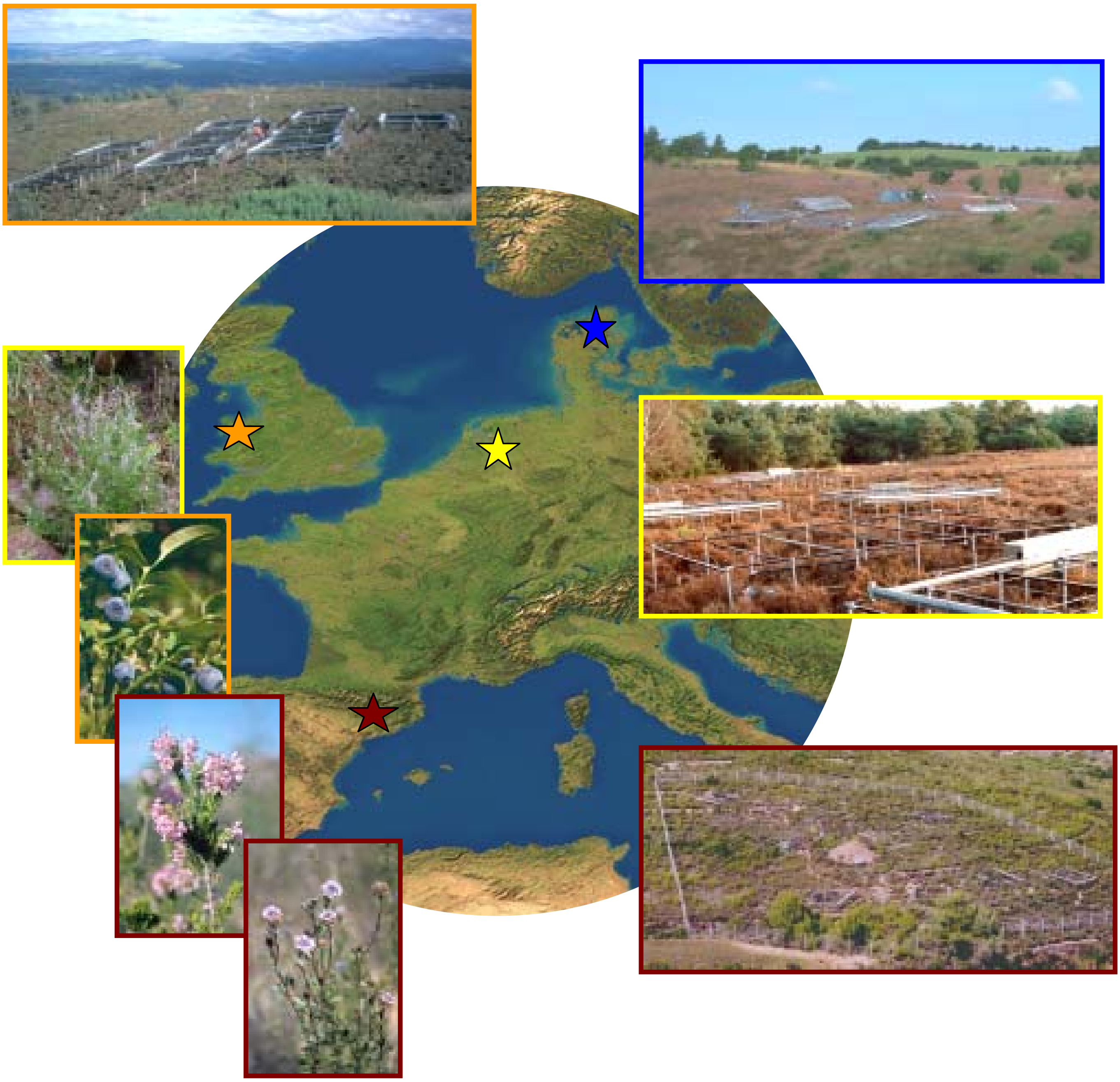
Plant ecophysiological responses to experimentally drier and warmer conditions in European shrublands

Laura Llorens i Guasch

Ph.D. thesis



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Plant ecophysiological responses to experimentally drier and warmer conditions in European shrublands

Memòria presentada per:

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Als meus avis

Als meus pares

Al Jacob

CONTENTS

GENERAL INTRODUCTION

What do we know about climate change and its biological effects?

Climate change

Greenhouse gases have the capacity to trap heat in the atmosphere by absorbing infrared radiation reflected by the earth’s surface. Atmospheric concentrations of greenhouse gases, such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), have increased dramatically since the beginning of the industrial revolution largely due to human activities such as fossil fuel combustion and land-use change (Fig. 1, IPCC 2001). It has been estimated that from 1765 to 1995 the added atmospheric burden of carbon dioxide and methane has been responsible for 61% and 23%, respectively, of the additional infrared radiation reaching the earth’s surface from the greenhouse effect (Bridgham et al. 1995).

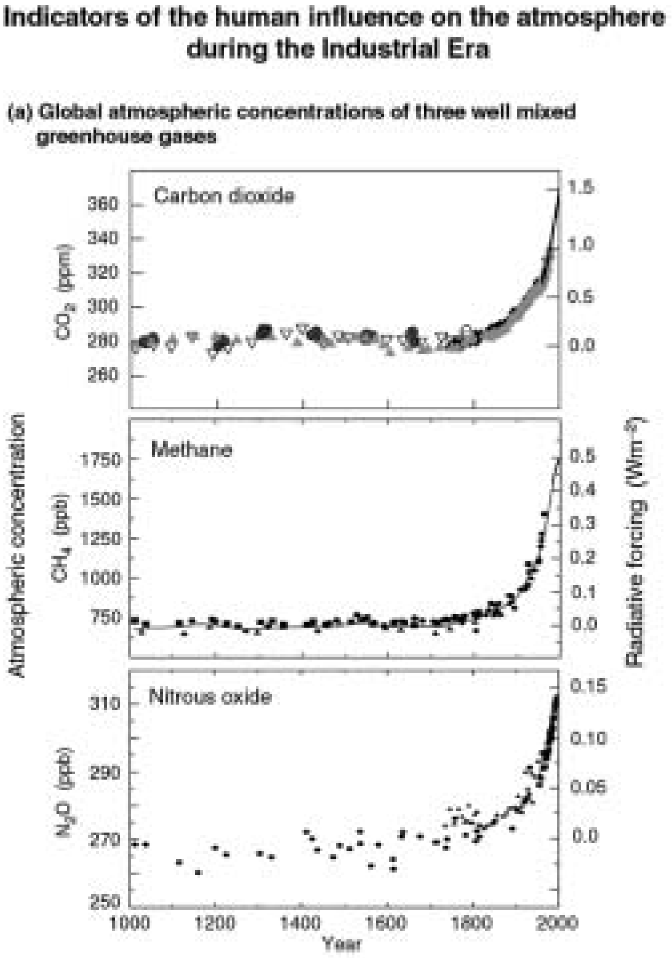


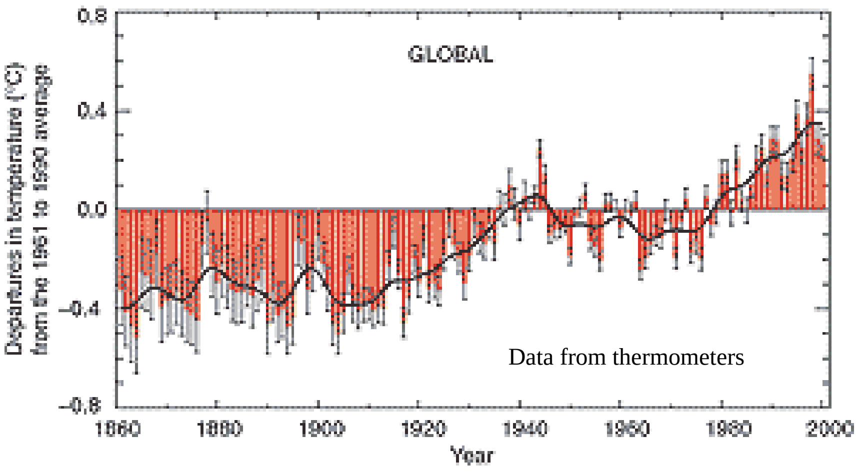
Fig. 1. Changes in the atmospheric concentrations of carbon dioxide (CO2), methane (CH4), and nitrous oxide (N2O) over the past 1000 years (from IPCC 2001).

Considerable evidence is now available showing that greenhouse gases have increased the global average surface temperature by 0.6ºC (± 0.2ºC) over the last century (Fig. 2). Indeed, most of Europe has experienced increases of about 0.8ºC on average. Minimum temperatures (i.e. nighttime temperatures) have increased at about twice the rate of maximum temperatures (0.2 versus 0.1ºC/decade) (Easterling et al. 1997, IPCC 2001).

Northern hemisphere temperature reconstructions from AD 1000 to 2000, based on a combination of tree-rings and ice cores, indicate that 20th century warmth has been anomalous, with the 1990s the warmest decade and 1998 the warmest year of the millennium (Mann et al. 1999; Fig. 2). Measurements of trapped air from a deep Antarctic ice core demonstrate that this recent increase is 5-10 times more rapid than any of the sustained changes in the ice-core record (IPCC 2001).

Variations of the Earth's surface temperature for:

(a) the past 140 years



(b) the past 1,000 years

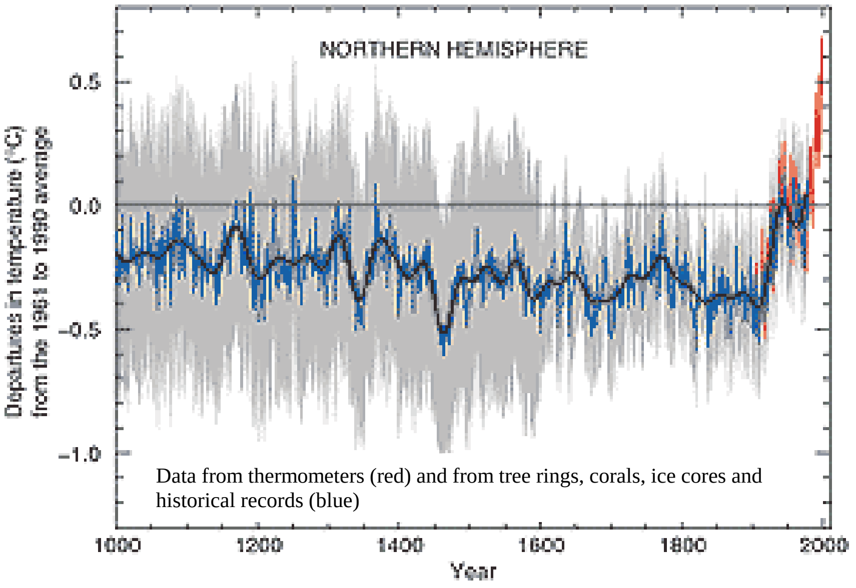


Fig. 2. Variations of the Earth's surface temperature over the last 140 years (a) and the last millennium (b) (from IPCC 2001).

Current predictions based on atmospheric general circulation models indicate that over this century the enhanced infrared radiation from increased atmospheric concentrations of greenhouse gases will result in a 1.4-5.8ºC increase in mean global surface temperature (Fig. 3). Increases in temperature will be accompanied by changes in rainfall patterns, which are more difficult to forecast. Even though, models predict an increase of about 20% in winter precipitation in high latitudes of Europe and a decrease in rainfall (specially in summer) in the Mediterranean region and central and Eastern Europe (Fig. 4).

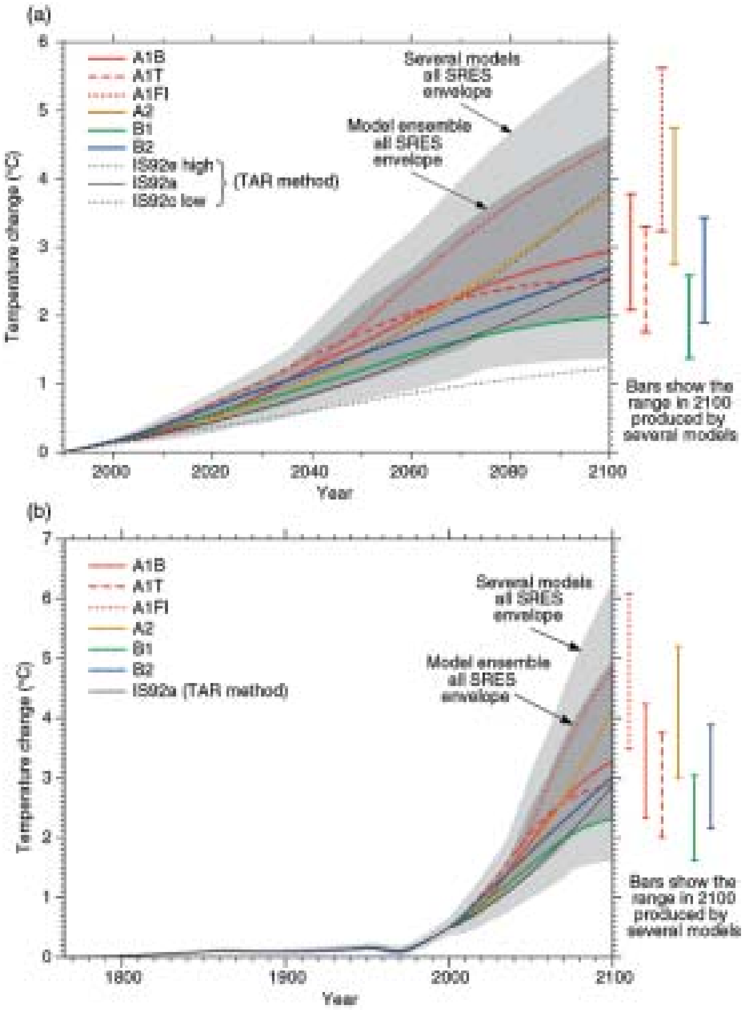


Fig. 3. (a) Global mean temperature projections for the six illustrative SRES scenarios. (b) Same as (a) but results using estimated historical anthropogenic forcing are also used (from IPCC 2001).

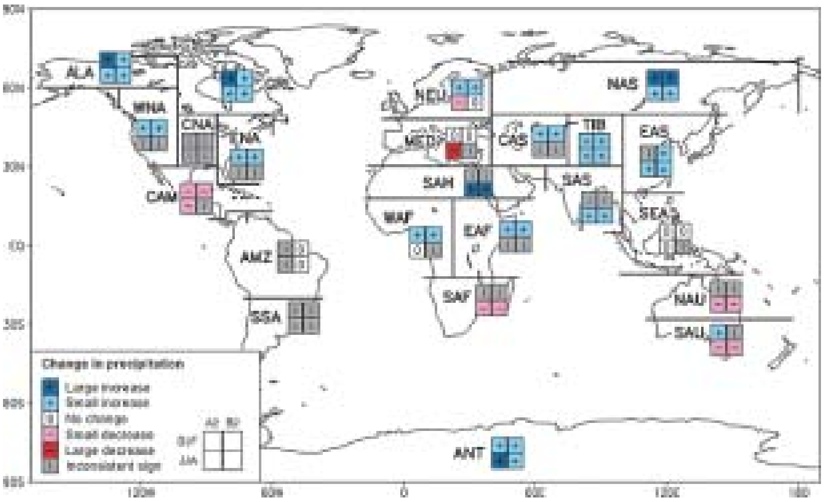


Fig. 4. Analysis of inter-model consistency in regional precipitation change. “Large increase” = greater than 20%; “Small increase” = 5-20%; “No change” = between -5 and +5%; “Small decrease” = between -5 and -20%; “Large decrease” = less than -20% (from IPCC 2001)

Biological effects of climate change

Evidence from analyses of long-term data sets indicate that the anomalous atmosphere and climate of the past few decades has already affected species physiology, the range and distribution of species, the phenology of organisms, and the composition and dynamics of communities (Hughes 2000, McCarty 2001, Walther et al. 2002, Parmesan & Yohe 2003). Some of the changes on plant species and communities documented till now are:

(1) *Effects on plant ecophysiology*: photosynthesis and hence plant growth and productivity are directly affected by atmospheric CO_2 concentration, temperature and water availability. Evidence is accumulating that plant growth in natural ecosystems has responded to recent trends in warming and to atmospheric changes. The increase in the amplitude of the annual oscillations in the atmospheric CO_2 between 1960 and 1994 has been related with and increase in the assimilation of CO_2 by land plants (Keeling et al. 1996). This idea is supported by satellite data showing increased plant growth in the Northern hemisphere during the period 1981-1999 (Myneni et al. 1997, Nemani et al. 2003). Tree-ring records at sites in both hemispheres indicate that increased growth rates have actually been occurring since the mid-19th century (Briffa et al. 1998). By contrast, several studies have found that tree growth in Alaska declined during the 20th century, being this decline attributed to temperature-induced drought stress (Barber et al. 2000, Lloyd & Fastie 2002). Increasing yields in conifer plantations since the 1930s (Cannell & Thornley 1998), and acceleration of turnover rates and biomass accumulation of tropical trees since the 1950s (Phillips & Gentry 1994, Phillips et al. 1998) have also been detected. Decreases in stomatal densities of plant leaves have also been related with the increase in the atmospheric CO_2 levels over the past 200 years (e.g., Woodward 1987, Peñuelas & Matamala 1990). Accordingly, analysis of the leaf $\delta^{13}C$ composition indicated that leaf water use efficiency increased through this period (Peñuelas & Azcón-Bieto 1992, Beerling & Woodward 1993).

(2) *Effects on species ranges and distributions*: A 3ºC change in mean annual temperature corresponds to a shift in isotherms of approximately 300-400 Km in latitude (in the temperate zone) or 500 m in elevation (Hughes 2000). Poleward and upward shifts of species ranges have occurred across a wide range of taxonomic groups and geographical locations during the twentieth century (Hughes 2000, McCarty 2001, Walther et al. 2002, Parmesan & Yohe 2003, Peñuelas & Boada 2003). Nevertheless,

range shifts vary greatly among and within species, implying differential dispersal abilities (Walther et al. 2002).

(3) *Effects on phenology*: the life cycles of many organisms are strongly influenced by temperature and precipitation. The study of phenological time series indicate that rising temperatures during the past century have advanced the timing of spring flowering and leaf unfolding in many species at northern latitudes both in Europe and the USA, whereas it has delayed the timing of leaf colouring and fall (see Peñuelas & Filella 2001 for a review; see also Menzel 2000, Roetzer et al. 2000, Menzel et al. 2001, Peñuelas et al. 2002). Therefore, the growing season has become longer. Satellite remote sensing data (Myneni et al. 1997, Nemani et al. 2003), as well as the study of the annual oscillations in the atmospheric CO_2 in Hawaii between 1960 and 1994 (Keeling et al. 1996), validate these ground observations on larger scales. Model results also agree with these observations (Osborne et al. 2000). The lengthening of the growing season in the Northern hemisphere is likely to contribute to the global increase in biospheric activity detected (Myneni et al. 1997). Differential changes in phenology in response to climate change among interacting species (e.g. plants and pollinators) have been observed (Peñuelas & Filella 2001, Walther et al. 2002, and references wherein).

(4) *Effects on species composition and dynamics of communities*: changes in the precipitation patterns, as well as in temperature, have been related to shifts in the species composition of ecosystems and even in global vegetation types (Brown et al. 1997, Alward et al. 1999). Increases in population size and distribution of invasive exotic species and agricultural pests in response to climate change have also been reported (McCarty 2001and references wherein).

Despite of studies of long-term data sets do present plausible arguments for recent climate change as a primary agent of the ecological changes observed, these studies cannot demonstrate that climate change has *caused* the changes in species and communities described above. Ecosystem level experiments, involving experimental manipulation of climatic factors, such as temperature or water, are essential to highlight causal relationships between climate and species or ecosystem changes. Over the last ten years, a growing number of temperature-manipulation experiments initiated around the world, using diverse methods to increase temperature (see below). Responses of plant productivity and other ecosystem processes to such experimental warming have been quite variable in magnitude and even in direction between sites and between years at a single site, with reported increases, decreases or no change (see references in

Rustad et al. 2001). Therefore, general response patterns have been difficult to identify. Consequently, researchers have begun to use specific statistical techniques, such as meta-analysis, to evaluate and synthesize data from a range of independent experiments (Gurevitch & Hedges 1999). Arft et al. (1999), for example, used meta-analysis to synthesize data from 13 circumpolar ecosystem warming research sites associated with the International Tundra Experiment (ITEX). Results from the ITEX synthesis showed a moderate but significant positive aboveground plant growth response to warming during the first 3 years of treatments, followed by no significant growth response for year 4 (Arft et al. 1999). Moreover, warming effects on plant growth differed between functional groups, with herbaceous forms having a stronger and more consistent vegetative growth response than woody forms. Results also indicated that key phenological events such as leaf bud burst and flowering occurred earlier in warmed plots throughout the study period. Reproductive effort and success increased in warmed plots in later years. Finally, whereas warmer, low arctic sites produced the strongest growth responses, colder sites showed a greater reproductive response. Thus, authors concluded that results of the meta-analysis suggested that the primary forces driving the response to warming vary across climatic zones, functional groups, and through time (Arft et al. 1999).

Results from a more recent meta-analysis across 32 ecosystem warming research sites in a wider range of biomes showed that 2-9 years of experimental warming in the range 0.3-6.0 ºC significantly increased soil respiration rates by 20%, net N mineralisation rates by 46% and plant productivity by 19% (Rustad et al. 2001). With the exception of aboveground plant productivity, which showed a greater positive response to warming in colder ecosystems, the magnitude of the response of these three processes to experimental warming was not generally significantly related to the geographic, climatic or environmental variables evaluated. These results are consistent with the conceptual model where warming directly increases rates of microbial processes including litter decomposition and N mineralization, thereby increasing the availability of nutrients, and, particularly in nutrient-limited ecosystems, increasing plant productivity. The increase in plant productivity may also be the result of a direct effect of warming on rates of net photosynthesis, or an extension of the growing season (Rustad et al. 2001).

In contrast to the substantial number of field warming experiments developed in the last decades, less field manipulation studies have addressed the effects of stronger and more frequent droughts on vegetation (e.g., Borghetti et al. 1998, Fay et al. 2000, 2002, Ogaya et al. 2003). Published studies on whole-ecosystem gas exchange in drought stressed ecosystems are also relatively rare, and were often short-term campaigns or based on modelling (e.g. Verhoef et al. 1996, Miranda et al. 1997, Law et al. 2000, Reichstein et al. 2002). Results of these studies have reported decreases in species growth rates in response to drought (Ogaya et al. 2003), as well as reductions in aboveground net primary productivity, soil CO_2 flux, and flowering duration with longer inter-rainfall intervals (Fay et al. 2000). Reichstein et al. (2002) found reductions in ecosystem respiration and light-saturated gross carbon uptake with drought.

Main concerns about climate change

The described changes in species physiology, distribution and phenology, as well as in species composition and dynamics of ecosystems have occurred with warming levels of only one half or even less of those expected over the 21st century (IPCC 2001). Hence, a clear need exists to understand how species and communities are reacting to global climate change to be able to predict and minimize the impact of future climatic changes. Nowadays, some of the main concerns in relation to climate change impacts on terrestrial ecosystems are:

(1) Although most of the species have changed in accordance with climate change predictions, there are species that are not responding, whereas others are changing in the opposite direction (e.g., Peñuelas et al. 2002). Contrasting effects of simulated climate changes on the ecophysiology of co-occurring species have often been reported (e.g. Chapin III & Shaver 1985, Harte & Shaw 1995, Chapin III et al. 1995, Chapin III et al. 1996, Day et al. 1999), as well as seasonal differences in response to climate change between and within species. Differential changes in phenology in response to climate change among interacting species (e.g. plants and pollinators) may lead to a decoupling in their life-cycle events (Peñuelas & Filella 2001, Walther et al. 2002, and references wherein), which, in its turn, might lead to changes in the species composition of ecosystems, community structure and ecosystem functioning (Peñuelas & Filella 2001).

(2) Because of the widespread loss and fragmentation of habitats, many areas, which may become climatically suitable with future warming, are remote from current distributions and beyond the dispersal capacity of many species. Consequently, species with low adaptability and/or dispersal capacity will be caught by the dilemma of

climate-forced range change and low likelihood of finding distant habitats to colonize, ultimately resulting in increased extinction rates (Walther et al. 2002). Thus, species with narrow temperature tolerances that grow slowly or have poor dispersal mechanisms are likely to be lost (Kirschbaum 2000).

(3) Increases in valued species may be offset by population increases in groups such as invasive exotics, disease vectors and agricultural pests (McCarty 2001). Negative effects of further increases in already abundant, aggressive species may overwhelm any benefits of climate change (McCarty 2001). It seems probable that at least some species will become extinct, either as a direct result of physiological stress or via interactions with other species (Hughes 2000). Changes in species composition and abundance will affect ecosystem functions (net primary productivity, carbon and nutrient cycling, energy fluxes) through changes in tissue chemistry, demography, turnover rates, and vegetation structure (physiognomy, leaf area index) (Shaver et al. 2000).

(4) Ecosystems are considered an important regulator of global climate, mainly providing a feedback concerning emissions of the greenhouse gas CO_2. The response of terrestrial vegetation to climate change has important implications: if elevated temperatures lead to longer growing seasons, then, net primary production and carbon sequestration may increase. The increase in vegetation growth and biomass will strengthen the C sink, which would act as a negative feedback on climate warming. However, a saturation of the response may occur and even terrestrial ecosystems may become a C source if elevated temperatures lead to increased plant and microbial nocturnal respiration rates without a compensatory increase in photosynthesis. To date, many studies have suggested that future global warming will lead to greater increases in heterotrophic soil respiration than in net primary production (the difference between gross photosynthetic production and plant autotrophic respiration), which will lead to further increases in atmospheric CO_2 concentrations (e.g., Smith & Shugart 1993, Wang & Polglase 1995, Woodwell et al. 1998). Recently, Cox et al. (2000) predicted that due to temperature related increases in respiration, ecosystems would become a net source of CO_2 by 2050.

The overall picture that emerges is that conservation scientists need to look at climate change as a current, not just a future, threat to species. Accurate predictions of how species and ecosystems will respond to climate change will assist in preparing for future conservation challenges.

Climate change experiments: the birth of CLIMOOR

Researches are using three main approaches for investigating the consequences of climate change on plant species and natural ecosystems:

(1) *Observational studies*, including:

(1.1) The description of the ecophysiological characteristics and/or other factors that determine the actual distribution of species (e.g., Peñuelas et al. 1998, Oliveira & Peñuelas 2002). This would include the studies along climatic gradients (e.g., Peñuelas et al. 1999).

(1.2) The study of past or present situations that are similar to the expected future climatic changes, which would include the study of past climates (e.g. Peñuelas & Matamala 1990, Peñuelas & Azcón-Bieto 1992, Beerling & Woodward 1993), or the study of extreme climatic events such as droughts (e.g. Peñuelas et al. 2000).

(1.3) The study of long-term data series (e.g., Fitter & Fitter 2002, Peñuelas et al. 2002).

(2) *Controlled experiments* (e.g., Gordon et al. 1999) or field experimental manipulations (e.g., Borghetti et al. 1998, Fay et al. 2002, Ogaya et al. 2003).

(3) *Modelling* (e.g., Osborne et al. 2000).

These three approaches are complimentary and are all needed to improve our understanding of the consequences of climate change on terrestrial ecosystems.

In particular, ecosystem-level experiments provide a useful and realistic tool to monitor the temporal sequence of ecosystem response to changes in one or several climatic factors. As commented above, a substantial number of field warming experiments have developed around the world in the last decades. Warming ecosystem manipulations have used different methods to increase temperature, such as electric heating cables buried in the soil, overhead infrared heaters, open- and closed-top field greenhouses, or the transplantation of vegetation/soil monoliths (see review in Shaver et al. 2000). However, these temperature-manipulation experiments have a number of important drawbacks (e.g., Shaver et al. 2000):

1) Most of these experiments involve unintended lateral effects on other environmental variables (e.g. light, humidity, soil structure, wind). Consequently, although these studies provide valuable insight into potential mechanisms driving plant response to climate change, most of them have restricted applicability to study whole ecosystem responses (Vitousek 1994).

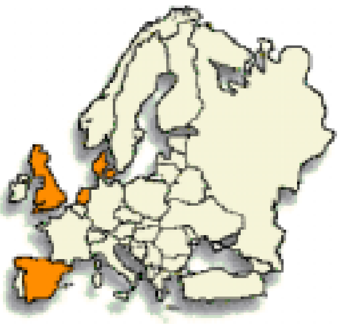
2) Most of these experimental manipulations simulate a diurnal increase in temperature rather than the predicted stronger increase in T_{min} (nighttime temperature) (IPCC 2001). As far as we know, there is only one field experimental study that specifically addresses the effects of nocturnal increases in temperature (Luxmoore et al. 1998).

3) To date, the majority of the climate experiments have been conducted in north temperate, boreal and arctic ecosystems, which are cool and moist. Since different abiotic factors constrain plant physiological activity in northern and southern countries, we would expect different responses of warmer and/or drier ecosystems, such as the Mediterranean ones, to climate change.

4) Finally, it is increasingly clear that long-term responses to climate change may differ greatly in both magnitude and direction from short-term responses (Shaver et al. 2000). Although experimental manipulations of climate conditions have provided useful insights on ecosystem response to climate change on annual to decadal time frames, these types of manipulations do not simulate the gradual change in climate that occurs on the decade to century scale (Shaver et al. 2000, Rustad et al. 2001). Studies along natural climatic gradients are thought to mirror these longer-term changes (decades to centuries) (Shaver et al. 2000).

Experimental field studies addressing the effects of stronger and more frequent droughts on vegetation have used rainout shelters (Fay et al. 2000) or plastic strips and funnels (Borghetti et al. 1998, Ogaya et al. 2003) to exclude natural precipitation. However, important microclimate impacts of the rainout shelters (reduction in PFD and restriction of net radiation fluxes, which increased soil temperatures) have also been reported (Fay et al. 2000).

Fig. 5. CLIMOOR sites



To overcome some of these drawbacks, it was developed a new experimental approach, within the frame of a European project called CLIMOOR (Climate driven changes in the functioning of heath and moorland ecosystems), which was initiated in 1998. The aim of the project was to investigate the effects of warming and drought on the functioning of heath and moorland ecosystems, using a novel manipulation technique to warm and dry these ecosystems, in combination with a geographical gradient approach. CLIMOOR manipulations started in March 1999 and were carried out at 4 sites: Mols

(Denmark, DK), Oldebroek (The Netherlands, NL), Clocaenog (United Kingdom, UK) and Garraf (Spain, SP) (Fig. 5). In each of these sites, temperature and precipitation manipulations were performed using automatically sliding curtains mounted on metal scaffolding 0.2 m above vegetation maximum height. Passive nighttime warming (0.6-2.0ºC) was achieved by avoiding IR dissipation at night over the whole year, simulating the mechanism of global warming due to greenhouse gas accumulation in the atmosphere (Fig. 6). Extended drought was attained by excluding rain over the growing seasons (spring-summer months in northern countries and spring and autumn months in Spain) (Fig. 7).

Fig. 6. Warming plot. An aluminium curtain automatically covers vegetation at night over the whole year

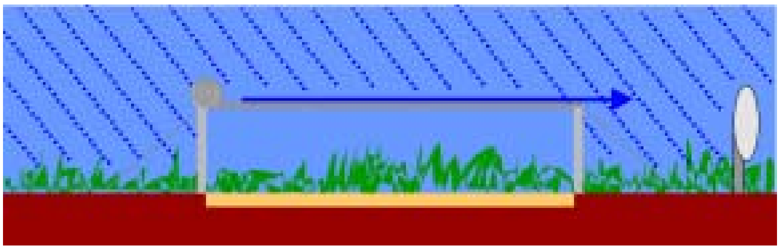


Fig. 7. Drought plot. A transparent curtain automatically covers vegetation during rainfall events in the growing season.

In summary, CLIMOOR experiment provides a suitable methodological approach to study the effects of climate change (warming and drought) on ecosystem processes, since:

1) It minimises unintended effects on wind, light and water balance, as demonstrated by measurements (Beier et al. 2003).

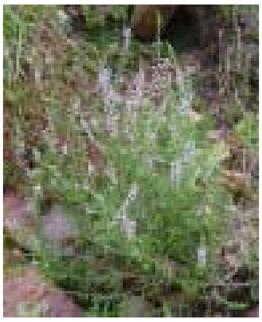
2) It increases minimum temperatures by means of a methodological approach that mimics the way climate change alter heat balance of ecosystems.

3) It assesses the effects of climate change on a Mediterranean ecosystem, which is an example of warm and dry ecosystem.

4) It combines field experiments with the study of ecosystem dynamics along a natural climatic gradient, which has been suggested to be a useful approach to distinguish relatively short-term, transient changes (annual to decadal time scale) from longer-term, near-equilibrium changes (decades to centuries time scale) (Shaver et al. 2000).

Heathland and shrubland ecosystems were chosen because they represent an important natural resource that will be very sensitive to the changes in climate and pollutant loading observed and predicted for the future (Anderson & Hetherington 1999). Shrubs with sclerophyllous, evergreen leaves characterize the vegetation of both heathlands in north-western Europe and shrublands exposed to a Mediterranean-type climate (Reader 1984). Specht (1979) attributed the dominance of sclerophyllous shrubs in areas with a Mediterranean-type climate to the occurrence of seasonal drought, while in north-western Europe, where rainfall is fairly evenly distributed throughout the year, low soil fertility may account for the dominance of sclerophyllous shrubs. Fires, which occur with regularity in both heathland and Mediterranean-type shrublands, help to maintain the dominance of shrubs by killing tree seedlings (Reader 1984). Grazing is another factor that helps to maintain shrub dominance, mainly in the northern heathlands. The four CLIMOOR sites are dominated by Ericaceous shrubs being the more common species *Calluna vulgaris* (L.) Hull (Ericaceae) (Fig. 8), *Deschampsia flexuosa* (L.) Trin. (Poaceae) and *Vaccinium myrtillus* L. (Ericaceae) in the three northern sites, and *Erica multiflora* L. (Ericaceae) and *Globularia alypum* L. (Globulariaceae) in the Spanish site (Table 1).

Fig. 8. *Calluna vulgaris*



	Garraf, SP	**Mols, DK**	**Oldebroek, NL**	**Clocaenog, UK**
Location	41º18'N, 1º49'E	56º23'N, 10º57'E	52º24'N, 5º55'W	53º03'N, 3º28'W
Climate	Mediterranean	Atlantic - dry	Atlantic - dry	Atlantic - wet
Altitude (m)	210	58	25	490
Precipitation 1998-2000 (mm)	455	758	1120	1675
Mean annual air temperature (°C)	13.9	9.4	10.1	8.2
Soil type	Petrocalcic calcixerept	Sandy podzol	Sandy podzol	Peaty podzol
N-input 1998-2000 (kg N ha^{-1})	10-15	13.2	30-40	20-25
Plant cover (%)	57	100	95	100
Main plant species	*Erica multiflora* *Globularia alypum*	*Calluna vulgaris* *Deschampsia flexuosa*	*Calluna vulgaris* *Deschampsia flexuosa*	*Calluna vulgaris* *Vaccinium myrtillus*

Table 1. Main characteristics of CLIMOOR sites

The Spanish CLIMOOR site

In Spain, the study was carried out in a dry shrubland (*Rosmarino-Ericion*) at the Garraf Natural Park, Barcelona, NE Spain (41º18'N, 1º49'E), at 210 m above sea level. The climate of the site is typically Mediterranean. The mean annual precipitation during the years 1999-2001 was 456 mm, whereas the mean annual air temperature was 13.9 ºC. The site, which is located on terraces from abandoned vineyard's fields, suffered big fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixerept (SSS 1998), thin (12-37 cm), with a loamy texture and abundant calcareous nodules. Currently the regenerating vegetation covers 50-60% with a maximum height of 70 cm. The dominant species are *Erica multiflora* (Fig. 9), *Globularia alypum* (Fig. 10), *Dorycnium pentaphyllum* and *Fumana ericoides,* whereas other common species are *Rosmarinus officinalis, Ulex parviflorus, Helianthemum syriacum, Brachypodium phoenicoides, Fumana thymifolia* and *Ampelodesmus mauritanica* (Fig. 11).

Fig. 10. *Globularia alypum*

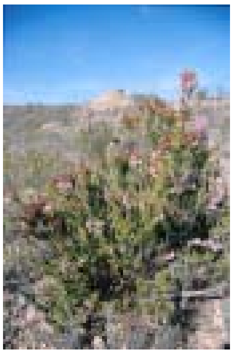


Fig. 9. *Erica multiflora*

Fig. 11. Garraf CLIMOOR site.

Evergreen shrubland is an important and widespread vegetation type throughout the Mediterranean region. Evergreen sclerophyllous shrublands (maquis) extend from semiarid to subhumid Mediterranean region and occupy more than 12 million hectares (Le Houérou 1974). In Spain, abandoned lands where cultivation ceased several decades ago, now support these semi-natural shrublands, which are presumed to represent only intermediate stages of an old-field succession (Haase et al. 1997). However, they do provide substantial cover in these otherwise sparsely vegetated landscapes, thus controlling runoff and soil erosion, and have become important grazing grounds for the local pastoralism (Haase et al. 2000). Woody perennial shrubs with several stout stems sprouting from a root stump (lignotuber or root-crown burl) dominate these sclerophyllous shrublands (Specht 1981). Although the essential oils and resins in the sclerophyllous canopy make the vegetation highly flammable during periods of drought, lignotubers (and other underground organs) have enabled the mediterranean plant communities to regenerate rapidly after fire (or other catastrophes) (Specht 1981). Despite of semiarid woody ecosystems comprise 16% of the world's terrestrial biomass, and contain 23-25% of the area and net primary productivity of the world's terrestrial surface (Atjay et al. 1979), they have not been extensively studied (Tognetti et al. 2000).

Objectives of the present PhD study

The work I present in this memory was developed in the frame of the CLIMOOR experiment, but was mainly focused on the study of the effects of our experimental warming and drought on the ecophysiology of the two dominant shrub species in the Spanish site: *Erica multiflora* and *Globularia alypum*. Both species are evergreen, sclerophyllous shrubs that typically occur in basic soils of the western Mediterranean basin, where they are common components of the coastal shrubland. Whereas there are several published studies on the ecology and phenology of *Erica multiflora* (e.g., Lloret & López-Soria 1993, Vilà & Terradas 1995, Vilà 1997, Vilà et al. 1998, Santandreu & Lloret 1999), we did not find any study published in a peer-reviewed journal on the ecophysiology of *Globularia alypum*.

The general objectives of the thesis were:

1) To obtain critical information on the biology and ecophysiology of the two Mediterranean species *Erica multiflora* and *Globularia alypum*.

2) To identify the periods of environmental stress for these two species and the main abiotic factors causing such stresses.

3) To test whether future warmer or drier conditions could affect the physiological performance, photosynthetic and water use strategies, growth or phenology of these two Mediterranean shrubs and whether this effect will vary among seasons or years.

4) To test whether future warmer or drier conditions will affect differently both species, and thus, whether they might lead to a change in their interactions, their competitive ability, and ultimately on their relative abundances.

5) To compare the effects that the studied climatic changes (warming and drought) could have on the physiological activity of *Erica multiflora* with the effects that these climatic changes could have on another Ericaceous shrub, *Calluna vulgaris*, that dominates the heathlands of the northern European countries.

6) To study whether warmer or drier conditions could change the level of physiological stress experienced by these shrubs during leaf development. To approach this aim we used *Vaccinium myrtillus* L., another common shrub of the northern heathlands.

Fig. 12. *Vaccinium myrtillus*

The first objective is addressed over the first four chapters of this PhD memory, since in these chapters I study extensively key traits of the biology and ecophysiology of both Mediterranean species, such as leaf gas exchange rates, shoot water potential, photosynthetic performance (by means of chlorophyll fluorescence measurements), growth or flowering phenology.

In **chapter 1** I describe and compare the diurnal and seasonal fluctuations in the photosynthetic performance and water relations of *E. multiflora* and *G. alypum* throughout two consecutive years. I try also to identify the periods of environmental stress for these species and the main abiotic factors causing such stresses, addressing thus the second objective of the PhD. To approach these aims I measured leaf gas exchange rates, shoot water potentials and chlorophyll fluorescence once each season, and leaf nitrogen and carbon concentrations, specific leaf area, and leaf $\delta^{13}C$ and $\delta^{15}N$ once a year. I measured leaf gas exchange rates because leaf gas exchange measurements (stomatal conductance and net photosynthetic and transpiration rates) are among the classical instantaneous methods to detect plant functional limitations imposed by environmental factors. Photosynthesis is the driving force of plant productivity and the ability to maintain the rate of photosynthetic carbon dioxide assimilation under environmental stresses is fundamental to the maintenance of plant growth and production (Lawlor 1995). Chlorophyll *a* fluorescence measurements allowed me to monitor the changes in the photochemical efficiency of photosynthesis, and thus, in the functioning and regulation of the photosynthetic apparatus, in a non-intrusive way (Schreiber et al. 1994). Measurements of leaf nitrogen content complement those of photosynthesis, because of the central role of this element in the biochemistry and photobiology of photosynthesis (Field & Mooney 1986, Rundel 1988): approximately 75% of the total nitrogen in a typical leaf may well be directly related to the process of photosynthesis (Field & Mooney 1986). The fractionation of carbon isotopes that occurs during photosynthesis is one of the most useful techniques for investigating the efficiency of CO_2 uptake (O’Leary 1988). Carbon isotope discrimination (Δ) – a measure of plant ^{13}C depletion as compared with atmospheric CO_2 – provides an integrative measure of water-use efficiency (the ratio CO_2 assimilation rate/leaf conductance) over time (Farquhar & Richards 1984, Farquhar et al. 1989). Finally, leaf $\delta^{15}N$ can be used to make inferences on the N source and on the relative activity of the different processes of the N cycle (supplies, retention, and losses) in the surrounding system (Peñuelas et al. 1999).

In the first 4 chapters, but specifically in chapters 2, 3 and 4 I address the third and fourth objectives of the PhD. In particular, in **chapter 2** I used the same variables than in chapter 1 (plus photosynthetic-response curves to light and CO_2) to compare the effect of warming and drought treatments on the ecophysiology of *E. multiflora* and *G. alypum*. Seasonal and yearly variations in treatment effects are discussed. Predicting the responses of leaf photosynthesis to climate change is fundamental to projecting the impact of global change on the future global carbon cycle and terrestrial biosphere (Bernacchi et al. 2001).

In **chapter 3**, I study whether drier or warmer conditions affect the annual or seasonal above-ground growth patterns of *E. multiflora* and *G. alypum* and their estimated annual stem biomass production over three years, 1999, 2000 and 2001. I also monitored the spring shoot elongation rates and annual leaf fall of both species in 2000. Growth measurements give us insight into longer-term responses of vegetation to environmental changes than instantaneous measurements, such as leaf gas exchange rates or fluorescence measurements.

In **chapter 4**, I assessed the impact of warmer and drier conditions on the flowering phenology of *E. multiflora* and *G. alypum*. I also studied the effect of warming and drought on flower production and flower duration of both species and on flower size of *E. multiflora*. Our major goals were: (1) to describe the flowering pattern of *E. multiflora* and *G. alypum*, and (2) to assess whether predicted warmer or drier conditions may change flowering patterns and flower production of these two Mediterranean species. Flowering is considered one of the most significant phenological stages by which the sensitivity to climate change can be evaluated (Spano et al. 1999). Moreover, flowering time is a trait which could be critical to plant success through its effect on reproductive processes such as pollination, total plant seed production and the timing of seed dispersal (Primack 1980, Augspurger 1981; Gross & Werner 1983, Gross & Werner 1983, Kelly & Levin 2000, Pilson 2000).

In **chapter 5**, I address the 5th objective of the PhD, i.e., to compare the effects of our experimental increase in temperature and drought on the photosynthetic performance of two Ericaceous shrub species along a north-south European gradient, which represents a gradient in the factors experimentally manipulated, temperature and precipitation. To approach this aim, I performed measurements of leaf gas exchange rates, chlorophyll *a* fluorescence and leaf carbon isotope ratio ($\delta^{13}C$) of *Calluna vulgaris* (in the 3 northern sites) and *Erica multiflora* (in the Spanish site) during the growing seasons of 1999 and 2000.

In **chapter 6**, I investigate the effects of warmer or drier conditions on the physiological stress experienced by bilberry (*Vaccinium myrtillus*) during leaf development. To assess physiological stress, I measured leaf gas exchange as a classical measure of instantaneous stress and leaf size and leaf fluctuating asymmetry (FA) as integrative measures of stress. FA is a measure of developmental instability. Developmental instability refers to the inability of a bilateral organ or organism to buffer its development against disturbances and to produce a predetermined phenotype (Moller & Swaddle 1997). FA is a particularly useful measure of developmental instability because, for bilaterally or radially symmetrical traits, the optimal phenotype is known. As stress during development may influence developmental precision, FA has been proposed as a potentially useful tool for monitoring stress levels in natural populations (Leary & Allendorf 1989, Graham et al. 1993). In fact, recently, several authors have suggested that measures of developmental instability, such as fluctuating asymmetry, may provide a more sensitive indicator of stress than traditional measures of performance (e.g. growth, fecundity, survival) because FA has a measurable response at a lower threshold of stress (Zvereva et al. 1997, Rettig et al. 1997).

Finally, in **chapter 7**, we report a more global view of the possible effects of drought or warming on ecosystem processes and functioning along the four European studied sites. This chapter integrates results on plant cover and biomass, plant ^{14}C uptake, stem and shoot growth, flowering, leaf chemical concentration, litterfall, and herbivory damage in the dominant plant species of each site.

Other work developed during the PhD, but not included in the present memory

CLIMOOR studies currently being concluded

Community responses to treatments in the Spanish site were also monitored throughout the study period, by measuring changes in plant cover, biomass and species composition. We used the pin point (or point quadrat) method (Jonasson 1988) to assess these changes. Results will be shown in a paper that is currently under preparation entitled "Warm- and drought-induced changes in the aboveground biomass and species composition of a Mediterranean shrubland" by Laura Llorens, Josep Peñuelas and Marc Estiarte.

Remote sensing vegetation indices NDVI and PRI were monitored seasonally in the Spanish site and were related with both community and individual plant biomass. Results are presented in the manuscript "Reflectance assessment of seasonal and annual changes in biomass and CO_2 uptake of a Mediterranean shrubland in response to experimental warming and drought" by Iolanda Filella, Josep Peñuelas, Laura Llorens and Marc Estiarte, submitted to Global Change Biology.

Other studies conducted during the pre-doctoral period

During the PhD period I also worked in the following papers:

- González-Solís J, JC Guix, E Mateos, L Llorens (2001) Population density of primates in a large fragment of the Brazilian Atlantic rainforest. Biodiversity and Conservation 10: 1267-1282.
- Llorens L, A Casinos, C Berge, M Majoral, FK Jouffroy (2001) A biomechanical study of the long bones in Platyrrhines. Folia Primatologica 72: 201-216.
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CHAPTER 1

Diurnal and seasonal variations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*

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Abstract

Diurnal and seasonal fluctuations in the photosynthetic performance and water relations of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum* were monitored throughout two consecutive years at Garraf Natural Park in NE Spain. We measured leaf gas exchange rates, chlorophyll fluorescence and shoot water potentials once each season. We also measured leaf nitrogen and carbon concentrations, leaf $\delta^{13}C$ and $\delta^{15}N$ and specific leaf area (SLA) once a year (August) on well developed mature leaves. *G. alypum* experienced seasonal fluctuations in their water potential, with the lowest values recorded in summer, whereas *E. multiflora* did not show significant differences in water potential among seasons. Moreover, we found lower water potentials in *G. alypum* than in *E. multiflora* throughout the entire study, suggesting that the latter behaved as a drought-avoiding species, whereas the former tolerated lower water potentials. In both species, maximum leaf gas exchange rates were observed in autumn and secondarily in spring; in contrast, photosynthetic and transpiration rates reached absolute minima in summer. The stronger fluctuations in water potential and leaf gas exchange rates found in *G. alypum* compared to *E. multiflora,* suggest that *G. alypum* is, *sensu* Levitt (1980), a water spender, whereas *E. multiflora* is a water conservative. This hypothesis is further supported by a higher integrated water-use efficiency (higher $\delta^{13}C$ values) and a higher degree of sclerophylly (lower SLA) in *E. multiflora* as compared to *G. alypum*. *G. alypum* showed higher leaf gas exchange rates and higher predawn potential photochemical efficiency (Fv/Fm) than *E. multiflora* during most of the study. In spring and autumn, predawn Fv/Fm values were within the optimal range, whereas we detected chronic photoinhibition in summer and winter in both species. However, whereas both species could maintain positive photosynthetic rates in winter, we found frequent negative values in summer, suggesting higher levels of stress during the drought period. These results together with the high correlations that we found between the net photosynthetic rates and several parameters of water availability (accumulated rainfall, soil moisture or midday water potential) provided further evidence of the key role of water availability in the regulation of the photosynthetic rates in these Mediterranean species. Warmer and drier conditions in the next decades, as a consequence of climate change, may alter the present, slight competitive advantage of *G. alypum* and the fitness of both shrub species within semi-arid Mediterranean environments.

