0	N60	N120	N0	N60	N120
R1	Nitrogen = 0 kg/ha	Nitrogen = 60 kg/ha	Nitrogen = 120 kg/ha			
R2						
R3						
R4						
R5						
4 = High CO₂				1= Control		
CO ₂ = 800-900 ppm Temperature = 20/10 °C (day/night)				CO ₂ = 400-450 ppm Temperature = 20/10 °C (day/night)		
	N0	N60	N120	N0	N60	N120
R1						
R2						
R3						
R4						
R5						

Fig. 3.2. The arrangement of treatments in the glasshouse for investigating the effect of climate change and nitrogen application on growth and N uptake of invasive weeds.

Leaf tissue nitrogen was determined for the plants applied with N. Three replications of test plants applied with 0 kg N ha⁻¹ (control) and 120 kg N ha⁻¹ (high N) were analyzed for N contents. The leaf tissue nitrogen was determined by

the method of digestion, distillation and titration as suggested by Bremner (1965). Digestion was the first step in this process. The leaves from all plant species were ground to fine powder. A 0.25 g of powdered sample was taken in a tube, and added with mercury catalyst and K₂SO₄ tablet as well as 3 ml of H₂SO₄ (98%). Then, it was added with a 2 ml of H₂O₂ (35%). The tubes were settled in the digestion set, and the process of digestion was started until the color of material turned green. The process of digestion was done within three hours and at a temperature below 410 °C. In digestion process, organic N is converted into ammonium sulphate. The digest was diluted to 25 ml of volume and stored in air tight plastic bottles. For distillation, 10 ml of aliquot was taken into distillation flask and distilled after adding 10 ml of 10 N sodium hydroxide (NaOH) until about 35 ml of distillate was received. Distillate was received in receiver flask containing 5 ml of mixed indicator (methyl red and bromocresol green) solution. The distillate was then titrated against 0.01 N H₂SO₄ to pinkish red end point (pH 5). Each batch of distillations was include with a distillation of 10 ml ammonium-N standard with 0.2 g MgO, 10 ml deionized water and with 0.2 g MgO to assure the recovery of ammonium-N standards to be at least 98%. The N in the plant material was calculated using the following formula;

$$N (\%) = \frac{[(V - B) \times N \times R \times 14.01 \times 100]}{Wt \times 1000}$$

Where,

V = volume of standard H₂SO₄ used for sample

B = volume of standard H₂SO₄ used for digest blank

R = ratio of the total digest volume to distillation volume

Wt = weight of plant material taken for digestion

N = normality of H₂SO₄ used for titration

For each of the weed species in either of the compartments, the data was recorded for plant height (cm), leaf length (cm), number of leaves, chlorophyll index, leaf area (cm²), fresh weight (g), and dry weight (g).

- Plant height was recorded with help of a transparent plastic meter rod. Plant height was recorded from the point where the plant trunk touches the soil surface up to the top point of plant (shoot or leave).
- Length of three longest leaves from each plant was recorded with the help of meter rod. The leaf length was recorded from the base of leaf to its tip and averaged.
- Number of leaves was counted manually for each plant in the experiment.
- Two readings of chlorophyll index were taken from each plant with the help of PlantPen NDVI 300 (Photon Systems Instruments, Czech Republic) chlorophyll meter and averaged.
- The weeds were harvested on 05 February 2014 and 2015 (in the first and second year of experiment); the fresh weight was recorded for each plant in the experiment using electric balance.
- The weeds were separated into leaves and shoots. The leaves separated from shoots were used to determine the leaf area with help of leaf area meter (CI-202 Portable Laser Area Meter, Bio-Science USA). Further, the harvested weed plants were put in paper bags, labeled and dried in oven (MemmertSchutzart DINEN 60529-IP20) for constant dry weight.

3.8. Effect of Herbicide (Glyphosate) Application on Control of Invasive Weeds under Different Climatic Conditions

Glyphosate is the most common herbicide used to control invasive species, hence, this herbicide was selected for this experiments. The efficacy of glyphosate was evaluated against four selected invasive weeds under four different climatic conditions. The invasive plants in all of the four climatic conditions (as described in section '3.4. Experimental conditions') were treated with different glyphosate doses when the weeds were at growth stage of shoot elongation (BBCH 31-33) according to Hess et al. (1997). The glyphosate rates used in the experiment were:

- 1) 0 g a.i. ha⁻¹ (untreated control),
- 2) 360 g a.i. ha⁻¹,

- 3) 720 g a.i. ha⁻¹,
- 4) 1080 g a.i. ha⁻¹,
- 5) 1440 g a.i. ha⁻¹ (recommended or standard dose), and
- 6) 2880 g a.i. ha⁻¹

(i.e. 0, 25, 50, 75, 100 and 200% of the recommended dose). The arrangement of treatments for this experiment has been presented in Fig. 3.3.)

Glyphosate (Roundup Star 441 g/l glyphosate potassium salt) was used to prepare herbicide doses included in the experiment. The herbicide doses were applied through an automatic application system which had calibration for delivering 200 L ha⁻¹ volume of spray. The herbicides were sprayed on 5th January in each year. The herbicide application was started with lowest dose (360 g a.i. ha⁻¹: 25% of recommended) and finished with highest doses (2880 g a.i. ha⁻¹: 200% of recommended). The desired dose of herbicide was filled in a 150 ml tube which was attached to herbicide applicator. Applicator was turned on to apply the herbicide. After herbicide treatment, the plants were put back to greenhouse under same conditions where they were grown.

3 = High CO₂ + elevated temperature							2 = Elevated temperature					
CO ₂ = 800-900 ppm Temperature = 25/15 °C (day/night)							CO ₂ = 400-450 ppm Temperature = 25/15 °C (day/night)					
H0	H25	H50	H75	H100	H200		H0	H25	H50	H75	H100	H200
R1	360 g ai/ha	720 g ai/ha	1080 g ai/ha	1440 g ai/ha	2880 g ai/ha							
R2												
R3												
R4												
R5												
4 = High CO₂							1 = Control					
CO ₂ = 800-900 ppm Temperature = 20/10 °C (day/night)							CO ₂ = 400-450 ppm Temperature = 20/10 °C (day/night)					
H0	H25	H50	H75	H100	H200		H0	H25	H50	H75	H100	H200
R1												
R2												
R3												
R4												
R5												

Fig. 3.3. The arrangement of treatments for investigating the effect of climatic conditions and herbicide application on control of invasive weeds.

The herbicide efficacy on weed plants in either of the climatic conditions was recorded as % effect (compared with untreated control) through visual observation (Brown et al. 2011). Fresh and dry weight of plants treated with herbicides were also determined. However, these were not used for constructing the dose-response curve. The reason was the biomass yielded in all treatments even from the plants which had completely died after glyphosate application. Hence, visual observations were used to explain the effect of herbicides on invasive weeds under different climatic conditions.

In the herbicide experiments too, the growth data of non-treated (control) plants was recorded. The parameters recorded were included plant height (cm), leaf length (cm), number of leaves, chlorophyll index, leaf area (cm²), fresh weight (g), and dry weight (g). The procedures to record this data has been mentioned in section 3.7.

3.9. Data Analysis

The significance of recorded data was checked by analysis of variance (ANOVA) technique performed with the help of software IBM SPSS Statistics 20.0 by using General Linear Model (GLM) (Field, 2013). The difference among the treatments was determined using Duncan's Multiple Range Test. Log-logistic equation was used to perform a non-linear regression in order to obtain dose-response curve for visual observation. The regression parameters for different invasive weeds in the experiments were determined using Sigma Plot^{®10} (Culpepper et al. 2006; Knezevic et al. 2007). Similarly, ED₅₀ and ED₉₀ of four weeds were also determined using Sigma Plot^{®10} (Culpepper et al. 2006; Knezevic et al. 2007). ED₅₀ and ED₉₀ were the doses of glyphosate required to control 50 and 90% of weeds, respectively.

4. RESULTS

4.1. Germination Test in the Screening Studies

The results of germination experiment have been presented in this section. Most of the weed species had a germination of above 50% (Table 4.1). Only three weeds (*Medicago sativa*, *Poa bulbosa*, *Potentilla recta*) had a germination percentage below 50% (Table 4.1).

Table 4.1: Germination percentage of different invasive weed species

No.	Weeds	Germination percentage		
		5 DAS*	9 DAS	13 DAS
1.	<i>Avena barbata</i>	0	62.2	67.7
2.	<i>Bromus tectorum</i>	45.6	93.3	93.3
3.	<i>Capsella bursa-pastoris</i>	12.2	64.4	65.6
4.	<i>Carduus nutans</i>	0	58.9	60.0
5.	<i>Cirsium vulgare</i>	0	53.3	51.1
6.	<i>Hordeum murinum</i>	30.0	73.3	80.0
7.	<i>Lactuca serriola</i>	0	58.9	63.3
8.	<i>Lolium multiflorum</i>	0	65.6	77.5
9.	<i>Medicago sativa</i>	9	42.2	44.4
10.	<i>Poa bulbosa</i>	0	18.7	35.6
11.	<i>Potentilla recta</i>	2.2	28.6	31.1

*DAS = Days after sowing

4.2. Determination of Test Species for Experiments (Screening Studies)

Results regarding the effect of ambient and high CO₂-concentrations on 11 invasive weed species have been presented in this section. The data recorded on 28-January, 12-February, 28-February and 15-March correspond to 3, 5, 7 and 9 weeks after transplanting (WAT) of weed seedlings. The results indicated that high CO₂-concentration increased the growth and biomass of many of the test species included in the initial experiment while some of the invasive weeds remained unaffected or affected negatively.

Dry and fresh weight of *Avena barbata* was similar at 3 and 5 WAT while, significantly increased by high CO₂-concentration (800 ppm) at 7 and 9 WAT (Fig. 4.1). Plant height of *A. barbata* was statistically similar for normal (400 ppm) and high CO₂. High CO₂ increased the chlorophyll index of *A. barbata* at third data recording.

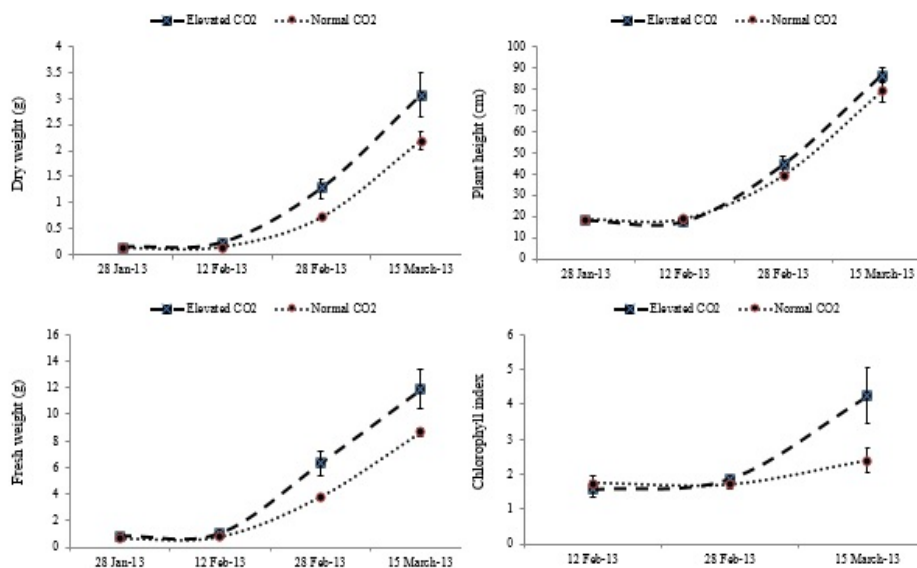


Fig. 4.1. Effect of normal and high CO₂-concentration on growth parameters of *Avena barbata*. The vertical bars on the lines are standard errors of means.