Introduction

Survival of a plant in a given environment depends largely upon its ability to photosynthesise at an adequate rate in order to grow faster than its competitors and balance its water loss to supply (Wuenscher and Kozlowski 1971). Rates of these two gas-exchange processes thus may play a major role in determining the outcome of competition among species and hence the species composition of plant communities (Wuenscher and Kozlowski 1971). In general, two contrasting water use strategies can be found in plants: prodigal and conservative (Passioura 1982). The prodigal (or water-spending) strategy is related to high stomatal conductance associated with high water losses, but also high carbon gain and thus growth (Heilmeier et al. 2002). A usual way to assess the potential competitive ability of plants and to get information on specific adaptations to the prevailing climate has been the study of diurnal and seasonal fluctuations in photosynthetic performance and water relations. In particular, many studies on Mediterranean species show seasonal adjustments in these parameters (e.g., Harley et al. 1987, Nunes et al. 1992, Oliveira et al. 1992, Tretiach 1993, Grammatikopoulos et al. 1995, García-Plazaola et al. 1997, Karavatas and Manetas 1999), as a consequence of the marked seasonality of the Mediterranean climate.

Mediterranean summer is characterised by low precipitation and high temperature, high irradiance and high water vapour pressure deficit (Di Castri 1973). Although the photosynthetic apparatus is largely unaffected by water limitations (e.g., Genty et al. 1987, Havaux 1992), the combination of high light and high temperature can be particularly inhibitory to photosystem II activity (Gamon and Pearcy 1990, Havaux 1992, Chaves et al. 1992). Indeed, many studies have described reductions in photochemical efficiency (e.g., Damesin and Rambal 1995, Valladares and Pearcy 1997, Matos et al. 1998) and low photosynthetic rates during summer drought due to stomatal control of water loss (e.g., Harley et al. 1987, Tenhunen et al. 1990, Damesin and Rambal 1995, Valladares and Pearcy 1997, Matos et al. 1998). However, co-occurring species often differ in their tolerance to drought (Duhme and Hinckley 1992, Castell et al. 1994). This variability is at least partly associated with differences in stomatal responses to dry conditions. Two drought adaptation strategies have been proposed: drought avoidance and drought tolerance (Levitt 1980). Drought avoidance is generally found in species with high stomatal sensitivity to drought. In these species, the high degree of stomatal control enables them to maintain high plant water potentials for extended periods of drought (Levitt 1980). In contrast, drought-tolerating species exhibit reduced stomatal sensitivity and, thus, simultaneous decreases in stomatal conductance and leaf water potential (e.g., Levitt 1980, Picon et al. 1996).

Although summer drought is generally considered the primary constraint to productivity and distribution of the Mediterranean vegetation (Di Castri 1973, Larcher 2000), some authors have suggested that winter cold stress also plays an important role in the development and distribution of Mediterranean evergreen species (Mitrakos 1980, Tretiach 1993). During Mediterranean winters, long rainless and cloudless periods can coincide with the coldest days (Kyparissis et al. 2000). Therefore, during such periods, photosynthesis may be limited directly by temperature or indirectly via hydraulic limitations on stomatal conductance (Oliveira et al. 1992, Grammatikopoulos et al. 1995). Indeed, low photosynthetic rates during winter (reviewed by Larcher 2000) and reductions of the efficiency of PSII under low temperatures (García-Plazaola et al. 1999, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001) have also been reported.

Global change effects on Mediterranean climate are likely to produce warmer and drier conditions and more frequent and stronger droughts (IPCC 2001) with consequent effects on vegetation. Since co-occurring Mediterranean species have often different climatic constraints, each species will likely respond differently to the climate change. To predict how climate change may affect species distribution or dominance and as a consequence, community structure and ecosystem functioning, it is essential to have a broad knowledge of which climatic factors are constraining physiological activity of each species and how these constraints are manifested temporally. Therefore, the aims of this study were a) to determine whether two co-occurring Mediterranean shrub species, *E. multiflora* and *G. alypum*, show different photosynthetic performance and/or different strategies of water use in response to the diurnal and seasonal variability in water availability and temperature, b) to identify the periods of environmental stress for these two species and the main abiotic factors causing such stresses. To approach these aims, we measured leaf water potentials, leaf gas exchange rates, chlorophyll fluorescence, specific leaf area (SLA), leaf nitrogen and carbon concentrations and leaf $\delta^{13}C$ (to study integrated plant water use) and $\delta^{15}N$ (to study plant N sources) throughout two consecutive years.

Material and methods

Site and species description

The study was carried out in a dry shrubland (*Rosmarino-Ericion*) next to the village of Olivella in Garraf Natural Park, Barcelona, NE Spain (41º18’N, 1º49’E) at 210 m above sea level and on a SSE slope (13º). The climate is typically Mediterranean, characterised by spring and autumn rainfalls, mild cool winters and hot and dry summers. The soil is a petrocalcic calcixerepts (SSS, 1998), thin (12-37 cm) and with a loamy texture and abundant calcareous nodules.

Erica multiflora L. and *Globularia alypum* L. are evergreen, sclerophyllous shrubs that typically occur in basic soils of the western Mediterranean Basin. They are two dominant shrubs of the studied shrubland. Both species resprout from underground organs after aboveground biomass removal. Vegetative growth occurs twice a year: in spring (from March to June) and autumn (from September to November). Flowering starts in August-September.

Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured every 1-2 weeks through the study period using Time Domain Reflectometry, TDR. Each sampling date, volumetric water content at 0-15 cm was estimated, using a cable tester (1502B, Tektronix, Beaverton, OR, USA), on 9 fixed sampling points distributed within the study site. Air temperatures during the sampling period were obtained from the nearest weather station (El Rascler, Begues) at 8 km from the study site. Air temperatures in the leaf chamber (PLC4B, ADC Inc., Hoddesdon, Hertfordshire, England) and photosynthetic photon flux density (PPFD) at the moment of the leaf gas exchange measurements were monitored with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England).

Shoot water potential

Water potentials of apical shoots were measured seasonally with a Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). Each sampling date, shoots of 3 plants (one shoot per plant) of *E. multiflora* and *G. alypum* were measured at predawn

(0230-0430 h in spring and summer and 0430-0630 h in autumn and winter, solar time) and midday (1100-1300 h, solar time).

Leaf gas exchange rates and chlorophyll (Chl) fluorescence

We measured leaf gas exchange rates and Chl fluorescence during 4-6 consecutive days in spring (May), summer (August), autumn (November) and winter (February) throughout two years (spring 1999 - spring 2001). We also measured Chl fluorescence in February 1999. Measurements were taken on one sunny shoot per plant, grown always in the spring of the current year.

Each season, leaf gas exchange rates were measured on 6-10 plants of *E. multiflora* and *G. alypum* in the morning (from 30 min after sunrise to 1130 h, solar time, at the latest) and in the afternoon (from midday to 1630 h, solar time, at the latest). Net photosynthetic rates (A), transpiration rates (E) and stomatal conductances were measured with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England). Branch tips with several leaves were introduced into the chamber of the system. All results are expressed on area basis, which was measured using ImagePC (v. α9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Water-use efficiency (WUE), defined as mmol of net CO_2 uptake per mol of H_2O transpired, was calculated by dividing instantaneous values of A by E.

Components of Chl fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). After a dark adaptation period of at least 30 min, we obtained minimum and maximum dark-adapted fluorescence (F_0, Fm) and Fv/Fm, where Fv=Fm-F_0. Fv/Fm has been used as a measure of the potential (or maximum) photochemical efficiency of PSII. Measurements were performed on 9-15 plants of *E. multiflora* and *G. alypum* at predawn and midday.

The actual photochemical efficiency of PSII in the light-adapted state was estimated as: $\Phi_{PS2} = \Delta F/Fm' = (Fm'-F)/Fm'$, where F is the steady-state fluorescence yield under the given environmental conditions, and Fm' is the maximum level of fluorescence obtained during a saturating flash of light (when all the PS2 traps are closed) under the same environmental conditions. From this index, we calculated the apparent electron transport rate (ETR) as:

$$ETR = \Delta F/Fm' \times PPFD \times 0.84 \times 0.5$$

where PPFD was the photosynthetic photon flux density incident on the leaf, 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electron required to the absorption of one quanta, as two photosystems are involved. We measured the ETR on 9-12 plants of *E. multiflora* and *G. alypum* at morning (0700-1000 h, solar time) and midday (1100-1400 h, solar time).

Isotope and elemental analyses

Carbon (C) and nitrogen (N) leaf concentrations and foliar $\delta^{15}N$ and $\delta^{13}C$ were determined on leaves from the current year collected in August of 1999 and 2000. Each year, we sampled 9 plants of *E. multiflora* and *G. alypum*. All analyses were carried out in an elemental analyser Carlo Erba EA1108 (Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany). The calibrations were performed using interspersed international isotopic standards of carbon and nitrogen (IAEA, Vienna, Austria). The elemental analysis calibration was performed using atropine (ThermoQuest Italia) as standard. The accuracy of the measurements was ± 0.15 ‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$.

Specific leaf area (SLA)

We calculated the SLA of the leaves of the same shoots in which we measured leaf gas exchange rates in summer (August). After measuring the total leaf area of the shoots (see above), we dried all the leaves in an oven at 70 ºC to constant weight. We calculated the SLA as the ratio of the area of the leaves divided by their dry weight (cm^2 mg^{-1}). Shoots were collected at the end of the sampling day.

Statistical analyses

We performed ANOVAs within each year, season and round to study the differences between the two species. Since multiple tests were performed with the same variables, significance level (p=0.05) was always adjusted for the number of statistical tests using a sequential Bonferroni correction to prevent against group-wide type errors. To investigate the differences among seasons, we performed ANOVAs for each species within each year and round, pooling the data from different years and/or rounds when the interactions were not significant. We used PPFD as a covariable in the ANOVAs whenever we analysed variables likely sensitive to instantaneous light levels. Pre-treatment measurements of the Chl fluorescence parameters (February 1999) were not considered in the analyses.

Reduced major axis (RMA) regressions were used to investigate the relationships among the studied variables and the different parameters related to water availability (accumulated rainfall, soil moisture and shoot water potential). Finally, to analyse the differences between the two species in the leaf nitrogen concentration, the C/N ratio or the stable isotopes composition, we performed ANOVAs with year and species as fixed factors.

Results

Environmental data

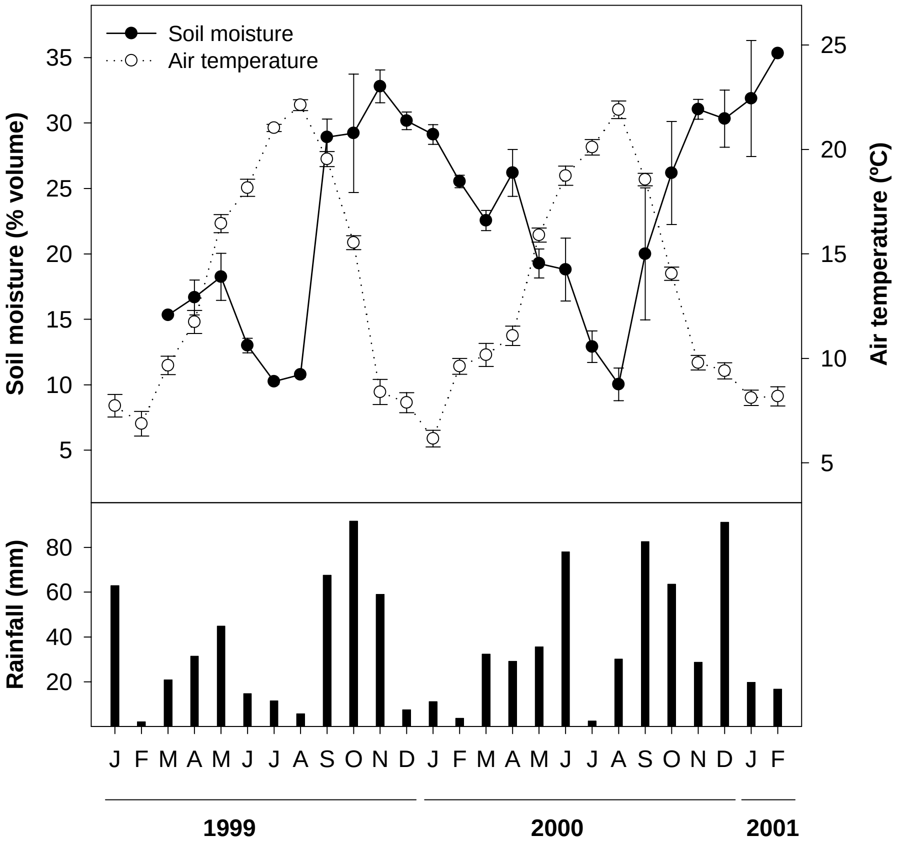

Fig. 1. Monthly soil moisture (% volume/volume), air temperature (ºC) and rainfall (mm) through the study period. Error bars indicate the standard error of the mean (soil moisture: n=2-6 days, except in March 1999 and February 2001 where n=1; air temperature: n=28-31 days).

The mean annual air temperature was 13.8 ºC in both 1999 and 2000. The maximum mean monthly air temperature occurred in August both years, whereas the minimum mean temperatures were reached in February in 1999 and in January in 2000 (Fig.1). Total precipitation was 420.5 mm in 1999 and 489 mm in 2000. Maximum monthly rainfall occurred in October in 1999 and in December in 2000. The driest month was February in 1999 and July in 2000 (Fig. 1).

The mean annual soil moisture (v/v) was 21% in 1999 and 23% in 2000. Maximum soil moisture occurred at the end of autumn and beginning of winter, whereas minimum soil moisture was registered in summer (Fig. 1).

Shoot water potential

G. alypum had lower water potentials than *E. multiflora* (Fig. 2), although seasonally the differences between the two species were only significant in summer 2000 at predawn (p=0.001) and midday (p=0.004) and in autumn 2000 (p=0.002) and winter 2000-01 (p=0.000) at predawn. Midday values were significantly lower than predawn values for both species (p<0.001).

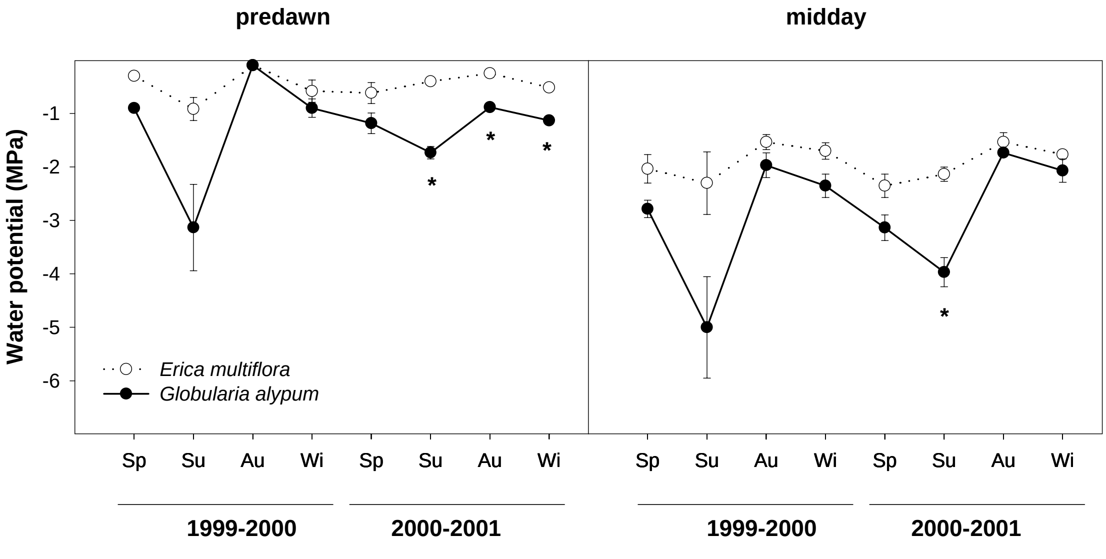

Fig. 2. Predawn and midday shoot water potential (MPa) of *E. multiflora* and *G. alypum* through the study period. Error bars indicate the standard errors of the mean (n=3, except in spring 1999 at predawn, where n=1). Significant differences between the two species (after sequential Bonferroni correction of the significance level p=0.05) are indicated by asterisks.

E. multiflora did not show differences in the water potential among seasons throughout the study period, either at predawn or at midday. In contrast, *G. alypum* showed a significant seasonal pattern with the lowest water potentials in summer both years at predawn and midday.

Leaf gas exchange rates

In the morning, *G. alypum* showed five times higher leaf net photosynthetic rates (A) than *E. multiflora* in spring 1999 and two times higher in autumn 2000 ($p<0.001$ in both cases) (Fig. 3). In the afternoon, net photosynthetic rates of *G. alypum* were higher (a mean of 2,5 times higher) than those of *E. multiflora* through all the study period (except in summer both years), although in spring and winter of the second year differences were not significant. Differences between morning and afternoon net photosynthetic rates of *E. multiflora* and *G. alypum* were almost significant ($p=0.08$). The highest net photosynthetic rates of the two species occurred in autumn, except for *G. alypum* in the first year, when autumn and spring values were similar. The lowest A values for both species and both years were in summer, although, in the second year, photosynthetic rates of *G. alypum* in summer were not statistically different from spring values.

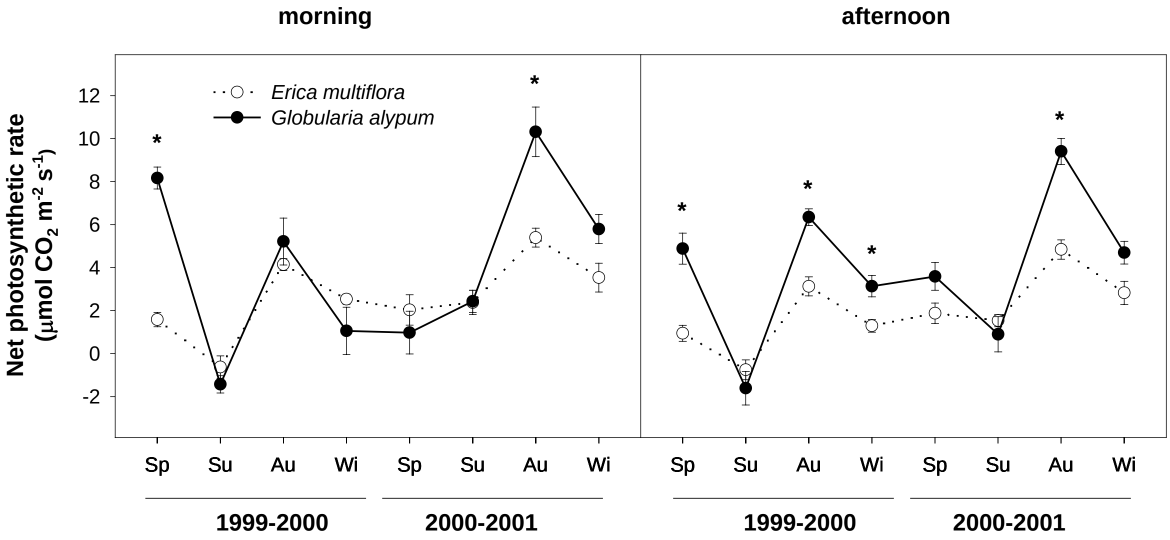

Fig. 3. Net photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) of *E. multiflora* and *G. alypum* through the study period in the morning and in the afternoon. Error bars indicate the standard errors of the mean (n=6-10). Significant differences between the two species (after sequential Bonferroni correction of the significance level $p=0.05$) are indicated by asterisks.

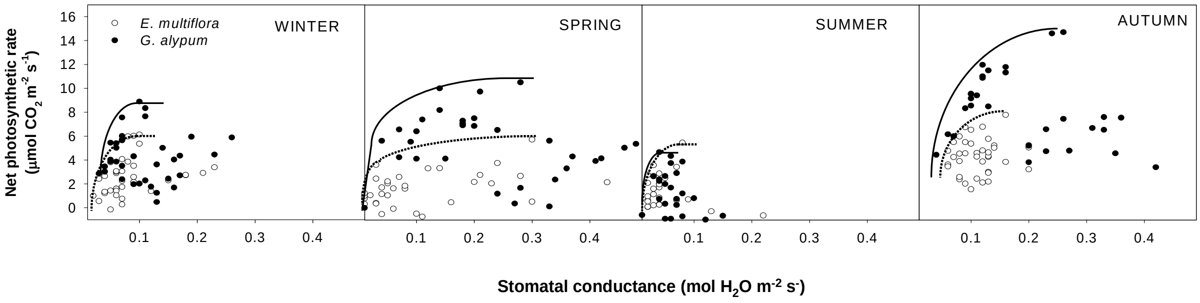

Fig. 4. Net photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) *versus* stomatal conductances (mol H_2O m^{-2} s^{-1}) in winter, spring, summer and autumn. Morning and afternoon values for the two years have been pooled. Lines were set to the upper surface of data clouds.

In spring and autumn, maximum net photosynthetic rates of *G. alypum* were higher than maximum rates of *E. multiflora* for a same stomatal conductance (Fig. 4). In winter, maximum leaf net photosynthetic rates in relation to stomatal conductances were also higher for *G. alypum* plants, although the differences between the two species were smaller than in spring and autumn. In summer, both species showed similar ranges of leaf net photosynthetic rates and stomatal conductances.

In spring and autumn, leaf transpiration rates of *G. alypum* were 2.4 times higher than those of *E. multiflora* in the morning and 1.7 times higher in the afternoon ($p<0.01$), except in autumn 2000 (Fig. 5). In winter and summer, differences were significant only in summer 2000 ($p<0.01$). In both species, transpiration rates were lower in the afternoon than in the morning ($p=0.001$) in all the seasons and years. The first year, leaf transpiration rates of *E. multiflora* (morning and afternoon) and *G. alypum* in the afternoon did not differ significantly among seasons. However, the first year in the morning, leaf transpiration rates of *G. alypum* were lower in winter and summer than in spring and autumn. The second year, morning transpiration rates of *E. multiflora* and *G. alypum* were higher in spring than in the other seasons ($p<0.001$).

G. alypum and *E. multiflora* showed similar WUE (A/E) values through all the study period, except in autumn 2000 ($p<0.001$ in the morning and in the afternoon) and in winter 2000 in the morning ($p=0.004$) (Fig. 5). There were no differences between morning and afternoon values of WUE in any of the two study species. *E. multiflora* and *G. alypum* showed the lowest WUE values in summer, except the second year in the morning, when there were no significant differences between spring and summer values.

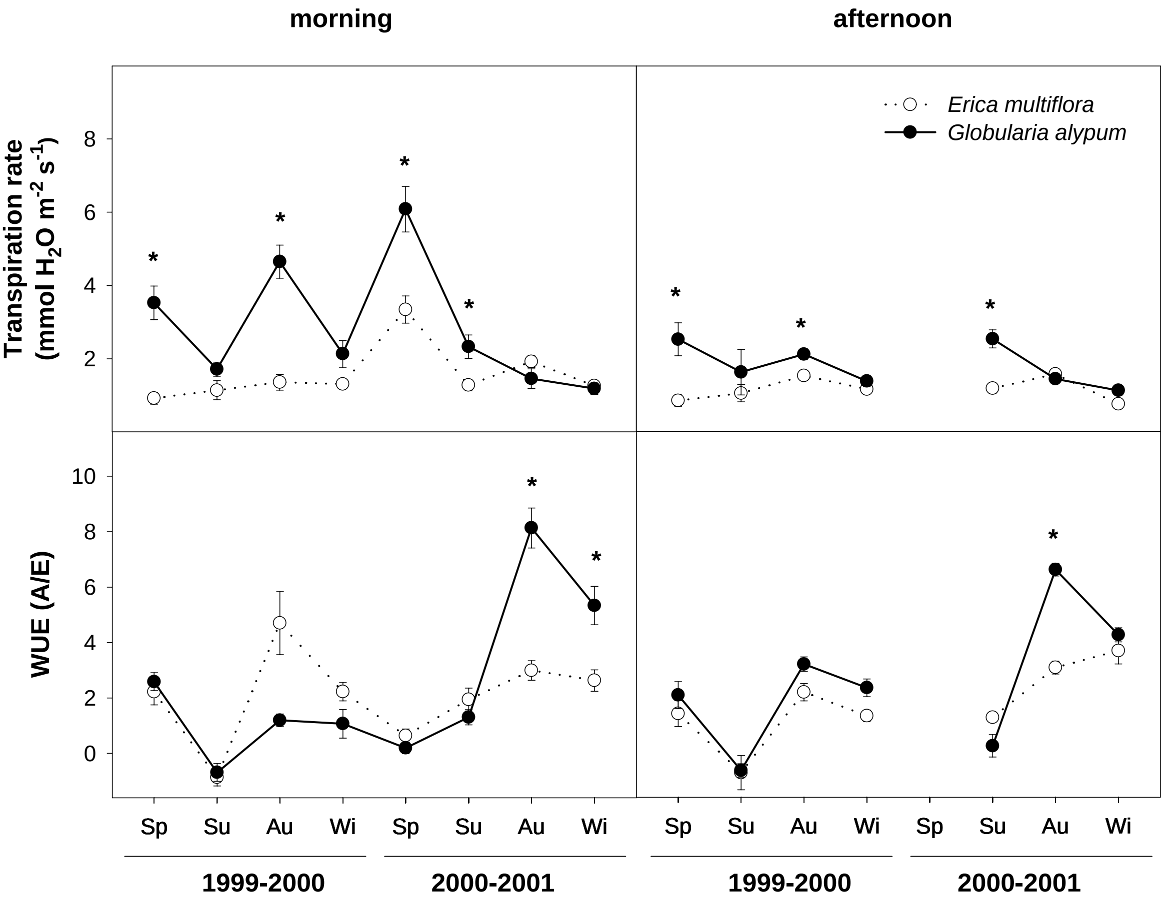

Fig. 5. Transpiration rates (mmol H_2O m^{-2} s^{-1}) and WUE (A/E, expressed as mmol of net CO_2 uptake per mol of H_2O transpired) of *E. multiflora* and *G. alypum* in the morning and in the afternoon through the study period. Error bars indicate the standard errors of the mean (n=6-10). Significant differences between the two species (after sequential Bonferroni correction of the significance level p=0.05) are indicated by asterisks.

Relationship between leaf net photosynthetic rates and temperature or water availability

When scatter plots are used to compare leaf net photosynthetic rates (morning and afternoon) *versus* temperature, several seasonal trends are highlighted (Fig. 6). There is a broad temperature optimum. *E. multiflora* and *G. alypum* usually showed their maximum net photosynthetic rates in autumn and their minimum ones in summer. Despite temperatures while measuring in winter were very similar to those of autumn, net photosynthetic rates in winter were lower than in autumn. Negative values of net photosynthetic rates were recorded in *E. multiflora* and *G. alypum* mainly in summer, although some negative values were observed also in winter and spring. Values were more negative for *G. alypum* than for *E. multiflora*.

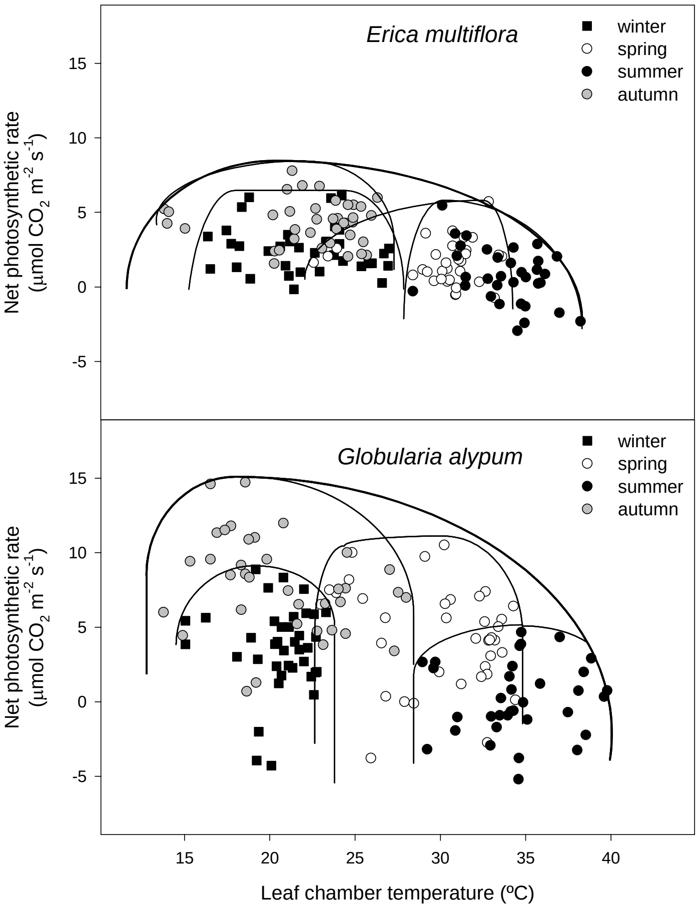

Fig. 6. Net photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) *versus* leaf chamber temperature (ºC) in winter, spring, summer and autumn. Morning and afternoon values for the two years have been pooled. Lines were set to the upper surface of data clouds.

The afternoon net photosynthetic rates of *G. alypum* and *E. multiflora* were significantly correlated with the accumulated rainfall over the 3 months previous to the measurements, the soil moisture and the midday water potential (Fig. 7). Morning net photosynthetic rates showed weaker correlations with the studied hydrologic parameters in the case of *G. alypum*, whereas the correlations were similar for *E. multiflora*.

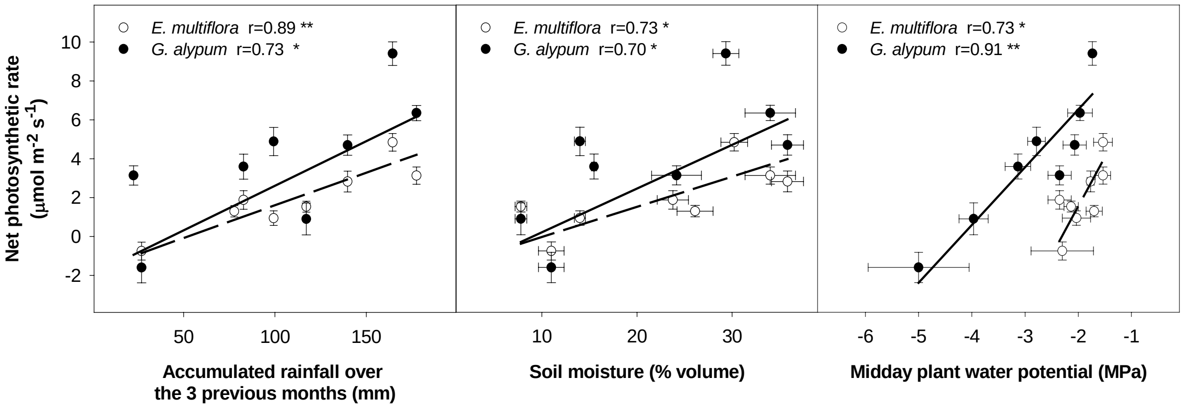

Fig. 7. Net photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) in the afternoon *versus* accumulated rainfall over the 3 months previous to the measurements (mm), the soil moisture (% volume/volume) and the shoot water potential at midday (MPa) for *E. multiflora* and *G. alypum* through the study period. Asterisks indicate significant correlations: * p<0.05, ** p<0.01.

Chl fluorescence

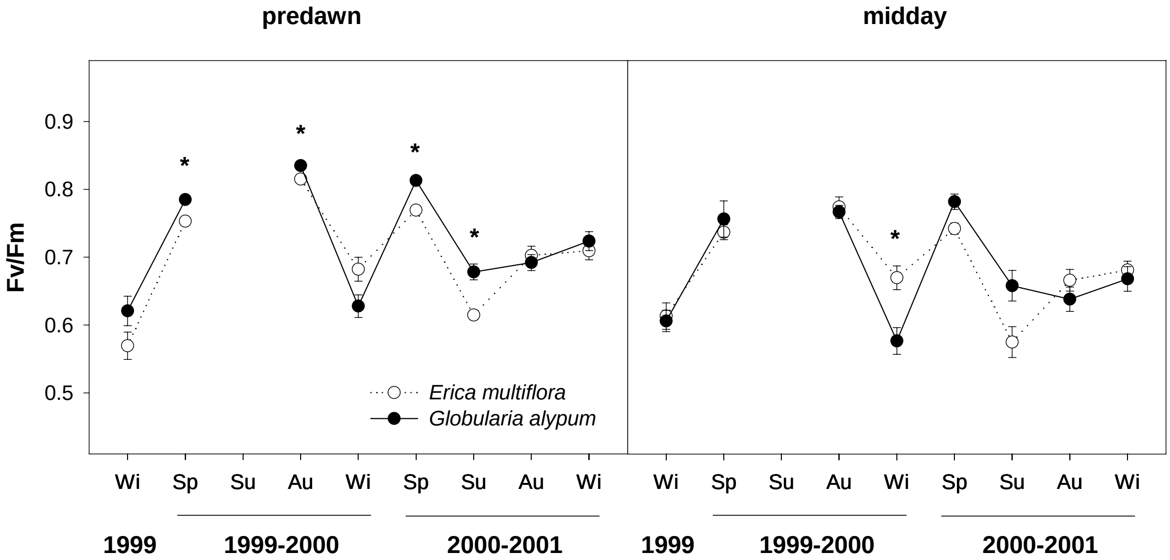

Fig. 8. Potential photochemical efficiency (Fv/Fm) of *E. multiflora* and *G. alypum* at predawn and midday through the study period. Error bars indicate the standard errors of the mean (n=9-15). Significant differences between the two species (after sequential Bonferroni correction of the significance level p=0.05) are indicated by asterisks.

At predawn, potential photochemical efficiencies of PS II (Fv/Fm) of *G. alypum* plants were higher than for *E. multiflora* in all seasons, except in winter (1999, 2000 and 2001) and in autumn 2000 (Fig. 8). At midday, both species showed similar values of Fv/Fm, except in winter 2000, where Fv/Fm values of *E. multiflora* were higher than those of *G. alypum* ($p=0.001$). Midday values of Fv/Fm were significantly lower than predawn values for both species in all the seasons ($p<0.001$). Spring and autumn Fv/Fm values were higher than winter and summer values in both species, at predawn and midday.

Apparent electron transport rates (ETR) of *E. multiflora* plants at midday were usually higher than those for *G. alypum*, although differences were statistically significant only in summer ($p=0.007$) and autumn ($p=0.004$) of the second year (Fig. 9). Midday ETR values of *E. multiflora* and *G. alypum* were higher in autumn and spring than in winter and summer.

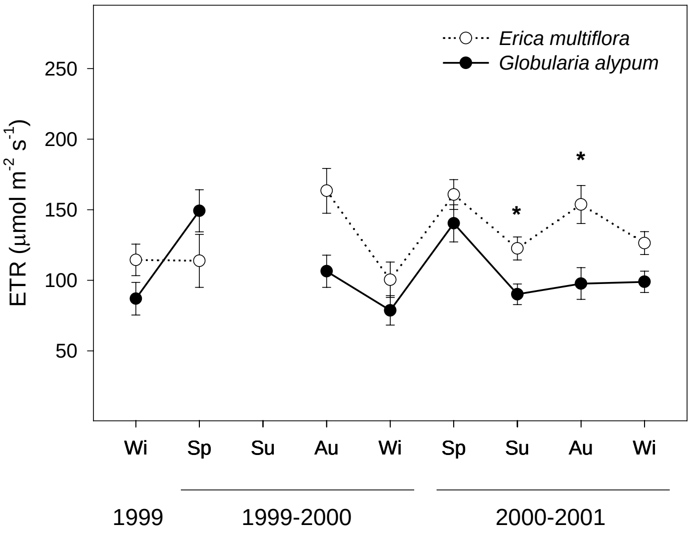

Fig. 9. Apparent electron transport rate (ETR) of *E. multiflora* and *G. alypum* at midday through the study period. Error bars indicate the standard errors of the mean (n=9-12). Significant differences between the two species (after sequential Bonferroni correction of the significance level $p=0.05$) are indicated by asterisks.

Specific leaf area (SLA) and foliar N concentration

Mature leaves of *G. alypum* had higher SLA values ($4.33 \times 10^{-2} \pm 3.6 \times 10^{-4}$ cm^2 mg^{-1}) than mature leaves of *E. multiflora* ($3.57 \times 10^{-2} \pm 2.6 \times 10^{-4}$ cm^2 mg^{-1}). The leaf nitrogen concentration was higher in *G. alypum* than in *E. multiflora* in both years ($p=0.004$) (Fig. 10). Leaf nitrogen concentration in 2000 was slightly higher than in 1999 for both species ($p=0.051$). *G. alypum* showed a lower C/N ratio than *E. multiflora* in both years ($p<0.001$) (Fig. 10). On the other hand, the C/N ratio was lower in both species in 2000 than in 1999 ($p=0.03$).

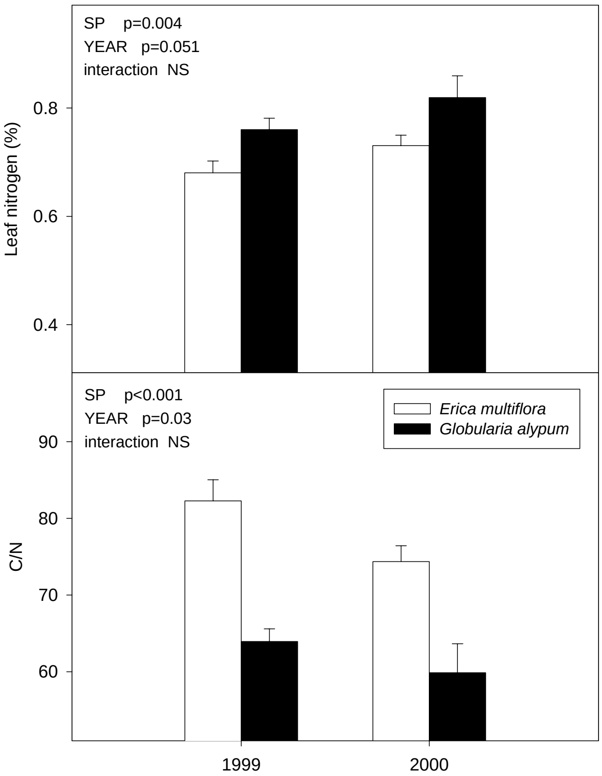

Fig. 10. Leaf nitrogen concentration (% dry mass) and C/N ratio for *E. multiflora* and *G. alypum* leaves at the end of summer in 1999 and 2000. Error bars indicate the standard errors of the mean (n=9).

Leaf stable isotopes

In both years, lower $\delta^{13}C$ values were found in *G. alypum* than in *E. multiflora* (p<0.001) (Fig. 11). There were no significant differences between years in the $\delta^{13}C$ values of both species. There were no significant differences between the two species or the two years in the leaf $\delta^{15}N$ values (Fig. 11).

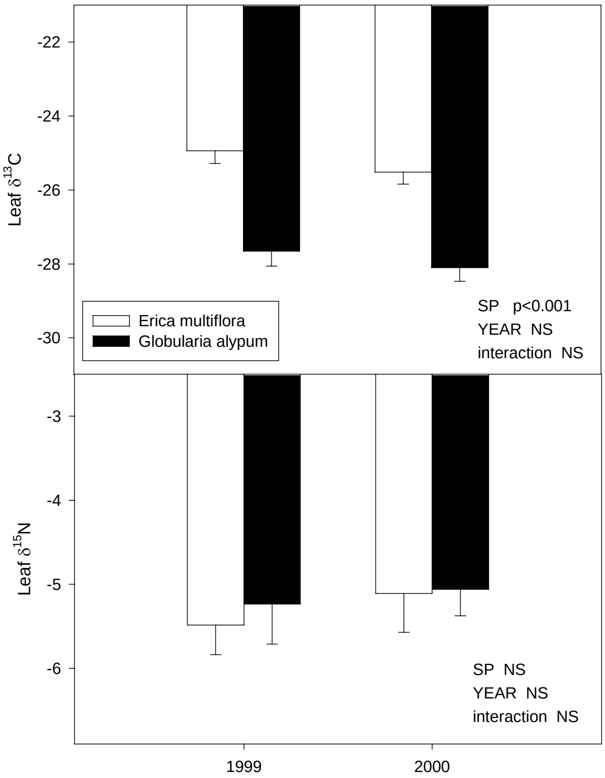

Fig. 11. Foliar $\delta^{13}C$ and $\delta^{15}N$ for *E. multiflora* and *G. alypum* leaves at the end of summer in 1999 and 2000. Error bars indicate the standard errors of the mean (n=9)

Discussion

Leaf gas exchange rates, as well as shoot water potential values, of *G. alypum* and *E. multiflora* were within the range reported for other Mediterranean shrubs that co-occur with them, such as *Pistacia lentiscus* or *Quercus coccifera* (Rhizopoulou et al. 1991, Llusià and Peñuelas 2000). *G. alypum* shrubs sustained more severe water deficits (lower shoot water potentials) than did *E. multiflora* through all the study period (Fig. 2). Moreover, *G. alypum* plants experienced seasonal fluctuations in their water potential, with the lowest values recorded in summer, whereas *E. multiflora* plants did not show significant differences in shoot water potential among seasons (Fig. 2). As a consequence, differences in water potentials between the two species were specially marked in summer.

Seasonal adjustments in leaf gas exchange rates were observed in *G. alypum* and *E. multiflora*. Photosynthetic and transpiration rates of both species reached absolute minima in the summer (with frequent negative values of photosynthesis), whereas they showed high photosynthetic and transpiration rates during the periods with high water availability (autumn and spring) (Figs. 3 and 5). Similar results have been reported for Mediterranean vegetation (e.g., Nunes et al. 1992, Castell et al. 1994, Damesin and Rambal 1995, Grammatikopoulos et al. 1995).

The marked reductions in gas exchange rates in summer in the absence of changes in shoot water potential indicates that *E. multiflora* behaved as a drought-avoiding species. In contrast, *G. alypum* is a more drought-tolerating species. The advantage of tolerating lower plant water potentials is that a strong gradient of water potential between the soil and the plant is produced, thus helping to maintain the flow of water into the plant (Dunn et al. 1976). However, decreases in plant water potential increases the risk of cavitation (Tyree and Sperry 1989), which can be responsible for the death of leaves and twigs during the summer (Correia and Catarino 1994).

The higher net photosynthetic (A) and transpiration (E) rates of *G. alypum* compared to *E. multiflora* through all the year (except in summer) was usually related to higher stomatal conductances (Figs. 3, 4 and 5). However, *G. alypum* had higher A than *E. multiflora* for a same stomatal conductance (Fig. 4). This could be related to a greater photosynthetic capacity in *G. alypum*, which agrees with the higher leaf nitrogen concentration that we found in this species (Fig. 10). A positive correlation between leaf nitrogen and rates of net photosynthesis has been demonstrated in many studies (e.g., Field and Mooney 1986, Evans 1989), since the photosynthetic apparatus and, specifically, Rubisco is a major sink for nitrogen in leaves (Field and Mooney 1986, Evans 1989).

It has been proposed that stomatal closure in part of the day in arid ecosystems would avoid dehydration and therefore major tissue damage (Pereira and Chaves 1995). Accordingly, we found that transpiration rates of the two study species were lower in the afternoon than in the morning through all the study period (Fig. 5). A higher leaf-to-air vapour pressure difference in the afternoon than in the morning (data not shown) may cause the afternoon depression in transpiration rates (Beadle et al. 1985). It has also been suggested that afternoon depression of leaf gas exchange rates may be associated with long periods of high photon flux density (Correia et al. 1990).

Relationship between net photosynthetic rates and temperature

In our study, *E. multiflora* and *G. alypum* showed a broad range of temperature optima for net photosynthesis (Fig. 6), which is similar to the range reported for a number of Californian and Chilean shrubs (15-30ºC, Oechel 1980) or for schlerophyllous shrubs of dry regions after compilation data from numerous authors (20-35ºC, Larcher 1995).

Our results are in agreement with the general idea that the range of temperatures more favourable to the carbon uptake coincides with the temperature limits during the period of growth in the Mediterranean region (Chaves et al. 1992). Traditionally this period has been identified as spring. However, the two studied species present two growing periods, one in spring and the other in autumn, and for both species the range of temperatures most favourable to CO_2 assimilation coincides with the latter. Climatic conditions in autumn (higher rainfall and soil moisture, lower temperatures and thus lower vapour pressure deficit) allow higher net photosynthetic rates than in spring. Moreover, the highest A values in autumn coincide with the flowering and fruiting period. Although temperatures on sunny sampling days of winter were also within the optimum temperature range, net photosynthetic rates were not as high as in autumn, probably due to the lower water availability and/or the suboptimal temperature events in winter.

Relationship between net photosynthetic rates and water availability

Several authors have pointed out situations where there is a lack of correlation between changes in leaf water potential and leaf gas exchange rates (Beadle et al. 1985, Reich and Hinckley 1989, Tenhunen et al. 1990, Matos et al. 1998, Giorio et al. 1999) and, thus, it has been suggested that stomata may respond to soil or root water status rather than to leaf water status (Reich and Hinckley 1989, Giorio et al. 1999). However, we found significant correlations between afternoon net photosynthetic rates and shoot water potentials at midday in both species (Fig. 7), although such correlations may be reflecting the significant correlations that we also found between shoot water potential at midday and soil moisture (see Fig. 7). Afternoon net photosynthetic rates were also highly correlated with the soil moisture and with the accumulated rainfall over the three months previous to the measurements in both species (Fig. 7), suggesting that water availability plays an important role in the regulation of net photosynthetic rates in the two studied species.

Water-use efficiency

Different authors suggested that efficient use of water is a conservative ecophysiological “strategy” that can be detrimental in a competitive water-limited environment (DeLucia and Heckathorn 1989, Read and Farquhar 1991). Accordingly, we found that the lowest instantaneous WUE (A/E) values were in summer (and spring in the second year) for both species (Fig. 5), in agreement with other studies on Mediterranean species that reported decreases in WUE as water deficit increased (e.g., Gratani et al. 2000, Moriana et al. 2002). It has been suggested that sometimes when stomata close, the ratio A/E falls sharply since net photosynthetic rates are reduced nearly to zero, but transpiration (mostly cuticular) continued because of the high evaporative demand (Larcher 1995). Our results contrast with those reported by a number of authors indicating that as water become less available, water-use efficiency increases in woody plants (e.g., Toft et al. 1989, Korol et al. 1999). Differences in the intensity of water stress may explain such contrasting results. Indeed, Chaves and Rodrigues (1987) reported that in *Vitis vinifera* the highest WUE was observed with mild water deficiency whereas, as stress increased, WUE fell.

Theory predicts that values of leaf $\delta^{13}C$ are positively related, via the ratio between the concentration of CO_2 in the leaf and in the air, to the integrated water-use efficiency over the growing season (WUEi) (Farquhar et al. 1989). *E. multiflora* leaves showed higher $\delta^{13}C$ than *G. alypum*, which corresponds to a higher WUEi (Fig. 11). However, instantaneous values of WUE measured in spring did not agree with this result, since we did not find differences between the two species (Fig. 5). Other studies have reported discrepancies between gas exchange and isotopic data (e.g., Vitousek et al. 1990, Gutiérrez and Meinzer 1994, Picon et al. 1996). Hence, scaling from instantaneous values of WUE may not reflect the integrated leaf gas exchange activity over the growth period (Gutiérrez and Meinzer 1994).

Higher WUEi in *E. multiflora* suggests that *E. multiflora* is more water conservative than *G. alypum*; lower transpiration rates of *E. multiflora* compared to those of *G. alypum* support this. Assuming plant water competition, the more prodigal use of water of *G. alypum* in spring (lower WUEi) would be advantageous because it allows *G. alypum* to use the water before the other species extract it and, as a consequence, to have higher photosynthetic rates. Accordingly, different studies have reported that low WUE plants tended to have higher net photosynthetic rates under high soil moisture conditions than high WUE plants (Ehleringer and Cooper 1988, Kolb and Davis 1994). However, these studies also showed that, when soil water is limiting (e.g., summer drought), low WUE plants are more susceptible to decreases in growth rate, water stress-induced embolism, shoot dieback and mortality (Ehleringer and Cooper 1988, Kolb and Davis 1994).

Finally, higher WUEi in *E. multiflora* than in *G. alypum*, coincides with a lower SLA of *E. multiflora* leaves; that is, *E. multiflora* leaves are more sclerophyllous than *G. alypum* leaves. Several authors have found increases in δ^{13}C and, thus, in the WUEi as the level of sclerophylly increased (Vitousek et al. 1990, Groom and Lamont 1997). On the other hand, higher leaf N concentration and net photosynthetic rates in *G. alypum* coincides with higher SLA, in agreement with previous studies demonstrating a positive correlation between these variables (Reich et al. 1997). SLA reflect a fundamental trade-off in plant functioning between a rapid production of biomass (high SLA) and an efficient conservation of nutrients (low SLA) (Garnier et al. 2001).

Photosynthetic efficiency

In our study, maximum predawn Fv/Fm values were within the optimal range reported by Björkman and Demmig (1987) and they occurred in spring of both years and in autumn of the first year, suggesting relatively low stress during these seasons (Fig. 8). Conversely, predawn values of Fv/Fm in summer and winter every year and in autumn 2000 were lower than 0.75, with a minimum of 0.57 ± 0.02 for *E. multiflora* and 0.62 ± 0.02 for *G. alypum* for winter 1999, in agreement with previous studies on Mediterranean vegetation that also reported lower Fv/Fm values during these seasons (e.g., García-Plazaola et al. 1997, Karavatas and Manetas 1999, Larcher 2000, Oliveira and Peñuelas 2001). Our results, thus, suggest the presence of chronic photoinhibition during summer and winter in the two study species, since no recovery of photochemical efficiency after nighttime was observed. Persistent reductions in PSII efficiency are attributed predominantly to the retention of de-epoxidised components of the xantophyll cycle (zeaxanthin and antheraxanthin) and their sustained engagement for energy dissipation activity, which may help to protect PSII from over-excitation and photodamage (e.g., Adams and Demmig-Adams 1995, García-Plazaola et al. 1999, Kyparissis et al. 2000). Nevertheless, sustained reductions of Fv/Fm values may also result from accumulation of non-functional PSII reaction centres and partial photoinactivation of PSII (Niyogi 1999). In winter, the relatively high assimilation rates of both species, favours the hypothesis that photoinhibition was due to photoprotection rather than photodamage. In contrast, the observed decreases of Fv/Fm in summer could be accompanied by extremely low photosynthetic rates (sometimes even negative ones, Fig. 3), suggesting possible damage to the photosynthetic apparatus.