Dry and fresh weigh of *Carduus nutans* was significantly increased by high CO₂-concentration at 7 and 9 WAT (Fig. 4.2). Plant height and chlorophyll index of *C. nutans* was unchanged by the CO₂ levels.

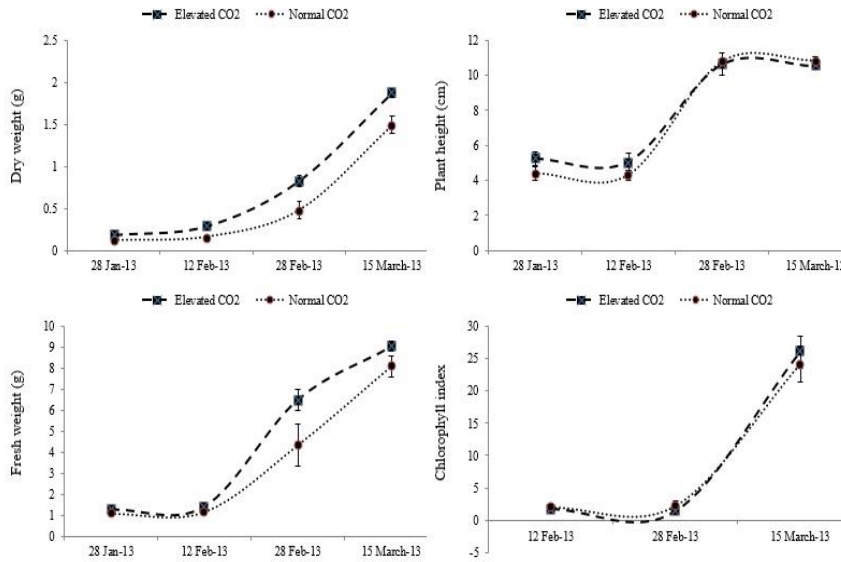


Fig. 4.2. Effect of normal and high CO₂-concentration on growth parameters of *Carduus nutans*. The vertical bars on the lines are standard errors of means.

Growth of *Cirsium vulgare* (including dry weight, fresh weight, and plant height) was statistically similar for normal and high CO₂ levels (Fig. 4.3). However, chlorophyll index of this weed was noted to be significantly increased by high CO₂-concentration at second data recording.

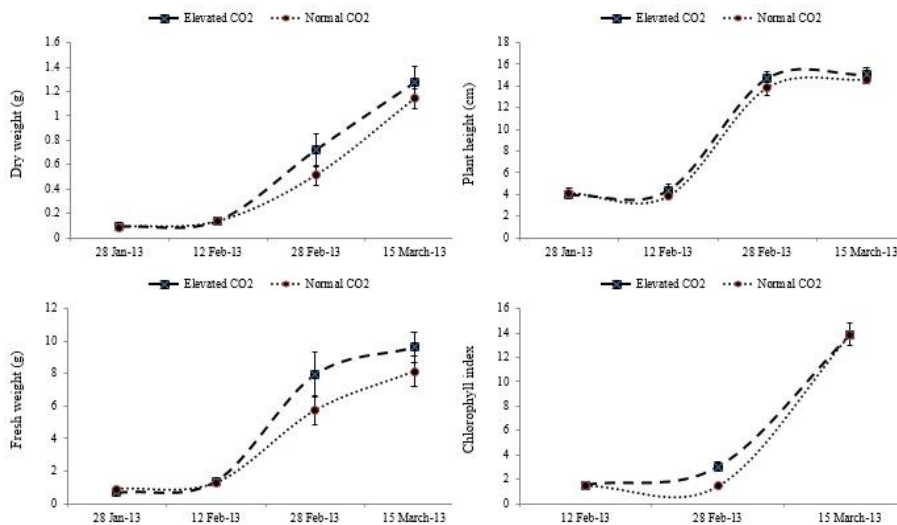


Fig. 4.3. Effect of normal and high CO₂-concentration on growth parameters of *Cirsium vulgare*. The vertical bars on the lines are standard errors of means.

Dry and fresh weights, and chlorophyll index of *Lolium multiflorum* were significantly increased by high CO₂ level at 7 and 9 WAT (Fig. 4.4). Plant height of this weed was negatively affected by high CO₂-concentration.

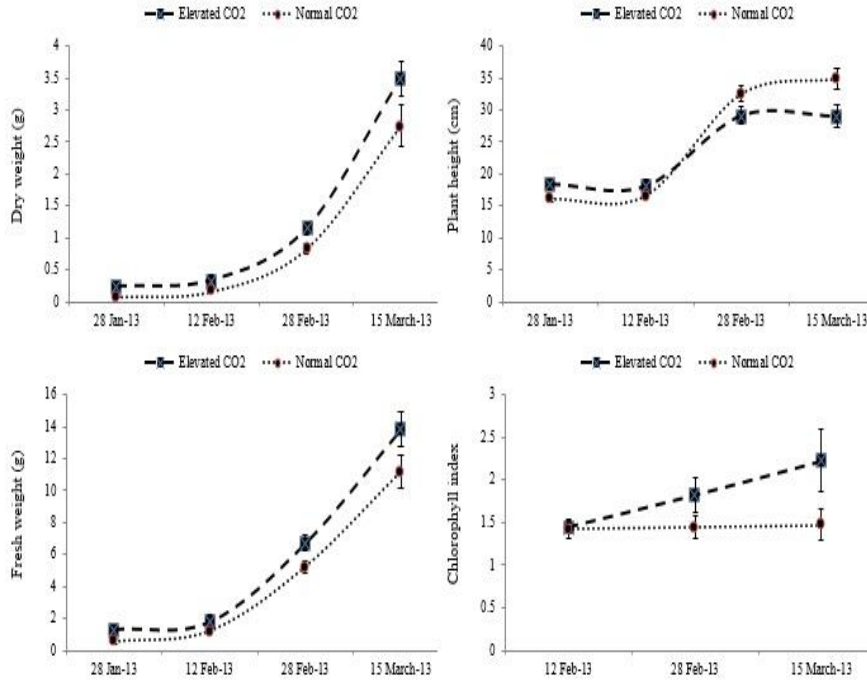


Fig. 4.4. Effect of normal and high CO₂-concentration on growth parameters of *Lolium multiflorum*. The vertical bars on the lines are standard errors of means.

Dry and fresh weights of *Medicago sativa* were increased by high CO₂ level only at 7 WAT (Fig. 4.5). Plant height of this weed was not affected by different CO₂ levels while chlorophyll index was significantly increased by high CO₂ levels during 5 and 7 WAT.

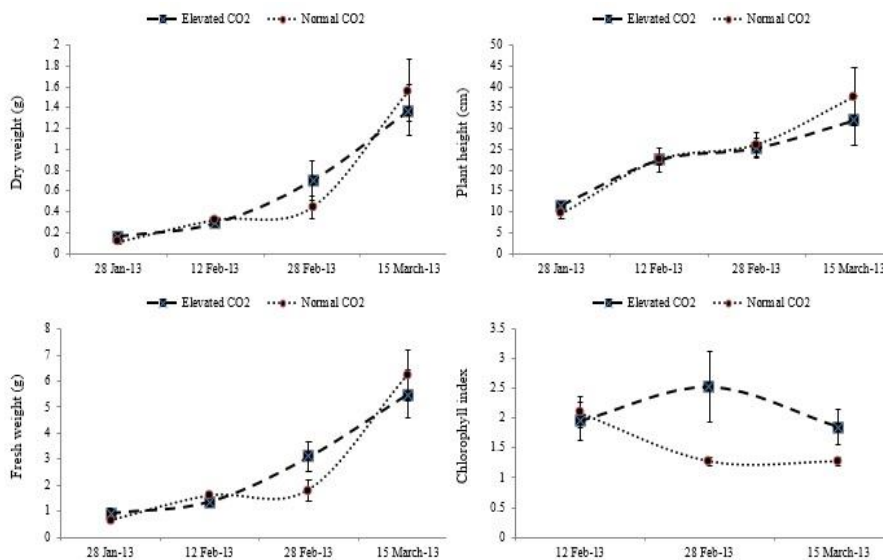


Fig. 4.5. Effect of normal and high CO₂-concentration on growth parameters of *Medicago sativa*. The vertical bars on the lines are standard errors of means.

Plant height, and dry and fresh weights of *Poa bulbosa* were significantly increased by high CO₂-concentration in general (Fig. 4.6). Besides chlorophyll index was also increased only at 9 WAT.

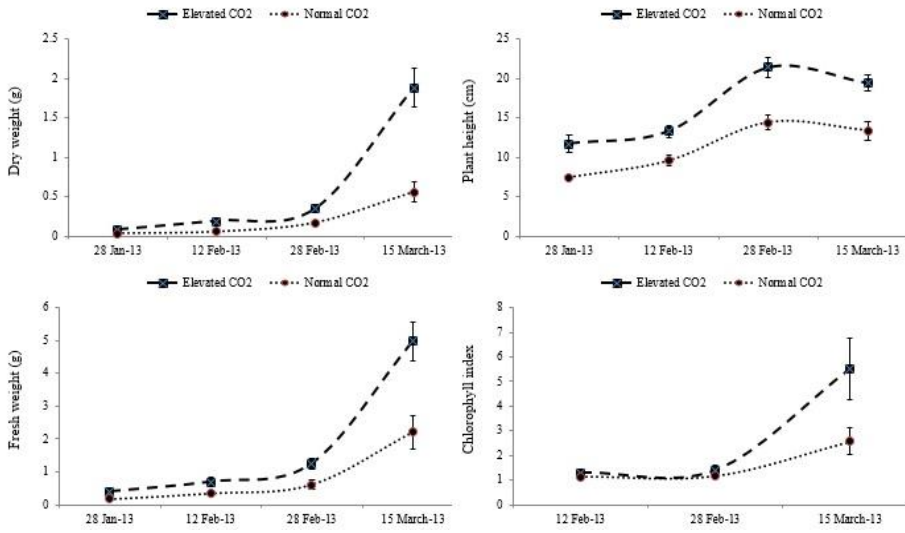


Fig. 4.6. Effect of normal and high CO₂-concentration on growth parameters of *Poa bulbosa*. The vertical bars on the lines are standard errors of means.

Dry weight of *Bromus tectorum* was increased under high CO₂-concentration at 7 and 9 WAT while fresh weight was increased only at 5 WAT (Fig. 4.7). Plant height was significantly increased by high CO₂-concentration in all stages while chlorophyll index was increased only at 7 WAT.

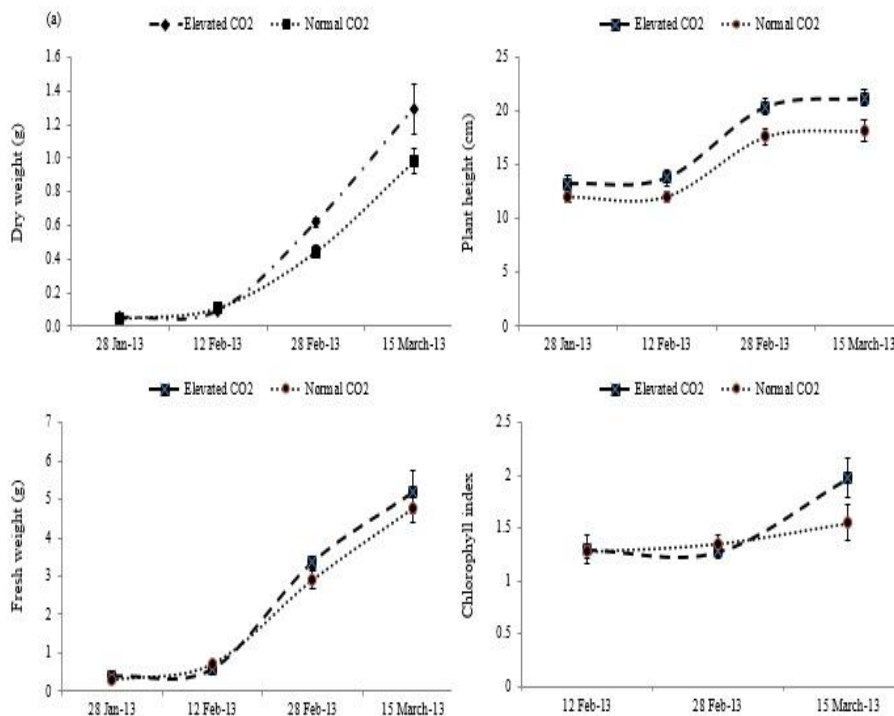


Fig. 4.7. Effect of normal and high CO₂-concentration on growth parameters of *Bromus tectorum*. The vertical bars on the lines are standard errors of means.

Dry weight of *Capsella bursa-pastoris* remained same under normal and high CO_2 ; however, fresh weight was significantly decreased by high CO_2 -concentration at 7 WAT (Fig. 4.8).

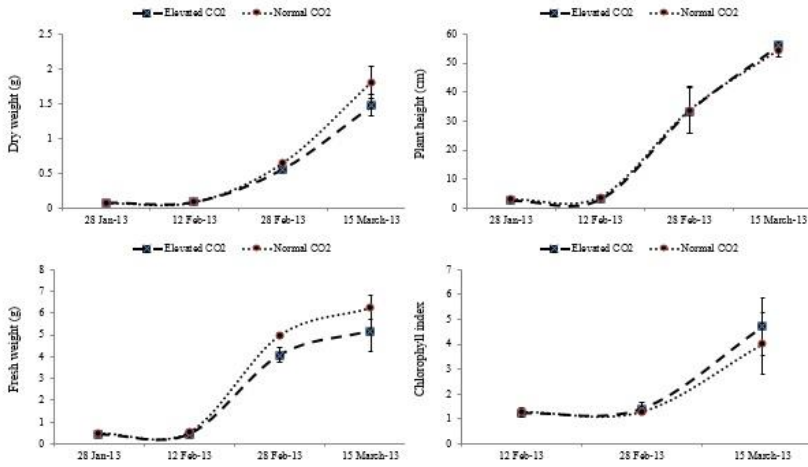


Fig. 4.8. Effect of normal and high CO_2 -concentration on growth parameters of *Capsella bursa-pastoris*. The vertical bars on the lines are standard errors of means.

Dry weight of *Hordeum murinum* was increased by high CO₂-concentration at 3, 5, 7 and 9 WAT (Fig. 4.9). Similarly, fresh weight of the same weed was significantly increased by high CO₂ at 3, 5, and 7 WAT. The plant height was increased by high CO₂-concentration at first two data recordings (3 and 5 WAT) while, chlorophyll index remained same under both CO₂ levels.

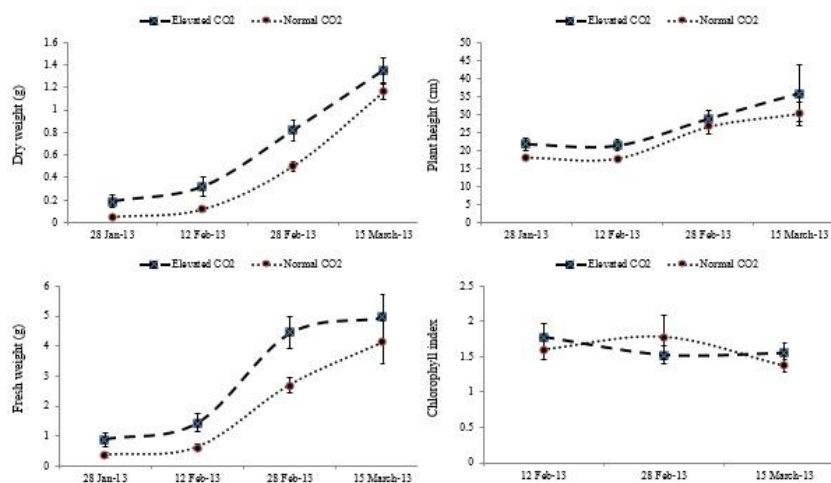


Fig. 4.9. Effect of normal and high CO₂-concentration on growth parameters of *Hordeum murinum*. The vertical bars on the lines are standard errors of means.

Dry and fresh weights of *Lactuca serriola* and *Potentilla recta* were increased by high CO₂-concentration at 7 WAT while, plant height and chlorophyll index were not affected by CO₂ levels (Fig. 4.10, 4.11).

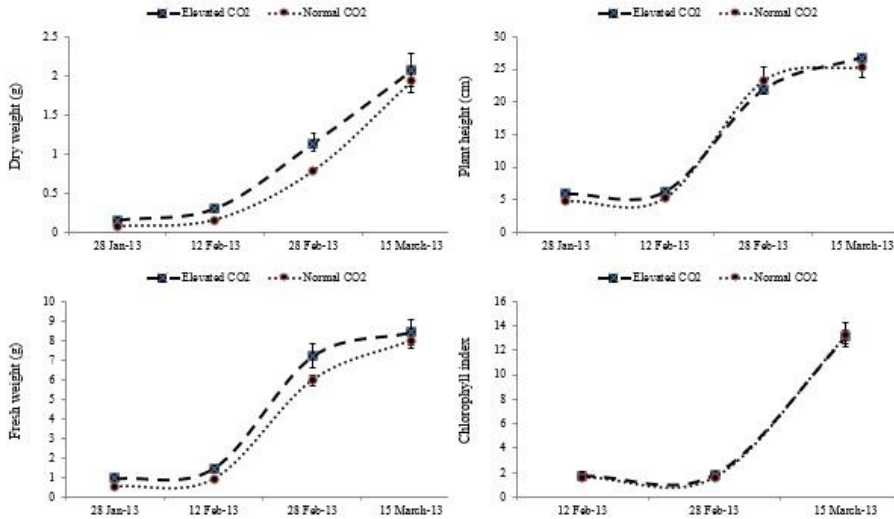


Fig. 4.10. Effect of normal and high CO₂-concentration on growth parameters of *Lactuca serriola*. The vertical bars on the lines are standard errors of means.

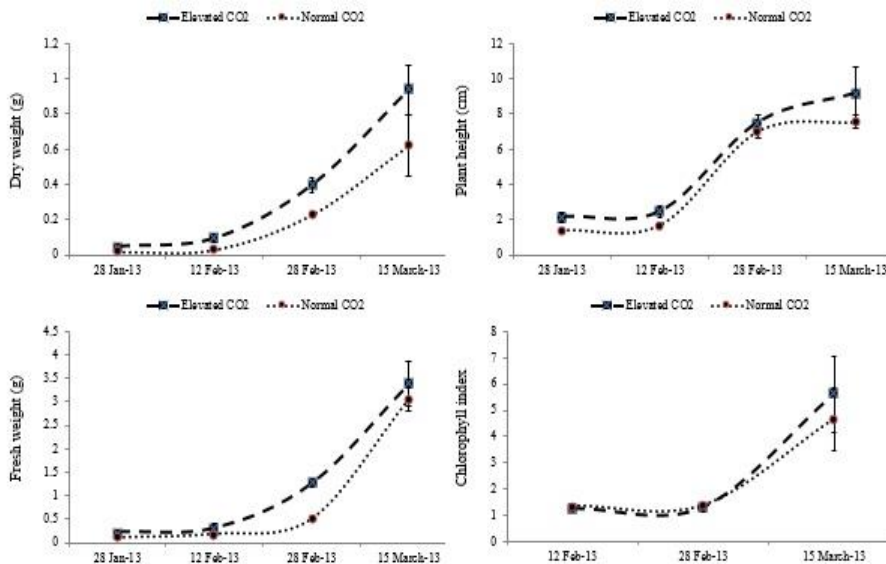


Fig. 4.11. Effect of normal and high CO₂-concentration on growth parameters of *Potentilla recta*. The vertical bars on the lines are standard errors of means.

Out of the tested invasive weeds, *A. barbata*, *B. tectorum*, *C. nutans*, *H. murinum*, *L. multiflorum*, *L. serriola*, *P. bulbosa* and *P. recta* were the weeds which responded positively (in terms of their growth) to high CO₂-concentration. Out of these, three weeds were randomly selected including *B. tectorum*, *H. murinum*, and *L. serriola*. Only three weeds were selected for detailed experiments because of the limited space available in glasshouse. *Capsella bursa-pastoris* was the weed which had responded negatively to high CO₂-concentration, hence, this weed too was included in the detailed experiments.

4.3. Effect of Nitrogen Fertilization and Climatic Conditions on Growth and Leaf Tissue N Contents of Weed Species

In the following, the results regarding the effects of CO₂-concentrations, temperature levels and N fertilization on growth and leaf tissue N contents of four invasive weed species have been presented.

4.3.1. *Bromus tectorum*

Analysis of variance (ANOVA) indicated that year, as a factor had a significant effect on dry weight, fresh weight, leaf length, and leaf area of *B. tectorum* (Table 4.2). Climatic conditions significantly affected dry weight, fresh weight, leaf length, plant height and leaf area of *B. tectorum* (Table 4.2).

Table 4.2. Analysis of variance (*p* values) for effect of climatic conditions and nitrogen fertilization on growth of *Bromus tectorum*

Factors	Dry weight	Fresh weight	C.I.	Leaf length	Plant height	No. of leaves	Leaf area	Leaf tissue N
Year	0.009	0.0001	0.076	0.0001	0.342	0.306	0.0001	0.641
Climate	0.0001	0.0001	0.159	0.0001	0.0001	0.603	0.0001	0.053
Nitrogen	0.363	0.002	0.941	0.335	0.851	0.936	0.0001	0.061
Year× climate	0.259	0.069	0.938	0.125	0.007	0.333	0.341	0.650
Year× nitrogen	0.08	0.001	0.567	0.879	0.143	0.098	0.241	0.828
Climate× nitrogen	0.961	0.446	0.371	0.828	0.297	0.745	0.0001	0.805
Year×climate × nitrogen	0.802	0.455	0.873	0.556	0.069	0.606	0.592	0.980

C.I. = chlorophyll index; No = number

Nitrogen application had a significant affect only on fresh weight and leaf area of *B. tectorum* (Table 4.2). Year×climate had a significant effect only on plant height, and year×nitrogen had a significant effect only on fresh weight of *B. tectorum* (Table 4.2). Climate×nitrogen had a significant effect only on leaf area of *B. tectorum* (Table 4.2). High CO₂-concentration and high CO₂-concentration+elevated temperature had a higher dry weight, fresh weight, leaf length, plant height, and leaf area than control and elevated temperature treatments (Table 4.3; Fig. 4.13-4.15).