Higher net photosynthetic rates in *G. alypum* than in *E. multiflora* in spring and autumn would create a higher photochemical sink for the electrons contributing to protect the photosynthetic apparatus against the accumulation of excessive excitation energy. This is in accordance with the higher potential photochemical efficiency of *G. alypum* compared to *E. multiflora* plants at predawn during most of the study period (except in autumn 2000 and winter). Midday Fv/Fm values for *E. multiflora* were suboptimal (lower than 0.75) through all the study period, except in November 1999, whereas values for *G. alypum* were lower than 0.75 in winter every year and in summer and autumn of 2000.

In C_3 plants, PSII activity is mainly partitioned between photosynthesis and photorespiration (Krall and Edwards 1992). However, despite the higher photosynthetic rates of *G. alypum*, we found similar or even lower apparent electron transport rates (ETR) in *G. alypum* than in *E. multiflora* (Fig. 9). These contradictory results may simple arise from the fact that photosynthetic rates are expressed by the total leaf area of the measured shoot, whereas the ETR values are expressed by the illuminated leaf area (which at least for *E. multiflora* never coincides with the total leaf area of the shoot). Alternatively, these results may indicate higher photorespiration rates in *E. multiflora*. Available data do not allow to sort out this question.

The results of our study suggest that, under the present climate conditions, *G. alypum* is able to take a greater advantage of periods with high water availability than *E. multiflora*, showing a higher carbon gain and a better photosynthetic performance. However, the predicted warmer and drier conditions in the next decades (IPCC 2001) will probably reduce such differences, lowering the present competitive advantage of *G. alypum*. Moreover, taking into account that both species, but in particular *E. multiflora*, showed suboptimal values of Fv/Fm at midday during most of the year, the drier and warmer conditions predicted for the next decades may decrease even more the photosynthetic performance of these two Mediterranean shrubs.

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CHAPTER 2

Ecophysiological responses of two Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*, to experimentally drier and warmer conditions

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Abstract

We used a new approach to experimentally dry and warm a Mediterranean shrubland. By means of automatically sliding curtains, we extended the drought period by excluding rain over the two growing seasons (spring and autumn), and we created passive warming by avoiding IR dissipation at night over the whole year. The aim of our study was to test how a future extended drought period and an increase in temperatures could affect the photosynthetic and water use strategies of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*, which are common species of the dry coastal shrublands. We monitored seasonally the shoot water potential, leaf gas exchange rates and chlorophyll *a* fluorescence of plants during two years (1999-2001). Additionally, we measured the photosynthetic-response curves to light and CO_2 in autumn 2001 and the foliar N concentration and leaf C and N stable isotopes in summer 1999 and 2000. Drought plants of both shrub species showed lower shoot water potentials, transpiration rates and stomatal conductances than control plants, although there was a high seasonal variability. Drought treatment reduced significantly the overall leaf net photosynthetic rates of *E. multiflora*, but not of *G. alypum*. Drought plants of *E. multiflora* also showed lower leaf net photosynthetic rates in response to light and CO_2 and lower carboxylation efficiency than controls, but there was no significant effect of drought on its overall PSII photochemical efficiency. Although warming treatment did not affect the leaf net photosynthetic rates of the two species overall the study, it increased significantly the carboxylation efficiency and leaf net photosynthetic rates of *G. alypum* plants in response to CO_2 levels in autumn 2001. In addition, warming treatment increased the potential photochemical efficiency of PSII (Fv/Fm) of both species (but especially of *G. alypum*) at predawn or midday and mainly in autumn and winter. Thus, our results suggest that drier conditions might decrease the annual productivity of these Mediterranean shrubs, particularly of *E. multiflora*, and that future warming could alleviate the present low temperature constraints of the photosynthetic performance of the two studied species, but especially of *G. alypum*, during the colder seasons. Ultimately, drier and warmer conditions in the near future may change the competitive relationship among these species in such Mediterranean ecosystems.

Introduction

Human activities are increasing atmospheric concentrations of greenhouse gases, which are projected to lead to regional and global changes in climate (IPCC, 2001). Over the last decade, a growing number of field experiments simulating some of the predicted climatic changes were initiated around the world (e.g. see Shaver *et al.*, 2000 for a review of warming experiments). However, to date, the majority of these climate experiments have been conducted in north temperate, boreal and arctic ecosystems. Thus, a clear need exists to explore the responses of warmer and drier ecosystems to climate change.

Current climate projections predict drier and warmer conditions for the Mediterranean basin in the next decades (IPCC, 2001). Till now, most of the techniques used to experimentally warm ecosystems alter other environmental factors (e.g. light, humidity, soil structure) and most of the experiments usually simulate a diurnal increase in temperature rather than the predicted stronger increase in T_{min} (nighttime temperature) (IPCC 2001). Thus, we used a new approach for the experimental drought and warming of a dry Mediterranean shrubland (Beier *et al.*, 2003). On one hand, we extended the drought period by excluding rain over the two growing seasons (spring and autumn). On the other hand, we created passive warming by avoiding IR dissipation at night over the whole year, which simulates the way climate change alters the heat balance of ecosystems.

Mediterranean summer drought is generally considered the primary constraint to the productivity and distribution of the Mediterranean vegetation (Larcher, 2000). Indeed, many studies have described reductions in photochemical efficiency and low photosynthetic rates during summer drought (e.g. Harley *et al.,* 1987; Tenhunen *et al.*, 1990; Damesin & Rambal, 1995; Valladares & Pearcy, 1997; Gratani *et al.*, 2000, Llorens *et al.* 2003b). In addition, temperature during Mediterranean summer may reach potentially-damaging thresholds for physiological processes (Epron, 1997), especially when drought-induced stomatal closure limits the ability of plants to avoid heat stress by transpirational cooling (Ladjal *et al.*, 2000). A marked decline in maximal rate of photosynthesis at saturating irradiance and high internal CO_2 concentration has been observed at temperatures ranging from 35 to 40 ºC (Tenhunen *et al.*, 1984; Niinemets *et al.*, 1999; Gratani *et al.*, 2000). Maximum summer air temperatures of 35 to 40 ºC frequently occur in the Mediterranean maquis (Larcher, 2000). Therefore, the increase in the duration and severity of drought, together with the indirect effects of future warming on evapotranspiration and soil dryness, may constraint severely the physiological activity of Mediterranean plants. Moreover, higher temperatures may significantly enhance leaf heat stress in summer, limiting growth and survival of plants due to severe restrictions on photosynthesis.

Conversely, a future warming may alleviate the physiological constraints that Mediterranean plants experience in winter. Mediterranean winters may be relatively cold, and often associated with long rainless and cloudless periods. Indeed, low photosynthetic rates (reviewed by Larcher, 2000) and reductions of the efficiency of PSII have been reported during winter (García-Plazaola *et al.*, 1999; Karavatas & Manetas, 1999; Larcher, 2000; Oliveira & Peñuelas, 2001; Llorens *et al.* 2003b). Some authors have suggested that winter cold stress also plays a relevant role in the development and distribution of Mediterranean evergreen species (Mitrakos, 1980; Tretiach, 1993).

Since co-occurring Mediterranean species often have different climatic constraints, each species will likely respond differently to the climate change. *Erica multiflora* and *Globularia alypum* are two co-occurring Mediterranean shrubs that differ in their photosynthetic and water use strategies to cope with seasonal variance in water availability and temperature (Llorens *et al*., 2003b). Whereas *E. multiflora* is a more water conservative species, *G. alypum* follows a prodigal or non-conservative water use strategy. The aim of our study was to test how future warming and extended drought events could affect the physiological performance and the photosynthetic and water use strategies of these two co-occurring Mediterranean shrubs. Particularly, we tested whether future warmer and drier conditions could have a different effect on the photosynthetic performance and water use of these shrubs depending on the season. Our hypotheses were the following:

- Drought would reduce leaf gas exchange rates and might result in enhanced photoinhibition (i.e. reduced photochemical efficiency of PSII). In addition, the decrease in water availability would raise both instantaneous (A/E) and integrated water-use efficiency, the latter shown by increased values of δ^{13}C of leaves. Plants in the drought treatment might reduce their foliar N concentrations (Heckathorn *et al*. 1997) and might show more positive foliar δ^{15}N values (Peñuelas *et al.* 2000).

- The effect of the warming treatment could be different depending on the season. In summer, warming treatment might decrease leaf gas exchange rates and thus, increase photoinhibition due to leaf overheating. Conversely, in winter, warming might stimulate leaf gas exchange rates, decreasing the risk of suffering photoinhibition. Instantaneous and integrated water-use efficiency might be expected to decrease through an increase in evaporative demand and larger water losses through transpiration. However, if warming reduces soil humidity, then, the reduction of transpiration rates could lead to increases in water-use efficiency. The nutrient availability (and the $\delta^{15}N$ values) will depend on the balance between the warming positive effect on mineralization and the negative effect of reduced soil moisture.

Materials and methods

Study site and species description

The study was carried out in a dry shrubland (*Rosmarino-Ericion*) in the Garraf Natural Park, Barcelona, NE Spain (41º18'N, 1º49'E), at 210 m above sea level and on a SSE slope (13º). The climate is typically Mediterranean, with mild temperatures and few but torrential rains during spring and autumn, cool winters and hot and dry summers. The site, which is located on terraces from abandoned vineyards, suffered large fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixerept (SSS, 1998), thin (12-37 cm), with a loamy texture and abundant calcareous nodules. Currently the regenerating vegetation covers 50-60 % with a maximum height of 70 cm.

Erica multiflora L. (Ericaceae) and *Globularia alypum* L. (Globulariaceae) are evergreen, sclerophyllous shrubs that typically occur in basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland. Both species resprout from underground organs after aboveground biomass removal. Vegetative growth occurs twice a year: in spring (from March to June) and autumn (from September to November). Flowering starts in August-September.

Experimental system

Two types of climatic manipulations were performed using automatically sliding covers (Beier *et al.*, 2003):

- Extended summer drought was induced by covering the plots with transparent and waterproof plastic curtains during all rain events over the two annual growing seasons, starting in March-April in spring and in September-October in autumn.

- Passive nighttime warming was achieved by covering the vegetation and soil during night with curtains made of a material refractory to the infrared radiation. Curtains drew over the vegetation at sunset and they were removed at sunrise (below and above 200 lux respectively). Thus, they retained a portion of the energy accumulated in the ecosystem during the light period, simulating the mechanism of global warming. Warming treatment started on March 16th 1999 and it was working all nights throughout the study, except during two periods (1-30 August 1999 and 1-26 January 2000), when the treatment was stopped due to mechanical problems or for calibration of system effects.

Covers were mounted on metal scaffolding 0.2 m above the vegetation (*ca* 0.8-1 m above the ground). Nine plots of 20 m^2 (4m x 5m) were established: 3 untreated controls, 3 warming and 3 drought plots. Plots were organized in three blocks (each block with one control, one drought and one warming plot). Control plots had similar scaffolding than warming and drought plots, but with no cover. The plots were open at the sides to allow free wind movement. Movement of the covers was controlled automatically by climatic conditions according to preset criteria (Beier et al. 2003). The automatic control of the covers minimized unintended side effects on the light regime, hydrology and wind (Beier et al. 2003). For instance, at night, warming covers were automatically removed when it rained in order to avoid influencing hydrological cycle. We assigned the outer 0.5 m of each study plot as a buffer zone with all measurements carried out in a central 12 m^2 area.

Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured on 3 fixed sampling points per plot, every 1-2 weeks through the study period, using Time Domain Reflectometry (TDR).

Air and soil temperatures (0, 2 and 10 cm depth) during the sampling period were obtained by means of temperature sensors RTD Pt100 1/3 DIN (Desin Instruments, Barcelona) located in the three plots (control, drought and warming) of one block. Temperatures were measured every 10 minutes, being recorded the average of three measurements of each sensor.

Shoot water potential

Water potentials of apical shoots were measured seasonally with an Scholander-type pressure bomb (PMS Instruments, Corvallis, OR, USA). Each sampling date, one shoot of *E. multiflora* and *G. alypum* per plot was measured at predawn (0230-0430 h in spring and summer and 0430-0630 h in autumn and winter, solar time) and midday (1100-1300 h, solar time).

Leaf gas exchange rates and chlorophyll (Chl) fluorescence

We measured leaf gas exchange rates and Chl fluorescence during 4-6 consecutive days in spring (May), summer (August), autumn (November) and winter (February) throughout two years (spring 1999 - winter 2001). Pre-treatment measurements (February 1999) were conducted in order to identify the variability between blocks, but they are not used in the statistical analyses. Measurements were taken on sun-exposed shoots grown in the spring of the current year.

Each season, leaf gas exchange rates were measured on 3-4 plants per plot (one shoot per plant) of *E. multiflora* and *G. alypum* in the morning (from 30 minutes after sunrise to 1130 h, solar time, at the latest) and in the afternoon (from midday to 1630 h, solar time, at the latest). Stomatal conductances (g_s), net photosynthetic rates (A) and transpiration rates (E) were measured with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England), which also measured air temperatures in the leaf chamber (PLC4B, ADC Inc., Hoddesdon, Hertfordshire, England) and incident photosynthetic photon flux density (PPFD) at the moment of the leaf gas exchange measurements. Branch tips with several leaves were introduced into the chamber of the system. All results are expressed on leaf area basis, which was measured using ImagePC (v. α9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Water-use efficiency (WUE), defined as mmol of net CO_2 uptake per mol of H_2O transpired, was calculated by dividing instantaneous values of A by E.

As complementary measurements, response curves of leaf net photosynthetic rates (A) to PPFD and to CO_2 were conducted with a field-portable infrared gas analysis system (model LI-6400; standard chamber 2 x 3 cm with 6400-02B light source and 9964-026 CO_2 source, Li-Cor Inc.) during five consecutive days in November 2001. Light response curves were carried out across the PPFD range 0-2000 μmol m^{-2} s^{-1} at 350 ppm of CO_2. CO_2 response curves were constructed across the CO_2 range 50-800 μmol mol^{-1} under saturating PPFD (1500 μmol m^{-2} s^{-1}). Leaf temperatures were fixed at 20 ºC. Measurements were performed in one plant of each species per plot (i.e. three plants per species and treatment).

Components of Chl fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). After a dark adaptation period of at least 30 min, we obtained minimum and maximum dark-adapted fluorescence (F_0, Fm) and Fv/Fm, where Fv=Fm-F_0. Fv/Fm has been used as a measure of the potential (or maximum) photochemical efficiency of PSII. Measurements were performed on 3-5 plants of *E. multiflora* and *G. alypum* per plot, at predawn and midday.

The actual photochemical efficiency of PSII in the light-adapted state was estimated as: Φ_{PSII} = ΔF/Fm' = (Fm'-F)/Fm', where F is the steady-state fluorescence yield under the given environmental conditions, and Fm' is the maximum level of fluorescence obtained during a saturating flash of light (when all the PSII traps are closed) under the same environmental conditions. From this index, we calculated the apparent electron transport rate (ETR) as:

ETR = ΔF/Fm' x PPFD x 0.84 x 0.5

where PPFD was the photosynthetic photon flux density incident on the leaf, 0.84 was the coefficient of absorption of the leaves, and 0.5 was the fraction of electron required to the absorption of one quanta, as two photosystems are involved (Krall & Edwards, 1992). We measured the ETR on 3-4 plants of *E. multiflora* and *G. alypum* per plot, at morning (0700-1000 h, solar time) and midday (1100-1400 h, solar time).

Isotope and elemental analyses

Leaf nitrogen (N) concentrations and foliar $\delta^{15}N$ and $\delta^{13}C$ were determined on current year leaves collected in August 1999 and 2000. We sampled 3 plants of *E. multiflora* and *G. alypum* per plot each year. All analyses were carried out in an elemental analyser EA1108 (Carlo Erba, Milano, Italy) coupled to a Delta C isotope ratio mass spectrometer with a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany). The calibrations were performed using interspersed international isotopic standards of carbon and nitrogen (IAEA, Vienna, Austria). The elemental analysis calibration was performed using atropine (ThermoQuest Italia) as standard.

Values are expressed relative to PDB standard for $\delta^{13}C$ and relative to atmospheric nitrogen for $\delta^{15}N$, according to the following equation:

$$\delta Z = (R_{sample}/R_{standard}-1) \times 1000$$

where Z = the heavy isotope of either nitrogen or carbon, and R = ratio of heavier to lighter isotope for the sample and standard ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). The accuracy of the measurements was ± 0.15 ‰ for $\delta^{13}C$ and ± 0.3‰ for $\delta^{15}N$.

Statistical analyses

Drought and warming effects were analysed always separately. To test the effect of treatments on soil moisture throughout the study period, we performed repeated-measures analysis of variance (ANOVAR) using monthly means per plot and with treatment as the independent factor. For each species and time of the day, we conducted analysis of variance (ANOVA) with the mean of ψ, g_s, A, E, Fv/Fm and ETR in each plot as dependent variables and with year (first: March 1999-February 2000 and second: March 2000-February 2001), season (spring, summer, autumn and winter) and treatment (control-drought or control-warming) as independent factors. We did not conduct ANOVAR because we randomly sampled plants in the different seasons. Significant differences between means, as well as significant interactions between factors, were identified taking $P \leq 0.05$ as the level of significance. When interactions were significant, we performed ANOVAs to analyse the effect of treatments within each year and/or season. In these analyses, since multiple tests were performed for the same variable, the significance level (P=0.05) was adjusted for the number of statistical tests using a sequential Bonferroni correction to prevent against group-wide type errors (Rice, 1989). To test the effect of treatments on the N concentration and the C and N isotope composition of the leaves, we performed ANOVAs for each species (using the mean values per plot), with treatment and year (1999, 2000) as independent factors.

To compare the photosynthetic response curves of *E. multiflora* and *G. alypum* to PPFD and CO_2 levels we used the comparison of fitted curves method (Potvin *et al.*, 1990). To linearize the curves we used the logarithmic transformation for PPFD values (x'=log (x+1)) and the root square transformation for CO_2 values ($x'=(x+0.5)^{1/2}$). We compared drought and warming curves with control curves separately for each species. In the PPFD-response curves, we determined also the dark respiration (Rd), as the mean of the values for the three plants per treatment recorded at PPFD=0. A-C_i curves were also plotted to determine the carboxylation efficiency for each treatment, which was estimated as the slope of each curve obtained by linear regression, since saturation was not achieved (Long & Hällgren, 1993).

Results

Environmental data

Rainfall in the first year of the study, from March 1999 to February 2000, was lower (370 mm) than in the second year, from March 2000 to February 2001 (511 mm) (Fig. 1). Drought treatment reduced significantly the soil moisture (% volume) throughout the study period a 23% on average in relation to control plots ($F_{1,4}$=14.1, *P*=0.02, Fig. 1), being the maximum reductions in autumn (up to 50% in October 1999). Warming treatment did not affect significantly the soil moisture throughout the study period, but it decreased it in February-March 2000 (Fig. 1). The monthly significant effects of treatments (after sequential Bonferroni correction) are depicted in Fig. 1.

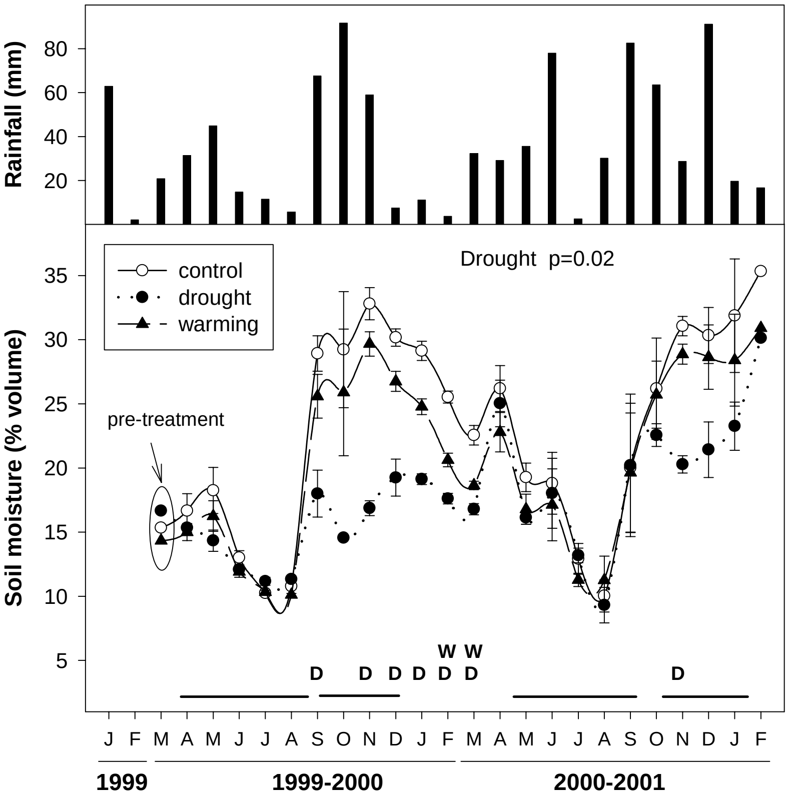

Fig. 1. Monthly rainfall (mm) and mean soil moisture (% volume/volume) of control, drought and warming treatments throughout the study period. Error bars indicate the standard error of the mean (*n*=2-6 days; values are averages of 3 plots). Lines above the months indicate the periods when drought treatment was working on. Global effects of treatments are depicted when significant ($P \leq 0.05$). D and W indicate significant differences (after sequential Bonferroni correction of the significance level *P*=0.05) between control and drought or control and warming treatments within each month.

Warming treatment increased minimum temperatures on average 0.7 ºC at air (20 cm above ground), 1.6 ºC at 2 cm depth and 1.15 ºC at 10 cm depth. The reduced heat loss in the warming plots at night increased also the diurnal temperatures in the air and soil compared to the control plots, showing a diurnal pattern with a maximum difference after sunrise and a minimum difference in the late afternoon (Fig. 2).

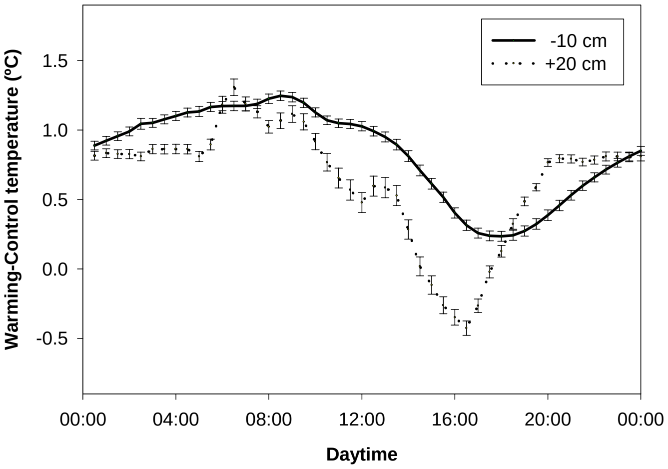

Fig. 2. Annual averages of air (20 cm above ground) and soil (10 cm depth) temperature (ºC) differences between warming and control plots (measured in one of the blocks from April 2000 to April 2001).

Shoot water potential (ψ)

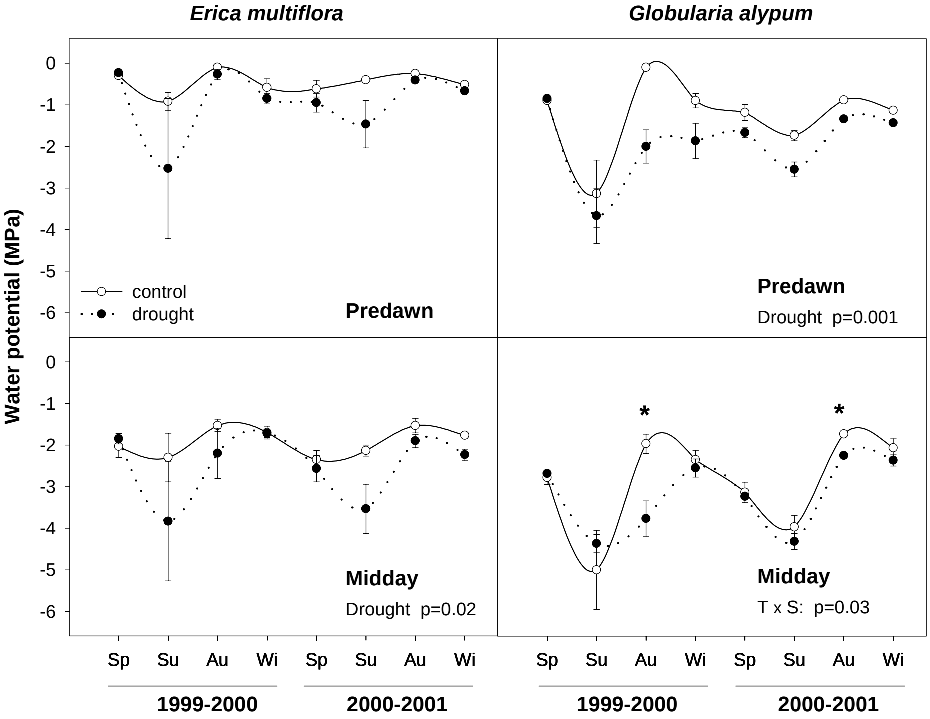

Fig. 3. Seasonal course of shoot water potential (MPa) of *E. multiflora* and *G. alypum* plants at predawn and midday for control and drought treatments. Error bars indicate the standard errors of the mean (*n*=3). Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as Sp=spring, Su=summer, Au=autumn, Wi=winter

Shoot water potential (ψ) values of *E. multiflora* plants in the drought treatment were lower than those of controls at midday ($F_{1,32}$=5.7, *P*=0.02; Fig. 3). Conversely, drought plants of *G. alypum* showed lower ψ values than controls through all the study period at predawn ($F_{1,28}$=13.0, *P*=0.001) and only in autumn at midday ($F_{1,8}$=22.2, *P*=0.002; Fig. 3). Warming did not produce any significant effect on shoot water potential (data not shown).

Leaf gas exchange rates

Drought plants of *E. multiflora* showed on average a decrease of 41% in their overall net photosynthetic rates (A) in the morning ($F_{1,32}$=12.2, *P*=0.001) and of 27% in the afternoon ($F_{1,32}$=5.4, *P*=0.03; Fig. 4). In contrast, drought treatment did not decrease significantly the overall A of *G. alypum* (Fig. 4).

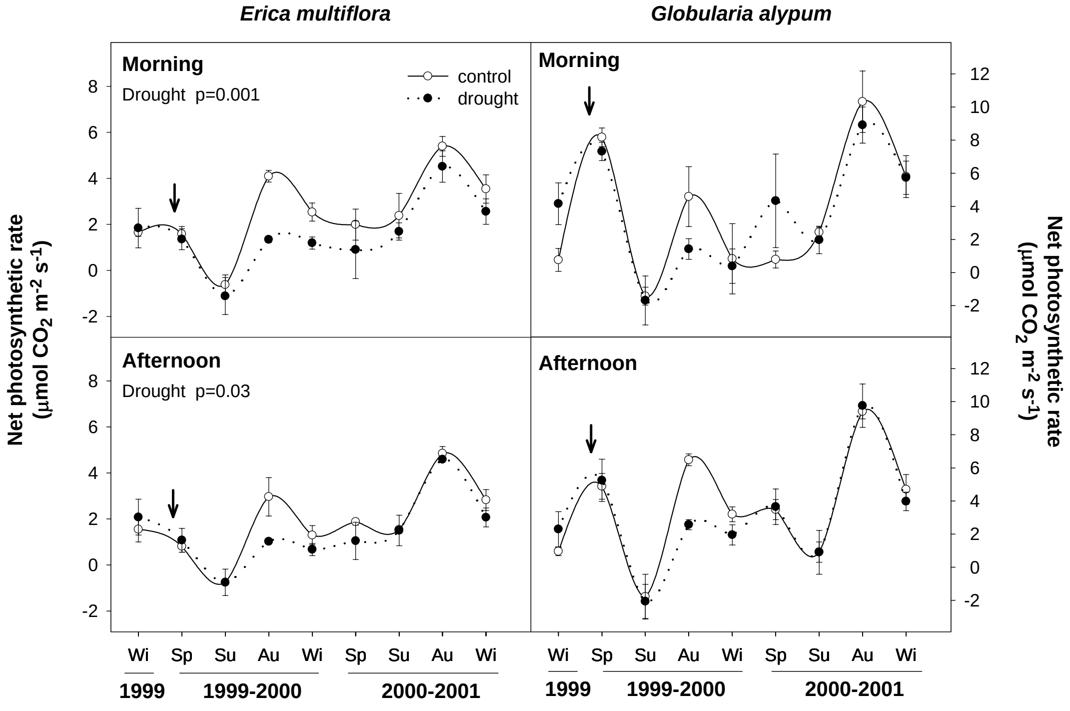

Fig. 4. Seasonal course of net photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) of *E. multiflora* and *G. alypum* plants, in the morning and in the afternoon, for control and drought treatments. Error bars indicate the standard errors of the mean (*n*=3). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as in Fig. 3.

There was also a global effect of drought treatment on leaf stomatal conductance of *E. multiflora* in the morning ($F_{1,32}$=15.5, P<0.001) and in the afternoon ($F_{1,32}$=5.7, P=0.02) and of *G. alypum* in the afternoon ($F_{1,32}$=6.3, P=0.02; Fig. 5). However, drought effect on stomatal conductance was not uniform throughout the study period since the interaction among treatment, year and season was always significant. Interactions were significant due to a stronger effect of drought treatment in autumn and spring (when the treatment was on).

Drought plants of *E. multiflora* and *G. alypum* showed a decrease of 24% in their overall leaf transpiration rates (E) in the morning ($F_{1,32}$=7.5, P=0.01 and $F_{1,32}$=8.2, P=0.007, respectively; Fig. 6). However, there was a significant interaction among treatment, year and season for *G. alypum* due to a stronger drought treatment in autumn 1999 (Fig. 1). In the afternoon, differences between control and drought plants were not significant.

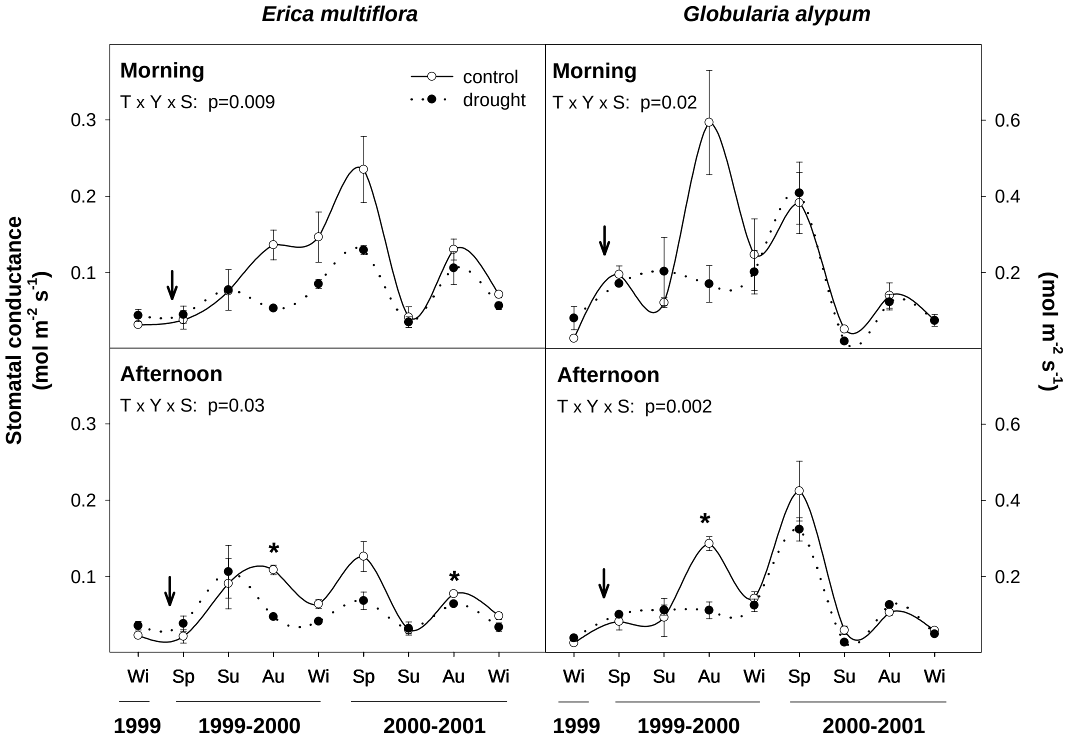

Fig. 5. Seasonal course of stomatal conductance (mol m^{-2} s^{-1}) of *E. multiflora* and *G. alypum* plants, in the morning and in the afternoon, for control and drought treatments. Error bars indicate the standard errors of the mean (n=3). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as in Fig. 3.

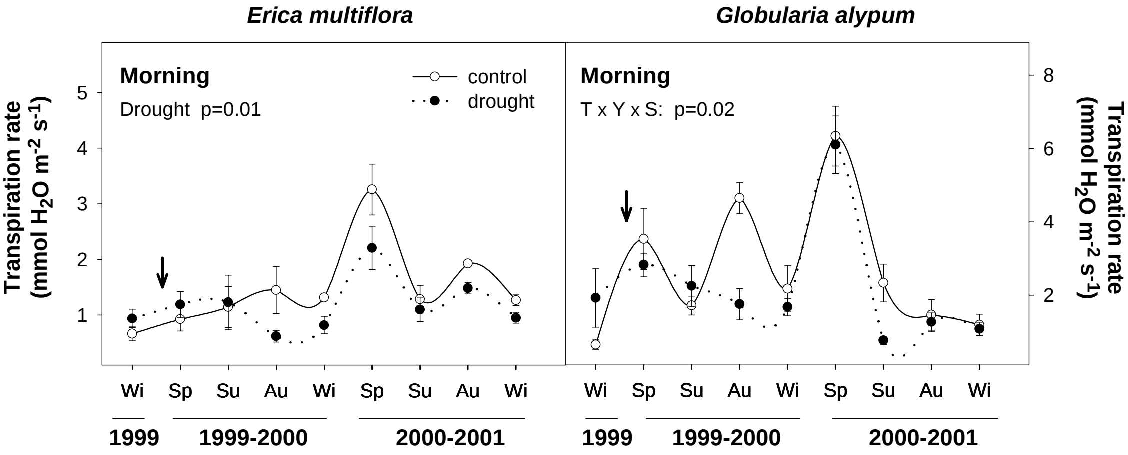

Fig. 6. Seasonal course of transpiration rates (mmol H_2O m^{-2} s^{-1}) of *E. multiflora* and *G. alypum* plants, in the morning, for control and drought treatments. Error bars indicate the standard errors of the mean (*n*=3). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as in Fig. 3.

Warming treatment did not affect significantly the leaf gas exchange rates of either *E. multiflora* or *G. alypum* plants (data not shown). Treatments neither affected significantly the instantaneous WUE (A/E) of plants (data not shown).

In autumn 2001, at leaf temperatures of 20ºC (± 0.05) and at 350 ± 0.05 ppm of CO_2, a photon flux density of 500 μmol m^{-2} s^{-1} was saturating, or very nearly so, for both study species (Fig. 7). Drought plants of *E. multiflora* showed significantly lower leaf net photosynthetic rates in response to PPFD than control plants ($F_{4,40}$=3.7, $P<0.05$), whereas drought did not affect the net photosynthetic rates of *G. alypum*. Warming did not affect either *E. multiflora* or *G. alypum* PPFD-response curves, in agreement with the two years data on leaf gas exchange rates. Dark respiration rates of *E. multiflora* plants (0.55 ± 0.2) were significantly lower (*P*=0.01) than those of *G. alypum* plants (1.96 ± 0.3). Whereas drought did not affect the dark respiration rates of *E. multiflora* plants, it slightly decreased the dark respiration rates of *G. alypum* plants (drought: 1.34 ± 0.03, control: 1.96 ± 0.3; *P*=0.08).

Drought plants of *E. multiflora* showed significantly lower net photosynthetic rates than control plants in response to CO_2 ($F_{4,34}$=24.6, $P<0.001$; Fig. 8). Conversely, warming plants of *G. alypum* showed significantly higher net photosynthetic rates than controls ($F_{4,34}$=10.7, $P<0.001$). A-C_i curves (data not shown) showed the same response to treatments than A-CO_2 curves, being the efficiency of carboxylation lower in drought plants of *E. multiflora* ($F_{1,38}$=10.2, *P*=0.003) and higher in warming plants of *G. alypum* ($F_{1,38}$=4.3, *P*=0.04) compared to controls.

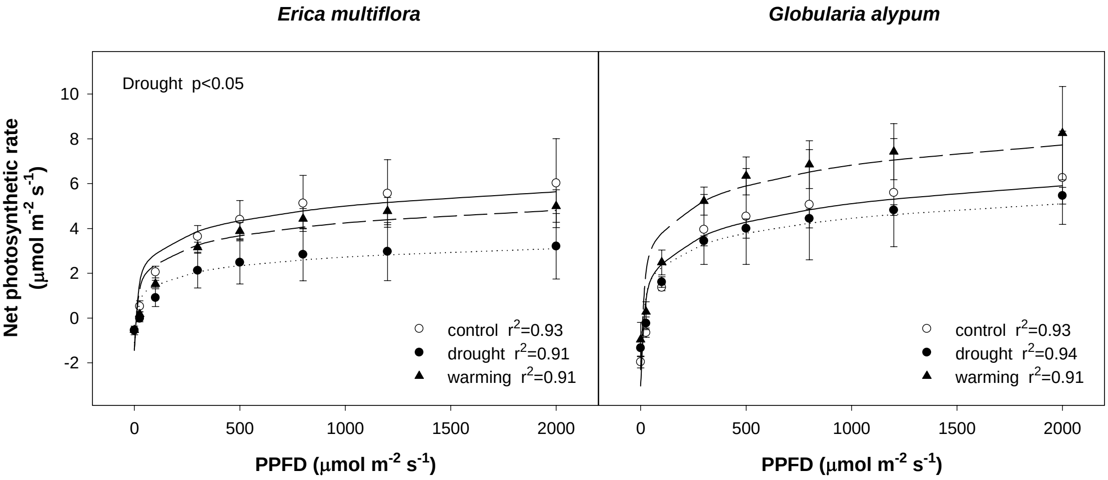

Fig. 7. Photosynthetic rate responses of *E. multiflora* and *G. alypum* shoots from control, drought and warming plots to different PPFD at ambient levels of CO_2 (3 plants per treatment). Differences in the light-response curves of drought and warming plants in relation to controls are depicted when significant (*P*≤0.05).

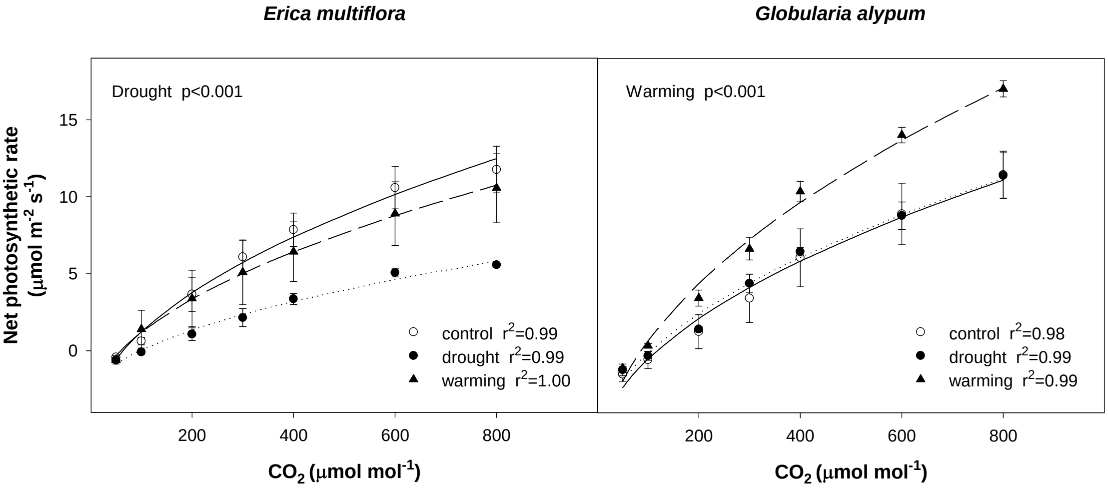

Fig. 8. Photosynthetic rate responses of *E. multiflora* and *G. alypum* shoots from control, drought and warming plots to different CO_2 concentrations at saturating PPFD (3 plants per treatment). Differences in the CO_2-response curves of drought and warming plants in relation to controls are depicted when significant (*P*≤0.05).

Chl fluorescence

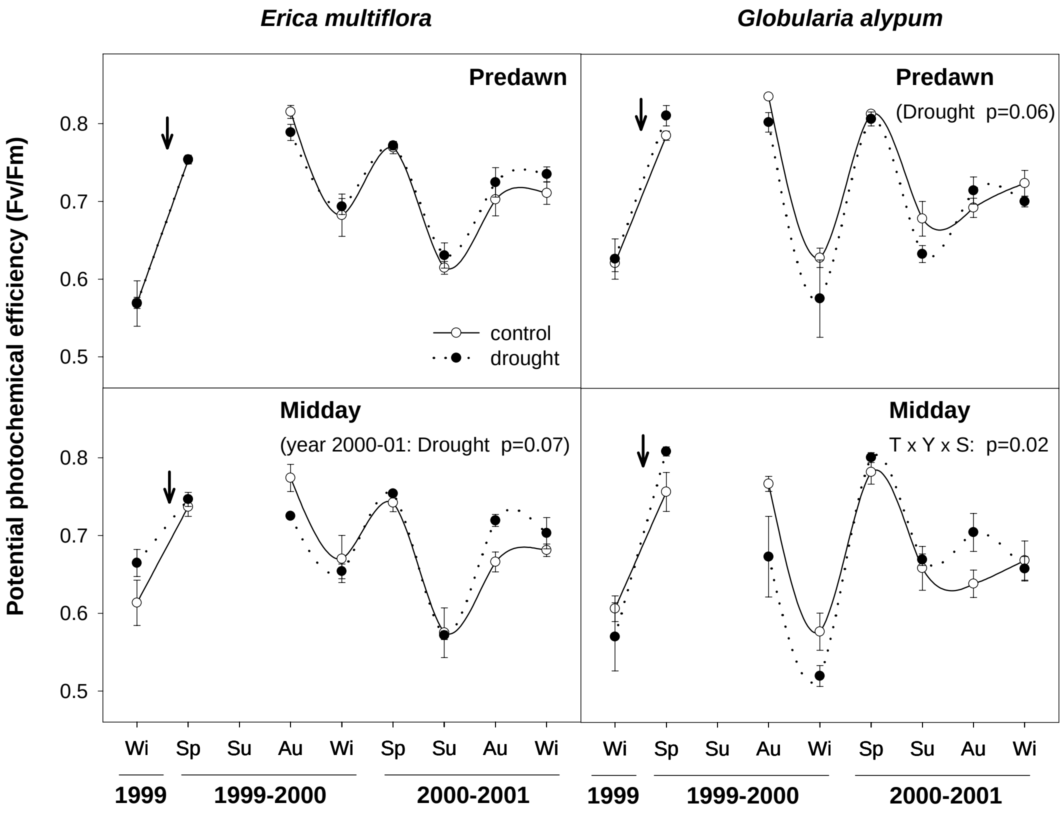

Fig. 9. Seasonal course of potential photochemical efficiency (Fv/Fm) of *E. multiflora* and *G. alypum* plants, at predawn and midday, for control and drought treatments. Error bars indicate the standard errors of the mean (*n*=3). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as in Fig. 3.

Drought plants of *E. multiflora* tended to show slightly higher Fv/Fm values than control plants in the second year at midday ($F_{1,16}$=3.7, P=0.07) (Fig. 9). Conversely, drought plants of *G. alypum* tended to present lower Fv/Fm values than control plants throughout the study period at predawn ($F_{1,28}$=3.7, P=0.06) (Fig. 9). Warming plants showed slightly higher Fv/Fm values than controls through all the study period at predawn in the case of *E. multiflora* ($F_{1,28}$=4.0, P=0.055) and at midday in the case of *G. alypum* ($F_{1,32}$=5.8, P=0.02) (Fig. 10).

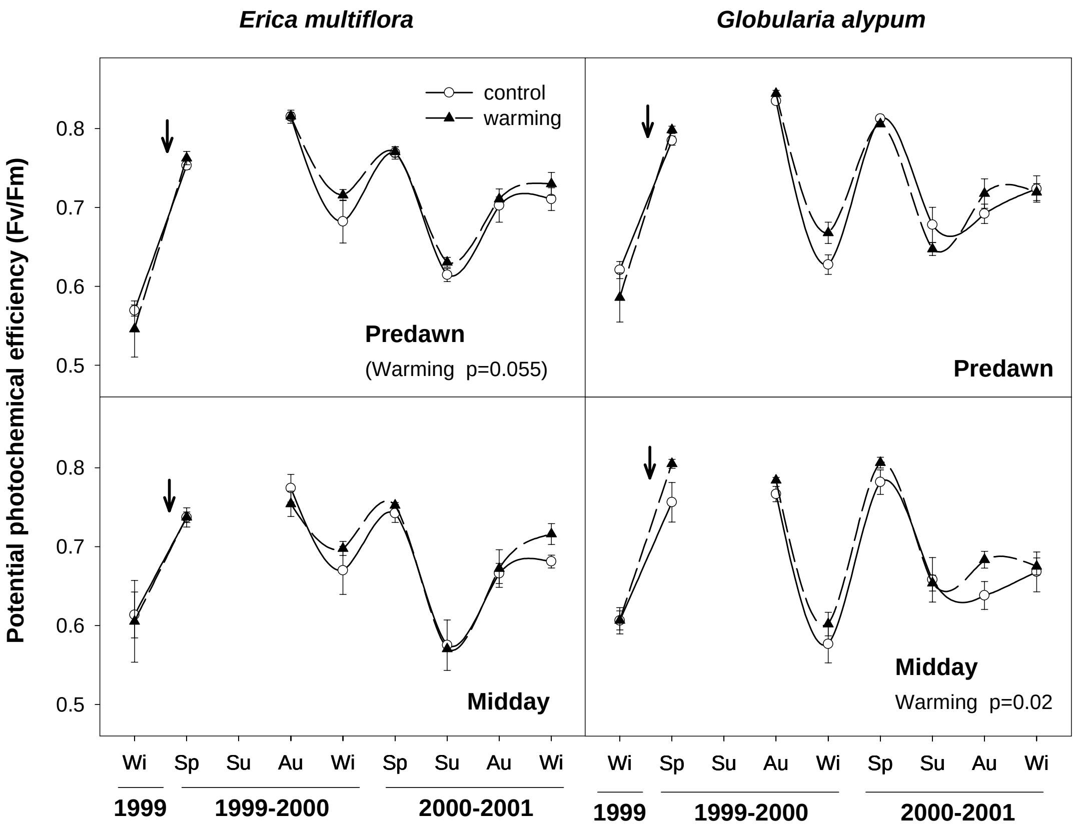

Fig. 10. Seasonal course of potential photochemical efficiency (Fv/Fm) of *E. multiflora* and *G. alypum* plants, at predawn and midday, for control and warming treatments. Error bars indicate the standard errors of the mean (*n*=3). Arrows indicate the start of the treatments. Significant interactions or treatment effects are depicted (T=treatment, Y=year, S=season). Seasons are abbreviated as in Fig. 3.

Drought treatment did not affect significantly the apparent electron transport rates (ETR) of *E. multiflora* (data not shown), whereas ETR of *G. alypum* plants in the drought treatment were lower (19%) than those of control plants only during the first year in the morning (drought: 100.8 ± 18.3 μmol m^{-2} s^{-1}, control: 124.4 ± 15.7 μmol m^{-2} s^{-1}; $F_{1,12}$=5.2, P=0.04) (data not shown). Warming did not have any significant effect on the ETR values of these two species. Moreover, treatments did not affect the ETR of *E. multiflora* and *G. alypum* plants (after sequential Bonferroni correction) within each season.

Leaf nitrogen (N) concentration

Leaf N concentration of drought plants was lower for *E. multiflora* and higher for *G. alypum* compared to controls, although differences were only marginally significant in both cases ($F_{1,8}$=4.5, P=0.07 and $F_{1,8}$=5.0, P=0.06, respectively) (Table 1). Warming did not affect the leaf nitrogen concentration either of *E. multiflora* or *G. alypum* plants (Table 1).

Table 1. Nitrogen concentration (% dry mass), δ^{13}C and δ^{15}N of *E. multiflora* and *G. alypum* leaves from control, drought and warming treatments at the end of summer in 1999 and 2000. Values are means ± se (n=3). Leaf nitrogen concentration of drought plants was slightly lower for *E. multiflora* ($F_{1,8}$=4.5, P=0.07) and slightly higher for *G. alypum* ($F_{1,8}$=5.0, P=0.06) compared to controls (significant differences with controls are highlited in bold). Treatments did not affect significantly leaf stable isotopes values.

		E. multiflora		***G. alypum***	
		1999	**2000**	**1999**	**2000**
N (%)					
	Control	0.68 ± 0.02	0.73 ± 0.03	0.76 ± 0.03	0.82 ± 0.06
	Drought	**0.59 ± 0.03**	**0.66 ± 0.06**	**0.85 ± 0.08**	**1.02 ± 0.09**
	Warming	0.63 ± 0.03	0.69 ± 0.04	0.80 ± 0.05	0.84 ± 0.03
δ^{13}C					
	Control	-24.9 ± 0.6	-25.5 ± 0.5	-27.6 ± 0.7	-28.1 ± 0.6
	Drought	-25.7 ± 0.6	-25.8 ± 0.4	-27.0 ± 0.9	-26.9 ± 0.4
	Warming	-25.7 ± 0.3	-25.8 ± 0.4	-27.6 ± 0.7	-28.0 ± 0.1
δ^{15}N					
	Control	-5.48 ± 0.6	-5.11 ± 0.8	-5.24 ± 0.8	-5.06 ± 0.5
	Drought	-5.48 ± 0.6	-4.57 ± 1.0	-4.33 ± 0.5	-4.40 ± 0.5
	Warming	-5.56 ± 0.7	-4.96 ± 0.6	-4.73 ± 0.7	-5.31 ± 0.4

Leaf stable isotopes

Neither drought nor warming treatment affected significantly the leaf δ^{13}C or δ^{15}N values of *E. multiflora* and *G. alypum* plants (Table 1).

Discussion

Drought treatment

Erica multiflora and *Globularia alypum* will experience lower shoot water potentials (ψ) if a longer and stronger summer drought occurs (Fig. 3). Lower values of ψ would produce a stronger gradient of water potential between the soil and the plant, thus helping to maintain the flow of water into the plant (Dunn *et al.*, 1976). However, decreases in plant water potential increase the risk of cavitation (Tyree & Sperry, 1989), which can be responsible for the death of leaves and twigs, especially during summer (Correia & Catarino, 1994). Stronger effects of drought on shoot water potentials of *G. alypum* compared to *E. multiflora* coincide with the more drought-avoiding strategy found in the latter (Llorens *et al*., 2003b).

Also in agreement with the more drought-avoiding strategy of *E. multiflora* (Llorens *et al*., 2003b), this species was more sensitive to soil drying than *G. alypum*, experiencing higher reductions in their leaf gas exchange rates to avoid dehydration (Figs. 4-6). Whereas drought treatment decreased significantly the net photosynthetic rates of *E. multiflora*, there was not a global effect of drought treatment on net photosynthetic rates of *G. alypum*. Despite the significant reduction in their photosynthetic rates, drought treatment did not change significantly the electron transport rates of *E. multiflora* plants. Higher photorespiration rates in drought plants of *E. multiflora* compared to controls were probably one of the main drivers accounting for the maintenance of its electron flow, since, in C_3 plants, PSII activity is mainly partitioned between photosynthesis and photorespiration (Krall & Edwards, 1992). Upregulation of Mehler reaction may also help to dissipate excess excitation energy in water-stressed plants, protecting the photosystem from photodamage (Krall and Edwards 1992, Osmond and Grace 1995, Asada 1999). In accordance with the results obtained throughout the two years of the study, drought plants of *E. multiflora* showed also significantly lower A than control plants in response to PPFD and CO_2 in autumn 2001 (Figs. 7, 8).

A low rate of net photosynthesis in plants suffering from water stress has been often reported, usually as a consequence of stomatal closure (reviews in Chaves, 1991; Yordanov *et al.*, 2000). However, the lower carboxylation efficiency (CE), i.e. lower Rubisco activity, that we found in the drought plants of *E. multiflora* compared to controls suggest that, in addition to a stomatal limitation, there was a non-stomatal limitation of photosynthesis. The decreases in Rubisco activity and net photosynthetic rates of *E. multiflora* plants as a response to drought were in agreement with the lower foliar N concentrations that we found in these plants compared to controls, although differences were only marginally significant (Table 1).

Decreases of foliar nitrogen concentration by drought have been attributed to three processes: (1) drought-induced retranslocation of shoot N to roots and rhizomes; (2) volatilization of foliar N; and (3) drought-related dilution of shoot N resulting from a greater impact of drought on soil N uptake than on growth (Heckathorn *et al.*, 1997). Since we observed lower initial decomposition rates in the drought plots compared to controls (Emmett *et al.,* 2003), decreases in the soil N availability and, thus, in the soil N uptake by drought plants of *E. multiflora* could explain their slightly lower foliar N concentrations. However, drought plants of *G. alypum* tended to have higher leaf nitrogen concentrations than control plants. Such unexpected result could be explained by a decrease in plant growth, which concentrated the nitrogen into less dry matter. Indeed, drought treatment decreased significantly the growth of *G. alypum* in relation to controls in spring 2000 (Peñuelas *et al.*, 2003). Additionally, drought plants of *G. alypum* could have developed more and/or deeper roots. Greater biomass allocation to root *vs* shoot have been correlated with higher shoot N concentration (%) and δ^{15}N values, suggesting that a larger root allocation allow plants to exploit more efficiently soil systems and to use more nitrogen from nitrogen-saturated soil sites (Lloret *et al.*, 1999). Accordingly, drought plants of *G. alypum* tended to have more positive leaf δ^{15}N values than controls (Table 1).

Drought treatment did not either affect the instantaneous (A/E) or the integrated (deduced from δ^{13}C values) water-use efficiency of *E. multiflora* or *G. alypum* plants, which indicates that leaf net photosynthesis and stomatal conductance (or leaf transpiration) changed proportionately. Although in many experimental and field studies drought has been shown to increase the instantaneous and/or the integrated water-use efficiency of woody plants (e.g. Ehleringer & Cooper, 1988; Morecroft & Woodward, 1990; Meinzer *et al.*, 1992), proportional decreases of leaf net photosynthesis and stomatal conductance (or leaf transpiration) have been also frequently reported (e.g. Schulze & Hall, 1982; Epron & Dreyer, 1993; Moriana *et al.*, 2002; Llorens *et al.*, 2003a).

An increase in drought has been related in many cases to a drop in potential photochemical efficiency of PSII (Fv/Fm), i.e. to an increase in photoinhibition (e.g. Björkman & Powles, 1984; Jagtap *et al.*, 1998). As far as photoinhibition is reversible within minutes to hours, it can be viewed as a protective mechanism that serves to dissipate excessive energy (Krause 1988; Osmond 1994). In our study, drought plants of *G. alypum* showed slightly lower Fv/Fm values than control plants throughout the study period at predawn (Fig. 9), but not at midday. Predawn depressions in PSII efficiency have been related to the retention of de-epoxidised components of the xantophyll cycle (zeaxanthin and antheraxanthin) overnight (Adams & Demmig-Adams, 1994; García-Plazaola *et al.*, 1999; Verhoeven *et al.*, 1999; Kyparissis *et al.*, 2000). The retention of zeaxanthin and antheraxanthin overnight would allow that energy dissipation could take place at a fairly high level upon exposure to direct sunlight after sunrise, when temperatures are typically the coldest and the enzymatic conversion of violaxanthin to antheraxanthin and zeaxanthin would be largely inhibited (Adams & Demmig-Adams, 1994). The retention of zeaxanthin and antheraxanthin overnight would be very sensitive to temperature, permitting reconversion of zeaxanthin to antheraxanthin and violaxanthin on warmer days when photosynthesis could presumably proceed at higher rates (Adams & Demmig-Adams, 1994). Sustained reductions of Fv/Fm values may also result from accumulation of non-functional PSII reaction centres and partial photoinactivation of PSII (Niyogi, 1999). However, the lack of differences in PSII efficiency between control and drought plants of *G. alypum* at midday suggest that the predawn depression in the Fv/Fm values in drought plants was related to energy dissipation activity rather than to damage to the photosystems.

A number of studies have reported a remarkable resistance of the photosynthetic apparatus to dehydration (e.g. Genty *et al.*, 1987; Gamon & Pearcy, 1990; Havaux, 1992; Epron, 1997) and several authors have demonstrated that water deficit may enhance the resistance of PSII photochemistry to superimposed constraints, such as high-temperature stress combined or not with photoinhibitory light (e.g. Havaux, 1992; Epron, 1997; Valladares & Pearcy, 1997; Ladjal *et al.*, 2000; Llorens et al. 2003a). Our results on *E. multiflora* support the latter two ideas, since drought did not affect or slightly enhanced (during the second year at midday) the Fv/Fm values of *E. multiflora* leaves (Fig. 9).

Warming treatment

The slight warming treatment (*ca* 1ºC in the night and in diurnal hours until midday) did not affect either the shoot water potential, nor the leaf gas exchange rates or the electron transport rates throughout the study period in any of the two studied species. Previous studies on the effects of warming on leaf gas exchange rates have reported contrasting results. Some authors did not find any effect of warming manipulations on leaf gas exchange rates (e.g. Wookey *et al.*, 1994; Nijs *et al.*, 1996; Loik *et al.*, 2000; Starr *et al.*, 2000), other authors found stimulated rates (e.g. Chapin & Shaver, 1996, Apple *et al.*, 2000), whereas others found decreases (Callaway *et al.*, 1994). Most of these studies have been performed in temperate and cold climates, where constraints of the physiological activity of plants are different than in the Mediterranean region. Moreover, comparisons among studies are difficult given the different characteristics and magnitude of warming treatments and the different sensitivity to temperature and optima for photosynthesis of each species.

In accordance with the lack of effects of warming treatment on leaf gas exchange rates, warming did not affect significantly either the instantaneous WUE or the leaf $\delta^{13}C$, i.e. the integrated WUE, of *E. multiflora* and *G. alypum* plants (Table 1). Warming neither affected the leaf $\delta^{15}N$ or the leaf N concentration (Table 1), which also agrees with the absence of warming effects on decomposition and mineralization rates in the soil (Emmett *et al.*, 2003).

In our study, warming plants of *E. multiflora* and *G. alypum* showed higher overall potential photochemical efficiency values (Fv/Fm) than control plants at predawn and midday, respectively (Fig. 10). Differences were especially evident in winter, and also in autumn for *G. alypum* plants, suggesting that episodic freezing or frosting events during these seasons might affect severely the photosynthetic performance of these two Mediterranean shrubs. Accordingly, our warming treatment reduced the number of days with frost ($T_{min} < 0$ ºC) by 50% for the period March 2000-2001 (22 days with frost in controls compared to 11 days in warming plots). Previous studies reported that low temperatures during winter constrain photosynthetic performance of some Mediterranean evergreen sclerophylls (e.g. García-Plazaola *et al.*, 1999; Karavatas & Manetas, 1999; Larcher, 2000; Oliveira & Peñuelas, 2001).

In spite of the absence of warming effects on A throughout the study period, plants of *G. alypum* in the warming plots had higher photosynthetic rates than control plants in response to PPFD and CO_2 in autumn 2001, although differences were significant only for the CO_2-response curves (Figs. 7, 8). During this season, *G. alypum* plants showed also higher CE, i.e. higher Rubisco activity, than control plants (data not shown, but see Fig. 8). Taking into account that warming did not alter the leaf N concentration, this result could be related with the higher photosynthetic performance that we found in the warming plants, mainly during the colder seasons.

The general absence of direct and indirect effects of the warming treatment on the studied instantaneous ecophysiological variables is probably due to the low increase of temperature achieved, especially during the diurnal hours (Fig. 2). However, the lack of effects of the warming treatment on instantaneous variables does not preclude an effect of warming on integrative variables, such as growth and flowering (Peñuelas et al. 2003).

Final remarks

Our results suggest that future drier conditions may decrease the annual productivity of these Mediterranean shrubs, although the magnitude of such decreases will be species-specific. However, leaf gas exchange responses (stimulation of photosynthesis, increase in water-use efficiency, decrease of transpiration) to the predicted rise in atmospheric CO_2 concentration (Körner 2000) might compensate the effects of drier conditions. On the other hand, warmer conditions in the immediate decades could alleviate the low temperature constraints on the photosynthetic performance of these Mediterranean species during the colder seasons, possibly increasing the length of the growth period. During summer, temperature increases of *ca* 1ºC (at night and in the morning) will not alter significantly the already depressed photosynthetic activity and performance of such Mediterranean shrubs.

Ultimately, drier and warmer conditions expected for the near future may change the competitive relationship among species of Mediterranean shrublands. Results of our study provide an example, since the two studied species, *E. multiflora* and *G. alypum,* showed different ecophysiological responses to our experimental drought and warming.

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CHAPTER 3

Contrasting growth changes in two dominant species of a Mediterranean shrubland submitted to experimental warming and drought

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Abstract

To understand the impact of climate change on growth of Mediterranean shrub species, we performed a field experiment from March 1999 to March 2002 using a new approach to prolong the drought period and to increase the night-time temperature. We studied whether drier and warmer conditions affect the annual or seasonal growth patterns of two common shrubs, *Erica multiflora* and *Globularia alypum*, which often co-occur in the Mediterranean shrublands. We measured annual growth in stem diameter and length of both species and we used these measurements to estimate annual stem biomass production for 1999, 2000 and 2001. We assessed plant seasonal (spring and autumn) growth measuring stem elongation in *E. multiflora* and the number and length of all new shoots of tagged stems in *G. alypum*. We also monitored the spring shoot elongation rates and annual leaf fall of both species in 2000. Control plants of *E. multiflora* showed significantly lower stem growth than *G. alypum* plants overall the study period. Stem growth of both species was higher in spring than in autumn. On average, drought treatment reduced 22% soil moisture, whereas warming increased temperature 0.7-1.6ºC. Drought treatment decreased 46% the annual stem elongation of *E. multiflora*, with the main effect being in spring. The decrease in water availability reduced 31% the annual stem diameter increment and 43% the annual stem elongation of *G. alypum* plants. New shoot growth of *G. alypum* was also strongly reduced. Allometrically estimated biomass production was decreased by drought in both species. Warming treatment produced contrasting effects on the growth patterns of *E. multiflora* and *G. alypum*. Warmer conditions increased on average 35% the stem basal diameter growth of *E. multiflora* plants, raising also their estimated biomass production. On the contrary, plants of *G. alypum* in the warming treatment showed a 14% lower annual stem growth in basal diameter and shorter shoots in spring compared to controls. Treatments did not affect the relationship between stem length and diameter, which suggest that there were no significant changes in stem biomass allocation between leaves and woody tissues. Drier and warmer conditions in the near future may lead to an increase in the competitive ability of *E. multiflora* compared to *G. alypum*.

Introduction

Climate projections predict drier and warmer conditions and more frequent and severe droughts in the Mediterranean basin in the next decades (IPCC 2001). Responses to such climatic changes may differ among species and cannot be readily generalized, since contrasting effects of simulated climate changes on the ecophysiology of co-occurring species have often been reported (Chapin and Shaver 1985, Chapin et al. 1995, Day et al. 1999). Hence, it is essential to improve our knowledge on the response of the different Mediterranean species to drier and warmer conditions to be able to predict the impact of climate change on Mediterranean ecosystems. Particularly, a proper understanding of how decreases in water availability and increases in temperature might affect the growth patterns of Mediterranean species will allow us to develop more reliable climate change scenarios of ecosystem functioning.

Water is the resource most often limiting plant growth in the Mediterranean-climate ecosystems. Indeed, a great influence of water availability on plant growth has been observed in Mediterranean regions (Mayor and Rodà 1994, Borghetti et al. 1998, Caritat et al. 2000, Costa et al. 2001, Royce and Barbour 2001, Cinnirella et al. 2002, Ogaya et al. 2003). Water scarcity may affect plant growth through direct effects on photosynthetic rates, mostly as a consequence of stomatal closure (reviews in Chaves 1991, Yordanov et al. 2000). We might also expect indirect effects of water scarcity on photosynthetic rates, because nutrient supply to the roots is under soil water control (Chapin 1980). In addition, water stress may result in accelerated leaf senescence, and in consequence, after a period of water stress, even if gas exchange rates are re-established, whole-plant growth may be reduced due to a decrease in the photosynthetic area per unit of dry weight (leaf area ratio, LAR) (Pereira 1995).

Temperature may influence plant growth through direct effects on metabolic rates, including photosynthesis, respiration, nutrient and water uptake, and resource utilization. Temperature is a key modifier of ontogenetic development rate (Morison and Lawlor 1999). Indeed, it has been found that warmer conditions accelerate organ initiation and expansion rates and shorten duration of organ growth and longevity (Yoshie and Fukuda 1994, Chapin and Shaver 1996, Morison and Lawlor 1999). Given the importance of temperature to the phenology of plants, climatic warming is likely to affect the timing of the onset and cessation of growth (Kramer 1995). In fact, the study of phenological time series has showed a lengthening of the growing season in North

America and Europe over the last century (Ahas 1999, Menzel and Fabian 1999, Menzel 2000, Schwartz and Reiter 2000, Peñuelas and Filella 2001), and particularly in the Mediterranean basin (Peñuelas et al. 2002), since spring events have advanced, whereas autumn events have been delayed (Menzel 2000, Peñuelas et al. 2002). In addition, indirect effects of temperature on decomposition of soil organic matter, mineralization of soil nutrients and soil moisture might also affect plant growth (Chapin et al. 1995, Jarvis and Linder 2000, Kirschbaum 2000). Because evapotranspiration rates are positively related to temperature, increased temperatures are likely to be associated with increased rates of water loss (Kirschbaum 2000, Lloyd and Fastie 2002). If temperature warms without a compensating increase in precipitation, plants may become increasingly water-stressed. Rising temperatures might thus lead to decreases in growth rate in water-limited ecosystems (Kirschbaum 2000, Lloyd and Fastie 2002).

Different responses of co-occurring species to climate change may alter their competitive interactions and lead to changes in the species composition of ecosystems. In the present study, we deal with the effect of a decrease in water availability (drought treatment) and an increase in temperature (warming treatment) on the annual and seasonal growth of two co-occurring shrubs, *Erica multiflora* and *Globularia alypum*, which are common species of the Mediterranean shrublands. In a previous study, we found that drought treatment reduced significantly the overall leaf net photosynthetic rates of *E. multiflora* plants compared to controls, but not of *G. alypum* (Llorens et al. unpublished). Warming treatment did not affect significantly the leaf net photosynthetic rates of these two species (Llorens et al. unpublished). However, measurements of instantaneous plant performance, such as leaf gas exchange rates, do not necessarily reflect the long-term effect of environmental changes. It has been established that processes that are integrated at annual time steps (e.g. growth) provide insight into longer term responses of vegetation to environmental changes and thus, are more useful than instantaneous physiological measurements in predicting decadal vegetation changes (Chapin and Shaver 1996). Hence, in the present study we conducted growth measurements to assess whether drier and warmer conditions (1) could modify the annual or seasonal growth patterns of the two studied species, and (2) could affect differently each species, which might alter the competitive balance between these two common Mediterranean species. To address these issues, we have performed a field experiment using a nonintrusive technique to experimentally dry and warm a

Mediterranean shrubland. From March 1999, we have extended the drought period each year by excluding rain over the two growing seasons (spring and autumn). We have also created passive warming by avoiding IR dissipation at night over the whole year, which simulates the way climate change alters the heat balance of ecosystems (Beier et al. 2003).

Materials and methods

Study site and species description

The study was carried out in a dry shrubland (*Rosmarino-Ericion*) at the Garraf Natural Park, Barcelona, NE Spain (41º18'N, 1º49'E), at 210 m above sea level and on a SSE slope (13º). The climate is typically Mediterranean. The site, which is located on terraces from abandoned vineyard's fields, suffered big fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixerept (SSS 1998), thin (12-37 cm), with a loamy texture and abundant calcareous nodules. Currently the regenerating vegetation covers 50-60% with a maximum height of 70 cm. The dominant species are *Erica multiflora*, *Globularia alypum, Dorycnium pentaphyllum, Rosmarinus officinalis, Fumana ericoides, Ulex parviflorus, Helianthemum syriacum, Brachypodium phoenicoides* and *Ampelodesmus mauritanica.*

Erica multiflora L. (Ericaceae) and *Globularia alypum* L. (Globulariaceae) are evergreen, sclerophyllous shrubs that typically occur in basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland. Both species resprout from underground organs after aboveground biomass removal. Vegetative growth occurs twice a year: in spring (from March to June) and autumn (from September to November). Flowering starts in August-September.

Experimental system

Two types of climatic manipulations were performed using automatically sliding curtains (*ca* 20 cm above vegetation maximum height) (Beier et al. 2003):

- Extended summer drought was induced by covering the plots with transparent and waterproof plastic curtains during all rain events over the two growing seasons, from March-April to July-August and from September-October to December-January.

- Passive night-time warming was achieved by covering the vegetation and soil during night with curtains made of a material refractory to the infrared radiation. The curtains retained a portion of the energy accumulated in the ecosystem during the light period, simulating the mechanism of global warming due to the accumulation of greenhouse gases. The warming treatment started on March 16th 1999 and was on over all the study, except during three periods (August 1999, January 2000 and September-October 2001), when the treatment was stopped for calibration of system effects or due to mechanical problems. The curtains were automatically removed during rain night events in order to avoid influencing the hydrological cycle.

Nine plots of 20 m^2 (4m x 5m) were established in the study site: 3 untreated controls, 3 warming and 3 drought plots. Plots were organised in three blocks (each block with one control, one drought and one warming plot). Control plots had a similar scaffolding than warming and drought plots, but with no curtain. The study plots were open at all sites. We considered the outer 0.5 m of each study plot a buffer zone and we conducted all the measurements in the central 12 m^2 area.

Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured on 3 fixed sampling points per plot, every 1-2 weeks, using Time Domain Reflectometry (TDR). Air (20 cm above ground) and soil temperatures (0, 2 and 10 cm depth) were obtained by means of temperature sensors RTD Pt100 1/3 DIN (Desin Instruments, Barcelona) located in the three plots (control, drought and warming) of one block. Temperatures were measured every 10 minutes, being recorded the average of three measurements of each sensor.

Plant annual growth

Before treatment applications, 5-7 living stems of 4-5 plants of *E. multiflora* and *G. alypum* were tagged in each plot. In total, we labelled 235 stems of *E. multiflora* and 268 of *G. alypum*. Basal diameters and maximum lengths of the tagged stems were measured in winter each year from 1999 to 2002. To minimize the impact of noncircular stem cross sections, stem diameter was the mean of two perpendicular measurements taken at the stem base with a digital caliper to the nearest 0.01 mm. To allow re-measuring, a line was painted with a permanent marker on the exact point of the stem where diameter had been measured. Maximum stem length was measured from the stem base to the more distant point at the end of the longest branch. Annual stem growth was calculated as the difference between consecutive winter measurements.

Plant seasonal growth

Stem growth of *E. multiflora* is mainly apical, whereas lateral shoot growth may account for a large proportion of stem growth of *G. alypum*. Thus, we used different parameters to assess the seasonal stem growth of each species. In *E. multiflora*, we used the maximum stem length to evaluate seasonal growth, whereas in *G. alypum* we measured the number and length of all the new shoots of each tagged stem. We calculated the total shoot growth per stem of *G. alypum* as the sum of the length of all the new shoots. Seasonal growth was calculated as the difference between non-growing season (winter and summer) measurements.

Shoot elongation rates

At the beginning of the experiment we labelled three shoots from three different stems of 5 plants of each species in each plot. Thus, we tagged 15 shoots of each species in each plot, 135 in total per species. At the beginning of May 2000 we measured the spring length of these shoots and we repeated the measurements after one month. We calculated the shoot elongation rate as: (final length-initial length)/number of days.

Biomass allometric relationships

Allometric relationships between stem biomass (dry weight) and stem diameter or length were calculated for both species, using plants collected from the surrounding area outside the plots. Total stem biomass was measured after drying the plant material in an oven at 70ºC until constant weight. Since multiple stepwise regression analysis showed that both, stem diameter and stem length, explained a significant part of the variability in stem biomass, we used allometric equations including both variables to estimate the annual stem biomass increments of the tagged stems of each species for 1999, 2000 and 2001.

Environmental influence on plant growth

The influence of soil moisture on annual stem diameter growth of both species in the different treatments was analysed by means of Pearson correlations using the annual means per plot of 2000 and 2001 (N=6 for each treatment). We did not use data from 1999 because we had no soil moisture records until mid-March.

Leaf fall

We monitored the leaf fall of 4-8 plants of *E. multiflora* and 9-12 plants of *G. alypum* per plot during the year 2000. Plant litterfall was collected bimonthly by means of open collectors (4.4 cm of diameter) located under each selected plant. Samples were dried to constant weight and afterwards leaves were separated and weighted.

Statistical analyses

Statistical analyses were performed by means of repeated-measures analysis of variance (ANOVAR, GLM procedures in SPSS 10.0.6) with the initial stem diameter, length or biomass as a covariate. To compare annual stem growth between the two species or between years or seasons for each species, we used only control plants. In the analyses of treatment effects, the factor “block” was included to assess whether treatment effects were homogeneous among the blocks. To test the effect of treatments on seasonal growth parameters, we performed ANOVAR for each species for spring and autumn separately. Data on stem length and biomass of both species and on length, number and total growth of *G. alypum* shoots were log transformed to achieve normality. To analyse the effect of treatments on shoot relative growth rates and leaf fall of each species, we performed ANOVAs with treatment and block as factors. Drought and warming treatments were always compared with control treatment separately. In all the analyses, we used means per plant.

Results

Environmental data

Total annual rainfall was 420, 489 and 458 mm in 1999, 2000 and 2001, respectively. Mean annual air temperatures were slightly lower in 1999 and 2000 (13.8ºC both years) than in 2001 (14.0ºC).

Drought treatment reduced the soil moisture a 22% on average throughout the study period (Fig. 1). The maximum reductions occurred in autumn (up to 50% in

October 1999). Warming treatment had a slight effect on soil moisture, reducing it on average 8% throughout the study period (Fig. 1).

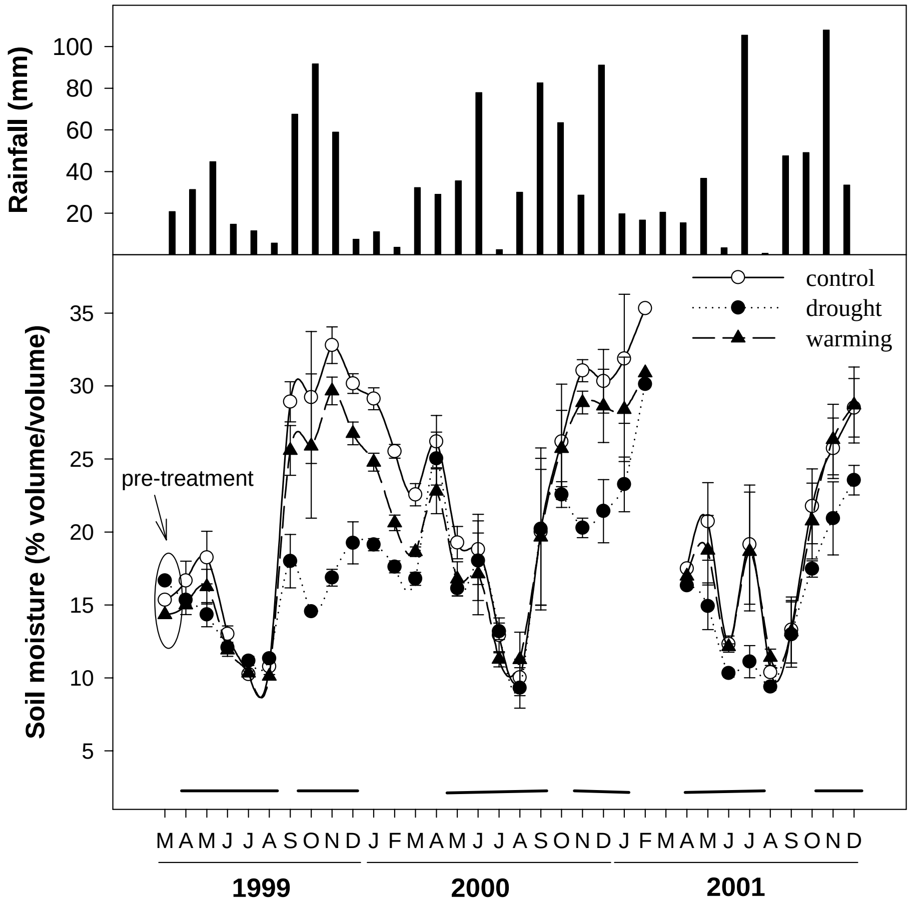

Fig. 1. Monthly rainfall (mm) and mean soil moisture (% volume/volume) of control, drought and warming treatments throughout the study period. Error bars indicate the standard error of the mean (N=2-6 days; values are averages of 3 plots). Lines above the months indicate the periods when drought treatment was working.

Warming treatment increased minimum temperatures on average 0.7 ºC at air, 1.6 ºC at 2 cm depth and 1.1 ºC at 10 cm depth. The reduced heat loss in the warming plots at night also increased the diurnal air and soil temperatures, with a maximum after sunrise and a minimum in the late afternoon (Fig. 2).

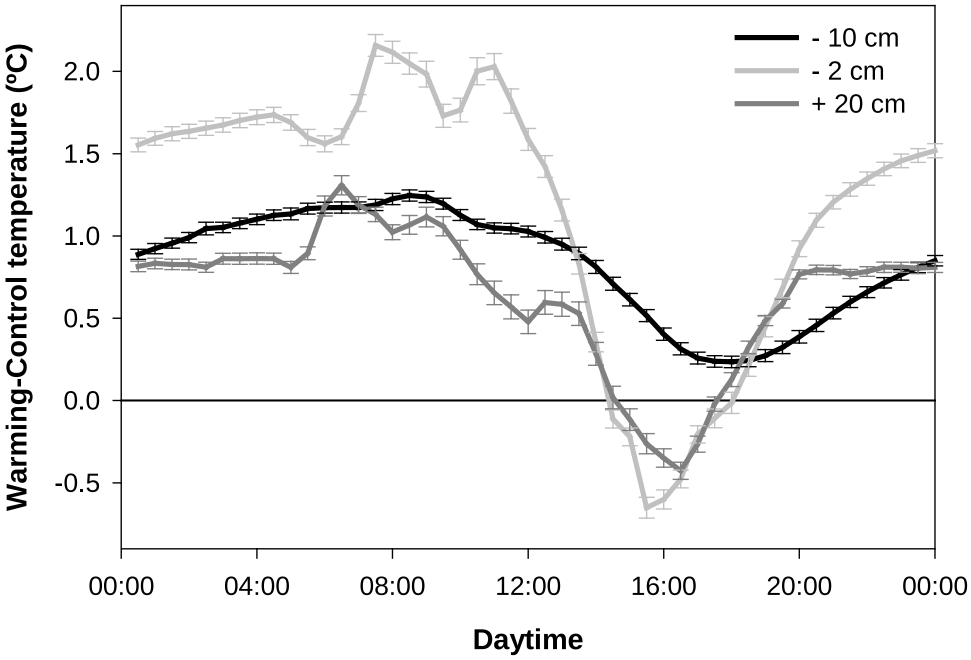

Fig. 2. Annual averages of air (20 cm above ground) and soil (2 and 10 cm depth) temperature (ºC) differences between warming and control plots (measured in one of the blocks from April 2000 to April 2001). Error bars indicate the standard error of the mean (N=365).

Annual stem growth in basal diameter

Control plants of *E. multiflora* showed significantly lower annual stem growth in basal diameter than control plants of *G. alypum* throughout the study period ($F_{1,21}$=11.1, p=0.003; Fig. 3). There were no significant differences in the stem diameter increment of control plants of *E. multiflora* among years, whereas control plants of *G. alypum* showed increasing diameter increments from 1999 to 2001 (Fig. 3).

Overall annual stem diameter growth of *E. multiflora* plants was not significantly affected by drought (Fig. 3). In contrast, plants of *G. alypum* in the drought treatment showed a 31% reduction in annual stem diameter growth compared to controls throughout the study period ($F_{1,21}$=9.7, p=0.005) (Fig. 3). In the warming treatment, annual stem diameter growth was overall a 35% higher in *E. multiflora* plants ($F_{1,22}$=7.1, p=0.01) and a 14% lower in *G. alypum* plants ($F_{1,22}$=6.7, p=0.02) compared to controls (Fig. 3).

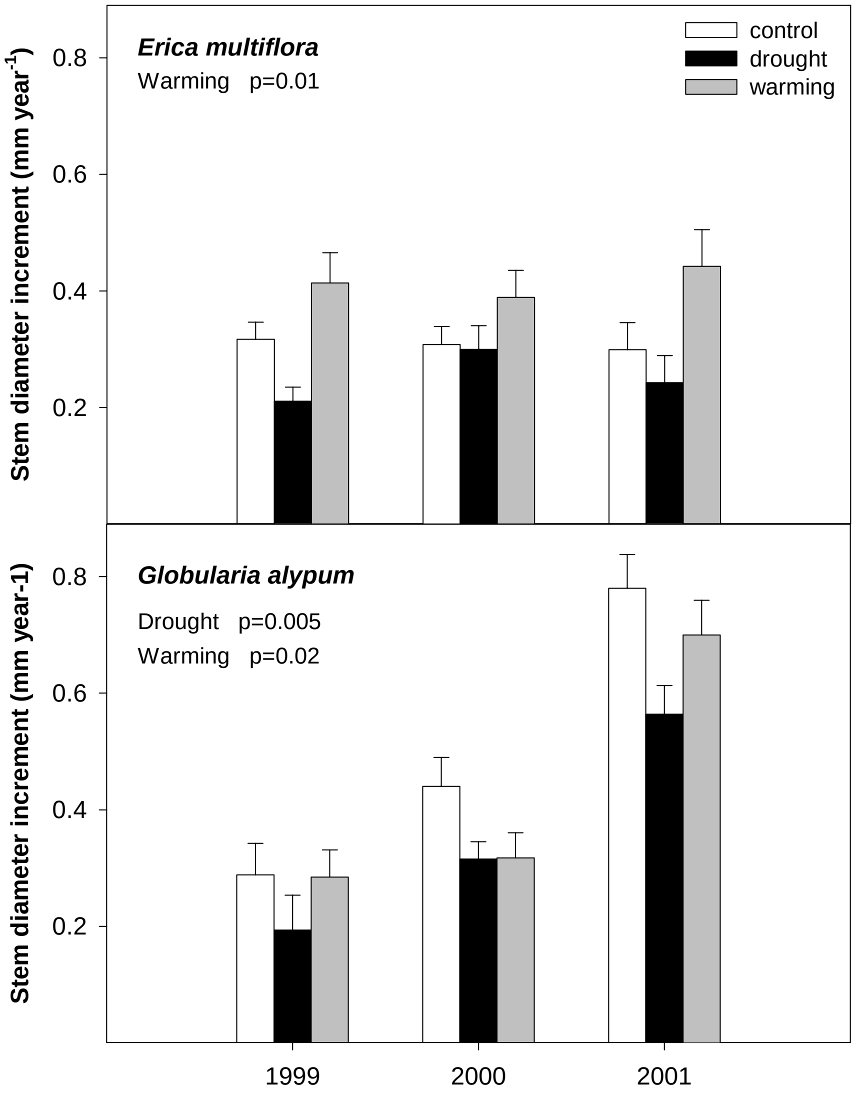

Fig. 3. Diameter increment of *E. multiflora* and *G. alypum* stems in 1999, 2000 and 2001 for control, drought and warming plants. Overall treatment effects are depicted when significant. Error bars indicate the standard error of the mean (N=14-16).

Annual stem elongation

Control plants of *E. multiflora* showed significantly lower annual stem elongation than control plants of *G. alypum* throughout the study period ($F_{1,22}$=15.8, p=0.001) (Fig. 4). Stem elongation of control plants of both species was significantly higher in 1999 than in the other two years. Control plants of *E. multiflora* showed also a higher stem elongation in 2000 than in 2001 (Fig. 4).

Overall, *E. multiflora* and *G. alypum* plants showed significant reductions of 46 and 43% respectively in their annual stem elongation in the drought plots compared to controls ($F_{1,22}$=13.5, p=0.001 for *E. multiflora* and $F_{1,23}$=9.2, p=0.006 for *G. alypum*;

Fig. 4). Warming treatment did not affect significantly the annual stem elongation of any of the two studied species (Fig. 4).

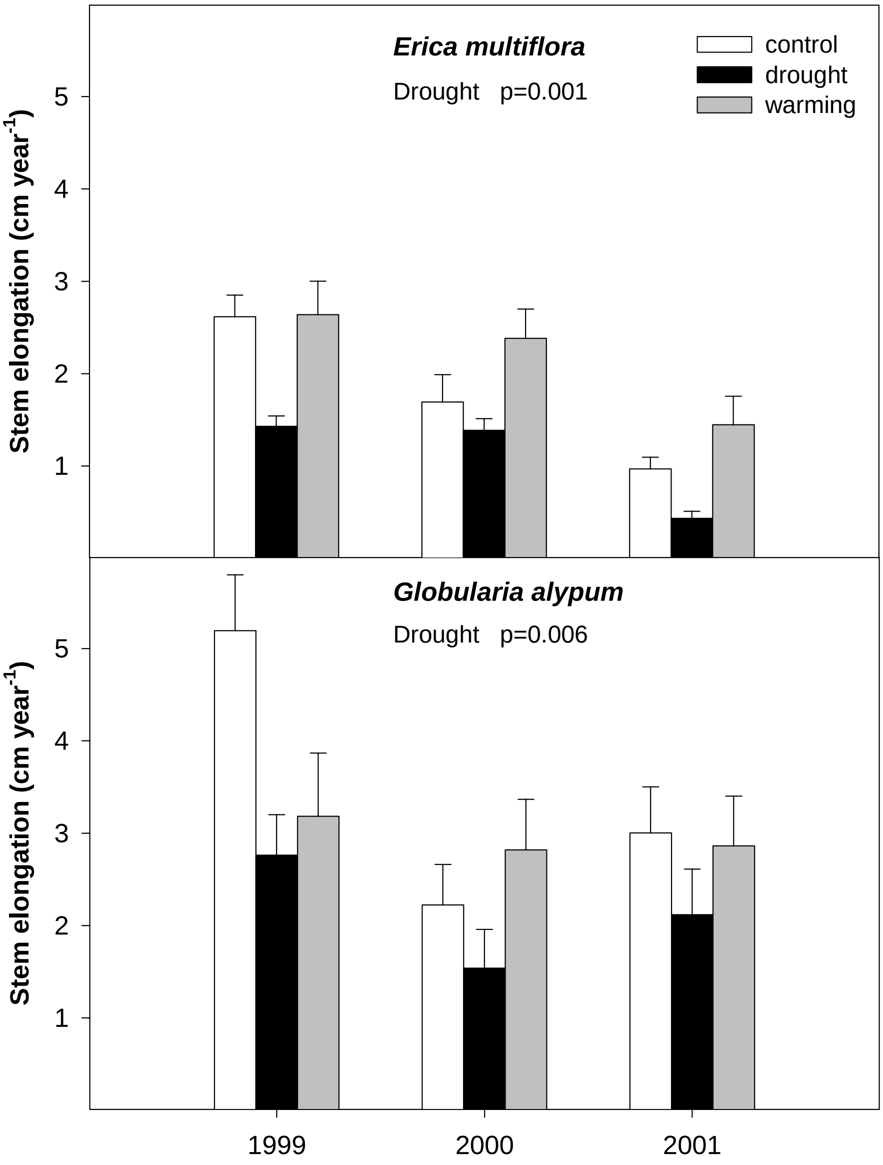

Fig. 4. Stem elongation of *E. multiflora* and *G. alypum* in 1999, 2000 and 2001 for control, drought and warming plants. Overall treatment effects are depicted when significant. Error bars indicate the standard error of the mean (N=14-16).

Stem length versus stem diameter

Control plants of *E. multiflora* and *G. alypum* showed a similar relationship between stem length and stem diameter throughout the study period (Fig. 5). The stem length/diameter ratio tended to decrease in subsequent years in both species (Fig. 5).

Drought and warming treatments did not affect significantly this relationship in any of the two studied species (Fig. 5).

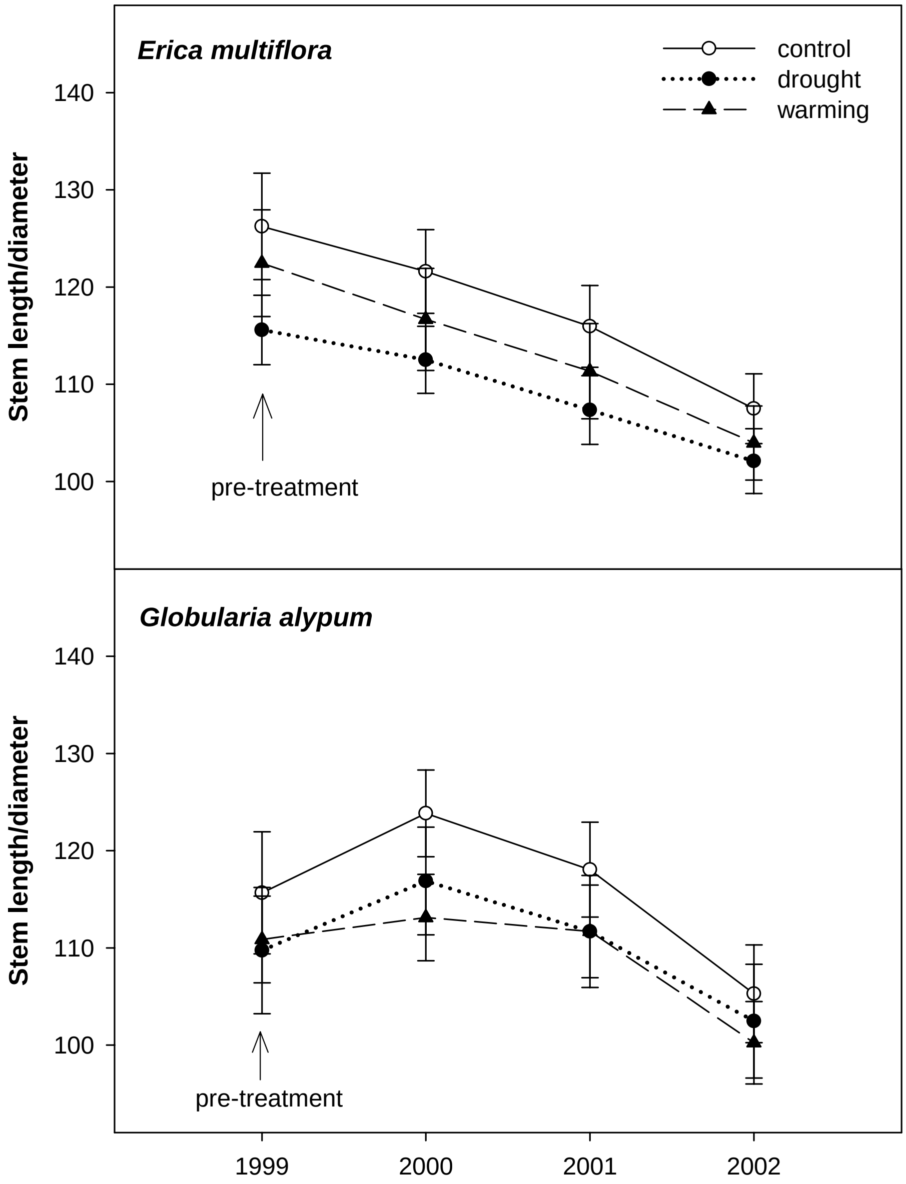

Fig. 5. Stem length/diameter of *E. multiflora* and *G. alypum* in winter 1999, 2000, 2001 and 2002 for control, drought and warming plants. Error bars indicate the standard error of the mean (N=14-15).

Seasonal stem elongation of *E. multiflora* plants

Stem elongation of control plants of *E. multiflora* was significantly higher in spring than in autumn ($F_{1,25}$=46.3, $p<0.001$) (Fig. 6).

Stem elongation of *E. multiflora* plants was significantly lower in the drought treatment than in control plots in spring ($F_{1,22}$=9.9, p=0.005), but not in autumn (Fig. 6). No significant effect of warming was found.

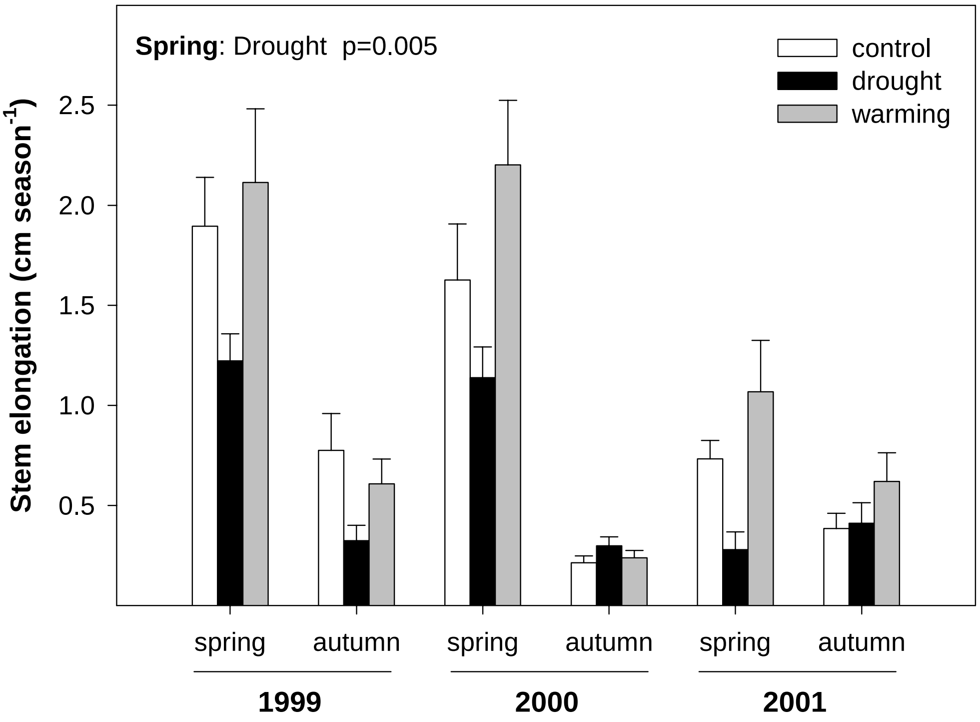

Fig. 6. Stem elongation of *E. multiflora* in spring and autumn of 1999, 2000 and 2001 for control, drought and warming plants. Effects of treatments in spring and/or autumn are depicted when significant. Error bars indicate the standard error of the mean (N=14-15).

Seasonal shoot growth of *G. alypum* plants

Control plants of *G. alypum* showed significantly higher mean length and number of new shoots per stem and thus, total new shoot growth in spring than in autumn (p<0.001 in all the analyses) (Fig. 7).

Drought reduced significantly the mean length of the shoots produced by *G. alypum* plants throughout the study period ($F_{1,22}$=46.6, p<0.001), with significant effects both in spring ($F_{1,23}$=12.6, p=0.002) and autumn ($F_{1,22}$=28.4, p<0.001) (Fig. 7). Conversely, warming treatment reduced the mean length of the new shoots of *G. alypum* only in spring ($F_{1,22}$=7.0, p=0.01) (Fig. 7).

Drought significantly decreased the mean number of shoots per stem of *G. alypum* plants compared to controls throughout the study ($F_{1,22}$=14.9, p=0.001; Fig. 7).

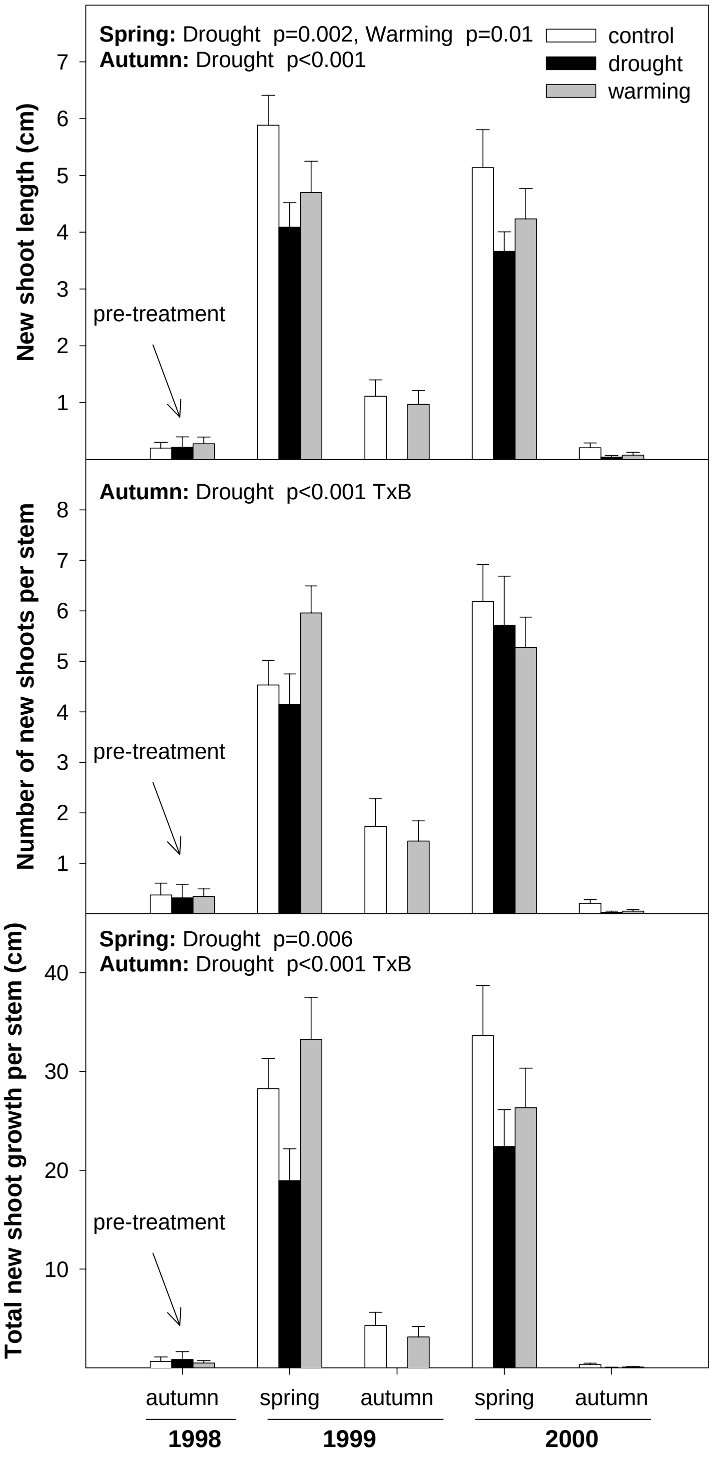

Fig. 7. Mean shoot length, number of shoots and total shoot growth of *G. alypum* in autumn 1998 and spring and autumn of 1999 and 2000 for control, drought and warming plants. Effects of treatments in spring and autumn are depicted when significant. TxB indicates that the interaction between treatment and block was significant. Error bars indicate the standard error of the mean (N=14-15).