Table 4.3. Effect of climatic conditions on growth of *Bromus tectorum* (2-years' average data)

Climatic conditions	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	3.9b	34.1b	41.6	29.9b	41.8b	196	3244.1c	4.49
Elevated temperature	3.9b	35.1b	41.9	30.8b	42.0b	185	2963.5c	4.56
High CO ₂ +elevated temperature	5.0a	44.5a	43.3	36.6a	50.5a	187	3683.9b	3.93
High CO ₂	4.6a	44.9a	43.4	36.4a	49.6a	178	4345.8a	4.23
MSE	0.94	32.6	NS	13.7	15.5	NS	630.2	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

Chlorophyll index, number of leaves and leaf tissue N were statistically similar for *B. tectorum* plants grown under either of the climatic conditions. Nitrogen application [both, medium (60 kg ha⁻¹) and high dose (120 kg ha⁻¹)] significantly increased the fresh weight and leaf area of *B. tectorum*, while rest of the parameter were not influenced by nitrogen application (Table 4.4).

Table 4.4. Effect of nitrogen fertilization on growth of *Bromus tectorum* (2-years' average data)

Nitrogen rates	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	4.1	37.0b	42.4	32.8	46.1	188.1	3132.8b	4.1
Medium dose	4.4	41.0a	42.5	34.0	46.0	184.2	3782.1a	-
High dose	4.4	41.0a	42.7	33.5	45.7	187.5	3763.0a	4.5
MSE	NS	32.6	NS	NS	NS	NS	630.2	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

A combination of high CO₂-concentration+high N dose had greatly increased the leaf area of *B. tectorum* compared with the control and other treatments in the experiment (Fig. 4.12). The high N dose in climatic conditions other than high CO₂-concentration did not provided a leaf area as high as in high CO₂-concentration (Fig. 4.12).

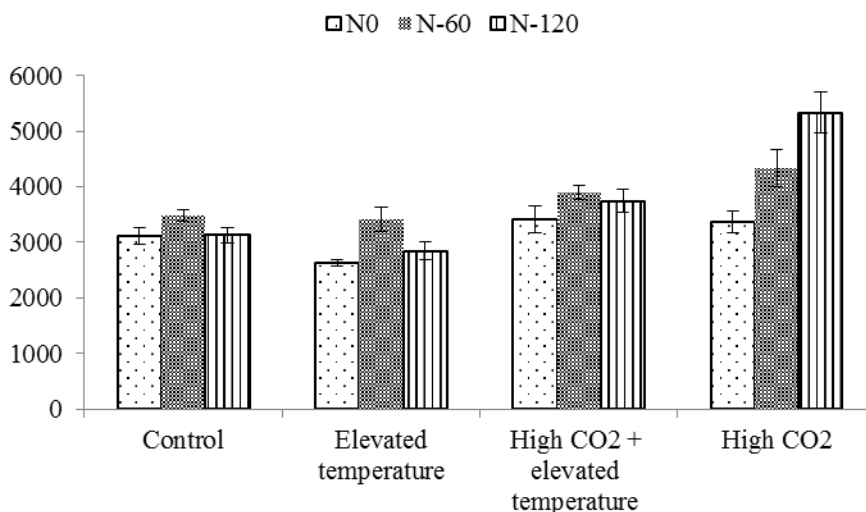


Fig. 4.12. Interactive effect of nitrogen fertilization and climatic conditions on leaf area of *Bromus tectorum* (2-years' average data); The vertical bars on the histograms are standard errors of means.



Fig. 4.13. Growth of *Bromus tectorum* under normal (left) and high CO₂-concentration (right)



Fig. 4.14. Growth of *Bromus tectorum* under ambient conditions (left) and high CO₂-concentration+elevated temperature (right)



Fig. 4.15. Growth of *Bromus tectorum* under normal (left) and elevated temperature (right)

4.3.2. *Capsella bursa-pastoris*

ANOVA indicated that year as a factor had a significant effect on dry weight, fresh weight, plant height, leaf area and leaf tissue N of *C. bursa-pastoris* (Table 4.5). Climatic conditions had a significant effect on dry weight, fresh weight, chlorophyll index, leaf length, plant height, number of leaves and leaf area of *C. bursa-pastoris*. Nitrogen application significantly affected the dry weight, fresh weight, chlorophyll index, leaf length, number of leaves and leaf area of *C. bursa-pastoris* (Table 4.5). Year \times climate had a significant effect only on chlorophyll index of *C. bursa-pastoris*, while climate \times nitrogen had a significant effect on dry weight of *C. bursa-pastoris*.

Table 4.5. Analysis of variance (*p* values) for effect of climatic conditions and nitrogen fertilization on growth of *Capsella bursa-pastoris*

Factors	Dry weight	Fresh weight	C.I.	Leaf length	Plant height	No. of leaves	Leaf area	Leaf tissue N
Year	0.005	0.0001	0.845	0.178	0.002	0.77	0.0001	0.002
Climate	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.865
Nitrogen	0.002	0.0001	0.008	0.001	0.17	0.0001	0.0001	0.532
Year× climate	0.273	0.292	0.0001	0.132	0.123	0.184	0.903	0.667
Year× nitrogen	0.382	0.319	0.55	0.69	0.78	0.86	0.081	0.869
Climate× nitrogen	0.0001	0.077	0.916	0.891	0.093	0.058	0.095	0.993
Year× climate× nitrogen	0.963	0.956	0.44	0.983	0.99	0.99	0.724	0.976

C.I. = chlorophyll index; No = number.

Control and high CO₂-concentration treatments had a higher dry weight, fresh weight, chlorophyll index, leaf length, plant height and leaf area than elevated temperature and high CO₂-concentration+elevated temperature (Table 4.6; Fig. 4.17-4.19). A higher chlorophyll index, leaf length, plant height and number of leaves were recorded in elevated temperature treatment than high CO₂-concentration+elevated temperature treatment (Table 4.6; Fig. 4.17-4.19).

Table 4.6. Effect of climatic conditions on growth of *Capsella bursa-pastoris* (2-years' average data)

Climatic conditions	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	5.03a	52.8a	39.2ab	27.7a	64.3a	36.5a	1331a	3.86
Elevated temperature	3.45b	37.0b	38.5bc	24.5bc	39.0b	30.6b	892.4b	3.84
High CO ₂ + elevated temperature	3.64b	37.8b	36.8c	23.3c	32.8c	21.4c	831.1b	3.76
High CO ₂	5.09a	53.5a	40.7a	26.6ab	66.7a	29.2b	1106a	3.76
MSE	1.13	93.8	8.0	14.7	81.5	46.4	354.4	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

Nitrogen application had significantly increased the dry weight, fresh weight, chlorophyll index, leaf length, plant height, number of leaves and leaf area of *C. bursa-pastoris* (Table 4.7). Highest dry weight and fresh weight were noted for high N dose followed by medium and control dose of N.

Table 4.7. Effect of nitrogen fertilization on growth of *Capsella bursa-pastoris* (2-years' average data)

Nitrogen rates	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N%
Control	3.9c	38.6c	37.7b	23.8b	48.9b	25.8b	877.1b	3.8
Medium dose	4.2bc	44.9b	39.4a	25.7ab	49.1b	30.1a	1012.0b	-
High dose	4.7a	51.9a	39.4a	27.0a	54.1a	32.4a	1231.5a	3.8
MSE	1.13	93.8	8.05	14.7	81.5	46.4	354.4	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

Chlorophyll index, leaf length and number of leaves were higher (and statistically similar) in high and medium N doses compared with control treatment. Plant height and leaf area were improved only by high N dose, while medium N dose and control had similar plant height and leaf area (Table 4.7).

A combination of high CO₂-concentration+high N dose had the highest dry weight of *C. bursa-pastoris* compared with any other treatment combination in the experiment (Fig. 4.16).

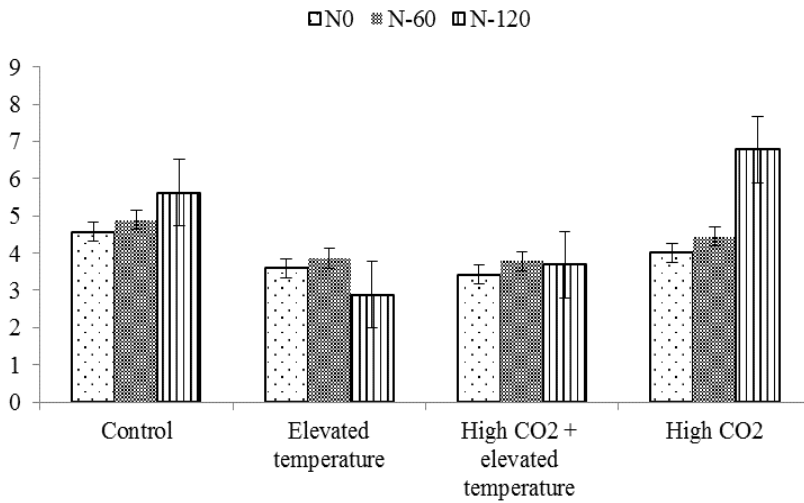


Fig. 4.16. Interactive effect of nitrogen fertilization and climatic conditions on dry weight of *Capsella bursa-pastoris* (2-years' average data); The vertical bars on the histograms are standard errors of means.



Fig. 4.17. Growth of *Capsella bursa-pastoris* under normal (left) and high CO₂-concentration (right)



Fig. 4.18. Growth of *Capsella bursa-pastoris* under ambient conditions (left) and high CO₂-concentration+ elevated temperature (right)



Fig. 4.19. Growth of *Capsella bursa-pastoris* under normal (left) and elevated temperature (right)

4.3.3. *Hordeum murinum*

Year as a factor had a significant effect on number of leaves and leaf tissues N of *H. murinum* (Table 4.8). Climatic conditions significantly affected the dry weight, fresh weight, chlorophyll index, leaf length, plant height and leaf area of *H. murinum*. Nitrogen had a positive influence only for plant height, leaf area and leaf tissue N of *H. murinum* (Table 4.8). Year \times climate had a significant effect on fresh weight, leaf length and plant height of *H. murinum*.

Table 4.8. Analysis of variance (p values) for effect of climatic conditions and nitrogen fertilization on growth of *Hordeum murinum*

Factors	Dry weight	Fresh weight	C.I.	Leaf length	Plant height	No. of leaves	Leaf area	Leaf tissue N
Year	0.18	0.77	0.27	0.06	0.80	0.001	0.623	0.016
Climate	0.0001	0.0001	0.0001	0.0001	0.0001	0.165	0.0001	0.142
Nitrogen	0.316	0.191	0.99	0.195	0.004	0.347	0.002	0.014
Year × climate	0.218	0.045	0.901	0.002	0.001	0.671	0.308	0.112
Year × nitrogen	0.151	0.831	0.761	0.119	0.684	0.704	0.705	0.398
Climate × nitrogen	0.313	0.174	0.634	0.686	0.811	0.540	0.262	0.214
Year × climate × nitrogen	0.630	0.97	0.537	0.869	0.926	0.289	0.836	0.647

C.I. = chlorophyll index; No = number.

A higher (and statistically similar) dry weight, fresh weight and plant height were noted for *H. murinum* plants grown under high CO₂-concentration and high CO₂-concentration+elevated temperature compared with elevated temperature and control treatments (Table 4.9; Fig. 4.20-4.22). The latter two treatments (elevated temperature and control) had a statistically similar dry weight, fresh weight and plant height for *H. murinum*. The highest leaf length and leaf area of *H. murinum* plants were noted for CO₂-concentration treatment followed by high CO₂-concentration+elevated temperature treatment (Table 4.9). Elevated temperature treatment had a higher leaf area of *H. murinum* than control while these two treatments had a statistically similar leaf length (Table 4.9). Leaf tissue N concentration was statistically similar for all climatic conditions.