Such reduction was due to a significant decrease in the number of shoots in autumn ($F_{1,22}$=41.1, p<0.001), since differences in spring were not significant. However, the effect of drought in autumn was not the same in all the blocks ($F_{1,22}$=7.5, p=0.003), since there was a significant reduction in two of them and no significant change in the other. Warming did not have any significant effect on the mean number of shoots per stem (Fig. 7).

Overall, drought treatment significantly reduced the total shoot growth per stem ($F_{1,22}$=33.4, p<0.001), with significant reductions both, in spring ($F_{1,23}$=9.4, p=0.006) and autumn ($F_{1,22}$=43.7, p<0.001) (Fig. 7). However, in autumn, the effect of drought differed among blocks ($F_{1,22}$=6.4, p=0.006), with a significant reduction of shoot growth in drought plots of two blocks and no significant change in the other. Warming did not significantly affect the total shoot growth per stem of *G. alypum* plants (Fig. 7).

Shoot elongation rates in spring 2000

While drought did not affect significantly the elongation rates of new shoots of *E. multiflora* and *G. alypum*, warming increased the shoot elongation rates of *E. multiflora*, although the effect was only marginally significant ($F_{1,24}$=3.2, p=0.08) (Fig. 8).

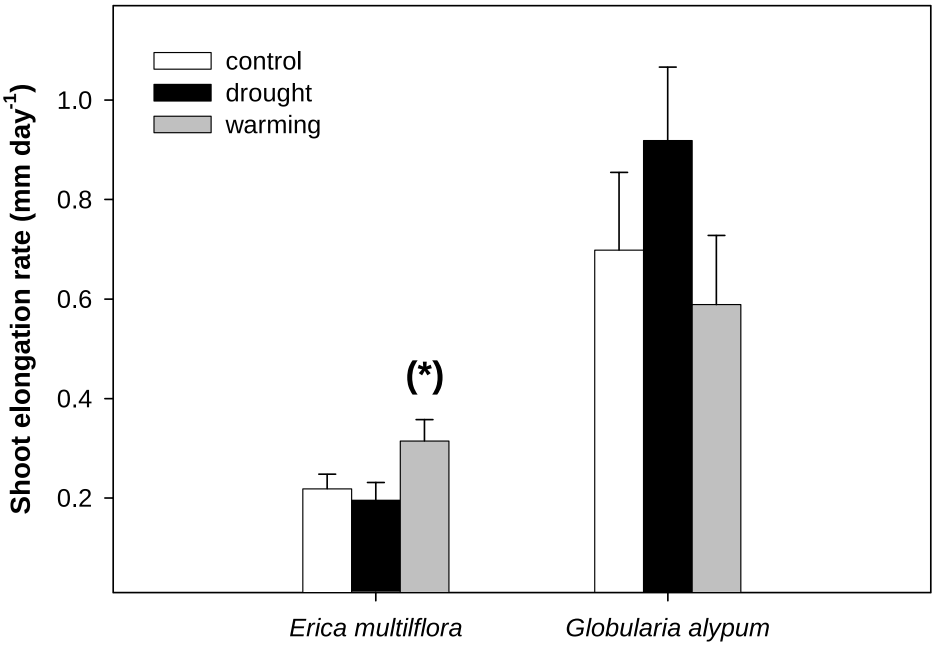

Fig. 8. Shoot elongation rate (mm day^{-1}) of *E. multiflora* and *G. alypum* in spring 2000 for control, drought and warming plants. (*) indicates marginally significant (p<0.1) differences between treatment and control plants. Error bars indicate the standard error of the mean (N=15).

Annual stem biomass increments

Allometric relationships between stem biomass and stem diameter or length were highly significant, being the correlations with stem diameter stronger than with stem length (Table 1). As the allometric equations including both variables, stem diameter and length, had slightly stronger correlation coefficients, we used both variables to estimate stem biomass.

Table 1. Allometric relationships between stem biomass (B) and stem diameter (D) and length (L) in *E. multiflora* (N = 40) and *G. alypum* (N = 100).

	Species	**Allometric relationship**	r^2	P
Stem diameter				
	E. multiflora	log B = -0.506 + 2.324 logD	0.947	<0.001
	G. alypum	log B = -0.897 + 2.619 logD	0.926	<0.001
Stem length				
	E. multiflora	log B = -4.738 + 2.086 logL	0.839	<0.001
	G. alypum	log B = -5.408 + 2.280 logL	0.640	<0.001
Stem diameter + length				
	E. multiflora	logB = -1.73 + 1.79 logD + 0.57 logL	0.961	<0.001
	G. alypum	logB = -2.11 + 2.23 logD + 0.54 logL	0.942	<0.001

Plants of both species showed a lower production of annual stem biomass in the drought treatment than in the control treatment throughout the study period ($F_{1,22}$=5.7, p=0.03 and $F_{1,22}$=5.3, p=0.03 for *E. multiflora* and *G. alypum*, respectively; Fig. 9). Conversely, warming enhanced the annual stem biomass production of *E. multiflora* plants ($F_{1,22}$=4.1, p=0.05), whereas it did not significantly affect the estimated annual stem biomass increment of *G. alypum* (Fig. 9).

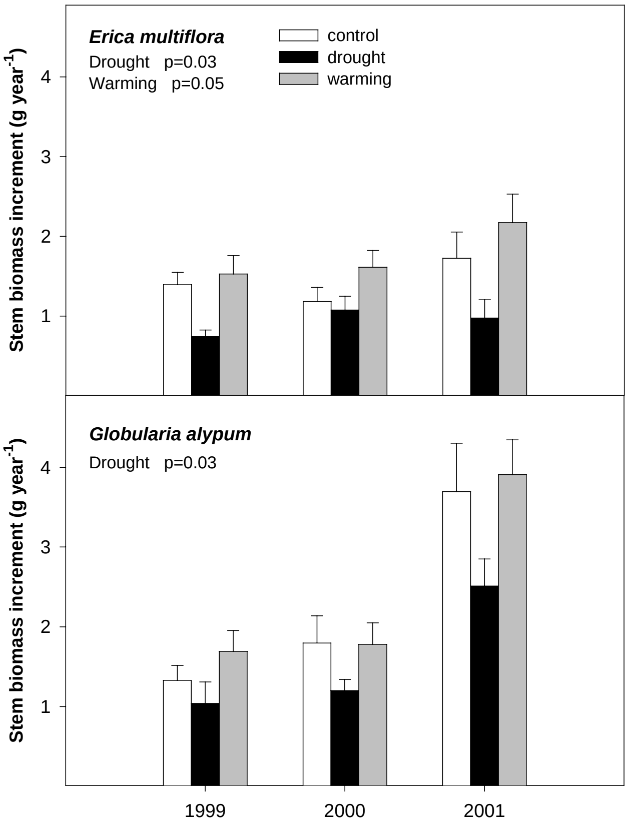

Fig. 9. Stem biomass increment of *E. multiflora* and *G. alypum* in 1999, 2000 and 2001 for control, drought and warming plants. Overall treatment effects are depicted when significant. Error bars indicate the standard error of the mean (N=14-16).

Environmental influence on annual stem diameter growth

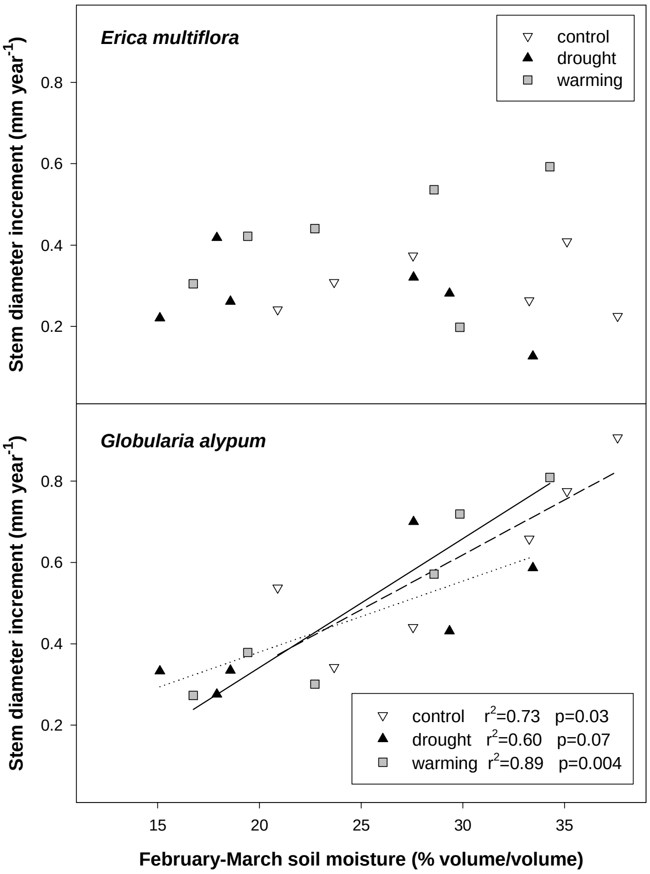

Fig. 10. Annual diameter increment *versus* mean soil moisture of February and March for control, drought and warming plots in 2000 and 2001 (N=6 for each treatment).

Annual stem diameter increments of *E. multiflora* did not significantly correlate with soil moisture. Conversely, annual stem diameter increments of *G. alypum* plants showed positive significant correlations with mean soil moisture throughout the first six months of the year, and mainly with the mean soil moisture of February and March (Fig. 10).

Leaf fall

Our treatments did not significantly affect the leaf fall of any of the two studied species (Fig. 11).

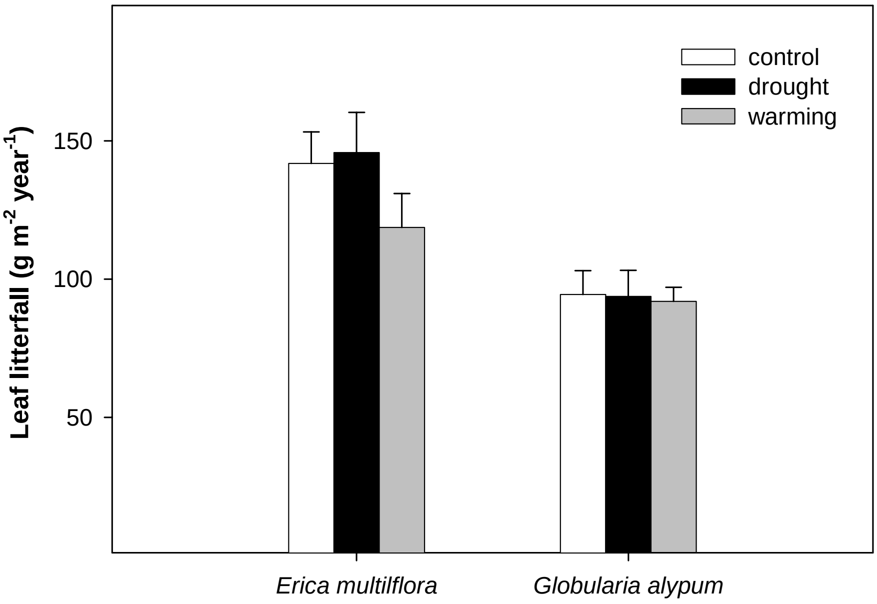

Fig. 11. Annual leaf litter production (g m^{-2} year^{-1}) below *E. multiflora* and *G. alypum* plants in 2000 for control, drought and warming treatments. There were no significant differences between treatment and control plants. Error bars indicate the standard error of the mean (N=19-23 for *E. multiflora* and N=30-33 for *G. alypum*).

Discussion

Species comparison

In a previous study we reported lower photosynthetic rates of *E. multiflora* compared to *G. alypum* during most of the year (Llorens et al. 2003). Accordingly, in the present study, we have found lower annual stem growth of control plants of *E. multiflora* compared to *G. alypum* throughout the study period (Figs 3, 4, 8, 9). Interestingly, despite the lower stem growth of *E. multiflora* in relation to *G. alypum*, there were no significant differences between the two species in the relationship between stem length and stem diameter (Fig. 5), suggesting common allometric constraints on this relationship.

We previously described *E. multiflora* as a water conservative species and *G. alypum* as a water spender species (Llorens et al. 2003). Accordingly, now we have found no significant dependence on soil moisture of annual stem diameter growth of *E. multiflora*, whereas stem diameter increments of *G. alypum* were significantly correlated with volumetric soil water content, being the strongest correlations with the average soil moisture of February and March (Fig. 10). This result suggests that *E. multiflora* probably relies more on the reserves stored during winter to supply seasonal growth in spring, whereas *G. alypum* depends more on concurrent carbon and nutrient uptake. Higher soil moisture during February and March 2001 (34% in 2001, 24% in 2000) might account for the higher values of stem diameter growths of control plants of *G. alypum* in 2001 compared to the previous two years (Fig. 3).

Seasonal growth comparison

Seasonal stem growth of the two studied species was higher in spring than in autumn (Figs 6, 7), despite precipitation was usually lower in spring (Fig. 1), as well as photosynthetic rates of these species (Llorens et al. 2003). Lower growth in autumn might be a consequence of reserve depletion in summer (Larcher and Thomaser-Thin 1988), since these shrubs show null or very low net photosynthesis during this season (Llorens et al. 2003). In contrast, mild temperatures allow net photosynthesis and probably carbon reserves accumulation during winter (Larcher and Thomaser-Thin 1988, Llorens et al. 2003) and these reserves are likely to support the comparatively strong stem and shoot development observed in spring, as it has also been suggested for other Mediterranean sclerophylls (Oliveira et al. 1994). Resource competition between growth and reproduction in autumn might also contribute to the higher growth observed in spring. Reproductive organs are usually strong sinks for carbohydrate and nitrogen reserves (Woodward et al. 1994). Thus, the costs associated with the development of reproductive structures could have reduced the allocation to other functions, such as vegetative growth, in autumn (Bloom et al. 1985, Woodward et al. 1994).

Drought treatment effects

Drier conditions reduced the annual and seasonal growth of *G. alypum*, as it has been reported for other Mediterranean species (e.g., Borghetti et al. 1998, Lebourgeois et al. 1998, Ogaya et al. 2003). The imposed drought also reduced the annual stem elongation of *E. multiflora* plants, as a consequence of spring rather than autumn decreases (Figs. 4, 6). However, drought did not significantly affect the annual stem diameter growth of *E. multiflora* (Fig. 3), in accordance with the lower dependence on soil moisture of this species compared to *G. alypum* (Fig. 10 and Llorens et al. 2003). The observed reductions in stem growth in both species would have decreased their annual stem biomass production (Fig. 9), assuming that drier conditions did not modify the allometric relationship between stem biometry and biomass. Reductions in the stem lengthening and biomass of *E. multiflora* plants under the imposed drier conditions are in agreement with the observed decrease in their leaf net photosynthetic rates (Llorens et al. unpublished). Particularly, we observed an overall 34% reduction in their leaf net photosynthetic rates in front of the overall 46% decrease in the stem elongation found in the present study.

In contrast, the lower annual growth of *G. alypum* plants in the drought treatment compared to controls can not be explained by decreases in the leaf net photosynthetic rates, since drier conditions did not affect significantly the leaf net photosynthetic rates of this species overall the study period (Llorens et al. unpublished). This apparent discrepancy reflects a complex relationship between these two processes. Besides the large difference in the time scales between instantaneous photosynthetic measurements and long-term growth, growth is controlled by several other processes in addition to carbon acquisition (Xiong et al. 2000). For instance, differences in plant leaf area may change growth rates without important changes in photosynthetic rates (Pereira 1995). Since it has been reported that water stress may result in accelerated leaf senescence (Pereira 1995), a greater leaf fall in *G. alypum* plants in the drought treatment, might result in a lower total leaf area and thus, might explain the observed reduction in growth without any change in leaf net photosynthetic rates. However, we did not find any significant effect of our drought treatment on the annual leaf fall of *G. alypum* plants in 2000 (Fig. 11). Drought-induced-limitation in growth of *G. alypum* does not either seem to be due to reduced N or P acquisition associated with water shortage, since we previously found that N and P foliar concentrations tended to increase in *G. alypum* plants under drought treatment (Peñuelas et al. 2003). Alternately, a greater carbon allocation to roots in drought plants of *G. alypum* might explain a lower carbon investment on aboveground growth in this species. Further more detailed studies on gas exchange, growth patterns and carbon allocation are warranted to disentangle this apparent contradiction between growth decrease and unchanged photosynthetic rates.

Warming treatment effects

Warming produced contrasting effects on the annual stem diameter growth of these species. While warming increased the annual stem diameter growth of *E. multiflora* 35% on average, it decreased 14% the annual stem diameter growth of *G. alypum* overall the study period (Fig. 3). Assuming no changes in carbon allocation patterns, plants of *E. multiflora* under our experimental increase in temperature would produce a higher annual stem biomass than controls (Fig. 9). In contrast, warming treatment would not have affected the stem biomass production of *G. alypum* plants, despite its negative effect on stem diameter growth. Other studies have also reported contrasting effects of warming on the growth patterns of co-occurring species (e.g., Chapin and Shaver 1985, 1996, Day et al. 1999, Weltzin et al. 2000).

Positive growth responses to temperature increases have often been reported (e.g., Coleman and Bazzaz 1992, Graglia et al. 1997, Arft et al. 1999, Jarvis and Linder 2000, Ro et al. 2001, Theurillat and Guisan 2001), although most of warming experiments have been performed in cool and moist regions. Enhanced diameter growth of *E. multiflora* plants at warming plots might result from higher relative growth rates (RGR). Indeed, we found that plants of *E. multiflora* in the warming treatment tended to have higher shoot elongation rates than controls (Fig. 8), as it has also been reported for other shrub species in experimentally heated plots in a Rocky Mountain meadow (Harte and Shaw 1995). Higher shoot elongation rates in *E. multiflora* plants at warming plots were not the result of higher leaf net photosynthetic rates or higher specific leaf area (SLA), since there were no significant differences in such parameters between warming and control plants (Llorens et al. unpublished). Greater biomass allocation to leaves might explain the slightly higher growth rates in warming plants of *E. multiflora* (Xiong et al. 2000). However, our experimental increase in temperature did not modify the relationship between stem length and diameter of *E. multiflora* plants (Fig. 5), which suggests that there were no significant changes in biomass allocation between leaves and woody tissues. A lengthening of the growing season might also have contributed to the observed higher annual stem growth of *E. multiflora* plants under warmer conditions. In fact, it has been predicted an advance of the onset of spring growing season by up to six days per 1ºC increase in winter air temperature (Menzel and Fabian 1999), which is similar to the temperature rise achieved in our warming treatment. Peñuelas et al. (2002) reported that leaves of deciduous species unfold on average 16 days earlier and fall 13 days later in a nearby area after a temperature increase of 1.4ºC over 49 years.

Warming reduced significantly the annual diameter growth of *G. alypum* plants compared to controls, but did not affect either their annual stem elongation nor their estimated annual stem biomass production. Effects of warming on leaf gas exchange rates, SLA (Llorens et al. unpublished) or leaf fall (Fig. 11) were not significant and thus, can not explain the reduction in annual diameter growth of *G. alypum* plants. Decreases in growth with increasing temperature have been reported, usually as a consequence of increased heat damage or temperature-induced drought stress (Barber et al. 2000, Kirschbaum 2000, Lloyd and Fastie 2002). In our study, warming treatment reduced the soil moisture on average 8% compared to control treatment. Particularly, soil moisture was significantly lower in the warming plots compared to controls during February and March 2000. Accordingly, the highest reduction in diameter increment of *G. alypum* plants under warming was observed in 2000. Thus, we suggest that the higher sensitivity to soil moisture found in *G. alypum* compared to *E. multiflora* might explain the reduction in basal diameter growth of *G. alypum* in the warming plots. Although we can not exclude the possibility that increases in dark respiration of *G. alypum* plants in the warming treatment might have contributed to their reduction in growth (Pereira 1995), several studies have concluded that the ratio of respiration to photosynthesis does not deviate significantly from constancy over a broad range of temperatures (e.g., Gifford 1994, 1995, Körner 1996, Waring et al. 1998).

Our warming treatment also reduced the mean length of new shoots of *G. alypum* plants in spring (Fig. 7). Shoot length of *G. alypum* plants was, in general, more sensitive to our experimental changes of climatic conditions than the number of shoots. Pre-formation of buds in the previous spring could explain that the effect of treatments on the number of shoots was not evident until autumn 1999 or even spring 2000. The likely dependence of the number of shoots in a particular growing season on the biomass of shoots in the preceding growing seasons, would act as an important buffer against exceptionally severe growing seasons, as it was found for the number of leaves in *Cassiope tetragona* (Callaghan et al. 1989). Thus, similar shoot growths can be attained in different years by small numbers of long shoots or large numbers of short shoots, according to weather conditions. Such mechanism which buffers annual variation in growth could be of considerable importance in the Mediterranean-climate areas, where there is a large unpredictability in the precipitation patterns. Thus, significant decreases in the number of shoots and in the total shoot growth per stem of *G. alypum* plants can be expected from consecutive adverse growing seasons.

Final remarks

Our results highlight the complex relationships between environmental factors and growth. Studies of growth patterns should be performed on distinct parameters, since we have found different effects of our treatments on the distinct measures of growth. Such differences might reflect acclimation responses, such as changes in biomass allocation, which would remain hidden studying only one parameter.

In addition to the complexity of the responses at the plant level, there is a complex response at the species level. Whereas, in general, drier conditions reduced growth of both species, our experimental slight increase in temperature produced contrasting effects on the growth patterns of the two studied species. While warming treatment increased the stem diameter growth and the estimated biomass production of *E. multiflora* plants, warming decreased the stem diameter growth of *G. alypum*. Thus, future warmer conditions might favour the former species, whereas they might be detrimental for the latter one.

Finally, our results are based on only three years and it has been shown that long-term responses may be different from short-term responses (Chapin et al. 1995, Arft et al. 1999, Shaver et al. 2000). Moreover, germination rates and seedling responses might differ from mature plant responses, although preliminary results for these species also point to a more negative effect of drought on *G. alypum* seedling establishment compared to *E. multiflora* (Lloret et al. unpublished). Longer-term studies including demographic issues are thus warranted to obtain more robust predictions on future changes in species composition in these Mediterranean shrublands. However, results presented here point to changes in the competitive ability of these two currently dominant species in response to the predicted climate change for the next decades.

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CHAPTER 4

Experimental evidences of future warmer and drier conditions affecting flower phenology, production, life span and size of two co-occurring Mediterranean shrubs, *Erica multiflora* and *Globularia alypum*

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To be submitted

Abstract

We aimed to assess the impact of drier and warmer conditions on flowering traits of two common species of the coastal Mediterranean shrublands, *Erica multiflora* and *Globularia alypum*, which flower in autumn and winter. We performed a field experiment over two years using a novel and non-intrusive technique to prolong the drought period or to create passive nighttime warming. On average, drought treatment reduced soil moisture 29% during the first year (March 1999-February 2000) and 17% during the second year (March 2000-February 2001). These drier conditions delayed most of the studied flowering phenophases in both species and years, being the delay stronger for *G. alypum* than for *E. multiflora*. Moreover, drought treatment decreased the functional flower production and tended to extend the flower life span in both species. Whereas drought plants of *E. multiflora* showed a 22 days longer flowering period, drought plants of *G. alypum* showed an 11-12 days shorter flowering period. Drought plants of *E. multiflora* had smaller flowers than controls. Results suggest that water is a crucial resource for these species during the flowering period. In addition, they are in agreement with the existence of a trade-off between allocation of resources to floral construction versus floral maintenance. Warming treatment increased minimum temperatures 0.7-1.6ºC on average. The only significant effect of our warming treatment on flowering traits of *E. multiflora* was a reduction in flower size. By contrast, in the first year, warmer conditions delayed 14 days the appearance of functional spring-bud flower heads of *G. alypum*, whereas they advanced 7 days the appearance of functional autumn-bud flower heads. Enhanced temperatures also decreased the number of *G. alypum* plants with functional spring-bud flower heads by 20.2%, whereas it increased the number of *G. alypum* plants with functional autumn-bud flower heads a 28.5%. Our results suggest that warmer temperatures in the Mediterranean could beneficiate species whose flower bud production depends on late autumn or winter growth. The observed effects of drier or warmer conditions on flowering traits of these two species suggest that predicted climate change might have important effects on individual plant reproductive success, plant interactions with other organisms, plant population dynamics, and ecosystem functioning.

Introduction

Phenology, the study of the seasonal timing of life cycle events, has found new relevance in research into global climate change (Schwartz 1998, Menzel et al. 2001, Peñuelas and Filella 2001). The influence of climate changes can be examined by using the annual variation of starting dates of life cycle events or phenophases (Roetzer et al. 2000, Menzel et al. 2001). Phenophases are mainly driven by environmental factors, among which weather conditions are the most important (Menzel 2000). In particular, phenophases of most organisms are strongly influenced by temperature and precipitation (Peñuelas et al. 2002). Since climate projections predict drier and warmer conditions and more frequent and severe droughts in the Mediterranean basin in the next decades (IPCC 2001), we would expect alterations in the phenology of Mediterranean species as a consequence of climate change.

Flowering is considered to be one of the most significant phenological stages by which the sensitivity to climatic change can be evaluated (Spano et al. 1999). The time at which plant species flower is determined by both genetic and environmental factors, and by their interaction (Fitter et al. 1995). It has been found that climatic factors in temperate and cool climates may play a major, more direct role in the induction and performance of flowering than genetic factors (e.g., Walkovszky 1998, Tyler 2001). Three major climatic factors have been identified as cues that initiate onset of flowering: temperature, photoperiod and rainfall (Rathcke and Lacey 1985). Most temperate woody species and some perennial herbs flower in response to temperature (e.g., Schemske et al. 1978, Rathcke and Lacey 1985, Fitter et al. 1995, Diekmann 1996, Maak and Von Storch 1997, Galán et al. 2001). Thus, raised temperatures may affect the seasonal timing of flowering events. In fact, the study of phenological time series indicate that rising temperatures during the past century have advanced the timing of spring flowering in many species at northern latitudes both in Europe and the USA (see Peñuelas and Filella 2001 for a review; see also Menzel 2000, Roetzer et al. 2000, Menzel et al. 2001). Also experimental increases in temperature advanced onset of flowering in arctic and alpine species (e.g., Alatalo and Totland 1997, Welker et al. 1997, Sandvik and Totland 2000, Sandvik 2001). In the Mediterranean area, a few studies have also shown advances of spring flowering dates by warming (Osborne et al. 2000, Peñuelas et al. 2002). Most phenological studies have been performed on species that flower in spring, whereas only a few studies deal with climate change effects on autumn phenophases (mainly on leaf colouring and fall), whose shifts seem to be less pronounced and smaller than in spring (Menzel and Fabian 1999, Menzel 2000, Menzel et al. 2001, Peñuelas et al. 2002). To our knowledge, no experimental study deals specifically with the effects of climate change on flowering phenology of species that flower in autumn.

Several authors have found great differences of phenological responses to climatic warming among species (e.g., Fitter et al. 1995, Peñuelas et al. 2002). Such differences might be related to species differences in the degree of flowering dependence on temperature and photoperiod. Species with a high sensitivity to temperature and little to photoperiod would be expected to undergo marked changes in flowering time, whereas others, more strictly controlled by photoperiod, would be expected to show little change (Fitter et al. 1995). Species lacking phenological adaptability may require a stronger signal or may be unable to adapt to climate warming (Bradley et al. 1999). Bradley et al. (1999) speculated that species without phenological adaptability might experience greater stress or even extinction during extended climate change. Because flowering time is determining reproductive success and hence fitness of most plant species (O’Neil 1999, Kelly and Levin 2000), differential shifts in flowering time among species may well alter community composition (Fitter et al. 1995).

In seasonal tropical forests, flowering is often induced by rainfall (Augspurger 1981, Lieberman 1982, Reich and Borchert 1982, Rathcke and Lacey 1985). Rainfall also stimulates flowering of some desert plants (Fox 1990, Abd El-Ghani 1997), although it has also been reported that, in deserts, the drying soil, which indicates the end of the growing period, causes annuals to flower (Rathcke and Lacey 1985). In the Mediterranean area, phenological studies have shown a small effect of precipitation on flowering phenology (Spano et al. 1999, Picó and Retana 2001, Peñuelas et al. 2002). However, other reproductive traits, such as flower/inflorescence number or size, may be strongly affected by plant water status, since an input of water from vegetative portions of the plant is required for floral bud expansion, flower opening, nectar production and turgor maintenance in floral organs under evapotranspirational demand (Galen et al. 1999). Thus, water loss through inflorescences may place extreme demands on plant water status in arid environments (Nobel 1977, Galen et al. 1999).

One approach to considering the consequences of climate change on flowering phenology of naturally occurring plant populations is to manipulate temperature or water experimentally. However, till now, most of the techniques used to experimentally warm (e.g., heat-resistance ground cables, overhead infrared lamps, field chambers, greenhouses; see Shaver et al. 2000 for a review) or dry (e.g., rainout shelters; Fay et al. 2000) ecosystems or ecosystem components alter additional environmental conditions (e.g. light, humidity, soil structure, wind). Moreover, most of these experiments have been performed in cool and moist ecosystems. And, in addition, warming experiments usually simulate a general diurnal increase in temperature rather than the observed increase in T_{min} (nighttime temperature) (IPCC 2001). Thus, to reduce these drawbacks, we have performed a field experiment using a non-intrusive technique to warm and dry a Mediterranean shrubland. Warming has been achieved by reducing the loss of IR-radiation from the soil-vegetation surface to the atmosphere at night, imitating the effect produced by greenhouse gases accumulation and resulting in an increase in T_{min}, or nighttime warming. A longer and stronger drought period has been attained by excluding rain during the growing season (Beier et al. 2003).

Erica multiflora L. and *Globularia alypum* L. are common components of the coastal Mediterranean shrubland and they are two dominant species in our study site. They are evergreen, sclerophyllous shrubs that flower in autumn and winter. The major goals of our study were (1) to describe the flowering patterns of these two co-occurring species, and (2) to assess whether predicted warmer or drier conditions may change their flowering patterns, flower production, and/or flower life-span. We also studied the effect of warming and drought on flower size (corolla length) of *E. multiflora*. Since the date to flowering is a time-dependent trait, we used survival analysis (also known as “failure time analysis”) to evaluate whether our experimental drier or warmer conditions altered the time distribution of the different flowering phenophases of these species. To our knowledge, this is the first experimental field study examining the possible impact of expected future warmer or drier conditions on the flowering phenology of Mediterranean species (thus, on species already adapted to a warm and dry environment). This is also the first experimental study focused on investigating the effects of such climatic changes on the flowering phenology of species that flower in autumn and winter.

Materials and methods

Study site and species description

The study was carried out in a dry shrubland (*Rosmarino-Ericion*) in the Garraf Natural Park, Barcelona, NE Spain (41º18'N, 1º49'E), at 210 m above sea level and on a SSE slope (13º). The Garraf hills are a calcareous formation 35 km south of Barcelona. Their latitude and location near the seashore result in a typical Mediterranean climate. The site, which is located on terraces from abandoned vineyards, suffered large fires in the summers of 1982 and 1994. The soil is a petrocalcic calcixerept (SSS, 1998), thin (12-37 cm), with a loamy texture and abundant calcareous nodules. Currently, the regenerating vegetation covers 50-60% with a maximum height of 70 cm. The dominant species are *Erica multiflora*, *Globularia alypum*, *Dorycnium pentaphyllum*, *Rosmarinus officinalis, Fumana ericoides, Ulex parviflorus, Helianthemum syriacum, Brachypodium phoenicoides* and *Ampelodesmus mauritanica.*

Erica multiflora L. (Ericaceae) and *Globularia alypum* L. (Globulariaceae) are evergreen, sclerophyllous shrubs that typically occur in basic soils of the western Mediterranean Basin, where they are common components of the coastal shrubland. Plants of both species consist of many shoots up to 1.5 m high, arising from a burl. Vegetative growth occurs twice a year: in spring (from March to June) and in autumn (from September to November).

Erica multiflora flowers (3-6 mm) are hermaphrodites, bell-shaped, pendulous and grouped in elongated inflorescences (1-8 cm), generally located at the terminal position of branches. Each inflorescence is composed of between one and *ca* 150 flowers. Flower buds start to appear in August-September. Small nectaries are present at the inner bases of the corolla of *E. multiflora* flowers, being insects the main pollination vectors in this species (Santandreu and Lloret 1999).

Globularia alypum flowers are also hermaphrodites and are grouped in flower heads (10-30 mm of diameter) located in a terminal or sometimes in a lateral position. Some flower head buds are formed during the previous spring, whereas others are formed in autumn. Spring-bud flower heads are dependent on spring growth and start to open in August, whereas autumn-bud flower heads depend on autumn growth and thus, do not start to open until the end of October.

Experimental system

Two types of climatic manipulations were performed using automatically sliding covers (Beier et al. 2003):

- Extended summer drought was induced by covering the plots with transparent and waterproof plastic curtains during all rain events over the two growing seasons, starting in March-April in spring and in September-October in autumn.
- Passive nighttime warming was achieved by covering the vegetation and soil during night by means of aluminium curtains. The curtains drew over the vegetation at sunset and were removed at sunrise (below and above 200 lux respectively). Thus, curtains reflected infrared radiation from the soil-vegetation surface at night, retaining a portion of the energy accumulated in the ecosystem during the light period, which mimics the mechanism of global warming. Warming treatment started on March 16th 1999 and it was working all nights throughout the study, except during two periods (1-30 August 1999 and 1-26 January 2000), when the treatment was stopped for calibration of system effects or due to mechanical problems.

Covers were mounted on metal scaffolding 0.2 m above vegetation maximum height (*ca* 0.8-1 m above the ground). Nine plots of 20 m^2 (4m x 5m) were established: 3 untreated controls, 3 warming and 3 drought plots. Control plots had similar scaffolding than warming and drought plots, but with no curtain. The plots were open at the sides to allow free wind movement. They were organized in three blocks (each block with one control, one drought and one warming plot). The automatic control of the covers minimised unintended side effects on the light regime, hydrology and wind (Beier et al. 2003). For instance, at night, warming covers were automatically removed when it rained to avoid influencing hydrological cycle. We assigned the outer 0.5 m of each study plot as a buffer zone with all measurements carried out in a central 12 m^2 area.

Environmental data

Precipitation was registered at the study site with a standard rain gauge. Soil moisture was measured on 3 fixed sampling points per plot, every 1-2 weeks, using Time Domain Reflectometry (TDR). Air (20 cm above ground) and soil temperatures (0, 2 and 10 cm depth) were obtained by means of temperature sensors RTD Pt100 1/3 DIN (Desin Instruments, Barcelona) located in the three plots (control, drought and warming) of one block. Temperatures were measured every 10 minutes, being recorded the average of three measurements of each sensor.

Flowering phenology

Before treatment applications, we labeled 4-11 plants of *E. multiflora* and 10-12 plants of *G. alypum* per plot. In total, we monitored 23 plants of *E. multiflora* in control plots, 19 in drought plots and 23 in warming plots. For *G. alypum*, we monitored 34 plants in control and drought plots and 31 in warming plots. During the flowering seasons of 1999 and 2000, we estimated the flower production of *E. multiflora* (the percentage of branches with flowers in relation to the total number of branches) and the percentage of functional flowers or flower heads (number of open flowers or flower heads with visible stamens in relation to the total number of flowers or flower heads) of each labeled plant of both species. We did not estimate flower production in *G. alypum* since it was maximum (practically all branches had flower heads) in all plants both years. For this species, we distinguished between spring-bud and autumn-bud functional flower heads. Monitoring measurements were conducted once a week during the most active flowering period and biweekly during the less active flowering period. In 1999, we additionally estimated the percentage of senescence flowers in *E. multiflora* or senescence spring-bud flower heads in *G. alypum* (number of flowers or spring-bud flower heads with dried petals and stamens in relation to total production). Estimations were categorized as: 0, 1-25%, 26-50%, 51-75%, 76-100% (Gómez 1993, Pavón and Briones 2001). The length of the flowering period was computed for each plant as the number of days from the appearance of the first functional flower or flower head until the first date with no functional flower or flower head. Only plants that had functional flowers or flower heads were used to calculate the flowering period.

Flower production

In December both years, we measured the length of all the inflorescences of 3-4 tagged stems from 4-5 plants of *E. multiflora* in each plot. Inflorescence length was determined from the point of insertion of the lowest flower to the tip of the uppermost flower. To estimate the number of flowers of each inflorescence, we measured the length of 51 inflorescences from different *E. multiflora* plants of a surrounding area outside the plots and after removing all their flowers we counted them. We thus obtained an allometric equation relating inflorescence length with number of flowers.

At the end of the flowering season of 1999, we counted the number of autumn-bud flower heads produced by each tagged stem of *G. alypum*. During the flowering season of 2000, weekly or biweekly (as for the phenological records), we counted the total number of functional flower heads of 3 plants of *G. alypum* per plot.

Life span of individual flowers of E. multiflora and flower heads of G. alypum

At the beginning of November 1999, we marked one closed flower per plant in 4-10 plants of *E. multiflora* per plot. At the beginning of August 2000, we marked one closed spring-bud flower head per plant in 10 plants of *G. alypum* per plot. In both years, the phenological stage of each tagged flower or flower head was monitored weekly. We calculated the life span of an individual flower of *E. multiflora* or a flower head of *G. alypum* as the number of days that the flower or flower head was functional, i.e. showed functional stamens.

Corolla length of *E. multiflora* flowers

We measured the corolla length of 10 flowers per plant (from different inflorescences) of 5 plants per plot with an electronic calliper to the nearest 0.01 mm.

Statistical analyses

By translating time-to-event into time to achieve different flowering phenophases, we applied the techniques of survival time analysis to assess whether drier or warmer conditions affect the flowering phenology of *E. multiflora* and *G. alypum*. In particular, we used survival analyses to study the treatment effects on: (1) the onset of the flowering period (i.e., the time of the appearance of >1% of functional flowers or flower heads) in both years, (2) the onset of flower senescence (i.e., the time of the appearance of >1% of senescence flowers or flower heads) in 1999, (3) the position of the flowering peak (i.e., the time when plants achieved more than 75% of functional flowers or flower heads) in both years, and (4) the end of the flowering period of *E. multiflora* (i.e., the time when plants had more than 75% of senescence flowers) in 1999. In *E. multiflora*, we also analyzed the time when plants had more than 75% of flowered branches in both years to assess maximal flower production. Survival time was defined as the number of days from 1 January each year until the event of interest (onset of flowering, flowering peak, etc.) occurred. When the event did not occur before the end of the study, its survival time was considered to be the last day of the study, and the individual was flagged as right censored. Data censored contribute to the analysis until the point of censoring, thus allowing the retention of the partial information that may be available (Vermerris and McIntyre 1999). We used Kaplan-Meier (or product-limit) nonparametric method for the computation of survival curves and the log rank (Cox-Mantel) statistics to test for differences between treatments and controls (Kleinbaum 1996). Drought and warming curves were always compared separately with control curves.

To analyze the effect of treatments on the length of the flowering period, the life span of flowers (or flower heads), or the variables related to flower production we performed ANOVAs within each year with treatment and block as factors. The factor “block” was included in the analyses to assess whether treatment effects were homogeneous among blocks. When the effect of block and the interaction between block and treatment were not significant, we removed block from the analysis. To test the effect of treatments on the maximum number of functional flower heads (spring plus autumn) of *G. alypum* counted in a single census within the flowering season of 2000, we always used maximum values divided by the number of stems of each plant to account for variation due to plant size. In this case, we transformed data logarithmically to reach normality. To evaluate whether treatments affected the final number of functional autumn flower heads of *G. alypum* in 1999, we performed Mann-Whitney tests, since data did not meet assumptions of normality and equality of variance.

Results

Environmental data

Total annual rainfall was 420 and 489 mm in 1999 and 2000, respectively. Mean annual air temperature was 13.8ºC both years.

On average, drought treatment reduced soil moisture 29% during the first year (from March 1999 to February 2000) and 17% during the second year (from March 2000 to February 2001) of the experiment (Table 1). The maximum reductions occurred in autumn (up to 50% in October 1999). Warming treatment had a slight effect on soil moisture, reducing it on average 11.0% in the first year and 8.4% in the second year compared to control plots (Table 1).

Table 1. Mean soil moisture and standard errors for control, drought and warming treatments (N=3 plots per treatment) in the first (March 1999-February 2000) and second year of the experiment (March 2000-February 2001). Yearly values per plot were obtained as the average of the mean monthly values.

	1st year		**2nd year**	
	AVG	**SE**	**AVG**	**SE**
CONTROL	21.7	2.4	23.7	2.2
DROUGHT	15.5	0.8	19.7	1.6
WARMING	19.3	2.0	21.7	2.0

Warming treatment increased minimum temperatures on average 0.7 ºC at air, 1.6 ºC at 2 cm depth and 1.1 ºC at 10 cm depth (Table 2). The reduced heat loss in the warming plots at night also increased the diurnal air and soil temperatures, with a maximum after sunrise and a minimum in the late afternoon (Table 2).

Table 2. Annual averages of soil (10 cm depth) and air (20 cm above ground) temperature (ºC) differences between warming and control plots at different time intervals. Data were measured from April 2000 to April 2001 in one of the blocks.

	Soil		**Air**	
HOUR	AVG	SD	AVG	SD
00:30 to 04:00	1,00	0,60	0,91	0,53
04:30 to 08:00	1,18	0,65	1,12	0,65
08:30 to 12:00	1,15	0,64	0,92	0,98
12:30 to 16:00	0,72	0,72	0,21	1,17
16:30 to 20:00	0,25	0,70	0,19	0,49
20:30 to 24:00	0,66	0,63	0,87	0,49

Flowering patterns of control plants of E. multiflora and G. alypum

A 91% of *E. multiflora* marked plants in 1999 and a 95% in 2000 showed more than 75% of flowered branches (Fig. 1). These maximum percentages were achieved on julian day 335 in 1999 (November 22) and 360 in 2000 (December 25) (Fig. 1). The flowering pattern of *E. multiflora* control plants is characterized by a highly synchronous peak of flowering, which starts at the end of September (julian day *ca* 272-274) and finishes the last week of March (Fig. 2). During this period, 100% of the individuals (N=23 in 1999 and N=22 in 2000) had functional flowers (>1%) in both years (0% censored data in Table 3, see also Fig. 2). Before this important flowering peak, there was a smaller one starting the second week of August in both years, in which less than 20% of *E. multiflora* plants had functional flowers. Time between onset of flowering and 100% of plants with functional flowers (>1%) varied from 58 days in 1999 to 81 days in 2000. However, discarding the initial small peak, the time was 21 days in 1999 and 26 days in 2000. The 100% of plants with functional flowers (>1%) was achieved on October 13 in 1999 and on October 26 in 2000. Only 40-50% of the plants had more than 75% of functional flowers in relation to total flowers (Fig. 2). The peak of the maximum number of plants with >75% of functional flowers was sharp, highly synchronous, and it was strikingly achieved in the same sampling week both years (October 28 in 1999 and October 26 in 2000).

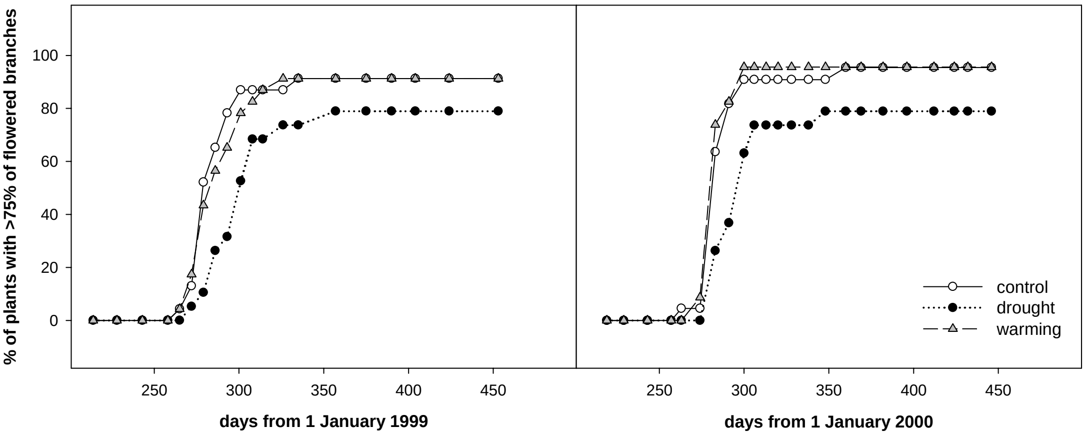

Fig.1. Percentage of *E. multiflora* plants with more than 75% of flowered branches in relation to the total number of branches of each plant in the flowering seasons of 1999 and 2000. Sample sizes are: control (N=23 in 1999, N=22 in 2000), drought (N=19) and warming (N=23). See tables 3 and 4 for statistical details.

G. alypum control plants showed a great difference in their flowering pattern between the two studied years (Fig. 3). The first year, the flowering pattern of control plants of *G. alypum* was clearly bimodal, with the first flowering period corresponding mainly to spring-bud flowers and the second one to autumn-bud flowers. The first flowering period lasted from the second of August until *ca* the 28th of October (julian day 301), whereas the second one lasted since the 28th of October until the last week of March. The maximum number of control plants with functional flower heads (82%) occurred during the first flowering period, particularly, the 29 of September. During the second flowering period, the maximum percentage of control plants with functional flowers (>1%) was 59%. In the second year, the flowering pattern of *G. alypum* was basically unimodal, although there were two additional small peaks, before and after the large flowering peak. The maximum number of control plants with functional flower heads (88%) occurred later (at October 9th) than in 1999. In the last flowering peak (corresponding mainly to autumn-bud flowers) a maximum of 15% of plants had functional flower heads. In 1999, there were two peaks of plants with >75% of functional flower heads. The first one had its maximum on the 29th of September (21% of plants), whereas the maximum of the second one was on the 8 of February (18% of plants). By contrast, in 2000, there was only a narrow period when plants had more than 75% of functional flower heads, with a maximum of 32% of plants the 17th of October. In 1999, 91.2% and 61.8% of control plants had functional spring-bud and autumn-bud flower heads, respectively (see % of censored data in Table 4). On the contrary, in 2000, all control plants had functional spring-bud flower heads, whereas only 14.7% of control plants had functional autumn-bud flower heads (see % of censored data in Table 4).

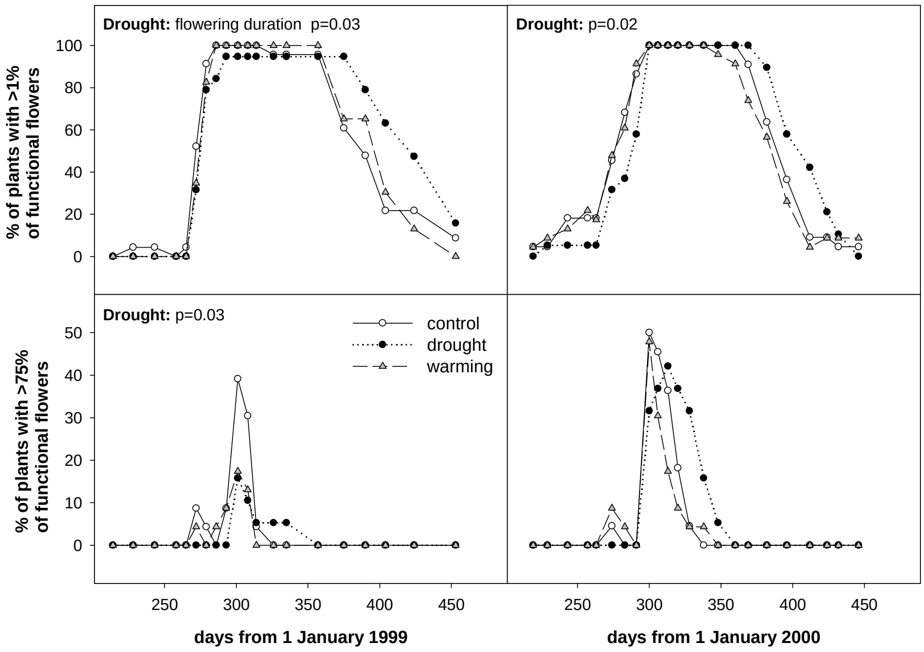

Fig.2. Percentage of *E. multiflora* plants with more than 1% and 75% of functional flowers in relation to the total number of flowers per plant in the flowering seasons of 1999 and 2000. Sample sizes are: control (N=23 in 1999, N=22 in 2000), drought (N=19) and warming (N=23). See tables 3 and 4 for statistical details.

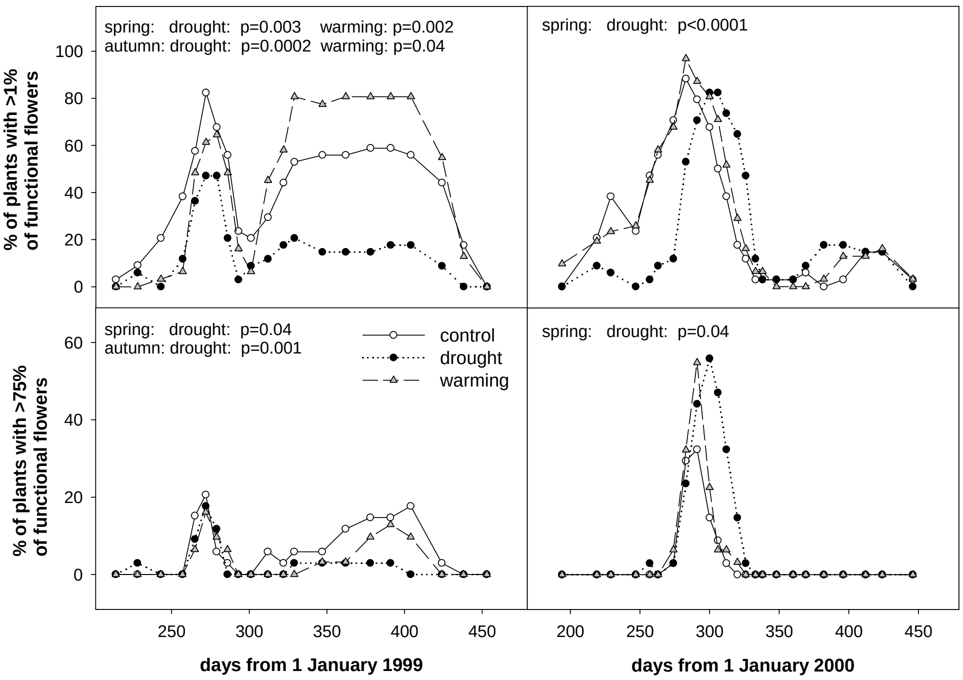

Fig.3. Percentage of *G. alypum* plants with more than 1% and 75% of functional flower heads in relation to the total number of flowers per plant in the flowering seasons of 1999 and 2000. Sample sizes are: control (N=34), drought (N=34) and warming (N=31). See tables 5 and 6 for statistical details.

Treatment effects on flowering phenology

E. multiflora

In both years, drought treatment decreased significantly the number of *E. multiflora* plants with more than 75% of flowered branches (79%) compared to controls (91% and 95% in 1999 and 2000, respectively) (Table 3 and Fig. 1). In addition, median time at >75% of flowered branches was earlier for control plants than for drought plants in both years (Table 3 and Fig.1). However, all plants had functional flowers (>1%) (Table 3). The appearance of the first functional flower was significantly delayed by drought in 2000, but not in 1999 (Table 3 and Fig. 2). In 2000, Kaplan-Meier estimates showed the overall median time at first functional flower appearance in control plots to be on julian day 283 (October 9), whereas in drought plots was 8 days later (October 17). Differences in time at first (>1%) senescence flower between control and drought plants of *E. multiflora* were statistically significant, although only data for 1999 was available (Table 3 and Fig. 2). Half of the sampled control plants had senescence flowers (>1%) the day 293 (October 20), whereas in this date only 31% of drought plants had more than 1% of senescence flowers. Drought plants were less likely to achieve more than 75% of functional flowers than controls in 1999 (Table 3 and Fig. 1), although censoring was present in all treatments. In 1999, the end of the flowering period (i.e., more than 75% of senescence flowers) was also later in the drought plants of *E. multiflora* than in controls (Table 3). Warming did not affect significantly any of the studied flowering phenophases of *E. multiflora* (Table 3 and Figs 1, 2).

Table 3. Kaplan-Meier estimates of median times for different flowering phenophases and log-rank statistics for the comparisons between survival probability curves of control (N=23 in 1999 and 22 in 2000) and drought (N=19) or warming (N=23) plants of *Erica multiflora* in 1999 and 2000. In all logrank tests *df*=1. Significant differences are highlighted in bold type.

	Median ± s.e.	**% censored**	**X^2**	**P**
1999				
>75% of flowered stems				
Control	279 ± 2.8	8.7		
Drought	**301 ± 4.7**	**21.0**	**5.97**	**0.01**
Warming	286 ± 5.5	8.7	0.13	0.72
Onset of flowering				
Control	279 ± 2.3	0		
Drought	279 ± 3.0	0	1.26	0.26
Warming	286 ± 4.1	0	0.94	0.33
Onset of flower senescence				
Control	293 ± 1.5	0		
Drought	**301 ± 2.8**	**0**	**4.42**	**0.03**
Warming	293 ± 2.4	0	0.01	0.92
Flowering peak				
Control	301 ± 3.6	39.1		
Drought	**-**	**68.4**	**4.62**	**0.03**
Warming	-	65.2	2.55	0.11
End of flowering				
Control	335 ± 5.3	0		
Drought	**375 ± 4.7**	**0**	**11.6**	**0.0007**
Warming	335 ± 3.3	0	0.14	0.71
2000				
>75% of flowered stems				
Control	283 ± 3.5	4.5		
Drought	**300 ± 3.8**	**21.0**	**6.76**	**0.009**
Warming	283 ± 1.3	4.3	0.23	0.63
Onset of flowering				
Control	283 ± 4.1	0		
Drought	**291 ± 23.9**	**0**	**5.77**	**0.02**
Warming	274 ± 6.9	0	0.68	0.41
Flowering peak				
Control	300 ± 6.2	13.6		
Drought	313 ± 5.1	26.3	2.52	0.11
Warming	300 ± 5.6	21.7	0.05	0.82

G. alypum

Whereas all control plants of *G. alypum* had functional flowers (>1%) both years, 32.3 and 8.8% of drought plants of *G. alypum* in 1999 and in 2000 respectively did not show any functional flower (Table 4).

Table 4. Kaplan-Meier estimates of median times for different flowering phenophases and log-rank statistics for the comparisons between survival probability curves of control (N=34) and drought (N=34) or warming (N=31) plants of *Globularia alypum* in 1999 and 2000. In all logrank tests *df*=1. * We only used plants that had functional flowers (N=34 for controls, N=24 for drought and N=31 for warming plants). Significant differences are highlighted in bold type.

	Median ± s.e.	**% censored**	**X^2**	***P***
1999				
Onset of flowering				
Control	265 ± 5.5	0		
Drought	**272 ± 6.8**	**32.3**	**11.4**	**0.0007**
Warming	**301 ± 5.7**	**0**	**8.40**	**0.004**
Onset of spring-bud flowering				
Control	265 ± 4.1	8.8		
Drought	**272 ± 4.0**	**35.3**	**8.66**	**0.003**
Warming	**279 ± 5.4**	**29.0**	**9.40**	**0.002**
Onset of autumn-bud flowering				
Control	329 ± 29.1	38.2		
Drought	**-**	**82.3**	**14.0**	**0.0002**
Warming	**322 ± 4.3**	**9.7**	**4.13**	**0.04**
*Onset of spring-bud flower senescence**				
Control	286 ± 3.0	0		
Drought	279 ± 2.9	0	0.65	0.42
Warming	286 ± 3.8	0	2.39	0.12
>75% functional flower heads				
Control	347 ± 29.1	38.2		
Drought	**-**	**73.5**	**7.0**	**0.008**
Warming	-	54.8	2.15	0.14
>50% funct. spring-bud flower heads				
Control	272 ± 5.1	44.1		
Drought	**-**	**67.6**	**4.19**	**0.04**
Warming	279	48.4	0.32	0.57
>50% funct. autumn-bud flower heads				
Control	-	61.6		
Drought	**-**	**94.1**	**10.05**	**0.001**
Warming	404	48.4	0.70	0.40
2000				
Onset of flowering				
Control	247 ± 8.7	0		
Drought	**300 ± 7.4**	**8.8**	**19.7**	**<0.0001**
Warming	257 ± 4.9	0	0.57	0.45
Onset of spring-bud flowering				
Control	247 ± 8.7	0		
Drought	**291 ± 3.2**	**8.8**	**25.1**	**<0.0001**
Warming	257 ± 4.6	0	1.42	0.23
Onset of autumn-bud flowering				
Control	-	85.3		
Drought	-	82.3	0.18	0.67
Warming	-	87.1	0.03	0.85
>75% functional flower heads				
Control	-	64.7		
Drought	**300 ± 4.01**	**35.3**	**4.38**	**0.04**
Warming	291	45.2	2.14	0.14
>50% funct. spring-bud flower heads				
Control	291 ± 9.9	44.1		
Drought	291 ± 5.5	32.3	0.25	0.61
Warming	283 ± 3.6	38.7	0.43	0.51

Both years, the time until the appearance of the first functional flower was longer in *G. alypum* plants in the drought plots than in controls (julian days: 272 and 300 in drought plots *vs.* 265 and 247 in control plots in 1999 and 2000 respectively; Table 4). Drier conditions also increased significantly the number of plants without functional spring-bud flower heads in both years (35.3% and 8.8% in drought plots *vs.* 8.8% and 0% in control plots in 1999 and 2000 respectively; Table 4) and without functional autumn-bud flower heads in 1999 (82.3% in drought plots *vs.* 38.2% in control plots; Table 4). In 2000, we did not detect any significant effect of our drought treatment on the production of functional autumn-bud flower heads, probably because of the high percentage of control plants (85.3%) that did not develop this type of flowers (Table 4 and Fig. 3). In 1999, more plants of *G. alypum* in the drought treatment (73.5%) than in controls (38.2%) did not achieve more than 75% of functional flower heads; however, in 2000, we found the opposite trend (drought: 35.3% *vs.* control: 64.7%; Table 4 and Fig. 3). Drier conditions decreased the chance that *G. alypum* plants had more than 50% of spring or autumn-bud functional flower heads compared to controls in 1999 (Table 4). In 2000, the number of functional autumn-bud flower heads was so small that we could not perform the statistical analysis.

All *G. alypum* plants in the warming treatment had functional flower heads (>1%) both years (Table 4). However, warming plants of *G. alypum* showed a 29-days later median time of first functional flower head appearance and a 14-days later median time of first functional spring-bud flower head appearance compared to control plants in 1999 (Table 4). In this year, warming decreased the number of plants with functional spring-bud flower heads (71%) compared to controls (91.2%), whereas it increased the number of plants with functional autumn-bud flower heads (warming: 90.3% *vs.* control: 61.8%; Table 4 and Fig. 3). Moreover, warming plants had later functional spring-bud flower heads (median 279) than controls (median 265), but earlier functional autumn-bud flower heads (median: warming 322, controls 329) (Table 4 and Fig. 3). By contrast, warming did not have any significant effect on the flowering phenology of *G. alypum* plants in 2000 (Table 4 and Fig. 3). Warming did never affect significantly the maximum number of functional flower heads (Table 4).

Flowering period duration

The mean flowering duration of control plants of *E. multiflora* was 117 days in both years (± 6.8 in 1999 and ± 5.4 in 2000; Fig. 4). *E. multiflora* plants in the drought treatment showed a longer flowering period than controls in 1999 (drought mean: 139 ± 5.1, $F_{1,35}$=5.1, *P*=0.03), whereas we did not find a significant effect of our drought treatment on the length of the flowering period of *E. multiflora* plants in 2000 (Fig. 4). We did not find any significant effect of warming on the duration of the flowering period of *E. multiflora* plants (Fig. 4).

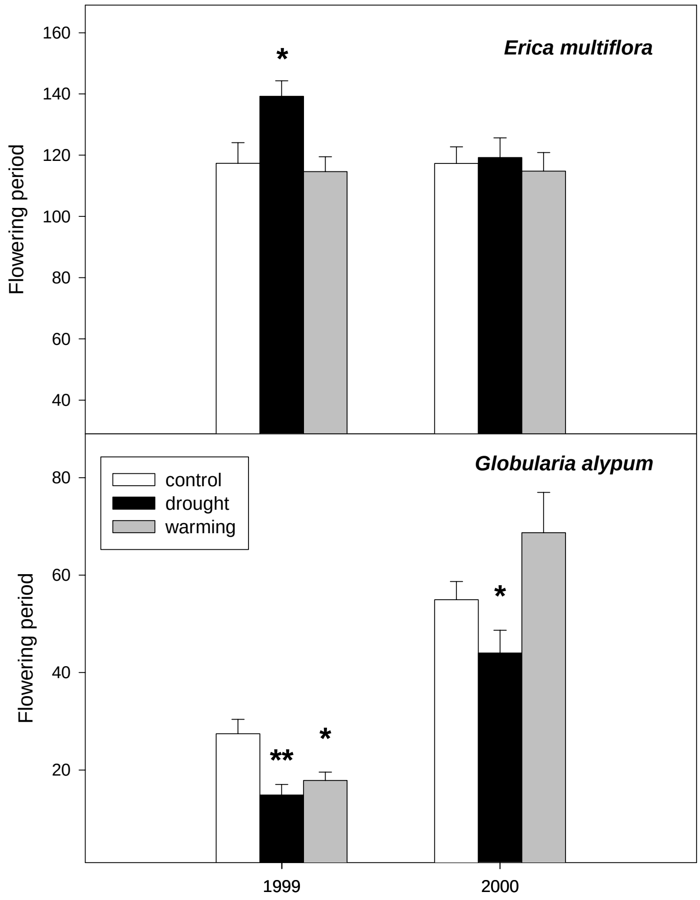

Fig. 4. *E. multiflora* flowering period and *G. alypum* flowering period of spring-bud flowers in 1999 and 2000 in control (N=23, 22 for *E. multiflora* and N=30, 34 for *G. alypum* in 1999 and 2000 respectively), drought (N=18, 19 for *E. multiflora* and N=22, 31 for *G. alypum* in 1999 and 2000 respectively) and warming plots (N=23, 23 for *E. multiflora* and N=22, 31 for *G. alypum* in 1999 and 2000 respectively).

The mean flowering duration of *G. alypum* spring-bud flower heads in control plots was 27 ± 3.0 days in 1999 and 55 ± 3.7 days in 2000 (Fig. 4). For autumn-bud flower heads, mean flowering duration in control plants was 97 ± 7.3 days in 1999 and 25 ± 8.6 days in 2000 (data not shown). In total, *G. alypum* actual flowering period in control plots lasted 124 days in 1999 and 80 days in 2000. Drought plants of *G. alypum* showed a 11-12 days shorter flowering period of spring-bud flower heads compared to controls in both years ($F_{1,46}$=11.9, *P*=0.001 in 1999 and $F_{1,59}$=6.9, *P*=0.01 in 2000). Plants under warmer conditions also had a shorter flowering period (minus 10 days in relation to controls) of spring-bud flower heads in 1999 ($F_{1,46}$=4.8, *P*=0.03). We did not find any significant effect of treatments on the length of the flowering period of autumn-bud flower heads (data not shown), although this could be because we had a low sample size of functional autumn-bud flower heads in both years. The interaction between treatment and block was not significant in any case.

Flower production

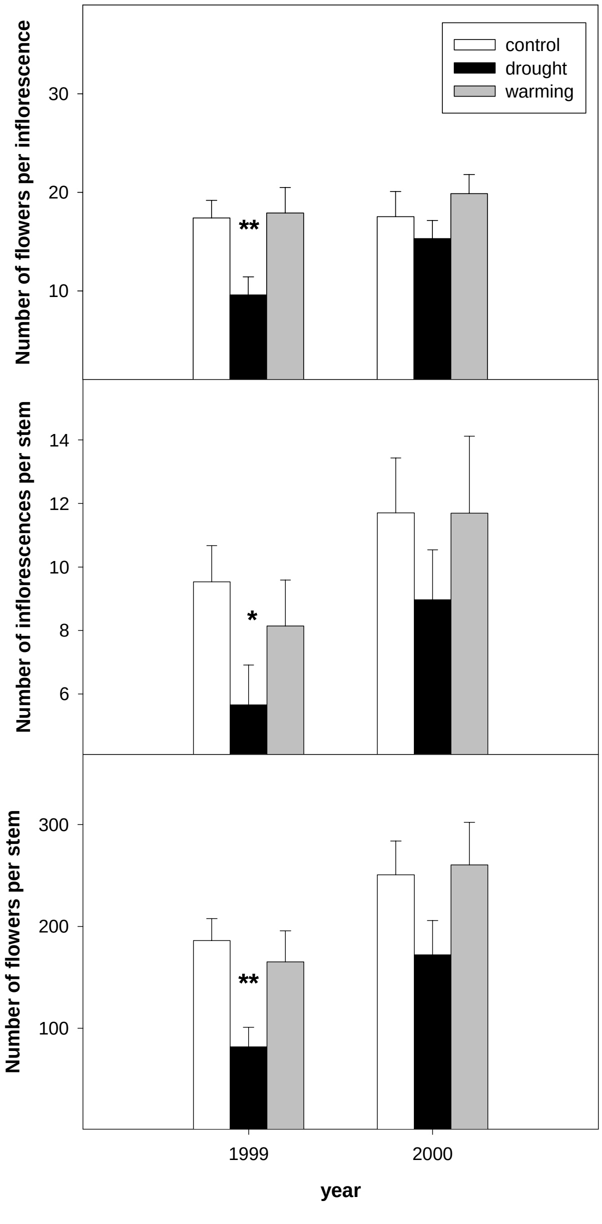

Fig. 5. Flower production of *E. multiflora* plants in control (N=14), drought (N=15) and warming (N=14) plots in 1999 and 2000.

Mean inflorescence length was lower in plants of *E. multiflora* in the drought plots than in controls in both years, although differences were significant only in 1999 ($F_{1,20}$=7.0, p=0.01 in 1999 and $F_{1,22}$=3.1, p=0.09 in 2000) (data not shown). The mean number of flowers per inflorescence ($F_{1,23}$=10.01, p=0.004), inflorescences per stem ($F_{1,23}$=5.2, p=0.03) and flowers per stem ($F_{1,23}$=14.06, p=0.001) were significantly lower in *E. multiflora* plants in the drought plots compared to controls in 1999, but not in 2000 (Fig. 5). We did not find any significant effect of warming on flower production variables (Fig. 5). The interaction between treatment and block was never significant.

Any of our tagged stems of *G. alypum* plants in the drought treatment had functional autumn-bud flower heads in 1999. This year, control plants of *G. alypum* had a median of 0.3 (range:0-5.3) autumn-bud flower heads per stem, whereas plants in the warming treatment had a median of 1.0 (range: 0.1-6) (data not shown). Thus, in 1999, drought reduced significantly (Mann-Whitney *U*=40, p<0.001) the number of functional autumn-bud flower heads of *G. alypum* plants, whereas the effect of warming was not significant. In 2000, neither blocks nor treatments affected significantly the maximum number of functional flower heads per stem that plants of *G. alypum* had in a sampling date (means ± se were 2.3 ± 1.2 for controls, 1.6 ± 0.5 for drought and 2.6 ± 0.9 warming plants). In this year, autumn-bud flower heads were a percentage very small of these total functional flower heads (median was 0 in the three treatments).

Life span of individual flowers of E. multiflora or flower heads of G. alypum

In 1999, we measured the life span of *E. multiflora* flowers. In the drought treatment, *E. multiflora* flowers tended to have a longer life-span than controls ($F_{1,18}$=3.38, p=0.08), whereas warmer conditions did not have any significant effect on life-span of *E. multiflora* flowers (Fig. 6).

In 2000, we measured the life span of the spring-bud flower heads of *G. alypum*. While these flower heads had a longer life-span in drought plots compared to controls ($F_{1,38}$=5.64, p=0.02), warming did not have any significant effect on their life-span (Fig. 6).

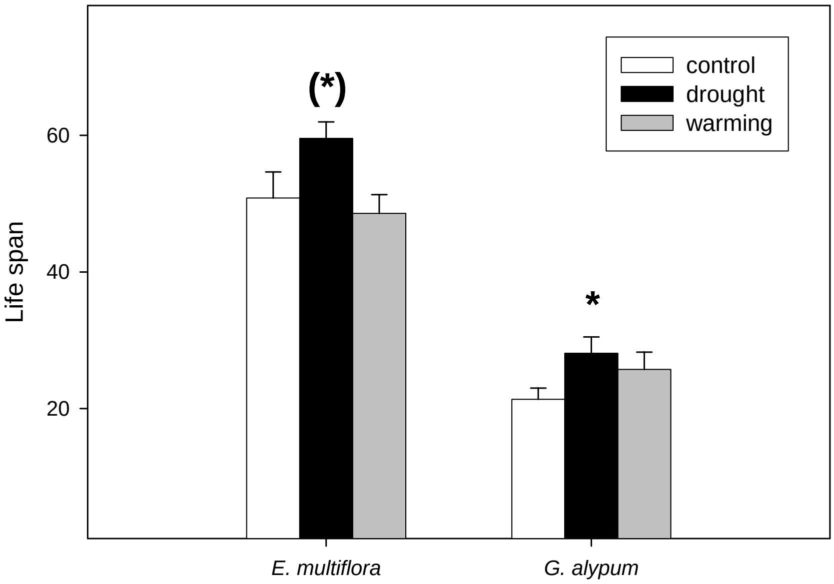

Fig. 6. Life span of *E. multiflora* flowers in 1999 and of *G. alypum* spring flower heads in 2000 for control (N=11 for *E. multiflora* and N=21 for *G. alypum*), drought (N=9 for *E. multiflora* and N=19 for *G. alypum*) and warming plants (N=16 for *E. multiflora* and N=19 for *G. alypum*).

Corolla length of *E. multiflora* flowers

In 2000, we measured the corolla length of *E. multiflora* flowers and we found that *E. multiflora* flowers had shorter corollas in both treatments, drought ($F_{1,24}$=22.99, p<0.001) and warming ($F_{1,24}$=5.99, p=0.02), compared to controls (Fig. 7). There was no significant interaction between treatments and blocks.

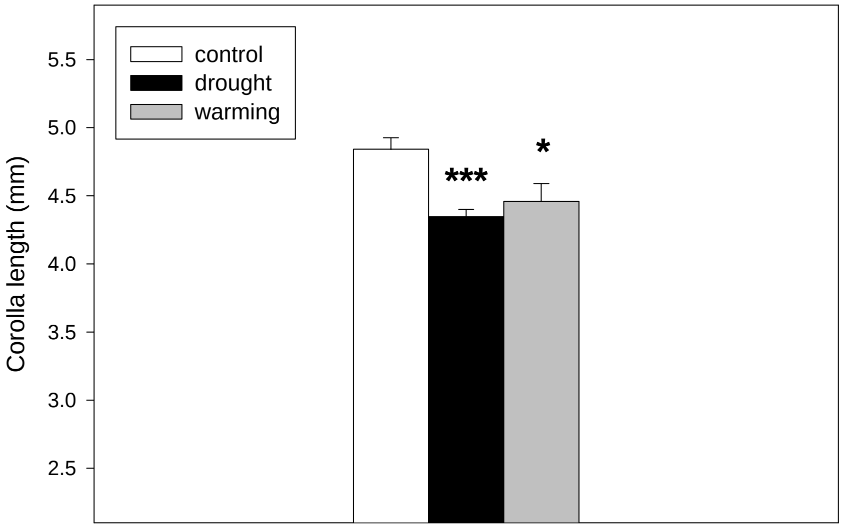

Fig. 7. Corolla length (mm) of *E. multiflora* flowers of control, drought and warming plants (N=15 for each treatment) in 2000. * p<0.05 *** p<0.001.

Discussion

Flowering patterns

In the Mediterranean Basin, most plant species reach their blooming peak in spring and have characteristically short flowering periods of one-three months (Floret et al. 1989, Bosch et al. 1997, Castro-Díez and Montserrat-Martí 1998). *Erica multiflora* and *Globularia alypum* are two exceptions to both flowering characteristics, since they have their blooming peak in autumn (end of September-October) and flower for nearly 7 months in our study site (from September to March). It has been suggested that extended flowering in Mediterranean environments might act as a mechanism to compensate for reproductive failure in some parts of the season (Picó and Retana 2001). In species with extended flowering periods, surplus flower production may serve as a buffer when adverse weather conditions or competition from other flowering species reduces pollen flow (Stephenson 1981). Moreover, extended duration of flowering could allow individuals to track and accumulate resources needed for seed maturation in environments where resources are either temporally unpredictable or sparse (Rathcke and Lacey 1985).

The strong and highly synchronous between years peak of functional flowers of *E. multiflora* suggests a disadvantage to out-of-season individuals, possibly due to low water availability early in the season and pollination reduction late in the season. In a situation in which out-of-season individuals are at a disadvantage, there would be a strong stabilizing selective pressure in favor of synchrony (Fenner 1998).

In contrast to *E. multiflora*, control plants of *G. alypum* showed a large interannual variation in their flowering pattern. While control plants of *G. alypum* had a bimodal amplitude of flowering in the first year, they showed a basically unimodal flowering pattern in the second year. Such difference was mainly due to the different production of functional autumn-bud flower heads between years. While a 61.8% of *G. alypum* plants had functional autumn-bud flower heads in 1999, this percentage was 14.7% in 2000. Because autumn inflorescences of *G. alypum* emerge from new growth, this difference was a consequence of the highest number of new shoots produced by control plants of *G. alypum* in autumn 1999 (1.7 ± 0.5 new shoots/stem) compared to autumn 2000 (0.2 ± 0.07 new shoots/stem) (Llorens et al. unpublished data). The higher amount of rainfall registered during the growth period of autumn 1999 in relation to autumn 2000 (October rainfall: 92 mm in 1999 *vs.* 64 mm in 2000) was probably the cause of the higher shoot growth in autumn 1999. Nevertheless, a lower production of functional spring-bud flower heads in 1999 than in 2000 might also contribute to a higher production of functional autumn-bud flower heads in the first year. If this was the case, the bimodal flowering pattern of *G. alypum* would allow plants to achieve higher fecundity during the second blooming peak when flowering or fruiting partly or completed failed in the first blooming peak. Alternatively, interannual differences in flowering might be caused by a long-term internal rhythm. Costs associated to the high growth and flower production in autumn 1999 might have constrained growth and flower production of *G. alypum* plants in autumn 2000. Since production and maintenance of reproductive tissues have an energetic cost, a high level of reproduction at one point in time may draw down reserves and consequently constrain future reproduction (e.g., Snow and Whigham 1989, Primack and Hall 1990, Sandvik 2001).

The high overlapping of the flowering period of these two entomophilous species suggests a strong competition for pollinators between them. However, it has been reported that different plant species may, in fact, facilitate each others' pollination by attracting more pollinators to an area because of greater total food reward (Ollerton and Lack 1992, Fenner 1998). In our study site, despite of the overlapping of the flowering periods of *E. multiflora* and *G. alypum*, their flowering peaks (>75% of functional flowers) did not coincide (Figs. 2 and 3).

Treatment effects on flowering phenology

Previous studies on Mediterranean plant species showed a small effect of precipitation on the beginning of flowering (Spano et al. 1999, Picó and Retana 2001, Peñuelas et al. 2002). By contrast, we found a strong effect of drier conditions on the flowering phenology of the two studied Mediterranean shrubs. Indeed, drought delayed most of the flowering phenophases of both species in both years, although the delay was, in general, stronger for *G. alypum* than for *E. multiflora* (Tables 3, 4 and Figs. 2, 3). For instance, drought delayed significantly the onset of flowering of *E. multiflora* only in 2000, whereas it delayed the onset of flowering of *G. alypum* in both years (Figs. 2, 3). Moreover, in 2000, drought delayed the onset of flowering of *E. multiflora* 8 days (probably less since this is the interval between sampling dates), whereas it delayed the onset of flowering of *G. alypum* 53 days considering total functional flower heads (i.e. without distinguishing between spring and autumn-bud flower heads) or 44 days considering functional spring-bud flower heads. Thus, our results suggest that water is a crucial resource for these species during the flowering period, especially for *G. alypum*, and are in agreement with the delayed flowering caused by moisture limitation reported in a desert annual by Fox (1990). Lower leaf net photosynthetic rates in drought plants compared to controls (Llorens et al. 2003b) might also explain the observed delays in the onset of flowering if plants require accumulating a certain amount of carbohydrates to flower, as it has been suggested for some species (Rathcke and Lacey 1985). A delay and/or an extension of the vegetative growth period might also delay the onset of flowering of *E. multiflora* and the onset of autumn-bud flowering of *G. alypum*.

We did not find any significant effect of warming on the timing of the different flowering phenophases of *E. multiflora*, whereas enhanced temperatures modified in opposite directions the onset of spring-bud and autumn-bud flowering of *G. alypum* plants in 1999. In this year, while warmer conditions delayed the onset of flowering of

G. alypum plants (29 days considering the time to first functional flower head or 14 days considering the time to first functional spring-bud flower head), they advanced the appearance of functional autumn-bud flower heads by 7 days (or less since this is the interval between consecutive measurements) (Table 4 and Fig. 3). By contrast, warming did not have any significant effect on the flowering phenology of *G. alypum* plants in 2000 (Table 4 and Fig. 3). On one hand, the observed delay in the onset of flowering of *G. alypum* plants in the warming treatment in 1999, but not in 2000, is probably related to a greater reduction of soil humidity by warming in 1999 than in 2000. Considering August, September and October, average soil humidity difference between control and warming plots was 2.5 ± 0.6% in 1999 and –0.03 ± 0.6% in 2000. In previous studies, we showed a higher sensitivity to soil humidity of *G. alypum* compared to *E. multiflora* plants (Llorens et al. 2003a, b). On the other hand, the earlier onset of autumn-bud flowering by warming in 1999 and not in 2000 was probably due to the colder temperatures registered during November 1999 (mean temperature: 8.4 ± 0.6 and 9.8 ± 0.3ºC in 1999 and 2000 respectively). Nevertheless, the low sampling size in 2000, due to the high number of plants without functional autumn-bud flowering, might have hidden treatment effects in this year.

Treatment effects on flowering period length

Drought plants of *E. multiflora* showed a delay in the onset of flower senescence (Table 3) and as a consequence they had a longer flowering period than controls in 1999 (Fig. 4). This delay might be caused by a later production of new flowers (onset of flowering was not delayed) or by the lengthening of flower life span. Although we cannot discard the first hypothesis, we found that *E. multiflora* flowers tended to have a longer life span in the drought treatment than in control plots (see below). By contrast, we did not find any significant effect of drought treatment on the flowering period duration of *E. multiflora* plants in 2000 (Fig. 4), since drier conditions delayed both, the onset of flowering (Table 3) and the onset of flower senescence in this year (not measured, but deduced from Fig. 2). It has been suggested that, within individuals, extended duration of flowering may be advantageous for reducing the uncertainty of pollination (Rathcke and Lacey 1985). However, O'Neil (1999) demonstrated that in *Lythrum salicaria* the extent to which longer flowering duration was advantageous was dependent on the date of flowering initiation, since later in the flowering period the number of pollinator visits abruptly decreased, independently of the density of plants in flower. Santandreu and

Lloret (1999) reported that insect visitors are also less abundant at the end of the flowering period of *E. multiflora*, probably as a consequence of low temperatures. Therefore, a longer flowering period in *E. multiflora* plants in the drought treatment might not be advantageous, at least from the point of view of pollination.

In contrast to *E. multiflora*, drought treatment reduced the spring-bud flowering period of *G. alypum* plants in both years (Fig. 4), because it delayed the onset of flowering more than the onset of spring-bud flower senescence (Table 4 and Fig. 3). Nevertheless, the life span of *G. alypum* spring-bud flower heads was significantly increased by drought (see below). This suggests that *G. alypum* plants in the drought plots produced less spring-bud flower heads through the flowering period than controls (see below). Since drought treatment did not modify significantly the length of autumn-bud flowering period, the total length of the flowering period of *G. alypum* plants was shorter in the drought plots than in controls in both years. These results are in accordance with those of Steyn et al. (1996), who found that drought stress shortened the flowering period of five Namaqualand ephemeral species. They are also in accordance with the reported negative correlation between the initiation of flowering and flowering duration of *Lythrum salicaria* (O'Neil 1997).

Whereas we did not find any significant effect of warming treatment on the length of the flowering period of *E. multiflora* plants, warming reduced the length of the spring-bud flowering period of *G. alypum* plants in 1999 (Fig. 4). Such reduction was due to a delay in the onset of spring-bud flowering of *G. alypum* plants, without a change in the onset of senescence (Table 4). Since there was no effect of warming on the length of the autumn-bud flowering period, the total length of the flowering period of *G. alypum* plants was shorter in the warming treatment than in controls in 1999. By contrast, we did not find any significant effect of warming on the length of the flowering period of *G. alypum* plants in 2000. The different effect of warming between years was probably related to the stronger effect of this treatment on soil humidity in 1999 (see above). Dunne et al. (2003) reported that experimental warming extended flowering duration (due to an advance in the timing of flowering) of early flowering species, but not of late flowering species, of a subalpine meadow.

Treatment effects on flower production

Compared to controls, plants of *E. multiflora* and *G. alypum* showed a lower production of functional flowers under our experimental drier conditions. In general, the effect of drought treatment on flower production of both species was stronger in 1999 than in 2000, which was probably related to the fact that 1999 was drier than 2000 (table 1) and moreover, drought treatment was stronger in the first year. During the months of September, October and November, drought plots had on average 14 ± 1.4% lower soil humidity than controls in 1999, whereas the difference was only of 5 ± 1.9% in 2000. Decreases in flower production with decreases in water availability have been shown in other studies (Herrera 1991, Steyn et al. 1996, Tyler 2001) and are in agreement with the idea that the total amount of water needed to produce and sustain flowers may represent a high cost for the plant (Nobel 1977, Reich and Borchert 1982). Moreover, reductions in photosynthesis by drought (Llorens et al. 2003b) might also contribute to decrease the production of flowers. Floral organs draw carbon, nutrients and water from the vegetative portion of the plant, not only during their initial growth and expansion, but also continuously over the flower's life span (Galen 1999).

Till now, most of the temperature-elevation experiments have been performed on arctic, alpine or subalpine ecosystems and have often found increases in the number of flowers or seeds per plant (e.g., Wookey et al. 1993, Alatalo and Totland 1997, Welker et al. 1997, Sandvik and Totland 2000, Sandvik 2001), although decreased flowering by warming has also been reported (De Valpine and Harte 2001). In our study, we did not find any significant effect of warming treatment on the flower production of *E. multiflora* plants (Table 3, Figs 1, 2, 5), whereas warming effects on flower production of *G. alypum* plants were dependent on the year and the type of flower head. In 1999, enhanced temperature decreased the number of *G. alypum* plants with functional spring-bud flower heads (control: 91.2% *vs.* warming: 71%), whereas it increased the number of *G. alypum* plants with functional autumn-bud flower heads (control: 61.8% *vs.* warming: 90.3%). The negative effect of warming on the number of functional spring-bud flower heads in autumn 1999 was not related to differences in shoot production between warming and control plants in spring 1999, since warming plants showed a higher number of new shoots than controls (5.9 ± 0.5 *vs.* 4.5 ± 0.5 new shoots per stem in warming and control plots, respectively). Since *G. alypum* new shoots always produce a terminal flower bud, these results indicate that there were a higher number of spring-buds aborted in warming plots compared to controls in autumn 1999. The increased spring-bud flower abortion by warming in 1999 but not in 2000 might be related to the higher temperatures registered in 1999 compared to 2000 during the period of spring-bud flower formation (average of mean temperatures of April and May was 14.1ºC in 1999 and 13.5ºC in 2000), or during the summer (average of mean temperatures of July and August was 21.6ºC in 1999 and 21.0ºC in 2000). It has been shown that temperature increases beyond the range to which plants are adapted may cause heat damage on warm days, which can decrease reproduction (Sandvik and Totland 2000). Alternatively, the lower soil moisture in warming plots during the months of August, September and October of 1999 and not of 2000, might have acted direct and/or indirectly (e.g., through the observed reduction in the length of the spring-bud flowering period) reducing the spring-bud flower head development in this year.

Since there were no differences in autumn shoot production between control and warming plants in 1999 (controls: 1.7 ± 0.5 *vs.* warming: 1.4 ± 0.4 new shoots per stem), the higher number of plants with functional autumn-bud flower heads in the warming plots would indicate that autumn shoots developed more functional flowers in warming plots than in controls in 1999. The observed advance in the onset of autumn-bud flowering in warming plots compared to controls in 1999 (and not in 2000) might have allowed a higher production of functional autumn-bud flower heads in warming plots in this year. Lower temperatures in November 1999 than in 2000 (mean temperature: 8.4 ± 0.6 and 9.8 ± 0.3ºC in 1999 and 2000 respectively) might explain the different effect of warming treatment on functional autumn-bud flower production between years. Moreover, the lower spring-bud flower head production in warming plots in 1999 might have induced the observed increase in autumn-bud flower head production in this year, if we consider that the bimodal flowering of *G. alypum* may result from a mechanism to compensate reproductive failure early in the season.

Flower life span

Floral longevity (the length of time that flowers remain open and functional) is an important plant trait that influences the amount of pollen received and disseminated by a plant and hence, its reproductive fitness (Primack 1985). In our study, mean life span of flowers of *E. multiflora* control plants was 50.8 ± 3.8 days, whereas it was 59.5 ± 2.4 for flowers of *E. multiflora* plants in the drought treatment. Spring-bud flower heads of *G. alypum* also showed a longer life span in the drought plots compared to controls (21.4 ± 1.6 days in controls *vs.* 28.1 days in drought plots). Thus, longer-lived flowers under drier conditions might counteract the scarcity of pollinators that plants may encounter due to delays in the onset of flowering. However, maintenance of flowers has a cost for the plant (Ashman and Schoen 1997). In addition to carbon cost of flower maintenance and nectar production, there may be a maintenance cost associated with water loss due to flower transpiration and/or nectar production, and this could be substantial (Nobel 1977, Ashman and Schoen 1994, 1997). In particular, nectar production may use up to 37% of a plant's available energy (Southwick 1984, Pyke 1991) and its secretion is dependent on water availability of the soil (Zimmerman 1983). Thus, experimental evidence supports floral longevity as an adaptation that balances rates of pollen receipt and removal against the cost of floral maintenance (Ashman and Schoen 1994, 1997).

A possible indirect consequence of floral longevity is reduced floral production (Ashman and Schoen 1994, Schoen and Ashman 1995). It has been proposed that there would be a fixed pool of resources for flowering and hence a trade-off between allocation of resources to floral construction versus floral maintenance (Ashman and Schoen 1994, Schoen and Ashman 1995). Our results are in agreement with the existence of such trade-off, since we found that drought plants had a lower number of flowers (thus, lower construction costs), but their flowers had a longer life span (thus, higher maintenance costs) than controls. Therefore, a reduction in the number of flowers, together with an increase in their life span, seems to be a more advantageous strategy for plants growing under lower water availability than for instance, a decrease in flower life span without changing the number of flowers.

Compared to our results, Santandreu and Lloret (1999) reported a shorter flowering period of *E. multiflora* (September to December) and a shorter life span of individual flowers of this species (about 2 weeks) in a Mediterranean locality 30 Km north of Barcelona. This locality is colder (mean monthly temperature is 10.2ºC at the nearest weather station) and more humid (mean annual precipitation is 928 mm) than our study site (Garraf). The longer flowering period and flower life span of *E. multiflora* plants in the drier site (Garraf) is in agreement with the increase in flowering period duration and flower life span that we observed in the drought plots compared to controls. Moreover, the higher mean number of *E. multiflora* flowers per inflorescence (43.4 ± 2.2) reported by Santandreu and Lloret (1999) compared to our results in Garraf (17.4 ± 1.8 and 17.5 ± 2.5 for control plants in 1999 and 2000, respectively) is also in accordance with the higher number of flowers per inflorescence that we found in *E. multiflora* plants in control plots compared to drought plots.

Flower size

It has been shown that in general, animal pollinators prefer large over smaller flowers (e.g., Young and Stanton 1990, Cresswell and Galen 1991, Galen 1999, Carroll et al. 2001), probably because nectar production correlates positively with flower size in a number of species (Stanton and Young 1994). Larger flowers result in increased visitation and pollen removal (Young and Stanton 1990, Cresswell and Galen 1991), mean seed weight per fruit (Sakai and Sakai 1995), and sometimes in greater percent seed set (Galen 1989). However, Galen et al. (1999) demonstrated that flowers with large corollas required greater water uptake during flower expansion and maintenance than flowers with smaller corollas. Therefore, they suggested that the cost of reproduction under drought might select for smaller, less conspicuous flowers, despite their disadvantage in pollination success. Indeed, in *Polemonium viscosum* populations of the Rocky Mountains, flower size decreases along a gradient of increasing aridity (Galen et al. 1987). When comparing flower size of *Polemonium viscosum* from two sites at different altitudes, Galen et al. (1987) found approximately 10% smaller corollas in the site with more water stressed (more negative leaf water potentials) plants. Artificially induced drought during flowering of *Epilobium angustifolium* led to a 33% decrease in flower size relative to controls (Carroll et al. 2001).

Our results support the conclusions of these previous studies, since we found that *E. multiflora* flowers were significantly smaller (had a shorter corolla length) in drought and warming treatments compared to controls. This suggests that the same corolla size may incur greater physiological costs for *E. multiflora* plants flowering under drier or warmer conditions than in control plots. Hence, the observed reductions in flower size probably diminish such costs, but might decrease pollinator visitation rates and, thus, plant reproductive success.

Final remarks

In the present study, we observed significant effects of drier or warmer conditions on the flowering phenology, flower production, life span and size of two co-occurring Mediterranean species, *Erica multiflora* and *Globularia alypum*. These changes might have important effects on individual plant reproductive success, interactions of the plants with other organisms, plant population dynamics, and ecosystem functioning (Peñuelas and Filella 2001, Dunne et al. 2003).

Flowering time is affecting reproductive success and hence fitness of most plant species, through its effect on reproductive processes such as pollination, total plant seed production, and the timing of seed dispersal (Primack 1980, Augspurger 1981, Gross and Werner 1983, Rathcke and Lacey 1985, Widén 1991, O'Neil 1999, Kelly and Levin 2000, Pilson 2000). Thus, delays in autumn flowering of *E. multiflora* and *G. alypum* might result in a reduction in their seed set and seedling establishment as a consequence of (i) reductions in pollination due to scarcity of pollinators late in the season, (ii) frost injuries to flowers, (iii) failure to produce mature fruits and seeds if frosts occur before maturation, (iv) resources limitation of reproductive output, and (v) seeds shed at an unsuitable time. The observed reductions in the abundance of flowering of both species under drier conditions might also reduce their reproductive success and ultimately their fitness. In fact, Lloret et al. (unpublished data) found a lower number of emerging seedlings of *G. alypum* under drought treatment, although drought effect on seed rain was not statistically significant.

The strong effect of drier conditions on flowering phenology, flower production, life span and size found here, supports an important role of water availability in determining year-to-year shifts in these parameters in these Mediterranean species, in contrast to the weak effects reported in previous studies (Spano et al. 1999, Picó and Retana 2001, Peñuelas et al. 2002). As previous studies were mainly focused on species that flower in spring, these contrasting results might imply a stronger effect of drier conditions on the phenology and flower production of species that flower in autumn and winter. Further studies are warranted to resolve this question. In contrast with colder temperate and artic ecosystems, and as expected for these already warm shrubland ecosystems, warming effects were not so strong and were more variable depending on the species. Nevertheless, our results suggest that enhanced temperatures in the Mediterranean could beneficiate species whose flower bud production depends on late autumn or winter growth. In conclusion, abiotic factors such as water availability and temperature might act as a source of selection on reproductive traits of these Mediterranean shrubs.

Our results also show that individuals of these two species may adjust to differences in resource status through plastic changes in allocation to flowering. It is generally assumed that phenotypic plasticity (i.e. the environmental modification of the phenotype) is an alternative to genetic differentiation for enhancing individual survival and reproduction in variable environments (Schlichting 1986, Aronson et al. 1993).

Plasticity may confer physiological buffering to adverse environmental conditions as well as an improved response to favourable conditions, and thereby maintain relative fitness over a range of naturally occurring environments (Thompson 1991, Aronson et al. 1993).

Our results support the idea that future decreases in water availability or increases in temperature will probably have different, even opposite, effects on flowering phenology of co-occurring species. The stronger effect of drier and warmer conditions on the flowering patterns of *G. alypum* compared to *E. multiflora* coincides with previous studies that show that *G. alypum* is a species more sensitive to these climatic changes (Llorens et al. 2003b, Peñuelas et al 2003). Such differential sensitivity to climate change between these two common species of the coastal Mediterranean shrublands, might well lead to a change in species composition and structure of these communities.

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CHAPTER 5

Effects of an experimental increase of temperature and drought on the photosynthetic performance of two Ericaceous shrub species along a North-South European gradient

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ECOSYSTEMS, in press

Abstract

Plant ecophysiological changes in response to climatic change may be different in northern and southern European countries, since different abiotic factors constrain plant physiological activity. We studied the effects of an experimental warming and drought on the photosynthetic performance of two Ericaceous shrubs (*Erica multiflora* and *Calluna vulgaris*) along a European gradient of temperature and precipitation (UK, Denmark, The Netherlands and Spain). At each site, passive warming treatment was applied during the night throughout the whole year, whereas drought treatment excluded rain events over 6-10 weeks during the growing season. We measured leaf gas exchange, chlorophyll *a* fluorescence and leaf carbon isotope ratio ($\delta^{13}C$) during the growing seasons of 1999 and 2000. Leaf net photosynthetic rates clearly followed a gradient from northern to southern countries in agreement with the geographical gradient in water availability. Accordingly, there was a strong correlation between net photosynthetic rates and the accumulated rainfall over the growing season. Drought plants showed lower leaf gas exchange rates than control plants in the four sites. Interestingly, although leaf photosynthetic rates decreased along the precipitation gradient and in response to drought treatment, drought plants were able to maintain higher leaf photosynthetic rates than control plants in relation to the accumulated rainfall over the months previous to the measurements. Drought plants also showed higher values of potential photochemical efficiency (Fv/Fm) in relation to controls, mainly at midday. The warming treatment did not affect significantly any of the studied instantaneous ecophysiological variables.

Key words: *Calluna vulgaris*, chlorophyll fluorescence, climatic change, drought, *Erica multiflora,* European transect, leaf gas exchange, stable isotopes, warming.

Introduction

Considerable evidence is now available showing that the global average surface temperature has increased by 0.6 ºC (± 0.2 ºC) since the late 19th century (IPCC 2001). Indeed, most of Europe has experienced increases of about 0.8 ºC on average. Global climatic models predict that over the period 1990 to 2100 the globally averaged surface temperature will rise by 1.4 to 5.8 ºC (IPCC 2001). Although future precipitation patterns are more uncertain, global climate change is likely to increase the risk of summer drought in central and southern Europe (IPCC 2001). These climatic changes may induce ecophysiological changes in plants that could affect their long-term performance (Michelsen and others 1996).

Over the last decade, a growing number of field experiments simulating some of the predicted climatic changes were initiated around the world (e.g. see Shaver and others 2000 for a review of warming experiments). However, until now most of the techniques used to experimentally warm ecosystems alter the environmental conditions (e.g. light, humidity, soil structure) and moreover, experiments usually simulate a diurnal increase in temperature rather than the predicted stronger increase in T_{min} (night time temperature) (IPCC 2001). Furthermore, whereas experimental manipulations provide short-term responses to climate change (annual to decadal scale), studies along natural climatic gradients are thought to mirror longer-term changes (decades to centuries) (Shaver and others 2000). To date, the majority of the climate experiments have been conducted in north temperate, boreal and arctic ecosystems. However, climate change responses of vegetation could be different in northern and southern countries of Europe, since different abiotic factors constrain plant physiological activity. Therefore, a clear need exists to also explore the responses of warmer and/or drier ecosystems, such as the Mediterranean ones, to climate change.

In Mediterranean-type regions, drought proves to be the climatic factor essentially responsible for the restriction of growth and survival of evergreen woody plants (Larcher 2000). The predicted extension of the drought period and the indirect effects of future warming, acting through changes in evapotranspiration and soil dryness, may constraint severely the physiological activity of Mediterranean plants. In addition, warming may increase the likelihood of heat stress in Mediterranean plants during summer. Conversely, European northern ecosystems may be particularly sensitive to climate change due to low temperatures constraints on biological activity

(Körner and Larcher 1988). Then, increases in minimum temperatures are expected to impact these ecosystems by alleviating such temperature constraints (Rustad and others 2001).

The aim of the present study was to test how future warming and drought events will affect the photosynthetic performance of two Ericaceous shrubs (*Erica multiflora* and *Calluna vulgaris*). *E. multiflora* was studied in northern Spain, whereas *C. vulgaris* was studied in Denmark, the UK and The Netherlands. The four studied sites represent a European gradient in temperature and precipitation. In addition, climatic manipulations were performed in each site in order to simulate the expected increase in drought and temperature. We extended the drought period by removing rain during the growing season and we warmed plots by avoiding IR dissipation at night over the whole year. We assessed photosynthetic performance of plants by means of leaf gas exchange and chlorophyll *a* fluorescence measurements during the growing season and through the analyses of leaf carbon isotope ratio ($\delta^{13}C$) at the end of the growing season.

Leaf gas exchange measurements (stomatal conductance and net photosynthetic and transpiration rates) are among the classical instantaneous methods to detect functional limitations in plants imposed by environmental factors. It is now well established that the rate of CO_2 assimilation in the leaves is depressed at moderate leaf water deficits or even before leaf water status is changed in response to a drop in soil water potential, mostly as a consequence of stomatal closure (see reviews Chaves 1991; Yordanov and others 2000). On the other hand, photosynthesis is temperature dependent and exhibits a temperature optimum. In response to small and short-term shifts in ambient temperature away from this optimum, photosynthetic activity decreases (Battaglia and others 1996).

Chlorophyll *a* fluorescence is one of the most important non-invasive method for monitoring changes in the functioning and the regulation of the photosynthetic apparatus (Schreiber and others 1994). The variable to maximum fluorescence ratio (Fv/Fm) of a dark-adapted leaf is a reliable measure of the *potential* photochemical efficiency of PSII and has been used as an estimate of the functional state of the photosynthetic apparatus at a given environmental situation (see Maxwell and Johnson 2000 for a recent review). Exposure of leaves to light levels in excess of what can be utilized in photosynthesis results in photoinhibition (Krause 1988), which is reflected by a reduction in the photochemical efficiency of PSII (Long and others 1994). As far as photoinhibition is reversible within minutes to hours, it can be viewed as a protective mechanism that serves to dissipate excessive energy (Krause 1988; Osmond 1994). In the long-term, chronic photoinhibition results in photon damage to the photosystem II reaction centre, the end point of which is the destruction of the functioning photosynthetic apparatus and the oxidation of chlorophyll (Osmond 1994).