Table 4.9. Effect of climatic conditions on growth of *Hordeum murinum* (2-years' average data)

Climatic conditions	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	5.54b	49.4b	46.6a	42.0c	63.2b	124.0	3502.5c	3.78
Elevated temperature	5.88b	48.5b	43.4ab	41.0c	62.8b	139.0	4150.0b	4.23
High CO ₂ + elevated temperature	7.09a	65.7a	46.0a	48.0b	71.9a	128.0	4380.5b	4.09
High CO ₂	6.94a	69.5a	45.2a	50.8a	74.9a	132.0	5224.8a	3.89
MSE	1.54	162.5	8.7	14.9	50.8	NS	628.1	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

Table 4.10. Effect of nitrogen fertilization on growth of *Hordeum murinum* (2-years' average data)

Nitrogen rates	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	6.2	55.3	45.3	44.8	65.2b	126.0	4042.1b	3.8b
Medium dose	6.3	59.2	45.3	45.2	68.9ab	133.0	4231.0b	-
High dose	6.6	60.3	45.4	46.3	70.6a	133.0	4670.2a	4.2a
MSE	NS	NS	NS	NS	50.8	NS	628.1	0.251

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number.; NS = non-significant.

Nitrogen application (both high and medium dose) had increased the plant height of *H. murinum* (Table 4.10). Similarly, a higher leaf tissue N concentration was recorded for high N dose (Table 4.10).



Fig. 4.20. Growth of *Hordeum murinum* under normal (left) and high CO₂-concentration (right)



Fig. 4.21. Growth of *Hordeum murinum* under ambient conditions (left) and high CO₂-concentration+elevated temperature (right)



Fig. 4.22. Growth of *Hordeum murinum* under normal (left) and elevated temperature (right)

4.3.4. *Lactuca serriola*

ANOVA indicated that year as a factor, had a significant effect on dry weight, fresh weight, chlorophyll index, plant height, number of leaves and leaf tissue N concentration of *L. serriola* (Table 4.11). Climatic conditions significantly affected the *L. serriola* plant growth parameters such as dry and fresh weights, leaf length, plant height, number of leaves, and leaf area of *L. serriola*. Similarly, N fertilization had significantly affected the dry and fresh weights, chlorophyll index, leaf length, plant height, number of leaves, leaf are and leaf tissue N concentration (Table 4.11). Year×climate had a significant effect on leaf length, number of leaves and leaf area while year×nitrogen had a significant effect only on dry weight, and number of leaves. Climate×nitrogen had a significant effect on dry weight, number of leaves and leaf area (Table 4.11).

Table 4.11. Analysis of variance (p values) for effect of climatic conditions and nitrogen fertilization on growth of *Lactuca serriola*

Factors	Dry weight	Fresh weight	C.I.	Leaf length	Plant height	No. of leaves	Leaf area	Leaf tissue N
Year	0.0001	0.0001	0.0001	0.339	0.01	0.0001	0.0001	0.0001
Climate	0.0001	0.0001	0.269	0.0001	0.016	0.0001	0.0001	0.421
Nitrogen	0.0001	0.0001	0.049	0.0001	0.002	0.0001	0.0001	0.042
Year× climate	0.658	0.18	0.064	0.0001	0.131	0.001	0.019	0.083
Year× nitrogen	0.004	0.132	0.508	0.532	0.563	0.001	0.362	0.402
Climate× nitrogen	0.0001	0.604	0.209	0.145	0.504	0.010	0.028	0.837
Year× climate× nitrogen	0.563	0.519	0.194	0.856	0.754	0.033	0.337	0.899

C.I. = chlorophyll index.

The highest dry weight was noted under high CO₂-concentration+elevated temperature followed by high CO₂-concentration, elevated temperature and control treatments, respectively (Table 4.12; Fig. 4.26-4.28). The plants grown under high CO₂-concentration had the highest fresh weight and leaf area followed by those grown under high CO₂-concentration+elevated temperature and elevated temperature, respectively. The plants under elevated temperature treatment had a higher fresh weight than the ones under control. Leaf length, plant height and leaf area were decreased by the elevated temperature compared to control treatment (Table 4.12).

Table 4.12. Effect of climatic conditions on growth of *Lactuca serriola* (2-years' average data)

Climatic conditions	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	7.28d	74.1d	38.4	31.2b	37.4ab	44.1bc	3716.4b	3.55
Elevated temperature	6.31c	63.6c	38.3	29.8c	36.2b	38.2c	2642.8c	3.69
High CO ₂ + elevated temperature	9.56a	89.3b	38.4	35.1a	38.6ab	48.3b	4088.4b	3.62
High CO ₂	8.41b	106.4a	37.5	35.0a	39.0a	57.2a	4553.9a	3.45
MSE	1.32	129.5	NS	4.16	13.3	128.6	521.0	NS

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

Table 4.13. Effect of nitrogen fertilization on growth of *Lactuca serriola* (2-years' average data)

Nitrogen rates	Dry weight (g)	Fresh weight (g)	C.I.	Leaf length (cm)	Plant height (cm)	No. of leaves	Leaf area (cm ²)	Leaf tissue N (%)
Control	6.8b	74.6b	38.0ab	32.8a	37.8ab	41.0b	2968.5c	3.5b
Medium dose	7.2b	83.3b	38.7a	33.8a	39.3a	45.0b	3880.7b	-
High dose	9.5a	92.1a	37.7b	31.4b	36.4b	54.0a	4401.8a	3.7a
MSE	1.32	129.5	3.78	4.16	13.3	128.6	521.0	0.125

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

High N dose increased the dry weight, number of leaves and leaf area of *L. serriola* over medium N dose and control treatments (Table 4.13). A higher leaf tissue N concentration was recorded for high N dose over control (Table 4.13).

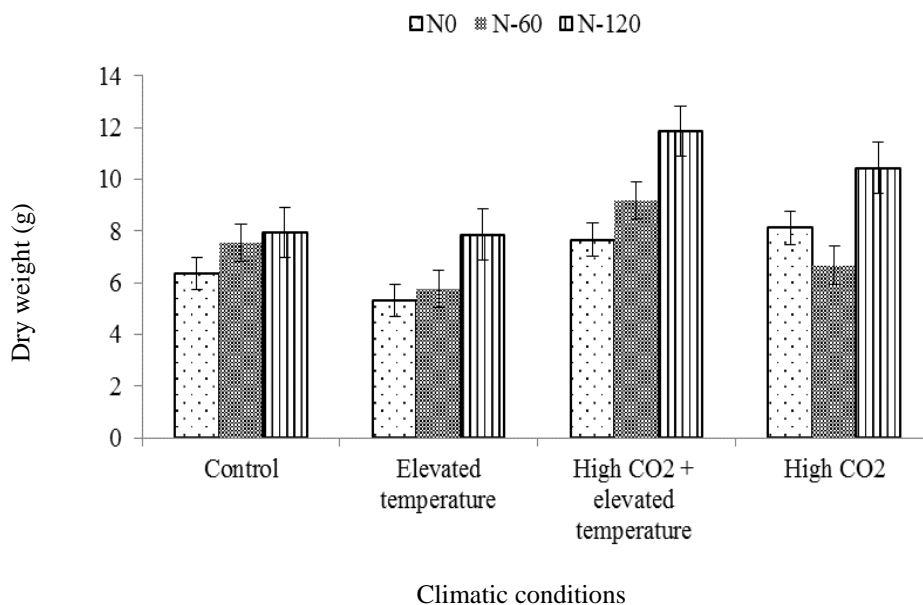


Fig. 4.23. Interactive effect of nitrogen fertilization and climatic conditions on dry weight of *Lactuca serriola* (2-years' average data); The vertical bars on the histograms are standard error of means.

A higher dry weight of *L. serriola* was noted when high CO₂-concentration or high CO₂-concentration+elevated temperature were combined with high N dose compared with the high N dose under other climatic conditions (Fig. 4.23). Similarly, high CO₂-concentration combined with high N dose had the highest number of leaves and leaf area compared with other treatment combinations in the experiment (Fig. 4.24-4.25).

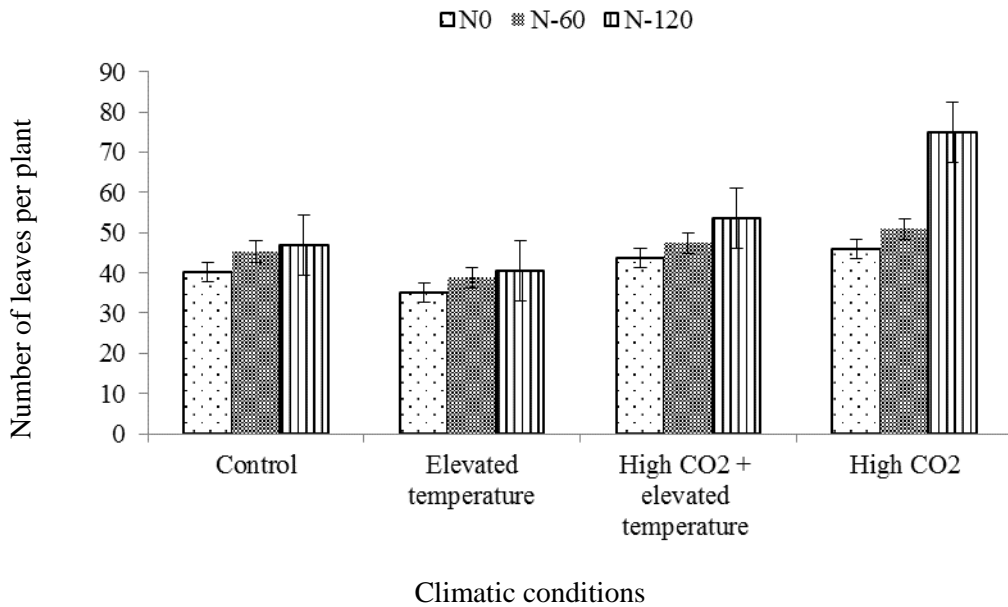


Fig. 4.24. Interactive effect of nitrogen fertilization and climatic conditions on number of leaves of *Lactuca serriola*; The vertical bars on the histograms are standard error of means.

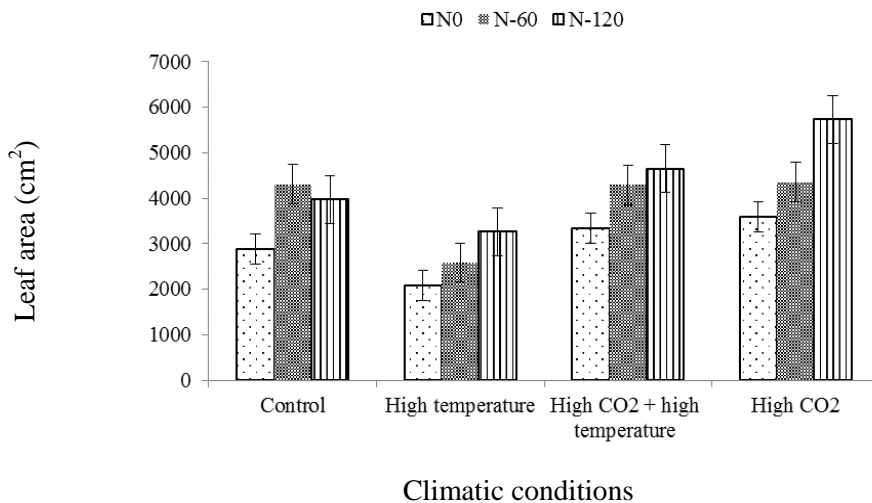


Fig. 4.25. Interactive effect of nitrogen fertilization and climatic conditions on leaf area of *Lactuca serriola* (2-years' average data); The vertical bars on the histograms are standard error of means.



Fig. 4.26. Growth of *Lactuca serriola* under normal (left) and high CO₂-concentration (right)



Fig. 4.27. Growth of *Lactuca serriola* under ambient (left) and high CO₂-concentration + elevated temperature (right)



Fig. 4.28. Growth of *Lactuca serriola* under normal (left) and elevated temperature (right)

4.4. Herbicide (Glyphosate) Activity under Different Climatic Conditions

The effect of herbicide application as influenced by the different CO₂-concentrations and temperature levels has been presented in this section. The response of invasive weeds in the experiment to applied herbicide under different climatic conditions was evaluated through visual observation. Weeds were observed daily after herbicide application, these started to wither 3-5 days after herbicide application, turned yellow nearly two weeks after spraying (personal observations). The analysis of variance indicated that all the weeds had responded similarly across the years to applied herbicide doses (Table 4.14). Similarly, climatic conditions, or climatic conditions×doses interaction had no effect on

control of weeds. However, different glyphosate doses gave a different control of weeds (Table 4.14). According to visual observations, standard dose (1440 g a.i. ha⁻¹) and double of the standard dose (2880 g a.i. ha⁻¹) completely controlled the weeds under all the climatic conditions (Table 4.14). Herbicide activity positively increased with increasing glyphosate doses. The doses of 720 and 1080 g a.i. ha⁻¹ had controlled the weeds by more than 80% but less than 100% under all the climatic conditions (Table 4.15).

Table 4.14. Analysis of variance (*p* values) of different doses of herbicides on weed control under different climatic conditions

Source	Variable	Significance
Year	<i>Bromus tectorum</i>	0.226
	<i>Capsella bursa-pastoris</i>	0.352
	<i>Hordeum murinum</i>	0.832
	<i>Lactuca serriola</i>	0.134
Climate	<i>Bromus tectorum</i>	0.940
	<i>Capsella bursa-pastoris</i>	0.499
	<i>Hordeum murinum</i>	0.344
	<i>Lactuca serriola</i>	0.701
Dose	<i>Bromus tectorum</i>	0.000
	<i>Capsella bursa-pastoris</i>	0.000
	<i>Hordeum murinum</i>	0.000
	<i>Lactuca serriola</i>	0.000
Year×Climate	<i>Bromus tectorum</i>	0.644
	<i>Capsella bursa-pastoris</i>	0.955
	<i>Hordeum murinum</i>	0.955
	<i>Lactuca serriola</i>	0.869
Year×Dose	<i>Bromus tectorum</i>	0.907
	<i>Capsella bursa-pastoris</i>	0.654
	<i>Hordeum murinum</i>	0.994
	<i>Lactuca serriola</i>	0.024
Climate×Dose	<i>Bromus tectorum</i>	0.987
	<i>Capsella bursa-pastoris</i>	0.302
	<i>Hordeum murinum</i>	0.477
	<i>Lactuca serriola</i>	0.996

Table 4.15. Effect of different herbicide doses on control of weeds (two years' average data)

Glyphosate doses (g a.i./ha)	<i>Bromus tectorum</i>	<i>Capsella bursa-pastoris</i>	<i>Hordeum murinum</i>	<i>Lactuca serriola</i>
360.0	61.9c	60.4d	48.9d	46.6d
720.0	94.8b	81.1c	82.3c	81.1c
1080.0	95.8b	97.1b	90.2b	92.5b
1440.0	100.0a	100.0a	100.0a	100.0a
2880.0	100.0a	100.0a	100.0a	100.0a
EMS	10.2	17.9	32.2	17.0

Since upper and lower limits of dose-response curves were similar for each species, only slopes and ED₅₀ and ED₉₀ values were provided in Table 4.16. Both *B. tectorum* and *C. bursa-pastoris* had a close ED₅₀ (311 ± 11.0 and 309 ± 40.0 , respectively) but a different ED₉₀ (605 ± 18.0 and 861 ± 69.0 , respectively). Both *H. murinum* and *L. serriola* had a very close values for ED₅₀ (376 ± 24.0 and 379 ± 20.0) and ED₉₀ (951 ± 44.0 and 940 ± 40.0). ED₅₀ values were similar for *B. tectorum* and *C. bursa-pastoris*, and *H. murinum* and *L. serriola*. Moreover, keeping in view the ED₉₀ values, *H. murinum* was the most difficult to control weed followed by *L. serriola*, *C. bursa-pastoris* and *B. tectorum*, respectively (Table 4.16).

Table 4.16. Dose-response curves for four invasive weed species grown under different climatic conditions (two years' average data)

Parameter	B	ED ₅₀ (ml/ha)	ED ₉₀ (ml/ha)
<i>Bromus tectorum</i>	3.4 ± 0.7	311 ± 11.0	605 ± 18.0
<i>Capsella bursa-pastoris</i>	1.7 ± 0.5	309 ± 40.0	861 ± 69.0
<i>Hordeum murinum</i>	2.1 ± 0.4	376 ± 24.0	951 ± 44.0
<i>Lactuca serriola</i>	2.1 ± 0.3	379 ± 20.0	940 ± 40.0

4.5. Effect of Climatic Conditions on Growth of Invasive Weeds (Non-Treated Control) in Herbicide Experiment

We also recorded the growth response of non-treated control (in herbicide experiment) to CO₂-concentration and temperature. The results for four weed species in the experiment are explained in the following.

4.5.1. *Bromus tectorum*

ANOVA indicated that year significantly affected chlorophyll index, leaf length, fresh and dry weights (Table 4.17). Climatic conditions had a significant effect on leaf length, plant height, fresh weight, dry weight and leaf area of *B. tectorum* (Table 4.17). Year×climate significantly affected fresh and dry weight of *B. tectorum* (Table 4.17). A higher leaf length, plant height, fresh weight, dry weight and leaf area were noted for *B. tectorum* plants grown under either high CO₂-concentration or high CO₂-concentration+elevated temperature than those grown under ambient conditions or elevated temperature (Table 4.18).

Table 4.17. Analysis of variance (p values) for effect of climatic conditions on *Bromus tectorum*

Factors	C.I.	No. of leaves	Leaf length	Plant height	Fresh weight	Dry weight	Leaf area
Year	0.0001	0.495	0.001	0.208	0.001	0.0001	0.0001
Climate	0.202	0.353	0.0001	0.0001	0.0001	0.0001	0.001
Year × climate	0.121	0.673	0.142	0.418	0.0001	0.0001	0.099

C.I. = chlorophyll index; No = number.

Table 4.18. Effect of different climatic conditions on growth and biomass of *Bromus tectorum* (2-years' average data)

Treatments	C.I.	No. of leaves	Leaf length (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)
Control	38.3	87.0	28.8b	38.7d	30.8c	3.3c	3132.7b
Elevated temperature	37.3	86.0	30.3b	42.6c	24.8d	2.8c	2433.2c
High CO ₂ + elevated temperature	38.9	82.0	37.4a	49.9b	35.7b	4.2b	3574.7ab
High CO ₂	36.7	99.0	40.3a	54.6a	40.7a	6.2a	3346.4a
EMS	NS	NS	9.89	7.25	4.99	0.35	213.7

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

4.5.2. *Capsella bursa-pastoris*

ANOVA indicated that year had a significant effect on chlorophyll index, plant height and dry weight of *C. bursa-pastoris* (Table 4.19). Climatic conditions had a significant effect on number of leaves, leaf length, plant height, fresh weight, dry weight and leaf area of *C. bursa-pastoris* (Table 4.19). Year×climate had a non-significant effect on all the parameters (Table 4.19).

Table 4.19. Analysis of variance (p values) for effect of climatic conditions on *C. bursa-pastoris*

Factors	C.I.	No. of leaves	Leaf length	Plant height	Fresh weight	Dry weight	Leaf area
Year	0.0001	0.302	0.612	0.031	0.899	0.0001	0.0001
Climate	0.227	0.0001	0.0001	0.0001	0.004	0.0001	0.0001
Year \times climate	0.72	0.393	0.885	0.541	0.185	0.381	0.195

C.I. = chlorophyll index; No = number.

Table 4.20. Effect of different climatic conditions on growth and biomass of *Capsella bursa-pastoris* (2-years' average data)

Treatments	C.I.	No. of leaves	Leaf length (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)
Control	39.2	34.0a	23.9b	63.2a	38.6b	5.5a	869.9a
Elevated temperature	40.1	27.0bc	24.1b	44.4b	38.9ab	3.3b	690.4b
High CO ₂ + elevated temperature	41.7	31.0ab	26.8ab	52.3b	31.5b	3.8b	673.9b
High CO ₂	39.2	24.0c	29.2a	62.8a	55.1a	5.3a	879.4a
EMS	NS	12.0	7.21	67.1	181.1	0.63	189

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; NS = non-significant.

Higher number of leaves were noted in treatments including control and high CO₂-concentration+elevated temperature compared with other treatments in the experiment (Table 4.20). A higher plant height, dry weight and leaf area were noted for high CO₂-concentration and control (ambient conditions) treatments (Table 4.20). *Capsella bursa-pastoris* plants in high CO₂-concentration and elevated temperature had a higher fresh weight than other treatments in the experiment (Table 4.20).

4.5.3. *Hordeum murinum*

ANOVA indicated that year had a significant effect on leaf length, fresh weight and dry weight of *H. murinum* (Table 4.21). Climatic conditions significantly affected leaf length, plant height, fresh and dry weights, and leaf area of *H. murinum* (Table 4.21). Year×climate had a significant effect on number of leaves, leaf length, fresh weight and dry weight of *H. murinum* (Table 4.21). *Hordeum murinum* plants grown under high CO₂-concentration+elevated temperature and high CO₂-concentration had a higher leaf length, plant height, fresh weight, dry weight and leaf area than the plants grown under ambient conditions and elevated temperature treatments (Table 4.22).

Table 4.21. Analysis of variance (*p* values) for effect of climatic conditions on *Hordeum murinum*

Factors	C.I.	No. of leaves	Leaf length (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)
Year	0.137	0.117	0.0001	0.142	0.0001	0.0001	0.017
Climate	0.362	0.423	0.0001	0.0001	0.0001	0.0001	0.0001
Year × climate	0.288	0.007	0.0001	0.385	0.0001	0.0001	0.701

C.I. = chlorophyll index; No = number.

Table 4.22. Effect of different climatic conditions on growth and biomass of *Hordeum murinum* (2-years' average data)

Treatments	C.I.	No. of leaves	Leaf length (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)
Control	44.6	114.0	41.9c	55.5b	33.7b	3.7b	3634.8c
Elevated temperature	46.7	119.0	46.3b	58.5b	34.8b	4.5b	4105.1bc
High CO ₂ + elevated temperature	47.1	126.0	55.6a	72.9a	55.1a	7.1a	4476.5ab
High CO ₂	45.5	134.0	57.9a	68.9a	49.3a	6.3a	4809.8a
EMS	NS	NS	7.06	15.79	32.1	0.55	333.3

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

4.5.4. *Lactuca serriola*

ANOVA indicated that year had a significant effect on chlorophyll index, number of leaves, leaf length, fresh and dry weights (Table 4.23). Climatic conditions had a significant effect on number of leaves, leaf length, plant height, fresh and dry weights, and leaf area (Table 4.23). *Lactuca serriola* plants grown under high CO₂-concentration+elevated temperature and high CO₂-concentration had a higher leaf length, plant height, fresh weight, dry weight and leaf area than the plants grown under control and elevated temperature treatments (Table 4.24).