During photosynthesis, plants discriminate against the heavier isotope of carbon (^{13}C) in ways which reflect plant metabolism and environment (Farquhar and others 1989). Environmental conditions influence the plant carbon isotope composition by affecting the ratio of intercellular and atmospheric CO_2 concentrations (ci/ca), which is controlled by stomatal conductance and the rate of CO_2 assimilation (Farquhar and others 1989). Therefore, the fractionation of carbon isotopes integrates information about how a plant regulates carbon dioxide and water fluxes over long periods, being $\delta^{13}C$ for C_3 plants positively correlated with the ratio of carbon assimilation to transpiration (water-use efficiency, WUE) (Farquhar and others 1989). Along continental-scale gradients elsewhere in the world, it has been shown that, for herbaceous and shrubs, increasing aridity can result in increasing stomatal closure and associated increase in $\delta^{13}C$ of leaves (Ehleringer and Cooper 1988).

We approached our aim of investigating climate change effects on the physiological activity of Ericaceous shrubs by testing the following hypotheses:

1) We expected to find decreasing photosynthetic performance of control plants with decreasing water availability, i.e. from northern to southern countries.

2) We hypothesised that a reduction in water availability during the growing season would decrease leaf gas exchange rates mainly in the Spanish site. The reduction in photosynthetic energy conversion under drier conditions may result in enhanced photoinhibition if leaves are exposed to excessive levels of radiation. Moreover, the extension of the drought period would rise both instantaneous (A/E) and integrated water-use efficiency, the latter shown by increased leaf $\delta^{13}C$ values.

3) Warming treatment would have a stronger impact in the three northern sites than in the Mediterranean site. In northern vegetation, temperature increases may stimulate photosynthetic rates directly due to warmer mornings or indirectly through increases in decomposition and nutrient mineralization and thus nutrient availability (Rustad and others 2001). The stimulation of photosynthesis may decrease the incidence of photoinhibition. On the contrary, in Mediterranean vegetation, positive direct effects of

warming on photosynthesis would be relatively smaller, because temperatures are already near the optimum for photosynthesis (Shaver and others 2000).

Methods

Study site and plant species

The present study was carried out at four sites: Garraf (Spain), Oldebroek (The Netherlands), Clocaenog (UK) and Mols (Denmark), spanning a gradient in temperature and precipitation (see Beier and others in this issue for the description of the sites). Garraf site is a Mediterranean shrubland with abundant *Erica multiflora* plants, whereas the three northerly sites are *Calluna vulgaris* heathlands. *Erica multiflora* L. and *Calluna vulgaris* (L.) Hull are two perennial shrubs of the Ericaceae family. *E. multiflora* is a short-leafed sclerophyllous shrub that typically occurs on basic soils of the western Mediterranean Basin (Vilà and Terradas 1995). *C. vulgaris* is an upland slow-growing species which typically occupies nutrient-poor acidic soil types in open situations (Iason and Hester 1993).

Experimental system

Two types of climatic manipulations of ecosystems were performed using automatically sliding roofs:

- Ecosystem warming: The roofs (reflective curtains) covered the vegetation and soil during night, reducing the loss of IR radiation, over the whole year. A rain sensor automatically removed the curtains during rain events.
- Extended summer drought: The roofs (transparent PE plastics) covered the vegetation during all rain events over 6-10 weeks of the growing season in the spring and summer.

Nine plots (20 m^2 per plot) were established in each site: 3 untreated controls, 3 warming and 3 drought plots. The warming treatment started in March-May 1999, whereas drought treatment was applied for a 6-10 week period between March and August each year (Beier and others 2003, this issue). During the growth period, the warming treatment increased the average air temperatures by 0.3-1.4ºC (mean 0.5ºC) and the soil temperatures by 0.4-1.1ºC (mean 0.8ºC) at all sites. During the drought period 60-90% of the water was removed, amounting to *ca* 30-70% of the total yearly water input (Beier and others 2003, this issue). Drought treatment reduced the soil water content by 50-80% at the peak of the drought (Beier and others 2003, this issue).

Measurements of leaf gas exchange rates and chlorophyll *a* fluorescence of *C. vulgaris* (in the three northerly sites) and *E. multiflora* (in Spain) were conducted during the growing season, at least one month after starting the drought treatment, in 1999 and 2000. Measurements were performed in 3-4 randomly selected plants in each plot, except in 2000 in Denmark, where no plants were available in some plots because a heather beetle attack removed all the *C. vulgaris* resprouts.

Leaf gas exchange measurements

Net photosynthetic (A) and transpiration rates (E) were determined on one sun exposed shoot, from the current year, per plant. The tips of non-flowering heather shoots (*ca* 2-3 cm) were used. Although we performed two rounds of measurements (one in the morning and the other in the afternoon) in each site each year, in the statistical analyses we used the average of the two rounds, since there were no significant differences between them. Measurements were performed with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England), which also monitored photosynthetically active radiation (PAR) and air temperatures in the leaf chamber. All results are expressed on area basis, which was measured using ImagePC (version α9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of a measured shoot. Instantaneous water-use efficiency (WUE), defined as mmol of net CO_2 uptake per mol of H_2O transpired, was calculated by dividing instantaneous values of A by E.

Chlorophyll a fluorescence measurements

Components of chlorophyll fluorescence were quantified with a portable modulated fluorometer PAM-2000 (Heinz Walz GmbH, Effeltrich, Germany). One sun exposed shoot from the current year per plant was measured *in situ*. After a dark adaptation period of at least 30 min, we obtained minimum and maximum dark-adapted fluorescence (F_0, Fm) and Fv/Fm, where $Fv=Fm-F_0$ (see Schreiber and others 1994 for methodology description). Fv/Fm is reflecting the potential (maximum) efficiency of photosynthetic energy conversion of PSII. We measured Fv/Fm in UK, The Netherlands and Spain in 1999 and in the four studied countries in 2000. Measurements were performed at predawn and midday.

The actual photochemical efficiency of PSII in the light-adapted state was estimated as: Φ_{PS2}=(Fm'-F)/Fm', where F is the steady-state fluorescence yield under the given environmental conditions, and Fm' is the maximum level of fluorescence obtained during a saturating flash of light (when all the PSII traps are closed) under the same environmental conditions (Genty and others 1989). The theoretical maximum Φ_{PS2} is 1 electron/photon absorbed. In practice, the maximum values obtained experimentally from fluorescence analysis are around 0.85 (Krall and Edwards 1992). We measured the Φ_{PS2} at midday in Denmark, The Netherlands and Spain in 1999 and in the four sites in 2000.

Leaf carbon isotope analyses

Leaf carbon isotope analyses were performed on leaf material collected in August-September in 1999 and 2000. Samples from each plot were combined, dried and ground. All analyses were carried out with an elemental analyser EA1108 (Carlo Erba, Milano, Italy) attached to a Delta C isotope mass spectrometer, and using a CONFLO II interface (Thermo Finnigan MAT, Bremen, Germany).

The isotopic composition of a sample ($\delta^{13}C_p$) was calculated as the ratio (‰):

$\delta^{13}C_p = ((R_{sample}/R_{standard})-1) \times 1000$

where R_{sample} and $R_{standard}$ are the ^{13}C:^{12}C ratios of the sample and the PeeDee Belemnite (PDB) standard respectively.

Statistical analyses

All the statistical analyses were performed using one value per plot, obtained from averaging the 3-4 plants measured per plot in the case of leaf gas exchange and Chl *a* fluorescence measurements and from composing material from different plants in the case of leaf $\delta^{13}C$. In the analyses of the variables sensitive to the instantaneous light (leaf gas exchange rates and Φ_{PS2}), we used PAR as a covariable in the ANOVAs. Differences were considered statistically significant at $p<0.05$.

To study the variability among countries, we performed ANOVAs with country (UK, Denmark, The Netherlands, Spain) and year (1999, 2000) as fixed factors. We used only control leaves in order to avoid the confounding effect of potential interactions between treatment and country. When the interaction between country and year was significant, we performed one way ANOVAs for each year separately. When the effect of country was significant, we compared values of the 4 countries by means of post-hoc Tukey tests.

To analyse treatment effects on the studied variables, we used ANOVA procedures, comparing drought and warming leaves with control leaves separately. Treatment (control-drought or control-warming), country and year were considered fixed factors in the ANOVAs. In the analyses of potential photochemical efficiency (Fv/Fm), round (predawn, midday) was also considered a fixed factor.

Pearson's correlations were performed between mean values of the studied variables for control leaves and the accumulated rainfall during the growing season (the three months previous to the measurements) for each country and year. On the other hand, to test whether treatments affected differentially the studied variables in relation to the accumulated rainfall over the growing season, we performed ANCOVAs comparing separately drought and warming leaves with control leaves and using the rainfall as the covariable.

Results

European transect

Climatological data

Table 1. Accumulated precipitation (mm) in control (C) and drought (D) plots and mean temperature (ºC) over the three months previous to the measurements in 1999 and 2000.

	Accumulated precipitation (mm)				**Temperature (ºC)**	
	1999		**2000**		**1999**	**2000**
	C	**D**	**C**	**D**		
UK	209	177	268	208	11.3	11
Denmark	240	30	179	38	15.1	12.5
Netherlands	144	28	268	109	16.7*	16.1
Spain	97	24	97	64	20.4	20.8

* Average only for May and July since June data were not available.

The gradient of accumulated rainfall over the growing season (the three months before the measurements) among the four studied countries was DK>UK>NL>SP in 1999 and UK=NL>DK>SP in 2000 (Table 1). In addition, the gradient of mean temperatures for the same period (May to July) was SP>NL>DK>UK both years (Table 1).

Leaf gas exchange rates

Differences among sites in leaf net photosynthetic rates of control plants (Table 2) followed the gradient of accumulated rainfall over the growing season experienced each year (Table 1). Differences among sites in leaf transpiration rates of control plants were also in accordance with the precipitation gradient in 1999 (Table 2). However, in 2000, control plants in Denmark showed the highest transpiration rates ($p<0.01$), whereas there were no significant differences in leaf transpiration rates among the other three countries. Values of instantaneous WUE were similar among countries in 1999 (Table 2). In 2000, control plants in UK showed the highest WUE values ($p<0.01$), whereas control plants in The Netherlands had higher values than in Denmark ($p=0.03$) and in Spain ($p=0.02$).

Table 2. Net photosynthetic rate (A), transpiration rate (E), WUE and potential photochemical efficiency (Fv/Fm) at predawn and midday for control plants in UK, Denmark (DK), The Netherlands (NL) and Spain (SP) sites in 1999 and 2000. Values are mean ± se (n=3, except for Denmark in 2000, where n=2). Significant differences ($p<0.05$) among countries are indicated by different letters.

	1999				**2000**			
	UK	**DK**	**NL**	**SP**	**UK**	**DK**	**NL**	**SP**
A (μmol CO_2 m^{-2} s^{-1})	4.93 ± 0.5^{a}	5.07 ± 0.8^{a}	2.84 ± 0.3^{ab}	1.41 ± 0.3^{b}	8.02 ± 0.8^{A}	3.63 ± 0.8^{BC}	5.84 ± 0.5^{AB}	1.48 ± 0.7^{C}
E (mmol HO_2 m^{-2} s^{-1}	4.63 ± 0.4^{a}	4.03 ± 1.1^{a}	2.74 ± 0.2^{ab}	0.92 ± 0.2^{b}	1.90 ± 0.2^{B}	5.47 ± 0.4^{A}	3.20 ± 0.3^{B}	2.90 ± 0.1^{B}
WUE (A/E)	1.13 ± 0.02^{a}	1.72 ± 0.7^{a}	1.12 ± 0.2^{a}	1.89 ± 0.2^{a}	4.79 ± 0.5^{A}	0.87 ± 0.2^{C}	2.34 ± 0.2^{B}	0.80 ± 0.05^{C}
Fv/Fm (predawn)	0.80 ± 0.003^{a}		0.80 ± 0.01^{a}	0.75 ± 0.005^{b}	0.78 ± 0.008	0.80 ± 0.002	0.78 ± 0.001	0.77 ± 0.008
Fv/Fm (midday)	0.78 ± 0.006^{a}	0.81 ± 0.003^{a}	0.77 ± 0.006^{b}	0.74 ± 0.01^{c}	0.75 ± 0.005	0.76 ± 0.002	0.74 ± 0.001	0.74 ± 0.001

Chlorophyll a fluorescence

Control plants in Spain had the lowest Fv/Fm values in 1999, at predawn and midday, whereas there were no significant differences between Fv/Fm values of control plants in UK and The Netherlands (Table 2). Fv/Fm values of control plants in Denmark in 1999 at midday were similar to those in UK and were higher than Fv/Fm values of control plants in The Netherlands or Spain ($p<0.05$). In 2000, there were no significant differences among the four sites in the Fv/Fm values of control plants, either at predawn or midday (Table 2).

Table 3. Actual photochemical efficiency (Φ_{PS2}) for control, drought and warming plants in the study sites. In Denmark, The Netherlands and Spain both years were pooled together since there were no significant interactions between treatment and year. # In UK, only data for 2000 was available. Values are means ± se (n=6, except for control and warming treatment in Denmark, where n=5 and for UK, where n=3). Significant differences among countries for control plants are indicated by different letters. Asterisks indicate significant differences between treatments and controls * $p<0.05$, (*) $p<0.1$.

	Control	**Drought**	**Warming**
UK $^{\#}$	0.51 ± 0.01	0.39 ± 0.05	0.38 ± 0.04
Denmark	0.53 ± 0.07^{a}	0.37 ± 0.05*	0.37 ± 0.05$^{(*)}$
Netherlands	0.39 ± 0.02^{b}	0.44 ± 0.03	0.41 ± 0.04
Spain	0.30 ± 0.03^{b}	0.27 ± 0.02	0.26 ± 0.03

When we pooled the data for the two study years, control plants in Denmark showed the highest actual photochemical efficiency of PSII (Φ_{PS2}), whereas there were no significant differences between the Φ_{PS2} values of control plants in The Netherlands and Spain (Table 3). However, when we used data only for the year 2000, in order to include UK in the analysis, control plants in UK showed the highest Φ_{PS2} values ($p<0.01$) (Table 3).

Leaf carbon isotope ratio ($\delta^{13}C$)

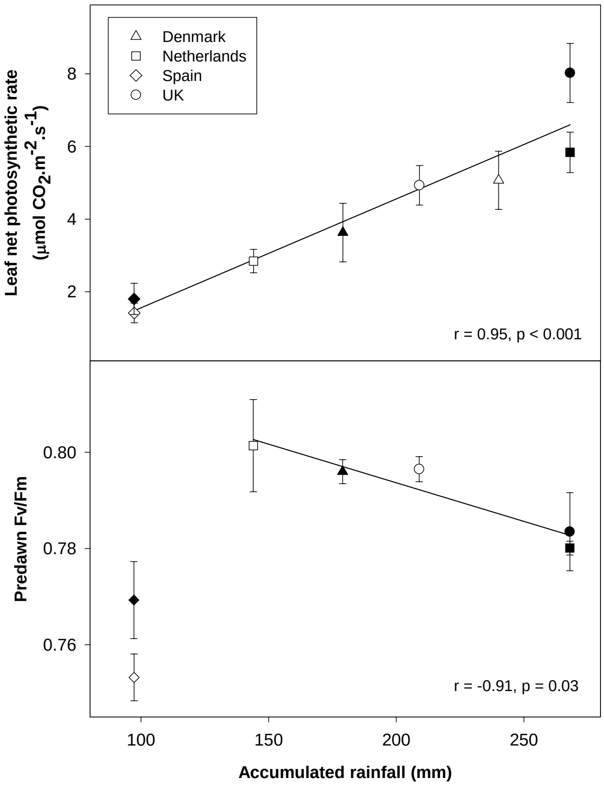
Both years, foliar $\delta^{13}C$ values of control plants were more positive in Spain than in the other three countries ($p<0.001$), while there were no significant differences among the three northern countries in the leaf $\delta^{13}C$ values of control plants (Table 4).

Table 4. Leaf δ^{13}C values in UK, Denmark, The Netherlands and Spain sites for control, drought and warming plants in 1999 and 2000. Both years were pooled together, since the interactions between treatment and year were not significant. Values are means ± se (n=6, except for warming treatment in Denmark where n=5). Significant differences among countries for control plants are indicated by different letters. Differences between control and treatments were not significant within each site, although there was a marginally significant global effect of warming (p=0.08).

	Control	**Drought**	**Warming**
UK	-28.53 ± 0.09^a	-28.43 ± 0.09	-28.88 ± 0.18
Denmark	-28.57 ± 0.30^a	-28.79 ± 0.41	-28.40 ± 0.37
Netherlands	-28.06 ± 0.19^a	-27.98 ± 0.12	-28.32 ± 0.11
Spain	-25.23 ± 0.36^b	-25.78 ± 0.31	-25.75 ± 0.23

Relationship between the studied physiological variables and the precipitation gradient

Fig. 1. Leaf net photosynthetic rates and potential photochemical efficiency (Fv/Fm) for control plants *versus* accumulated rainfall (mm) over the three months previous to the measurements for UK, Denmark, The Netherlands and Spain sites and in 1999 (white symbols) and 2000 (black symbols). Error bars indicate the standard errors of the mean of 3 plots values.



Leaf net photosynthetic rates of control plants were highly correlated with the amount of rainfall during the growth period (i.e. the three months previous to the measurements) within and across sites (r=0.95, p<0.001) (Fig. 1). This correlation was still significant when we considered only data from the three northern countries (r=0.90, p=0.01).

Neither the potential nor the actual photochemical efficiencies of PSII were significantly correlated with the accumulated precipitation over the growing season when we considered data for the control plants of the four sites. However, when we considered data only for the control plants of the three northern countries, the correlation between the predawn potential photochemical efficiency (Fv/Fm) and the accumulated rainfall was significant (r=-0.91, p=0.03) (Fig. 1).

Treatment effects

Leaf gas exchange rates

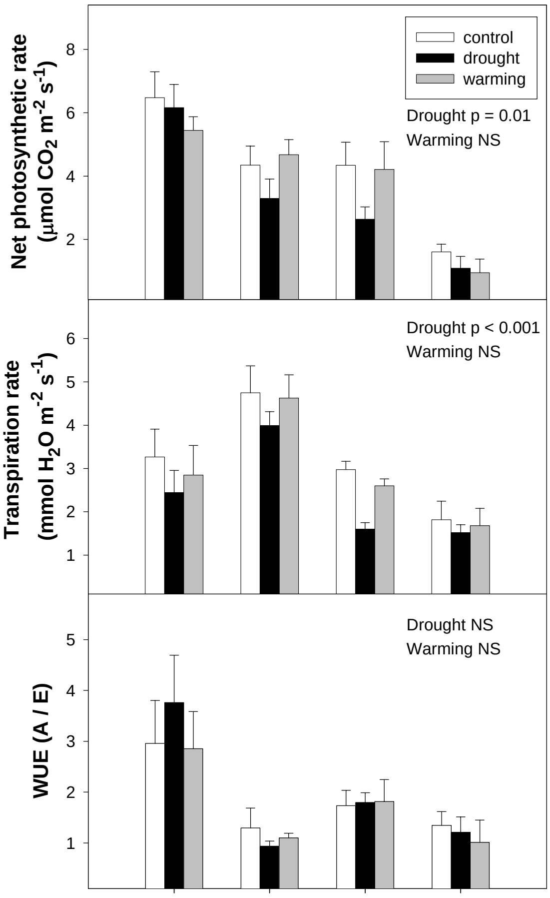


Fig. 2. Net photosynthetic rate, transpiration rate and WUE measured in 1999 and 2000 in control, drought and warming plants. Both years were pooled together, since the interactions between treatment and year were not significant. Error bars indicate the standard errors of the mean (n=6, except for warming treatment in Denmark, where n=5).

Drought plants showed lower leaf net photosynthetic ($F_{1,31}$=6.6, p=0.01) and transpiration rates ($F_{1,31}$=21.0, p<0.001) than control plants (Fig. 2). In contrast, we did not find any significant effect of warming on leaf gas exchange rates. Neither drought nor warming treatments affected significantly the leaf WUE of plants (Fig. 2).

Chlorophyll a fluorescence

Midday values of potential photochemical efficiency (Fv/Fm) were consistently lower than predawn values ($F_{1,72}$=121.8, p<0.001) in all the countries, treatments and years (Table 2 and Fig. 3). Drought plants showed slightly higher Fv/Fm values than control plants ($F_{1,48}$=4.4, p=0.04), mainly at midday (predawn: $F_{1,26}$=0.1, p=0.7, midday: $F_{1,26}$=6.6, p=0.02). In contrast, warming treatment did not affect the potential photochemical efficiency of plants (Fig. 3).

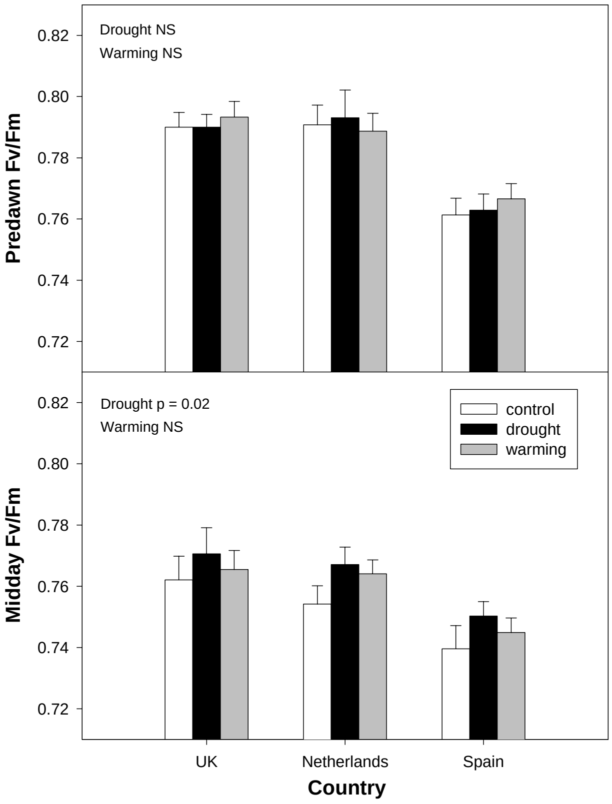


Fig. 3. Potential photochemical efficiency (Fv/Fm) at predawn and midday for control, drought and warming plants in UK, The Netherlands and Spain sites in 1999 and 2000. Both years were pooled together, since the interactions between treatment and year were not significant. Error bars indicate the standard errors of the mean (n=6).

Drought and warming plants showed lower Φ_{PS2} than control plants in Denmark ($F_{1,6}$=6.3, p=0.046 and $F_{1,5}$=5.6, p=0.06 respectively) (Table 3). In contrast, treatments had no significant effects on the Φ_{PS2} of plants in UK (only values for 2000), The Netherlands and Spain.

Leaf carbon isotope ratio ($\delta^{13}C$)

Drought treatment did not affect significantly the leaf δ^{13}C values of plants. Conversely, plants in the warming treatment showed more negative leaf δ^{13}C values than control plants, though differences were only marginally significant ($F_{1,31}$=3.3, p=0.08) (Table 4).

Treatment effects on the relationship between leaf net photosynthetic rates and accumulated rainfall over the growing season

Leaf net photosynthetic rates of control, drought and warming plants were significantly correlated with the amount of rainfall over the growing season (i.e. the three months previous to the measurements) (Fig. 4). Using the rainfall as a covariable in the analyses, drought plants showed higher leaf net photosynthetic rates than control plants ($F_{1,13}$=9.9, p=0.008), whereas there were no significant differences between warming and control plants (Fig. 4).

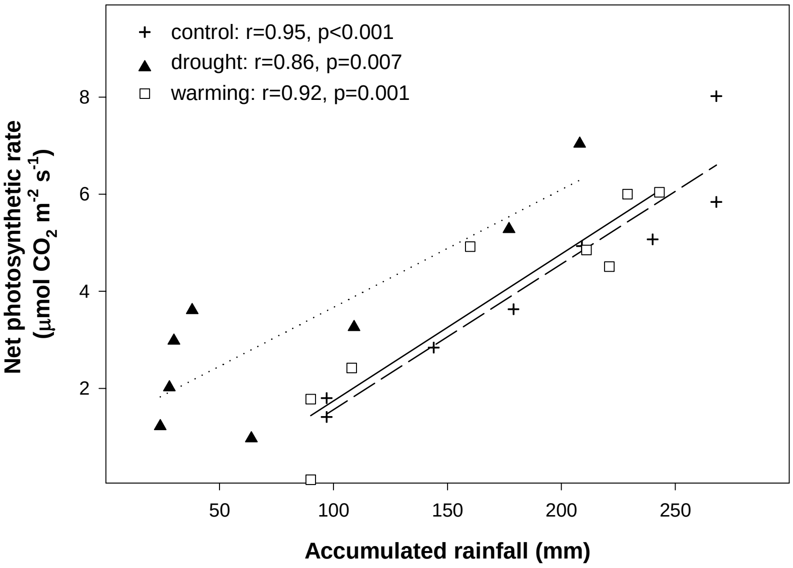


Fig. 4. Net photosynthetic rates *versus* accumulated rainfall for the three months previous to the measurements for control, drought and warming treatments in UK, Denmark, The Netherlands and Spain sites and in 1999 and 2000 (n=8 for each treatment).

Discussion

European transect

We examined the photosynthetic performance of Ericaceae shrubs along a European transect spanning a range in mean annual precipitation from 450 to 1700 mm and in mean annual temperature from 7 to 15ºC. Excluding the Spanish site (where we studied a different species), the range in mean annual precipitation for the three northern countries with *Calluna vulgaris* was 700-1700 mm and in mean annual temperature was 7-11ºC.

Leaf net photosynthetic rates of control plants followed a gradient from northern to southern countries, closely related to the accumulated rainfall over the growth period (Table 2 and Fig. 1). This result suggests that the amount of precipitation during the growing season was the main factor controlling differences in the photosynthetic rates among countries, regardless of other site characteristics, and in agreement with the observed general effect of drought treatment on photosynthetic rates (see below). In contrast, there was not a consistent gradient in the leaf transpiration rates or WUE of plants in both years (Table 2), since these two parameters were more affected by the vapour pressure deficit of the measuring day than net photosynthetic rates.

Our results also suggest that the accumulated rainfall over the growing season is an important factor controlling the differences in the predawn potential photochemical efficiency (Fv/Fm) of *C. vulgaris* plants within and across the three northern sites (Fig. 1). Interestingly, lower amounts of rainfall during the growing season in these three northern sites were correlated with higher predawn Fv/Fm values, which is in accordance with the higher Fv/Fm values that we found in drought plants compared to controls (see below). The fact that in Spain we studied a different species may explain the lack of correlation between predawn Fv/Fm values and accumulated rainfall when we used the data from the Spanish site.

Our results on leaf δ^{13}C (Table 4) are in accordance with the abundant literature reports showing that foliage of species from xeric habitats has more positive values of δ^{13}C than that from mesic habitats (e.g. Ehleringer and Cooper 1988; Garten and Taylor 1992; Meinzer and others 1992; Stewart and others 1995; Peñuelas and others 1999). Since theory predict that values of leaf δ^{13}C are positively related to the integrated water-use efficiency over the growing season (WUEi) (Farquhar and others 1989), the more positive values of leaf $\delta^{13}C$ of control plants in Spain would imply that these plants had higher WUEi than control plants in the other three countries. However, this result disagrees with the results that we obtained on instantaneous WUE (Table 2). Discrepancies between gas exchange and isotopic data have been reported in previous studies (e.g. Vitousek and others 1990; Picon and others 1996) and may arise from instantaneous WUE being highly dependent on meteorological conditions in the date of measurements, whereas leaf $\delta^{13}C$ is largely based on the long-term integration of WUE.

Drought effects

Photosynthesis at leaf level is one of the physiological processes more strongly affected by water shortage (Epron and Dreyer 1993). Accordingly, we found a general reduction in the net photosynthetic (A) and transpiration rates (E) in the drought plants compared to controls (Fig. 2), in agreement with previous studies on plants suffering from water stress (reviews in Schulze 1986; Chaves 1991; Yordanov and others 2000). However, we did not find any significant effect of drought treatment on the instantaneous WUE of plants, which means that photosynthetic and transpiration rates changed proportionally. In agreement with the results on instantaneous WUE, we did not find significant effects of drought treatment on $\delta^{13}C$ (Table 4) and, thus, on integrated WUE. Since drought did not decrease significantly the leaf nitrogen concentration (Peñuelas and others 2003, this issue) or the potential photochemical efficiency (Fv/Fm) of photosystem II (PSII), it appears that the reduction in leaf assimilation rates was mainly produced by stomatal closure.

Stomatal closure and the consequent reduction in the assimilation rate, may result in damage to the photosystem, since the absorption of light energy may be in excess of that required for carbon fixation (Jefferies 1994). Accordingly, an increase in drought has been related in many cases to a drop in potential photochemical efficiency of PSII (e.g. Björkman and Powles 1984; Epron and others 1992; Jagtap and others 1998). Nevertheless, some authors raise doubts about PSII sensitivity to water shortage, since a remarkable resistance of the photosynthetic apparatus to dehydration has been found (e.g. Gamon and Pearcy 1990; Havaux 1992; Epron and Dreyer 1993; Jefferies 1994; Epron 1997). In addition, some authors demonstrated that water deficit may enhance the resistance of PSII photochemistry to superimposed constraints, namely, high-temperature stress combined or not with photoinhibitory light (Havaux 1992;

Epron 1997; Yordanov and others 1999). The results of our study support the latter idea, since we found higher values of potential photochemical efficiency (Fv/Fm) in drought plants compared to controls, mainly at midday (Fig. 3). This pattern held across sites and years (see also Fig. 1), suggesting that it is a consistent result. Demonstration of a general increase in the stability of PSII to superimposed constraints caused by drought is of great ecophysiological significance because it is very likely that climate change will increase the frequency of hot and dry days, particularly in the Mediterranean countries.

According to the observed high stability of PSII in drought leaves, as shown by Fv/Fm values, we did not find significant effects of drought on Φ_{PS2} values in UK, The Netherlands and Spain (Table 3), in spite of the general decrease in leaf photosynthetic rates. This result suggests that drought plants from these countries may have higher photorespiration rates than controls. Higher photorespiration rates in drought plants would consume the excess of energy and would maintain high rates of electron transport, protecting the photosystem from photodamage (Krall and Edwards 1992; Kozaki and Takeba 1996).

Warming effects

Our warming treatment did not affect the leaf gas exchange rates of plants (Fig. 2). Previous studies about the effects of warming on leaf gas exchange rates have reported contrasting results. Some authors did not find any effect of warming manipulations on leaf gas exchange rates (e.g. Wookey and others 1994; Nijs and others 1996; Loik and others 2000; Starr and others 2000), other authors found stimulated rates (e.g. Chapin and Shaver 1996; Huxman and others 1998; Apple and others 2000), whereas others found decreases (Callaway and others 1994; Roden and Ball 1996). However, comparisons among studies are difficult given the different characteristics of warming treatments among the experiments and the different temperature sensitivities and optima for photosynthesis between species and ecotypes (Chapin and others 1995; Shaw and others 2000). Moreover, leaf gas exchange responses to temperature are often strongly influenced by other interacting factors, such as the internal plant water status and the water vapor pressure difference between the leaf and the surrounding air (Chaves 1991).

In our experiment, the lack of direct warming effects on leaf gas exchange rates is probably due to the fact that the increase in temperature during the day in the warming plots was too weak to produce significant differences in leaf gas exchange rates between warming and control plants. However, we did not detect indirect effects of warming treatment on leaf gas exchange rates via increased foliar nitrogen either, since leaf nitrogen concentrations were not significantly affected by the warming treatment (Peñuelas and others 2003, this issue).

In agreement with the lack of warming effects on leaf gas exchange rates, the warming treatment did not either affect the instantaneous WUE (Fig. 2) or the chlorophyll fluorescence parameters, i.e., the efficiency of PSII (Table 3 and Fig. 3). Warming plants tended to show more negative values of $\delta^{13}C$ than control plants (Table 4), which would imply lower integrated water-use efficiency (WUEi).

Water availability control of photosynthetic performance

The lower leaf net photosynthetic rates (A) of plants in the drought treatment compared to controls (Fig. 2) is consistent with the decrease in A of control plants associated with the reduction in the precipitation experienced along the geographical transect (Fig. 1). This result indicates that a consequence of the extension of the drought period will probably be a decrease in the annual productivity of these Ericaceous shrubs in a short-term, as well as in a long-term. Previous studies found approximately linear decreases in forest productivity with decreases in annual precipitation below 1500 mm (O'Neill and DeAngelis 1981). A close positive correlation between net primary productivity and precipitation was also reported across precipitation gradients in temperate grasslands (Lauenroth and Sala 1992) or among sites and years in deserts (Gutierrez and Whitford 1987).

Leaf net photosynthetic rates were significantly correlated with the accumulated rainfall over the growing season for plants in the drought and warming treatments (Fig. 4), in spite of the differences across sites and years in the intensity of these treatments. These results support the idea that precipitation over the growing season is really a major factor controlling A.

Interestingly, although A decreased in response to drought treatment, drought plants were able to perform higher photosynthesis than control plants in relation to the amount of water received over the previous months (Fig. 4). This could be related with the higher potential photochemical efficiency (Fv/Fm) that we found in drought plants in relation to controls (Fig. 3). Moreover, drought plants could have developed more and/or deeper roots, which might allow them to extract soil water more efficiently than control plants and/or to arrive to deeper and probably more permanent water sources.

Final conclusions and remarks

1) We found a decrease in the leaf net photosynthetic rates of plants from northern to southern countries, in agreement with the geographical gradient in water availability.

2) Drought treatment decreased the leaf gas exchange rates of plants in the four sites. However, drought plants had higher photosynthetic rates than controls in relation to the accumulated rainfall over the previous months. Moreover, plants in the drought plots showed higher potential photochemical efficiency than controls mainly at midday, suggesting an enhanced ability to cope with superimposed constraints, which, however, did not avoid a decrease in growth (Peñuelas and others 2003, this issue).

3) Warming treatment had no significant effects on the instantaneous photosynthetic performance of plants during the growing season in any of the four sites, which does not preclude possible significant differences when considering the integrated whole year plant productivity (Peñuelas and others 2003, this issue).

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CHAPTER 6

Developmental instability and gas exchange responses of a heathland shrub to experimental drought and warming

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Abstract

The effects of predicted climatic changes on the physiological stress of bilberry (*Vaccinium myrtillus* L.) were investigated in a field experiment in a heathland of N. Wales (UK). In the experiment drought was increased during the growing season by excluding precipitation, and night warming was created by reducing heat loss. To assess physiological stress, leaf size and leaf fluctuating asymmetry (FA) as integrative measures of stress during leaf development, and leaf gas exchange as a classical, instantaneous method to detect physiological stress were measured. Since leaf FA increased with increasing size, comparisons of asymmetries were made for relative rather than absolute asymmetry. Relative leaf FA was calculated as the absolute difference between leaf size (area or width) of left and right halves, corrected for trait size. Drought treatment increased the relative leaf area FA in 2000 and not in 1999, in agreement with a stronger treatment in 2000. Conversely, the warming treatment decreased the relative leaf area FA in 1999 and not in 2000, coinciding with the lower minimum temperatures in the growing season of 1999. Differences in water availability and temperature between years were related with changes in the relative leaf area FA, which were consistent with the treatment effects. In contrast, leaf gas exchange rates and leaf size showed no significant response to the environmental manipulations, although there was a slight decrease of photosynthetic values and leaf size in drought treatments in both years. Leaf FA therefore appeared to be a more sensitive indicator of physiological stress than leaf size or gas exchange measurements. Our results indicate that a future increase in the severity of drought during the growing season will increase physiological stress of *V. myrtillus*, whereas warming will decrease physiological stress during leaf development due to the alleviation of temperature constraints.

Introduction

Climate change from greenhouse gas emissions is predicted to raise the globally averaged surface temperature by 1.4 to 5.8 ºC over the period 1990 to 2100 (IPCC 2001). The raise in mean temperatures will be largely caused by the increase in minimum air temperatures, i.e. nighttime temperatures (IPCC 2001). Although future precipitation patterns are more uncertain, global climate change is likely to increase the risk of summer drought in central and southern Europe (IPCC 2001).

Warmer and drier conditions are likely to affect the physiological stress of plants. Stress is considered to be a significant and lasting deviation from favourable conditions, leading to abnormal demands and a destabilisation of vital processes (Larcher 2000). The detection and quantification of physiological stress in plants has been a major concern of ecophysiological studies. Measures of stress can be grouped into instantaneous physiological measurements, such as leaf gas exchange or Chl *a* fluorescence, and integrative physiological measures over larger temporal scales, such as growth, productivity, survival, or isotope content. Another integrative process is developmental instability, which refers to the inability of a bilateral organ or organism to buffer its development against disturbances and to produce a predetermined phenotype (Møller and Swaddle 1997). Recently, several authors have suggested that measures of developmental instability as fluctuating asymmetry (FA) may provide a more sensitive indicator of stress than traditional measures of performance (e.g. growth, fecundity, survival) because FA has a measurable response at a lower threshold of stress (Zvereva et al. 1997, Rettig et al. 1997).

Fluctuating asymmetry (FA) is a particularly useful measure of developmental instability because, for bilaterally or radially symmetrical traits, the optimal phenotype is known. Ideally, traits showing fluctuating asymmetry will have a normal frequency distribution of signed left-minus-right trait values with a mean of zero (Van Valen 1962, Palmer and Strobeck 1986). Such small deviations from perfect symmetry are considered to result from environmental or genetically based perturbations of the developmental process (Freeman et al. 1993, Møller and Shykoff 1999). As stress during development may influence developmental precision, FA has been proposed as a potentially useful tool for monitoring stress levels in natural populations (Leary and Allendorf 1989, Graham et al. 1993). Typically the greater the stress, the greater the fluctuating asymmetry (Palmer 1996).

In plants, environmental stress factors which have been associated with leaf developmental instability include high altitude (Wilsey et al. 1998), pollution (Zvereva et al. 1997), UV-B radiation (Midgley et al. 1998), competition (Rettig et al. 1997), herbivory (Lempa et al. 2000), pathogens (Møller 1999), high boron and low light (Roy and Stanton 1999), and electromagnetic fields (Freeman et al. 1999; see Møller and Shykoff 1999 for a recent review). To date few investigations have related developmental instability in plants with warming (Huether 1969) or drought (Hochwender and Fritz 1999, Alados et al. 2001), even though they are two important factors of predicted climatic change. To our knowledge only Valkama and Kozlov (2001) have investigated specifically the impact of both temperature and precipitation on developmental instability.

In contrast, the effects of changes in temperature or water availability on leaf gas exchange (stomatal conductance, photosynthetic and transpiration rates) have been extensively studied (reviews by Berry and Björkman 1980 and Yordanov et al. 2000). In general, stress produces a decrease in leaf gas exchange (Farquhar et al. 1989). Thus, it is expected that a reduction in water availability will lead to stomatal closure decreasing transpiration and photosynthetic rates. It is also well known that an increase in drought can cause a significant reduction in growth (Long and Hutchin 1991, Pereira and Chaves 1995). By reducing growth, plants can mitigate stress through a decrease of water and nutrient demands (Alados et al. 2001). Then, if water limitation occurs in the beginning of the growth cycle, leaf area is expected to be reduced (Nilsen and Orcutt 1996). In contrast, predicted elevated temperatures may increase leaf size and carbon assimilation and transpiration rates in sites where low temperatures constrain physiological activity of plants. Climate change effects on nutrient availability (Grogan and Chapin 2000) may also influence indirectly leaf growth and leaf gas exchange.

Recently, a number of field experiments simulating some of the predicted climatic changes have been conducted, mostly in arctic and subarctic ecosystems (see Shaver et al. 2000 for a review of warming experiments). However, most of the techniques used to experimentally warm ecosystems or ecosystem components (heat-resistance ground cables, overhead infrared lamps, vented and unvented field chambers, greenhouses) alter additional environmental conditions (e.g. light, humidity, soil structure, wind). Moreover, most of these experiments simulate a general diurnal increase in temperature rather than the observed increase in T_{min} (nighttime temperature) (IPCC 2001). To reduce these drawbacks, we have performed a field experiment using a non-intrusive technique to reduce the loss of IR-radiation from the Earth surface to the atmosphere. This approach imitates the effect produced by greenhouse gases accumulation and results in an increase in T_{min}, or nighttime warming. In addition, we have simulated prolonged drought events by excluding rain during the growing season (Beier et al. 2002).

The aim of the present study was to test whether predicted climatic changes (warming and prolonged drought) would produce physiological stress in *Vaccinium myrtillus*. In Europe, *V. myrtillus* has a vast distributional area extending from mid-latitude mountainous ranges to the arctic region (Gerdol et al. 2000) and is a characteristic species of moorlands. Moorlands, typically dominated by heather (*Calluna vulgaris* (L.) Hull), cover about 15% of the land area in the UK (Anderson and Hetherington 1999) and they are considered to be of high conservation value (Whitehead et al. 1997). Heather moorlands are under threat from changes in land use and management, the potential effects of atmospheric nitrogen deposition and climate change (Anderson and Hetherington 1999).

To detect environmental stress during the growing season in *V. myrtillus*, we used fluctuating asymmetry and leaf size as integrative measures of stress during leaf development, and leaf gas exchange measurements as an instantaneous measure of stress. We hypothesised that if water availability was a limiting factor for *V. myrtillus* development, our experimental water removal would increase stress conditions during leaf development, resulting in an increase in leaf fluctuating asymmetry and a decrease in leaf size. Moreover, fully developed leaves would decrease stomatal conductance and photosynthetic CO_2 fixation. On the other hand, we expected that increases in minimum temperatures would alleviate temperature constraints during leaf development, as biotic processes in such ecosystems are usually directly or indirectly constrained by low temperatures (Körner and Larcher 1988). Hence, we hypothesised that our warming treatment would increase leaf gas exchange rates and would decrease low temperature stress during *V. myrtillus* leaf development, reducing leaf fluctuating asymmetry and increasing leaf size.

Methods

Study site and plant species

The study was carried out in N. Wales (UK), within Clocaenog Forest at Craig Bron-banog (53º03' N 3º28' W), at 490 m altitude. Annual rainfalls were 1655 and 1826 mm in 1999 and 2000, respectively, whereas annual mean air temperatures were 8.3 and 7.7 ºC. The study site is in a heather moorland of approximately 15 ha surrounded by plantation forestry. The soil is an acid peaty podzol. The vegetation is dominated by mature *Calluna vulgaris* (L. Hull), *Vaccinium myrtillus* (L.) and *Empetrum nigrum* (L.), with very sparse *Deschampsia flexuosa* (L. Trin.).

Experimental design

Two types of climatic manipulations of the ecosystem were performed using automatically sliding roofs:

- Ecosystem drought: The roofs (transparent plastics) covered the vegetation during rain events over the growing season.
- Ecosystem warming: The roofs (reflective curtains) were unrolled across the experimental plots covering the vegetation and soil during night at a height of 0.6m, reducing the loss of IR radiation. A rain sensor removed the curtain during rain events to maintain the hydrological cycle.

Both drought and warming covers were removed during periods of high winds to prevent damage to the covers. Nine plots (20 m^2 per plot) were established: 3 untreated controls, 3 drought and 3 warming plots. Before measurements, drought treatment had excluded rain for c.a. 4-5 weeks each year, starting in June 18 in 1999 and in June 6 in 2000. Particularly, in 1999 drought treatment excluded 23% of 140 mm of accumulated rainfall over the growing season (June and July), whereas in 2000 drought treatment excluded 37% of 162 mm of accumulated rainfall. Warming treatment started in May 17th of 1999 and it was working overall the experiment period, increasing the temperature in c.a. 1 ºC compared to control plots.

Leaf sampling and asymmetry measurements

For measuring leaf asymmetry, we collected 10 fully developed, current year leaves per plot on 12-14 July 1999 and 2000. To avoid within plant pseudo-replication, all leaves were collected from different plants. Likewise, to minimise within-plant variation in asymmetry, we always sampled the fourth leaf from the apical end of the new shoot, unless this was damaged, eaten or missing, in which case we collected the third leaf.

Leaf asymmetry was calculated as the difference in leaf width (1) and leaf area (2) between the right (R) and left (L) halves of the adaxial part of a leaf:

(1) Right and left widths were the distances from the midrib to the right and left margin respectively, measured at the widest point of each leaf with a digital calliper to the nearest 0.01 mm.

(2) To measure right and left areas, we sliced each leaf down the middle of the mid vein and spread and fixed the two halves on a sheet. Thereafter, we digitised half leaf photocopies and determined their areas using ImagePC (v. α9 for Windows, Scion Co., Frederick, MD, USA).

Individual signed asymmetry was measured as L-R, where L is the trait value on the left side and R is the trait value on the right side. Absolute asymmetry (|L-R|) was computed as the absolute left-minus-right values of a particular leaf.

Leaf fluctuating asymmetry (FA) data analyses

Repeatability and measurement errors

The percent of total variance in a character that occurs among-individuals is often referred to as repeatability (Yezerinac et al. 1992). Repeatability of leaf width and leaf area asymmetry was obtained through a one-way analysis of variance with individuals as the independent factor and values of leaf FA as the dependent variable (Yezerinac et al. 1992, Merilä and Björklund 1995).

Conversely, the percent measurement error of a character is the percentage of the total variance attributable to the between sides (within-individual) variance due to the imprecision of the measurements. Measurement errors (ME) can be particularly important in fluctuating asymmetry measures, since differences between sides are often very small: generally <5% and often <1% of the size of the traits being measured (Palmer 1994). Because ME may substantially bias asymmetry estimates, an important initial step in analysing asymmetry is distinguishing variance in leaf asymmetry from ME (Palmer 1994, Swaddle et al. 1994). Thus, we estimated ME by measuring each leaf twice for each trait. Leaves were measured just after collection and re-measured several months later without reference to earlier measurements.

Leaf area and leaf width ME for each treatment (control, drought, warming) and year (1999, 2000) were estimated using two-way mixed model ANOVAs where side was the fixed factor and individual was the random factor (Palmer and Strobeck 1986, Palmer 1994, Merilä and Björklund 1995). The last procedure partitions out directional asymmetry (side effect) from non-directional asymmetry (interaction effect) and identifies whether the measurement error was smaller than other non-directional asymmetry.

Directional asymmetry and antisymmetry

Before interpreting our data, we followed Swaddle et al. (1994) and Palmer's (1994) suggestions to evaluate whether leaf asymmetry was FA rather than directional asymmetry or antisymmetry. As many cases of directional asymmetry and antisymmetry reflect normal development, i.e. they have a genetic rather than environmental origin, such asymmetries are usually not considered to be useful for measuring developmental instability (Leary and Allendorf 1989, Palmer 1994, 1996).

Directional asymmetry is characterised by consistently greater trait development on one particular side, either left or right, resulting in mean values of the distributions of right minus left sides (L-R) deviating from zero (Van Valen 1962, Palmer and Strobeck 1986). To test for directional asymmetry, we used two methods: (1) we utilized the mean squares generated by the two-way analysis of variance mentioned above, to determine if the leaf sides differed in size (Palmer 1994) and, (2) we performed one-sample *t*-tests on the signed differences (left-minus-right) for each treatment and year to determine whether the mean values differed from 0 (Swaddle et al. 1994).

Antisymmetry occurs when one side is consistently larger than the other, but the larger side may be either the right or left, at random, resulting in a platykurtic (broad-peaked or bimodal) distribution of L-R differences about a mean of zero (Van Valen 1962, Palmer and Strobeck 1986). To test for antisymmetry, we checked departures from normality of the distributions of L-R within treatments each year using Shapiro-Wilk tests (Zar 1996). We also examined the normality of the distributions determining whether skewness and kurtosis coefficients deviated from 0, which is the expected value for normal distributions (Palmer 1994, Zar 1996). A significant negative kurtosis indicates possible antisymmetry (Cowart and Graham 1999).

Leaf size

Improper interpretations of leaf FA may arise if leaf size differs among treatments and leaf FA co-varies with leaf size (Swaddle et al. 1994, Palmer 1994). To know whether leaf size (area or width) differed among treatments we performed ANOVAs comparing separately drought and warming leaves with control leaves, being treatment and year the fixed factors. We used one value of leaf size per plot (the average of 10 leaves) and year. To determine whether leaf size differed between years, we performed one way ANOVAs using only control leaves. Finally, to know if leaf FA co-varies with leaf size, we obtained non-parametric Spearman correlation coefficients for the two studied years between absolute leaf asymmetry (|L-R|) and leaf size ((L+R)/2) (n=90) (Palmer 1994).

FA indices

For both traits (leaf area and leaf width), we calculated a size-corrected or relative leaf FA index: |L-R|/size. To be sure that the correction was successful, we correlated again the size-corrected indices with size.

Typically, FA indices based on the unsigned left-minus-right character value have half-normal distributions, truncated at zero (i.e. highly skewed to the right) and with some zero values. Because of this, we applied a Box-Cox transformation to relative leaf FA indices (for leaf area and width) to normalise their distributions, before performing parametric analyses (Swaddle et al. 1994):

$$FA= (\text{relative } FA+0.001)^{0.4}$$

Data analyses

We used the statistical software of SPSS 10.0 (SPSS Inc., Chicago, Illinois) for all the statistical analyses. Since multiple tests were performed with the same variables, significance level (α=0.05) was always adjusted for the number of statistical tests using a sequential Bonferroni correction to prevent against group-wide type errors (Rice 1989). All tests were two-tailed.