Table 4.23. Analysis of variance (*p* values) for effect of climatic conditions on *Lactuca serriola*

Factors	C.I.	No. of leaves	Leaf length	Plant height	Fresh weight	Dry weight	Leaf area
Year	0.0001	0.001	0.0001	0.062	0.0001	0.0001	0.002
Climate	0.175	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Year × climate	0.073	0.005	0.078	0.151	0.0001	0.0001	0.330

C.I. = chlorophyll index; No = number.

Table 4.24. Effect of different climatic conditions on growth and biomass of *Lactuca serriola* (2-years' average data)

Treatments	C.I.	No. of leaves	Leaf length (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	Leaf area (cm ²)
Control	39.6	32.0b	22.2c	25.9c	60.2b	4.8b	2845.3c
Elevated temperature	40.4	34.0b	24.8b	26.9c	59.9b	5.1b	3240.0b
High CO ₂ + elevated temperature	41.2	47.0a	28.4a	32.6a	82.8a	8.9a	3499.1ab
High CO ₂	39.4	45.0a	28.1a	30.2b	85.1a	9.4a	3701.6a
EMS	NS	70.1	4.0	3.6	93.2	1.36	461.6

The means not sharing a letter in common in each column differ significantly from each other at $p \leq 0.01$; C.I. = chlorophyll index; No = number; NS = non-significant.

5. DISCUSSION AND CONCLUSIONS

Increased anthropogenic activities are the most important reasons for climate changes such as high CO₂-concentration in the atmosphere and global warming. This research work provides information regarding the response of invasive weed species (in their native range) to high CO₂-concentration and elevated temperature. The control and N uptake of invasive weed species under these conditions has also been discussed. The implications of this research have been discussed in the following.

5.1. Plant Growth and Biomass under High CO₂-Concentration, High CO₂-Concentration+Elevated Temperature and Elevated Temperature

In the preliminary studies, we evaluated the effect of high CO₂-concentration on growth of 11 weed species. Most of these weed species responded positively to increased CO₂-concentration in terms of their growth. Also, there were instances for no or negative response to high CO₂-concentration. Weeds including *Avena barbata*, *Carduus nutans*, *Lolium multiflorum*, *Poa bulbosa*, *Bromus tectorum*, *Hordeum murinum*, *Lactuca serriola* and *Potentilla recta* responded positively to high CO₂-concentration. *Cirsium vulgare* and *Medicago sativa* largely had a neutral response to high CO₂-concentration while *Capsella bursa-pastoris* had responded negatively to high CO₂-concentration in terms of its fresh weight.

In both the subsequent experiments (herbicide and nitrogen application), high CO₂-concentration improved the biomass and growth parameters of weeds including *B. tectorum*, *H. murinum*, and *L. serriola* while *C. bursa-pastoris* had responded differently to climatic conditions compared with the other weeds in the studies. In the same way, high CO₂-concentration+elevated temperature treatment had also improved the biomass and growth of these three weed species over control. Moreover, in most of the cases, sole application of high CO₂-concentration was found to be more advantageous for these weed species than a combination of high CO₂-concentration+elevated temperature. Leaf area was the parameter which had responded consistently to these two climatic conditions consistently across all the experiments for these three weeds. Leaf area was always found higher for high CO₂-concentration than high CO₂-concentration+elevated temperature. Occasionally, the other parameters were also noted higher for high CO₂-concentration than high CO₂-concentration+elevated temperature. These

included dry weight, fresh weight and plant height of *B. tectorum* in the herbicide experiment and number of leaves, plant height and fresh weight of *L. serriola* in nitrogen experiment. Across all the results for high CO₂-concentration and high CO₂-concentration+elevated temperature, dry weight of *L. serriola* was the only parameter which was higher in high CO₂-concentration+elevated temperature than sole high CO₂-concentration.

Most of the results from our studies are supported by the recent research work regarding influence of increasing CO₂-concentrations. For example, Ziska et al. (2005) mentioned that the biomass production in *B. tectorum* was sensitive to CO₂-concentrations. An increase in dry weight of this weed was recorded with the increasing CO₂-concentrations. Carbon is important component of body in all plants while CO₂ is the sole source from which plants obtain their carbon (Wardlaw, 1990). This carbon (in the form of CO₂) is converted to carbohydrates by the plants through the process of photosynthesis. However, the process of CO₂ fixation is accompanied with photorespiration in C₃ plants owing to limitation in CO₂-concentration (Black Jr., 1973). Hence, C₃ plants may benefit from the higher CO₂-concentration by decreasing the photorespiration (Johnson et al. 1993).

One important impact of high-CO₂ levels on plants is conservation of water in plant and soil surface through which they may improve growth activities of plants (Bader et al. 2013). In addition, the high CO₂-concentration improves the growth and number of fine roots which help the plants to absorb more water and nutrients (Wullschleger et al. 2002). Further, as observed in our studies, the high-CO₂ significantly improved the leaf area of plants (Kumar et al. 2012). This may be an effect of increased photosynthesis under high-CO₂ concentration (Kumar et al. 2012). Improved growth under high-CO₂ concentration may ultimately help the invasive weeds for their establishment and range expansion (Bellard et al. 2013). Several of other positive benefits of high CO₂-concentration may include decreased stomatal conductance, increased C uptake, improved plant productivity, increased root growth, increased nutrient and water use efficiency, and higher soil moisture contents (Wullschleger et al. 2002; Leakey et al. 2009; Marhan et al. 2010).

The results of our studies imply that a combination of high CO₂-concentration and elevated temperature had a positive effect on growth of weeds. However, such effects were probably due to high CO₂-concentration and high temperature had no

role in this growth improvement of weeds. This is due to the reason that sole warming had almost no positive influence on growth and biomass of weeds in the experiment. The benefits of high CO₂-concentration to C₃ plants are on record, the same was observed in our studies, while elevated temperature was not beneficial for the weeds in the experiments. Hence, we are of the view that increased growth of weeds observed in the treatment with high CO₂-concentration+elevated temperature was solely due to high CO₂-concentration and not the elevated temperature. High CO₂-concentration improves the growth of plants by increasing photosynthesis, reducing photorespiration and enhanced water availability through decreased number of stomata and cuticle thickness, and hence the improved growth, sink size and biomass production, while elevated temperature may negatively affect the growth of plants by increasing the rate of transpiration and evaporation, enhancing the plant metabolism and respiration (Zelitch, 1971; Flexas et al. 2002; Lawlor, 2002; Korres et al. 2016). The environment where these two factors were working in combination, probably the positive effect of high CO₂-concentration was stronger than the negative effect of elevated temperature, hence, we observed an increased growth under such environment.

As mentioned earlier, *C. bursa-pastoris* had responded differently to climatic conditions compared with the other three weed species in our studies. In general, high CO₂-concentration had a similar effect on growth and biomass of *C. bursa-pastoris* plants as of the 'control' treatment. The biomass and growth parameters for *C. bursa-pastoris* were similar for 'control' conditions and high CO₂-concentration treatment in both the herbicide and nitrogen experiments. Moreover, most of the parameters of *C. bursa-pastoris* were negatively affected by either the elevated temperature or elevated temperature+high CO₂-concentration treatments. The response of *C. bursa-pastoris* was interestingly different to high CO₂-concentration compared to one which in general, has been reported for C₃ plants (Fuhrer, 2003). Previously, Valerio et al. (2013) has reported the negative impacts of high CO₂-concentration and warming on another C₃ species. Increased photosynthetic activity, reduced transpiration and decreased respiration is the mechanism through which plants benefit from the high CO₂-concentration (Zelitch, 1971). Ultimately, the plant leaves have a different photosynthetic properties at high CO₂-concentration than at the normal CO₂ conditions (Drake and Leadley, 1991; Ainsworth and Long, 2005). However, it seems that such mechanisms and photosynthetic properties were not active in case of *C. bursa-*

pastoris, hence this weed did not express a positive growth response to high CO₂-concentration.

Many of the plants however gain photosynthetic properties to achieve higher resource utilization under high CO₂-concentration, the carbohydrates are produced in abundance. However, chloroplast may witness a physical damage due to overloading of carbohydrates in the leaves (Delucia et al. 1985; Sasek et al. 1985). This may be the other reason due to which *C. bursa-pastoris* plants did not expressed a positive response to high CO₂-concentration. Activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) in C₃ plants is highly important for truly benefiting from the high CO₂-concentrations in the surrounding environments. RuBisCo is involved in catalization of both the oxygenation (resulting in photorespiration) and carboxylation (resulting in CO₂ fixation in order to produce carbohydrates) of ribulose bisphosphate (RuBP) in the C₃ plants (Morison and Lawlor, 1999). The non-responsive or negative growth behavior of *C. bursa-pastoris* to high CO₂-concentration indicates that high CO₂ levels either did not affect the RuBisCo activity (probably due to lower CO₂ intakes) or there was more photorespiration than carboxylation for this weed (Morison and Lawlor, 1999).

In the herbicide experiment, elevated temperature had negatively affected the fresh weight and leaf area of *B. tectorum*, and biomass and growth parameters of *C. bursa-pastoris*. However, in the same experiment, elevated temperature had a positive effect on plant height of *B. tectorum*, leaf area of *H. murinum*, and leaf length and leaf area of *L. serriola*. In the N fertilization experiment, considering the data after statistical analysis (nevertheless, a similar trend was noted in the data of both the studies irrespective of the statistical significance), elevated temperature had no positive or negative effects on biomass, growth and leaf tissue nitrogen concentration of *B. tectorum* and *H. murinum* (grasses). However, the broadleaves (*C. bursa-pastoris* and *L. serriola*) had received a negative effect from elevated temperature on their biomass and growth. Elevated temperature however did not affect the leaf tissue nitrogen concentration of these weeds. Temperature is one among the key factors which impact the important life processes of plants starting from germination until decease. In general, elevated temperature had a negative or neutral effect on all the weed species in our studies compared with the ambient conditions. Recent literature supports the results of our studies. Importantly, the warming has been reported to be more favorable for C₄ than C₃ plants (Fuhrer, 2003). The same was observed in our studies where warming had almost no

advantage for C₃ plants, rather warming had occasionally harmed these plants. A global increase in temperature is not only a disadvantage for weeds (as indicated from our studies), but it also have negative impacts on crops (Olesen and Bindi, 2002; Peng et al. 2004). One important mechanism where the high temperature reduces the plant productivity is the reduced CO₂ assimilation through partial inactivation of photosynthetic enzymes (Conroy et al. 1994). The reduced intake of CO₂ owing to water stress as a result of high temperature might be the other reason of reduced photosynthetic activity (Cornic and Briantais, 1991). These were the probable reasons where temperature mostly had negative or neutral effect on plants in our experiments. Similarly, high temperature can increase the water requirements of plants owing to high rates of evapo-transpiration, while more energy will be required to accomplish this process, hence, a higher temperature may not witness a positive effect on plants, as was observed in our studies (Tubiello et al. 2007).

Increasing CO₂-concentration in the atmosphere has also consequences for weed-crop interactions (Valerio et al. 2011; Zeng et al. 2011), however, we have not included such studies in our experiments owing to resources limitation.

5.2. Response of Weeds to Nitrogen Fertilization under Different Climatic Conditions

Addition of N to environment in high quantities can result in environmental and ecological concerns (Galloway et al. 2008). We also determined the response of four (*B. tectorum*, *C. bursa-pastoris*, *H. murinum* and *L. serriola*) invasive weed species to nitrogen application under simulated climatic conditions. Determining the response of weed species to higher nitrogen levels has been important in the wake of increasing nitrogen concentration in the environment as a result of high nitrogen inputs in order to secure high food production (Bidwell et al. 2006; Vasquez et al. 2008; Zhang et al. 2012). The phenomenon has been termed as nitrogen pollution after accumulation of this element in the environment with high concentrations (Zhang et al. 2012). The four weed species responded differently to applied nitrogen in terms of their growth, biomass, leaf area and leaf tissue nitrogen concentration. Grasses (*B. tectorum* and *H. murinum*) and broadleaved weeds (*C. bursa-pastoris* and *L. serriola*) had a different response to nitrogen. Nitrogen fertilization positively affected only a few growth parameters of grasses that included fresh weight and leaf area for *B. tectorum*, and plant height, leaf area

and leaf tissue N of *H. murinum*. An interaction of climate×nitrogen was significant only for leaf area in *B. tectorum*.

In contrast to grasses, the broadleaved weeds (*C. bursa-pastoris* and *L. serriola*) mostly had a positive response to nitrogen fertilization. For example, all the growth and biomass parameters of these weeds were influenced significantly by nitrogen application except plant height and leaf tissue N in *C. bursa-pastoris*. Higher rates of nitrogen had increased the growth and biomass of *C. bursa-pastoris*, however, these had not increased the leaf tissue nitrogen concentration of this weed species. Similarly, higher rates of nitrogen had improved biomass, growth and leaf tissue nitrogen concentration of *L. serriola* (except chlorophyll index, leaf length and plant height of this weed which were affected negatively by high nitrogen dose). Importantly, the climate×nitrogen interactions had a positive influence only on dry weight of *C. bursa-pastoris* and dry weight, number of leaves and leaf area of *L. serriola*.

Usually the plant species have been reported to be non-responsive or express a lower response to high CO₂-concentration when grown under limited nitrogen conditions (Uprety and Mahalaxmi, 2000; Reddy et al. 2010; Franzaring et al. 2011). Hence, we had evaluated the effect of high-CO₂ on plant growth with no nitrogen limitation. However, the plants performed similarl as they had done in absence of nitrogen. Occasionally, it had increased the sink size (e.g. leaf area of *B. tectorum*) which helped to exert a synergistic effect of high CO₂ and nitrogen application on this weed. Such occasional synergistic effects were also noted for other weeds in our studies, for example dry weight of *C. bursa-pastoris* and dry weight, number of leaves and leaf area of *L. serriola* also received a synergistic effect from high N dose and high CO₂-concentration. Previous studies also report a positive response of *B. tectorum* to higher N concentration in the soil (Vasquez et al. 2008). Higher N concentration in the soil may help *B. tectorum* to enhance its competitiveness and invasiveness (Vasquez et al. 2008). Addition of high amounts of N to the environment will increase the fertilization of invasive weeds, and hence will increase the biomass gain of invasive weeds (Zhao et al. 2015). This gain in biomass will help the invasive plants to increase their invasion capacity. In addition to this, N accumulation in an environment can disturb its plant diversity thereby favoring certain species. Invasive plants may get benefited from high N concentration in the environment and use this advantage to occupy more space, decrease the density of other plant species and hence disturb the plant diversity in

that environment (Zavaleta et al. 2003). In the wake of such situations, better fertilizer management plans are required for reducing the escape of N into the environment.

5.3. Response of Weeds to Herbicide Application under Different Climatic Conditions

Climate changes such as high CO₂-concentration and warming has the potential to alter the efficacy of herbicides (Ziska, 2010). Results of our studies gave some interesting findings regarding effect of herbicides on control of invasive weeds under different climatic conditions. A difference in climatic conditions could not affect the activity of herbicide (glyphosate). Although, plants express a difference in their growth and biomass production under control and simulated environment (high CO₂-concentration and elevated temperature) in a single season, however, they may require several seasons to adapt these changes and express a different response to applied herbicides. Long-term and over the generation exposure of plants to high CO₂-concentration is more beneficial than a single season exposure (Marhan et al. 2010).

We can find similar examples from the recent literature where efficacy of herbicides was similar under normal and high CO₂-concentration. For example, the response of 23 weed species to herbicide application under high CO₂-concentration was evaluated in a study from Australia (Downey et al. 2012). The authors argued that the preliminary results shown a little or no increase in herbicide tolerance of weeds under high CO₂-concentration (Downey et al. 2012). Nevertheless, several studies have also reported a response of weeds to applied herbicides (under normal and simulated climatic conditions) different than our findings. This may be due to the reason that genetic material of weeds used in those studies had well adapted to changed environmental conditions and expressed a tolerance to applied herbicides under high CO₂-concentration. For example, *E. repens* plants expressed an improved tolerance against glyphosate under high CO₂-concentration (Ziska and Teasdale, 2000).

Most of the previous studies report a reduced efficacy of herbicides under high CO₂-concentration, while a neutral effect of high-CO₂ concentration on herbicide efficacy against weeds has also been reported in rare cases (Manea et al. 2011). A decrease in glyphosate activity occurs due to the dilution factor under high CO₂-

concentration (where growth of weed species is increased) (Ziska, 2014). The reduced herbicide efficacy owing to dilution is particularly important for weeds which have a belowground biomass (such as sedges or other weeds with rhizomes) or the perennial weeds which express a regrowth (Manea et al. 2011; Ziska, 2014). However, in our studies, no weed had a belowground biomass production or regrowth. This was the possible reason where glyphosate efficacy was not decreased under high CO₂-concentration. According to Ziska et al. (2004), the perennial weeds having a belowground growth such as a taproot or rhizome may express a tolerance against herbicide under high CO₂-concentration. *Cirsium arvense*, a perennial weed was presented as an example in this study which was tolerant to glyphosate under high CO₂-concentration owing to its belowground growth (Ziska et al. 2004). In previous studies, elevated temperature has the potential to decrease the sensitivity of weeds to glyphosate, however, such effects were associated with susceptibility or resistance of a weed to glyphosate (Nguyen et al. 2015). In contrast to glyphosate-susceptible weeds, the glyphosate-resistant weeds were found to decrease their herbicide sensitivity owing to a mutation which occurred only in resistant weed populations (Ghanizadeh et al. 2015; Nguyen et al. 2015). Hence, in our studies, elevated temperature did not decrease the sensitivity of weeds to glyphosate as the weeds in these studies were not herbicide-resistant (Vila-Aiub et al. 2013; Nguyen et al. 2015). In contrast to elevated temperature, low temperature may increase the sensitivity of resistant weeds to herbicides (Vila-Aiub et al. 2013; Ghanizadeh et al. 2015).

The results of our research work imply that glyphosate will be equally effective to control weed species in the experiment even after the weeds receive a benefit from changing climatic factor i.e. rising CO₂-concentration in the atmosphere. This is particularly important because glyphosate is a multipurpose non-selective herbicide which has been under use to control weeds in cropped and non-cropped areas (Robinson et al. 2012). Moreover, this herbicide has a particular importance for control of invasive weeds (Jabran and Dogan, 2015). It is a positive sign if this herbicide retains its efficacy against weeds under changing climatic factors (i.e. warming and high CO₂-concentration).

It is concluded that the high CO₂-concentration improved the growth and dry matter accumulation of invasive weeds, e.g. *Avena barbata*, *Carduus nutans*, *Lolium multiflorum*, *Poa bulbosa*, *Bromus tectorum*, *Hordeum murinum*, *Lactuca serriola* and *Potentilla recta*. Some of the weeds (such as *Capsella bursa-pastoris*)

were mostly un-affected or negatively affected by the climate changes like high atmospheric CO₂-concentration and warming. Nevertheless, some of the weeds were not affected by the climatic scenarios, e.g. *Cirsium vulgare*, *Medicago sativa*. The increasing temperature had a neutral or negative effect (a rare positive effect on a few parameters was also noted) on the growth of weed species in the experiment.

A combination of high CO₂-concentration+elevated temperature improved the growth of weeds; however, probably this increase resulted mainly from high CO₂ levels rather than temperature, because sole warming treatment had a neutral or negative effect on weed species, in contrast, the sole CO₂ (high concentration) stimulated the growth of test weeds. Applied herbicide (glyphosate) was equally effective to control weeds under all the climatic conditions i.e. control, high CO₂-concentration, elevated temperature and high CO₂-concentration+elevated temperature. Nitrogen fertilization had in general increased the growth and biomass of weeds in the experiment. Under certain instances, N had also helped to increase the photosynthetic structure, particularly under high CO₂-concentration. Rarely, a positive interaction of N fertilization and high CO₂-concentration was also recorded on the growth parameters (particularly leaf area) of *B. tectorum*, *C. bursa-pastoris* and *L. serriola*, which may improve the invasion capacity of these weed species in the future.

Our results further conclude that the invasive weed species even in their native range mostly take an advantage from changing climatic factors such as rising CO₂-concentration in the atmosphere. This may help these weed species to expand their range and become a threat for other vegetation. Particularly, the weeds like *Bromus tectorum*, *Hordeum murinum* and *Lactuca serriola* infest wheat and some other winter crops; the positive influence of high CO₂-concentration on these weeds can make them troublesome with a more likely chance to decrease the crop productivity. A neutral or negative impact of warming on invasive weeds and a very high effectiveness of herbicide under all climatic conditions may be considered as a positive sign. Similarly, N application had improved very few growth parameters of weeds, which indicates that invasive weeds may not gain big advantages from over fertilization of N in agricultural fields and its losses in the environment.

As a future work, similar studies are required to be conducted in real field conditions (e.g. using FACE i.e. free-air CO₂ enrichment) in order to document the influence of changing climate on invasive weeds on a large scale in a greater biogeographical region. Similarly, the surveys (repeated over the time) of weeds included in the experiment and modelling of the data (while including changing CO₂-concentration in the atmosphere and global warming, and the resultant increased growth as a factors) will help to have the idea about the future range expansion of these weeds? Further, the weeds taking an advantage from high CO₂-concentration may be kept under check so that these do not become a threat to other vegetation, crops and environment. Importantly, the mechanisms (including the enzymes' activities) behind the improved weed growth under high CO₂-concentrations (for three out of four invasive weeds species) are desired to be studied. Similarly, the exact mechanism for negative or neutral response of *C. bursa-pastoris* to high CO₂-concentration is also desired to be investigated. Photosynthetic and physiological functioning (including the activity of RuBisCo and other enzymes) of weeds expressing a negative or neutral response to high CO₂-concentration (such as *C. bursa-pastoris*) are desired to be studied.

The response of the populations of invasive weeds in the experiment collected from ranges other than their native are desired to be studied under different climatic conditions and compared with the native invasive populations. Further, the responses of the weeds in our studies are also desired to be investigated against the climatic changes other than CO₂-concentrations and temperature levels. These may include different precipitation levels, drought conditions and more levels of temperature.

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RESUME

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MS Thesis Utilization of allelopathic crop water extracts for reducing herbicide use in canola (*Brassica napus* L.)

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Research interests

Weed science	Invasive plants, allelopathy
Crop and weed management	Conservation agriculture