In all the statistical analyses, we used one value of leaf asymmetry per plot (the average of 10 leaves) and year. To examine the effect of treatments on leaf FA, we performed ANOVAs with treatment and year as fixed factors and leaf FA (area or width) as the dependent variable. For leaf area FA values, we found a significant interaction between year and treatment. Hence, we analysed separately the effect of treatments on leaf area FA for the two years. We tested the differences between drought and warming with control leaves separately. To compare leaf FA between years, we performed one way ANOVAs using only control leaves. Finally, we calculated the Spearman correlation coefficients between the average of leaf area and relative leaf area FA and the total rainfall received over the growing season (June and July) by each treatment each year (1999 and 2000).

Leaf gas exchange measurements

Leaf gas exchange rates were measured in 3-4 randomly selected plants in each plot on 12 July 1999 and 14 July 2000. Net photosynthetic rate (A) and stomatal conductance (g_s) were determined at midday-afternoon (12-17h solar time) on one shoot per plant. Only current year shoots in full sun and with fully expanded leaves were considered. Measurements were performed with a portable open-flow gas exchange system (ADC4, ADC Inc., Hoddesdon, Hertfordshire, England), which also monitored photosynthetically active radiation (PAR) and air temperatures in the leaf chamber. Water vapor pressure deficits between leaf and air (VPD) were calculated on the basis of relative humidity and chamber air temperature. All results are expressed on area basis. Leaf areas were measured using ImagePC (α9 for Windows, Scion Co., Frederick, MD, USA) from photocopies of all the leaves of each measured shoot.

Leaf gas exchange data analyses

In all the statistical analyses, we used one value per plot (the average of the 3-4 plants measured) and year. To test the effects of treatments on leaf gas exchange rates (photosynthesis and stomatal conductance), we performed ANCOVAs comparing drought and warming leaves with control leaves separately. Treatment and year were considered fixed factors, whereas photosynthetically active radiation (PAR) was the co-variable. To test the effect of year, we performed one way ANOVAs (since PAR was not significant as covariable) using only control leaves. We also calculated non-parametric Spearman correlation coefficients between leaf gas exchange values of plants from the different treatments and the total rainfall received by each treatment over the growing season (June and July) both years (1999 and 2000).

Results

Leaf fluctuating asymmetry

Repeatability and measurement errors

Repeatability of leaf width asymmetry measurements was 0.96 ($F_{179,180}$=44.9, p<0.001) and 0.99 for leaf area ($F_{179,180}$=240.9, p<0.001). The percentage of measurement error (ME) of leaf width and leaf area measurements within each treatment was less than 4.5% in both years. In all the ANOVAs the interaction between individual and side was significant (p<0.001), indicating that the ME was negligible compared to the variation between sides (asymmetry; Palmer 1994, Perfectti and Camacho 1999). The high repeatabilities and the relatively low levels of ME indicate that ME accounted for a minor part of the total variance in asymmetry and therefore asymmetries were measured with sufficient precision to allow the use of the average measure for each leaf in the following analyses.

Directional asymmetry and antisymmetry

Table 1- Summary of descriptive data (n=30) for the leaf asymmetry (R-L) of control (C), drought (D) and warming (W) plants calculated from leaf areas (cm^2) and leaf widths (mm). After sequential Bonferroni correction, no skewness or kurtosis values differed significantly from zero. Neither mean values differed significantly from zero.

	Year	**Treatment**	**Skewness**	**Kurtosis**	**Mean ± SE**
Leaf area FA	**1999**	**C**	-0.161	-1.338	-0.0178 ± 0.0123
		D	-0.516	1.053	-0.0128 ± 0.0108
		W	0.116	0.390	-0.0085 ± 0.0087
	2000	**C**	-0.262	0.745	0.0035 ± 0.0092
		D	-0.040	3.496	-0.0358 ± 0.0193
		W	0.034	-0.045	-0.0142 ± 0.0123
Leaf width FA	**1999**	**C**	-0.732	0.231	-0.0630 ± 0.0916
		D	-0.183	-0.151	-0.0950 ± 0.0710
		W	0.161	0.245	-0.0903 ± 0.0592
	2000	**C**	-0.665	0.260	0.0422 ± 0.0784
		D	-0.505	0.582	-0.1467 ± 0.0889
		W	0.679	2.267	-0.0465 ± 0.0925

We did not find any significant effect of leaf side in any of the two-way mixed model ANOVAs mentioned above, either for leaf width or for leaf area. Moreover, *t*-tests revealed that mean values of each treatment each year did not differ significantly from zero, indicating a lack of directional asymmetry (Table 1).

Left-minus-right distributions of leaf width and leaf area for each treatment and year did not deviate from normal distribution (p>0.05). Moreover, none of the skewness and kurtosis values differed significantly from zero (Table 1). Therefore, we concluded that observed deviations from symmetry are fluctuating asymmetry rather than antisymmetry.

Leaf size

Neither treatments nor year affected significantly leaf size (area or width), although there was a tendency to decrease leaf area and leaf width with drought (Fig. 1). The interaction between treatment and year was never significant, which indicates that the effects of treatments on leaf size followed the same trends both years.

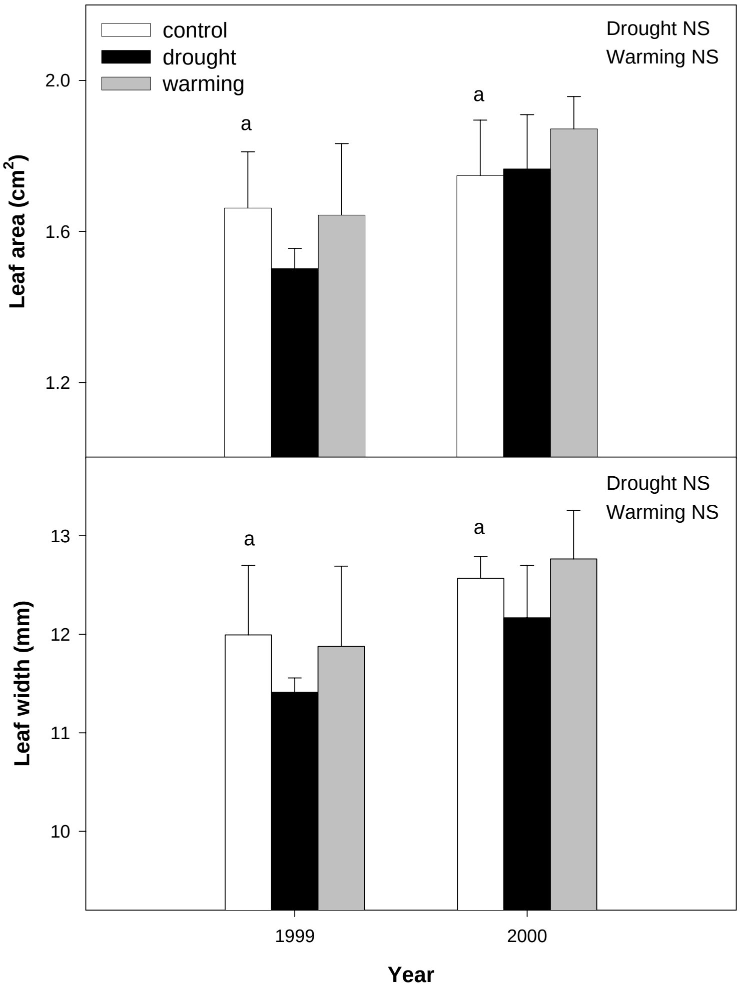

Fig. 1- Leaf size (L+R) expressed as leaf area (cm^2) and leaf width (mm) for control, drought and warming plants of *Vaccinium myrtillus* in 1999 and 2000. Error bars indicate the standard errors of the mean (n=3 plot averages of 10 leaves each). Significant differences in control plants between years are indicated by different letters. Statistical significance of differences between treatments and control are also depicted.

Absolute leaf asymmetry (|L-R|) was correlated with leaf size ((L+R)/2) for leaf area ($p<0.01$, both years) and for leaf width ($p=0.064$ in 1999 and $p=0.038$ in 2000). The positive values of the correlation coefficients both years indicated an increase in leaf FA with increasing size. Thus, comparisons of asymmetries were made for relative rather than absolute asymmetry (Møller and Swaddle 1997). No significant correlation was found between the relative index of fluctuating asymmetry and size in any of the studied traits.

Leaf fluctuating asymmetry

Mean relative (or size-corrected) FA values in *V. myrtillus* leaves were 0.056 (SE=0.004) for leaf width and 0.06 (SE=0.004) for leaf area. The Box-Cox transformation of the relative FA values succeeded in normalising their distributions (Shapiro-Wilk, in all groups $p>0.2$). Correlation between leaf area and leaf width normalised indices of FA was significant ($p<0.01$) with a Pearson's correlation coefficient of $r=0.51$ ($n=180$).

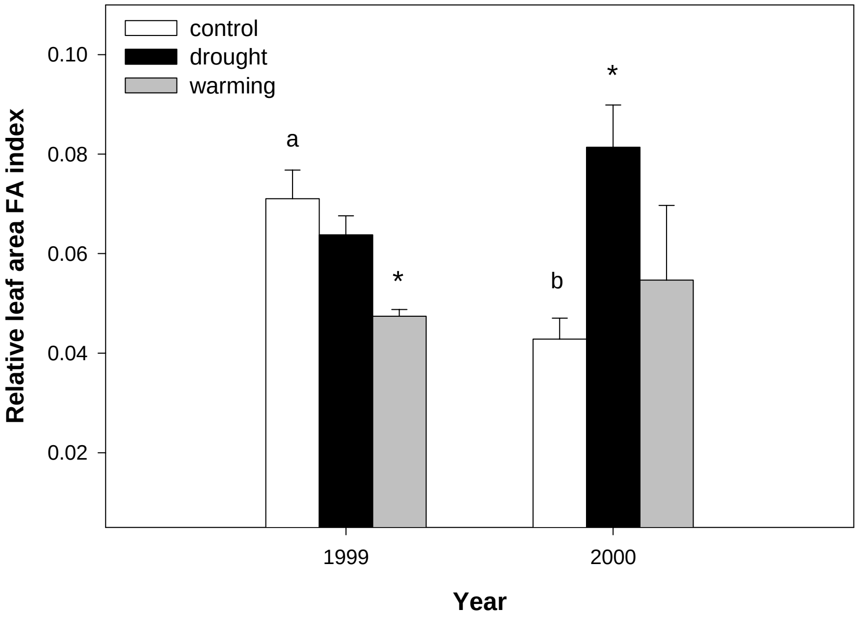

Fig. 2- Relative leaf area FA index for control, drought and warming treatments in 1999 and 2000. Error bars indicate the standard errors of the mean (n=3 plot averages of 10 leaves each). Significant differences ($p<0.05$) in control plants between years are indicated by different letters, whereas significant differences ($p<0.05$) between treatments and controls (within years) are indicated by an asterisk.

Values of relative leaf area FA (Fig. 2) for control leaves in 2000 were significantly lower than values in 1999 ($F_{1,4}$=21.2, p=0.01), whereas there were no differences in leaf width FA between years (data not shown). Treatments did not affect significantly leaf width FA and the interaction between treatment and year was never significant (data not shown). In contrast, although drought treatment did not affect leaf area FA values in 1999, drought leaves in 2000 showed higher leaf area FA values than control leaves ($F_{1,4}$=17.8, p=0.01). On the other hand, warming treatment decreased significantly leaf area FA values in 1999 ($F_{1,4}$=28.9, p=0.006), whereas no significant effect was found in 2000 (Fig. 2).

The leaf area did not correlate significantly with the accumulated rainfall received during the growing season by each treatment each year (r_s=-0.14, p=0.79, n=6). In contrast, the Spearman correlation between the relative leaf area FA index and the rainfall was significant (r_s=-0.829, p=0.04, n=6) (Fig. 3).

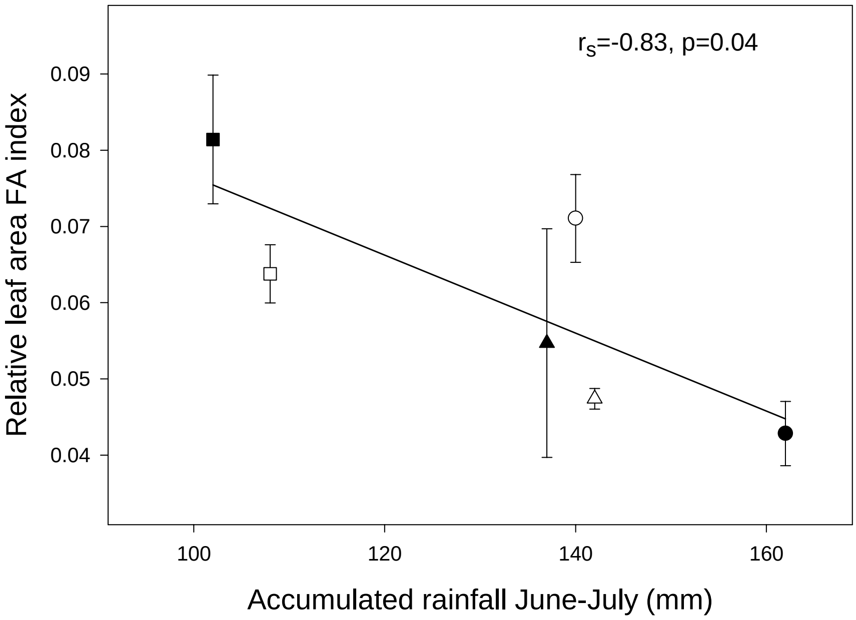

Fig. 3- Relative leaf area FA index in relation to accumulated rainfall (mm) over the growing season (June and July) for control (∗), drought (∀) and warming (6) treatments in 1999 (white symbols) and 2000 (black symbols). Each point is the mean of 3 plot averages of 10 leaves each. Bars are the standard errors of the mean.

Leaf gas exchange

Average values of photosynthetically active radiation (PAR) in the leaf chamber, leaf temperature and water vapor pressure deficit (VPD) were 1346 µmol m^{-2} s^{-1}, 31.4 ºC and 2.83 KPa in the 1999 measuring date, whereas they were 402 µmol m^{-2} s^{-1}, 14.3 ºC and 0.6 KPa in the 2000 measuring date. Thus, the sampling date in 1999 was much warmer, lighter and drier than in 2000. PAR, leaf temperature and VPD did not differ among treatments in any of the two years.

Leaf photosynthetic rates of control leaves (Fig. 4) were significantly lower in 1999 than in 2000 ($F_{1,4}$=8.72, p=0.04), whereas differences in stomatal conductance between years were marginally not significant ($F_{1,4}$=5.75, p=0.075) (Fig. 4). Environmental manipulations did not affect significantly leaf gas exchange rates, although there was a slight decrease of photosynthetic rates in drought treatment both years (Fig. 4). Interaction between treatment and year were never significant, i.e. the effects of treatments followed the same trends both years.

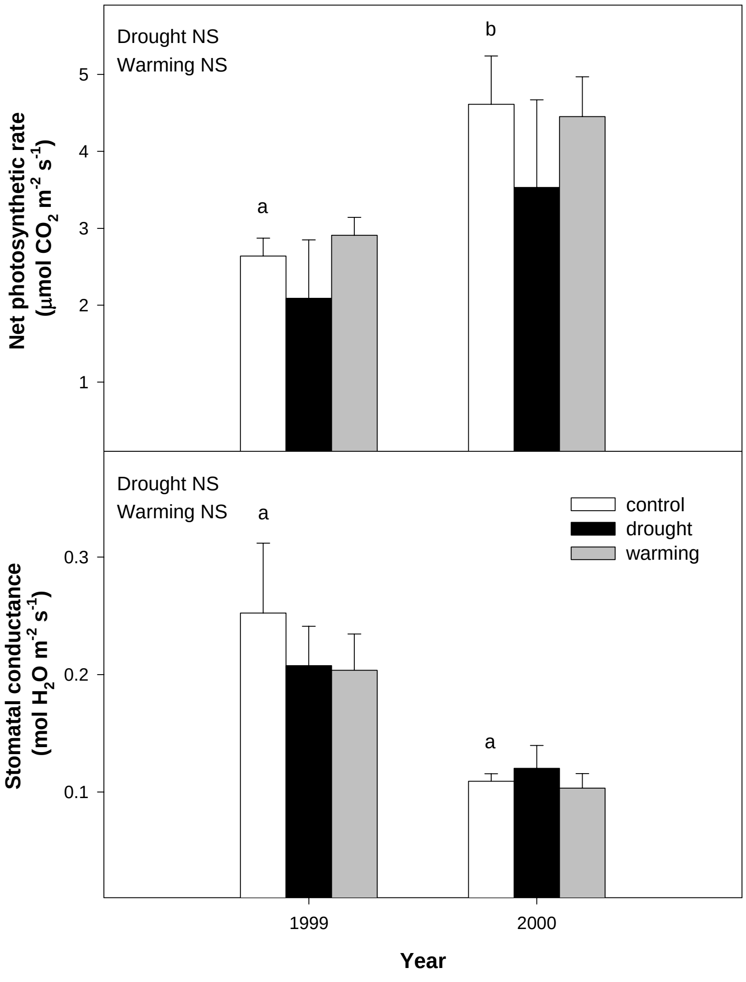

Fig. 4- Leaf net photosynthetic rate and stomatal conductance for control, drought and warming treatments in 1999 and 2000. Error bars indicate the standard errors of the mean (n=3 plot averages of 3-4 leaves each). Significant differences are indicated as in Fig. 1.

Spearman correlations between the average of leaf gas exchange values and the total rainfall received by each treatment over the growing season were not significant (r_s=0.37, p=0.47 for the leaf photosynthetic rates and r_s=-0.09, p=0.87 for the stomatal conductance; in both cases, n=6).

Discussion

The detected relative fluctuating asymmetry (FA) values in *V. myrtillus* (Fig. 2) were within the range of variability observed in previous studies of floral and foliar asymmetry (0.01-0.10; Møller and Eriksson 1994). Likewise, detected measurement errors (ME) of leaf area and leaf width were also within the range of variability (3%-20%) observed in previous studies (Møller and Shykoff 1999). Finally, repeatabilities of leaf asymmetries were high and comparable to those reported in similar studies, suggesting that methods to study FA were properly applied.

Drought effects

In relation to control plots, drought treatment was stronger in 2000 than in 1999, since the treatment excluded 37% of the accumulated rainfall over June and July in 2000 and 23% in 1999. Accordingly, whereas in 1999 we did not find any significant effect of drought treatment on relative leaf area FA, in 2000 drought leaves showed higher leaf area FA values than control leaves (Fig. 2). On the other hand, values of leaf area FA of control plants in 1999 were higher than in 2000, in agreement with the lower rainfall experienced by control plots during the growing season of 1999. Thus, our results suggest that a reduction in water availability increased stress conditions, affecting stability during leaf development, which is corroborated by the significant correlation between the relative leaf area FA values and the total rainfall over the growing season (Fig. 3).

To date, supporting evidence for an increase in leaf FA in response to decreased water availability during the growing season was limited. Hochwender and Fritz (1999) found that even though *Salix* plants developing in dry sites tended to present higher levels of FA than those in wet sites, the differences were not statistically significant. In another study, even though plants that were habituated to long periods of aridity tended to present greater developmental stability against extreme drought conditions than plants of the same species growing in more benign habitats, no significant differences of

FA between locations were observed (Alados et al. 2001). Furthermore, in a study over a period of 8 years, Valkama and Kozlov (2001) did not find any effect of the amount of precipitation on birch leaf FA.

It is well known that an increase in drought can cause a significant reduction in growth (Long and Hutchin 1991, Pereira and Chaves 1995) and a decrease in leaf photosynthetic rates, mostly as a consequence of stomatal closure (Chaves 1991). Our drought treatment tended to decrease leaf size (area or width) and leaf gas exchange (photosynthetic rates and stomatal conductance) of plants, though the differences with control plants were not statistically significant (Fig. 1 and Fig. 4). It is likely that the absence of drought effects on leaf gas exchange in 1999 were due to the low treatment intensity. In 2000, the still high water availability in drought plots and especially the low evaporative demand (low VPD, PAR and temperature during the measuring date) probably hindered any differentiation in stomatal conductance between treatments. When comparing control leaves between the two years, the lower leaf photosynthetic rates that we found in 1999 than in 2000 are in agreement with the lower rainfall registered in the growing season of 1999 and are consistent with the higher leaf asymmetry of control leaves in 1999.

Warming effects

In our study, warming treatment decreased leaf area FA during the first year (Fig. 2), suggesting a positive effect of nocturnal warming on the development of *V. myrtillus* leaves. Conversely, no effect of warming on leaf FA was found in 2000. Such differences between years were probably related with the differences in minimum temperatures during the growing season of both years. Indeed, the average of minimum temperatures for June and July in 1999 was 4.5 ºC, whereas in 2000 was 5.7 ºC. Thus, our results support the hypothesis that low temperatures throughout the growing season may be a stress factor for leaf development in *V. myrtillus*. Havström et al. (1993) pointed out that, during particularly cold years, temperature might be a limiting factor for the leaf growth, whereas during years with warmer summers, other factors (e.g., nutrients or water) can become limiting. The lower FA of control leaves in 2000 in relation to 1999 values, also match with the higher minimum temperatures experienced by leaves during June and July in 2000. Using a data set collected over a period of 8 years, Valkama and Kozlov (2001) found that asymmetry in birch leaves was highest during cold summers and lowest during warm summers.

In the present study, an increase of c.a. 1 ºC did not affect leaf size (area or width) of *V. myrtillus* (Fig. 1). Other studies have not either found significant effects of warming on leaf size (Wookey et al. 1994). In contrast, Callaghan et al. (1989) found a positive correlation between the temperature of the growing season and the leaf length in *Cassiope tetragona* from the arctic. Accordingly, an experimental warming of a sub-Arctic heath in Sweden increased leaf area in *V. myrtillus* (Parsons et al. 1994). Some studies have reported contrasting effects of increases in temperature on leaf size of different species. For example, whereas the graminoid *Eriophorum vaginatum* increased its leaf length after an experimental rise in temperature (+ 3.5 ºC) in tussock tundra of Alaska, the evergreen shrubs *Ledum palustre* and *Vaccinium vitis-idaea* were relatively insensitive to the treatment (Chapin and Shaver 1996). In addition, although *Cassiope tetragona* increased leaf length in response to an experimental increase in air temperature by 2-4 ºC at a sub-arctic fellfield site (1150 m a.s.l.), there was no change in leaf length of the same species in response to the treatment at a subarctic tree-line heath site (450 m a.s.l.) (Havström et al. 1993).

Finally, leaf gas exchange rates were not affected by warming treatment (Fig. 4). Several studies did not either find significant effects on leaf CO_2 assimilation and stomatal conductance after performing different climate warming manipulations (Wookey et al. 1994, Teskey 1997, Loik et al. 2000). On the contrary, other studies found significant effects of warming on photosynthesis, e.g. an increase of 3.5 ºC in air temperature during summer stimulated photosynthesis in *Eriophorum* and *Vaccinium* in arctic Alaska (Chapin and Shaver 1996). In our study, the absence of a direct warming effect on leaf gas exchange rates may be due to the fact that during the diurnal hours, when photosynthesis takes place, the warming treatment is very low (Beier et al. 2002).

Final remarks and conclusions

The ecophysiological significance of increases in leaf asymmetry, such the ones found in the present study, is difficult to assess, since the relationship between leaf asymmetry and fitness is not clear. Several authors have proposed different physiological consequences of leaf asymmetry, such as a less efficient use of light for photosynthesis among leaves on a branch (Møller 1995), a less even distribution of drag by wind causing an increase in damage during strong winds (Møller 1995), and a less even distribution of resources from the midvein throughout the leaf limiting leaf growth (Wilsey and Saloniemi 1999). None of these hypotheses have been demonstrated. However, Møller found that increased leaf FA in *Ulmus glabra* gave rise to higher infestation by leaf-mining insects (Møller 1995) and higher disease susceptibility (Møller 1999). In contrast, other authors failed to find a significant relationship between leaf asymmetry and some fitness components, such as reproductive effort, biomass accumulation or flower production (Roy and Stanton 1999, Andalo et al. 2000) while others have found equivocal results (Evans and Marshall 1996).

Our results support the idea that the measure of leaf FA provides a sensitive and inexpensive means to quantify developing stresses in plants in response to an increase in the severity of drought and warming. Thus, fluctuating asymmetry could be used as a tool for monitoring the impact of climatic change. In contrast, measurements of leaf size and instantaneous leaf gas exchange rates failed to provide evidence of physiological stress due to our environmental manipulations.

The reduction in water availability applied during the growing season increased the leaf fluctuating asymmetry in *V. myrtillus* indicating enhanced physiological stress during leaf development. On the other hand, the experimental warming reduced leaf fluctuating asymmetry, suggesting a decrease in the physiological stress during leaf development. However, a great inter-annual variability in the response of leaf FA to the treatments has been observed, which suggests that stressing environmental factors can change from one year to another. Differences between years supported the results obtained from the environmental manipulations.

Finally, this long-lived, slow-growing plant may not have acclimatised fully after only two years of manipulations. Therefore, measurements need to be continued if the long term effects of climatic change on the physiological stress of this species are to be elucidated.

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CHAPTER 7

Non-intrusive field experiments show different plant responses to warming and drought among sites, seasons and species in a North-South European gradient

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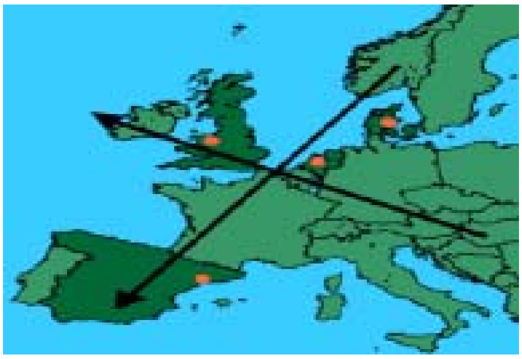
ECOSYSTEMS, in press



Abstract

We used a novel, non-intrusive experimental system to examine plant response to warming and drought across a climatic and geographical latitudinal gradient of shrub land ecosystems in four sites from northern to southern Europe (UK, Denmark, Netherlands, and Spain). In the first two years of experimentation reported here, we measured plant cover and biomass by the pin-point method, plant ^{14}C uptake, stem and shoot growth, flowering, leaf chemical concentration, litterfall and herbivory damage in the dominant plant species of each site. The two years of ca. 1 ºC experimental warming induced a 15% increase in total aboveground plant biomass growth in the UK site. Both direct and indirect effects of warming such as longer growth season and increased nutrient availability are likely to be particularly important in this and the other northern sites which tend to be temperature-limited. In the water-stressed southern site, there was no increase in total aboveground plant biomass growth as expected since warming increases water loss, and temperatures in those ecosystems are already close to the optimum for photosynthesis. The southern site presented instead the most negative response to the drought treatment consisting in a soil moisture reduction at the peak of the growing season ranging from 33% in the Spanish site to 82% in the Netherlands site. In the Spanish site there was a 14% decrease in total aboveground plant biomass growth relative to control. Flowering was decreased by drought (up to 24% in the UK and 40% in Spain). Warming and drought decreased litterfall in the Netherlands site (33 and 37% respectively) but did not affect it in the Spanish site. The tissue P concentrations generally decreased and the ratio N/P increased with warming and drought except in the UK site, indicating a progressive importance of P limitation as a consequence of warming and drought. The magnitude of the response to warming and drought was thus very sensitive to differences among sites (cold-wet northern sites were more sensitive to warming and the warm-dry southern site more sensitive to drought), seasons (plant processes were more sensitive to warming during the winter than during the summer), and species. As a result of these multiple plant responses, ecosystem and community level consequences may be expected.

Key words:

Calluna vulgaris, climate change, drought, *Erica multiflora*, European gradient, flowering, heathland, herbivory, plant biomass, plant chemistry, shrubland, warming

Introduction

Temperature and water are key factors that regulate biological processes. Global air temperatures have increased by 0.6 ºC during the 20thcentury and are predicted to increase between 1.4 and 5.8 ºC over this century, with the greatest increases expected in northern latitudes (IPCC 2001). These increases will be accompanied by changes in precipitation that are more difficult to forecast. However, many models predict increases of summer drought for some central and southern European regions (IPCC 2001).

Over the last ten years the need for information on the response of organisms and ecosystems to warming has been addressed by a growing number of temperature-manipulation experiments around the world. As a result, there are now substantial observational and experimental studies demonstrating the link between change in regional climate and biological processes in ecosystems. Arft et al (1999), Shaver et al (2000), and Rustad et al. (2001) have recently reviewed many of them. The responses of plant productivity and other ecosystem processes have been quite variable, with reported increases, decreases or no change (see references in Rustad et al. 2001). However, Antle et al (2001) have assembled a database of more than 2500 studies that addressed climate and either a physical or a biological process, and found that 80% of the studied species showing a change are changing in the manner expected with global warming, while 20% are changing in the opposite direction. Moreover, there is also a wide number of phenological observational, satellite and atmospheric data showing clear biological responses to warming both in northern and southern, cold-wet and warm-dry ecosystems (Peñuelas and Filella 2001; Peñuelas et al. 2002; Walther et al. 2002).

In spite of the relatively robust literature on the response of individual ecological processes to changing temperature, many questions remain unsolved: how will the relative sensitivity of respiration and photosynthesis affect net plant and ecosystem C storage and fluxes? Will the plant reproductive effort be affected? Will the plant nutrient uptake, use and content change? Will the litterfall change? Will the interactions plant-herbivore be altered? What will control or limit the magnitude of these responses? Will the responses remain constant over time? Will the responses strongly differ among species and ecosystems, or even seasons? For example, will higher latitude ecosystems be more affected than lower latitude ecosystems? Here we aimed to answer some of these questions relative to vegetation by measuring the responses of critical plant processes including C fixation, growth, cover and biomass, net primary productivity (NPP), flowering, chemistry, litterfall and herbivory, with a novel non-intrusive experimental approach that involves nighttime warming and drought at field scale. We manipulated temperature and precipitation in four shrubland ecosystems across a temperature and precipitation gradient from North to South Europe (Beier et al. 2003). The experimental approach was specifically chosen to overcome some of the main drawbacks of previous studies: intrusiveness of the methods, constant or diurnal more than nocturnal warming manipulations, a dominance of studies in cold and wet temperate and arctic ecosystems, lack of common protocols, and short-term (even though the results reported here are only for the first two years) and local limitations.

Most of the field experiments simulating climate change that have been reported used intrusive warming techniques that alter environmental conditions with unintended lateral effects and simulated diurnal increase of temperature (see references in Beier et al. 2003). As global warming is principally caused by a reduced loss of long wave IR-radiation from the Earth back into the outer atmosphere because of the greenhouse gas accumulation in the atmosphere, warming is expected to occur mainly at night. This expectation is supported by recent analysis of global temperature records showing that the observed global temperature increase of $0.6^{o}C$ has been due to larger increases during night compared to increases during the day (Easterling et al. 1997, Alward et al. 1999). We therefore studied the effects on vegetation of nighttime warming (also remaining most of the diurnal hours).

The great majority of studies on climate change effects have been conducted in northern temperate and arctic ecosystems, when the responses can be very different in southern warmer and drier ecosystems, since the factors constraining plant and ecosystem functioning strongly differ. The most limiting factor in northern European countries is temperature (Körner and Larcher 1988) whereas in southern European countries it is water availability (Larcher 2000). Therefore we studied the response to the warming and drought experimental treatments in northern and southern European sites.

To approach vegetation (and ecosystem) functioning response at larger spatial and temporal scales, we report local relatively short-term studies (we report results after two years of treatment here although the experiment is currently continuing), but across a natural climatic and environmental gradient in a geographical transect from North to

South Europe. With this geographical approach we aimed to take into consideration long-term responses of vegetation to environmental conditions (Chapin et al. 1995, Shaver et al 2000, Rustad et al. 2001). Long-term responses in vegetation patterns have shown close correspondence to distributions along environmental gradients, and therefore, different behavior of the sites across a climate gradient may be used as an indicator of long-term climate effects.

With this novel approach we examined the following specific hypotheses corresponding to the above listed questions:

1. *Vegetative growth.*

We hypothesized that most species would exhibit measurable significant increases in vegetative growth due to warming, whereas decreases were expected in response to drought. However, the response would depend on the species and the site. The response to warming would be greater in cold northern latitudes and the response to drought greater in drier southern latitudes since higher latitude ecosystems are typically strongly limited by temperature (Chapin et al. 1995), and lower latitude Mediterranean ecosystems are typically strongly limited by drought (Mitrakos 1980, Peñuelas 2000, Terradas 2001).

Warming was moreover expected to increase growth in the northern ecosystems because it increases the length of the seasonal and daily growing cycles (Peñuelas and Filella 2001). However, there was also the possibility of warming negative effects on plant growth in these northern sites since phenological growth advances (Peñuelas and Filella 2001) may result in increased frost damage risk (Ögren 1996). Another possible indirect positive effect of warming on plant growth may occur through increasing rates of soil matter decomposition and net mineralization resulting in greater nutrient availability.

Warming was, on the contrary, expected to decrease plant growth in water-stressed southern ecosystems since it increases water loss, and temperatures in those ecosystems are already close to the optimum (Shaver et al 2000, Larcher 2000). In the southern site, indirect effects of drought through reduced nutrient availability would also lead to decreased plant productivity.

2. Plant reproductive effort.

We hypothesized that flowering (sexual reproduction effort) would be enhanced by warming and decreased by drought in parallel with the effects on carbon fixation.

3. Litterfall

We hypothesized increased amounts as a result of increased growth and litter supply in response to warming and decreased amounts as a result of decreased growth in response to drought in northern sites. In the southern site we expected decreased amounts in both treatments as a result of the expected decrease in growth.

4. Plant chemistry

We hypothesized that increasing soil temperature ca 1 ºC would result in greater nutrient availability due to increased rates of decomposition and net mineralization. The contrary effects were hypothesized for drought treatments. The effects on tissue elemental concentrations will depend on the simultaneous growth change. The tissue concentration of condensed tannins, as representative of the carbon based secondary compounds with deterrent properties for herbivores and recalcitrance for decomposition, was expected to decrease with warming and associated higher nutrient availability and to increase with drought and associated lower nutrient and water availabilities (Peñuelas and Estiarte 1998).

5. Herbivory

We hypothesized that warming and consequent extension of growing season and winter survival would enhance herbivore development, abundance and activity, especially in the northern sites. Although direct impacts of precipitation changes on insect herbivores have been largely neglected in current research of climate change (Bale et al 2002), we here expected that drought would enhance the relative herbivory damage to the plants since less biomass was expected to be available for herbivores.

Since many of these hypotheses have been examined in specific research contexts or in meta-analyses of diverse studies mostly conducted in high latitude sites, our objective was to test them across a variety of sites and conditions in a large geographical gradient, at the ecosystem level, and with a common new non-intrusive method. We thus followed most of the recommendations for research on ecosystem response to climate change emanated from the scientific community devoted to this issue (see for example Rustad et al. 2001)

Material and methods

Sites and manipulations

Manipulations were carried out on shrublands dominated by Ericacea at 4 sites (Wales-UK, Denmark, Netherlands, Catalonia-Spain) spanning an European gradient in temperature and precipitation (Table 1 in Beier et al. 2003). In each site, we conducted field-scale nighttime warming and drought treatments and the response to the treatments was compared to control plots. Each type of manipulation was replicated 3 times at each site. Pre-treatment measurements were conducted in order to identify variability between sites or plots.

Warming treatment

The warming treatment was performed as nighttime warming by reflective curtains covering the vegetation at night (Figure 1 in Beier et al. 2003). Solar energy is accumulated in the ecosystem during the day and a fraction of the energy is re-radiated back to the atmosphere at night as long wave IR-radiation. The covering of the ecosystem with the reflective curtains reduces the loss of IR radiation. The warming plots are 20 m^2 (5x4 m) covered by a light scaffolding carrying the reflective aluminum curtain. The coverage of the study plots is activated automatically according to preset light (< 200 lux), rain and wind ($< 10\ m\ s^{-1}$) conditions (Beier et al 2003). In order to avoid influencing the hydrological cycle, the covers are automatically removed during rain events, triggered by rain sensors. The warming treatment has been applied since spring 1999 with an effect of warming the soil and plants in the order of ca. 1 ^{o}C (also during several diurnal hours) with slight variations depending on site, time of the year and meteorological conditions (Fig. 5 in Beier et al. 2003). Warming treatment increased the number of growing degree-days 10-20% at the northern sites, but did not affect it in the Spanish site (Beier et al 2003).

Drought treatment

The drought treatment was performed for a 2-month period in the spring/summer growing season in 1999 and 2000 (in the southern site an additional drought period was established in the autumn growing season; Table 2 in Beier et al. 2003) by covering the vegetation with waterproof, transparent covers. The drought plots are constructed similar to the warming plots except that the curtain material is a transparent plastic and that the moving of the curtains is only governed by the rain and wind. During the drought period the rain sensors activate the curtain to cover the plots whenever it rains and remove the curtains when the rain stops. The curtains are removed automatically if the wind speed exceeds 10 m s^{-1}. For the part of the year without drought treatment, the drought plots were run parallel to the control plots. Soil moisture decreased slightly in response to the warming treatment (up to 10% compared to the control plots in the Spanish and Dutch sites; Fig. 9 in Beier et al. 2003). The maximum decrease of soil moisture during the applied drought treatment ranged from 82% in the Netherlands to 33% in the Spanish site while air and soil temperatures were not affected. For the rest of the year, the drought treatment was off and therefore, the soil water content was not affected (Fig. 9 in Beier et al 2003).

"Untreated control"

Three untreated control plots with a similar light scaffolding as for the warming and drought treatments but without any curtain were installed for comparison.

Plant response

We conducted yearly monitoring of plant responses to the above described climate-manipulation treatments. Plant ^{14}C fixation, plant growth, plant cover and biomass, flowering (reproductive effort), plant chemistry, litterfall, and herbivory plant damage were the variables studied. The objective was to assess relative changes in plant performance among treatments, for the different sites and species. Since spatial variation was different from site to site and species to species, we used preliminary sampling to adjust sample sizes to our observed variability. We then applied the following measurements to all the experimental plots.

^{14}C allocation

The ^{14}C pulse labeling experiment was carried out with the dominant Ericacea species of each site (Gorissen et al. 2003). In Wales, Denmark, and the Netherlands this was *Calluna vulgaris* and in Spain *Erica multiflora*. Nine similar plants were selected outside the plots in each site and planted in cores. In each one of the nine plots it was placed one of these cores with plant in the soil in the fall of 1999. All plants in the Dutch site were eaten over winter, so no C allocation data from this site are available. The day before the ^{14}C-labelling, in spring 2000, the plants were sealed at the soil surface with a silicone sealant to achieve an airtight seal between the soil core headspace and the atmosphere. At labeling, a glass vial with a solution containing 5 MBq ^{14}C-labelled Na_2CO_3 was placed on top of the column. The shoot was subsequently enclosed in a polythene cover and fixed airtight around the top of the column. ^{14}C-CO_2 was released by injecting 20 ml 5 N HCl into the glass vial through a septum in the plastic cover. An equal amount of unlabelled CO_2 was released after 150 minutes to increase the uptake efficiency of ^{14}C. The labeling took place between 8 and 11 a.m. to prevent excessive temperatures in the plastic bag. All plants were harvested, dried and ground. Total carbon and ^{14}C content in shoots, roots, and soil were determined after wet digestion (see details in Gorissen et al. 2003).

Plant cover and biomass: pin-point method

The pin-point method was used for measuring plant frequency, and indirectly estimating plant cover, biomass and growth. These pin-point measurements were conducted annually each July. The vegetation response is mainly assessed by measuring species types and plant contacts and heights at a large number of points in each plot. This method enables, moreover, the assessment of changes in species composition, and species-specific growth to be recorded. A sharpened pin was lowered through the vegetation at a number of points. Each plant hit with the pin was counted and the plant species (or species group) recorded. At least 300 measurement points were used for each experimental plot. These points were arranged at 5 cm intervals along replicated 5 3-m long transect lines (in Spain) or 4 4-m long transect lines (in the Netherlands) or in subplots (in Denmark and Wales). For grasses, graminoids and herbs, the following contact types were differentiated: (a) green vegetative parts (leaves+stems), (b) standing dead vegetative parts, (c) reproductive structures (flowers, inflorescences, and fruits). For shrubs, the following were differentiated: (a) green leaves, (b) dead leaves, (c) stems, (d) reproductive structures [for *Calluna*, also (e) green shoots, (f) brown current-year shoots, besides (c) and (d)]. For each hit, the height above the ground was also recorded. For doing this, we used 1-cm marks along the pin or along a vertical rod held behind the pin in a way that did not disturb the vegetation. We expressed the total number of hits for each species (and for each plant part of each species, if relevant) per transect, and also as a proportion of the entire pin hits per transect. We also calibrated the pin-point measurements against absolute biomass using destructive sampling outside the plots. Biomass was regressed against hit numbers (or proportions), or (if hit heights were recorded) against hit numbers (or proportions) and heights.

Plant growth

The pin-point method does not provide yearly plant growth directly (though the frequency of hits was of course affected by plant growth and was also used to indirectly assess net aboveground biomass change). In two sites, the UK and Spain, direct measurement of plant growth was obtained by marking shoots and measuring them at intervals. A sample of 20-30 terminal shoots of the two most dominant site species were permanently marked in each plot. We measured the length of each shoot. In Spain, secondary growth (i.e. diameter increment) was also monitored. We marked a sample of 20-30 shoots and measured the basal diameter with digital calipers by taking 2 perpendicular readings at each one. We also marked the exact position where diameter had been measured to allow re-measuring at consecutive years. We calculated the increase in the basal diameter for two consecutive years (and the two different growing seasons in the third year in Spain). Thereafter, we also estimated plant growth by using allometric relationships between biomass and basal diameter and shoot lengths obtained with destructive sampling of plants outside the plots.

Flowering (Reproductive Effort)

The percentages of flowering shoots or flowering plants were measured in the pin-point campaigns. Moreover, in the UK and Spain we estimated for the dominant species the percentage of flowering of each plant establishing a scale from 0 to 4, (i.e., 0, 1-25, 26-50, 51-75, and 76-100% of flowering shoots relative to total plant shoots). In the Spanish site, we monitored the flowering of the two dominant shrub species (*Erica multiflora* and *Globularia alypum*) during flowering seasons. The two species have a different flowering pattern: whereas *Erica multiflora* presents a single flowering bloom, *Globularia alypum* has two to three blooms, in summer, autumn and winter. Flowering numbers were used as the best available indicator of reproductive effort

Plant chemical composition

We sampled leaves and shoots of upper canopy current-year growth when they were mature (July-August) every year. We separated them into flowers, leaves and stems, ground them to pass a fine mesh, and stored them until analyses. We analyzed C and N using the usual configuration (Peñuelas et al 2001a), P by ICP analyses (Peñuelas et al. 2001a); and foliar tannins by butanol (proantocyanidin) (Waterman and Mole, 1994) methods.

Plant litter production and quality

In each plot we installed 20-30 litterfall collectors (i.e. small pots with small holes allowing for drainage) randomly located under the plant canopy (or distinguishing between below plants of *E. multiflora* and *G. alypum* in the scattered vegetation of Spain). We collected the litter monthly (or every two or six months, depending on the site). We oven dried litter, we then ground it to pass a fine mesh and stored it until analyses. We analyzed C, N, P, and tannins by using the above-mentioned analytical methods.

Herbivory

In each site we measured the percentage of leaves damaged by herbivore feeding, either by differentiating the pin contacts (eaten vs. undamaged leaves) in the pin-point assessment (northern sites) or by randomly assessing 4 shoots in each one of 7 *E. multiflora* plants in each plot (in Spain).

Statistical analyses

We first conducted one way ANOVAs and post-hoc comparison tests with each measured plant variable as dependent variable and country as fixed factor. We thereafter conducted one way ANOVAs comparing warming and drought treatments separately with control treatment in each country. We used only the mean value of each variable for each plot, so that n = 3 per treatment because we considered the roof/plot as the smallest independent unit. We used as many sub-plots/ replicate samples as possible (usually 3 or more, up to 30, depending on the measured variable) to provide a good mean estimate for each plot. Once we had established the statistical significance of the treatment, we distilled the results of each measurement in each site in the form of magnitude percentage of the effect of the treatment (warming or drought). We compared values of treatment plots with values of control plots to best represent the relative response of plant processes to warming and drought on a common scale for all sites, species and variables.

Warming change $W= (Mw-Mc)/Mc$

Drought change D= (Md-Mc)/Mc

Where Mw, Md and Mc are the means of the warming, drought and control plots respectively. A treatment change not significantly different from zero indicates no treatment effect. Significant values above 0 indicate that the experiment had a positive effect on the variable; significant values below 0 indicate a negative effect. The variances around control, warming and drought treatments were calculated with standard methods (Sokal and Rolf 1995) and used to determine weighted average effects across sites and the confidence intervals around those effects. For those variables with incomplete data for all sites and for the two experimental years, we used the data from the second year (2000). However, for Denmark we had to use 1999 data since there was an extremely severe herbivore attack in 2000.

Results

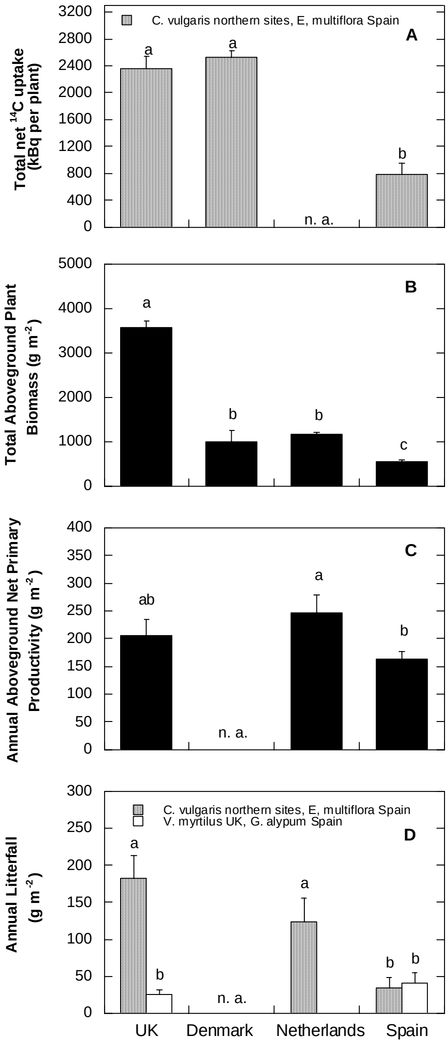


Fig. 1. Plant variables related to biomass: (A) plant ^{14}C fixation, (B) total aboveground plant biomass, (C) annual aboveground net primary productivity, and (D) annual litterfall in the control plots of the UK, Denmark, the Netherlands and Spain sites. Bars indicate the standard errors of the mean (n=3). Data from year 2000 (except for net primary productivity, where average of 1999 and 2000 is depicted). n.a. not available because of strong herbivory damage. Different letters indicate significantly different means (Tuckey post-hoc test of the ANOVA).

Total net ^{14}C uptake

Total net ^{14}C uptake by the plants was significantly smaller at the Spanish site than at the other sites (Fig. 1A). Warming treatment produced no significant effect on total net ^{14}C uptake in any site (Fig. 2A). Drought treatment significantly reduced the total net ^{14}C uptake by plants in Spain (95%) and Denmark (35%) sites (Fig. 2B).

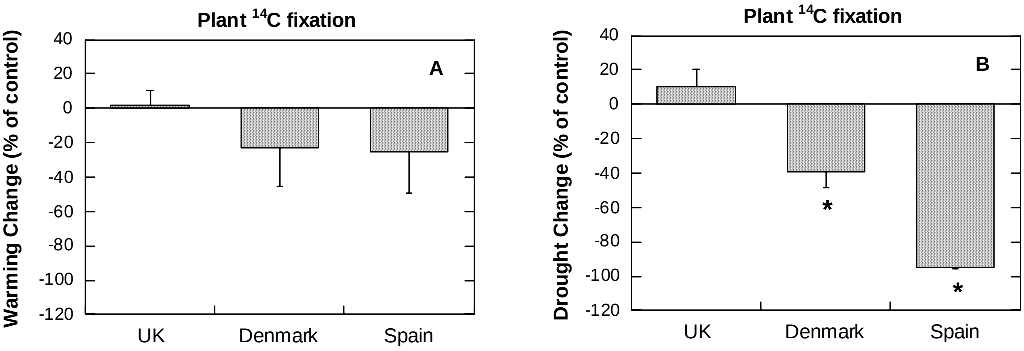


Fig. 2. Changes produced by the warming (A) and drought (B) treatments in the total plant net ^{14}C uptake by the Ericaceous C. vulgaris in the UK and Denmark and by the Ericaceous E. multiflora in Spain. Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * $p<0.05$ in the one way ANOVA conducted with actual net ^{14}C uptake values (KBq plant^{-1}) per plot.

Species annual growth

Plant growth, measured as diameter increase (*E. multiflora* and *G. alypum* in Spain) or shoot length (*C. vulgaris and V. myrtillus* in the UK), was not significantly affected by the warming treatment in the two studied species of the UK and in one of the species of Spain, the Ericacea *E. multiflora*. However, in the Spanish site, the growth of the other dominant species, *G. alypum*, was significantly inhibited (32%) by the warming treatment (Fig. 3A). Apart from these species-specific responses to warming, season-specific responses were also found for each species. Warming increased the diameter growth of *E. multiflora* by 84% in the period January-June, but not in the warm period July-December (Fig. 3C). Drought treatment decreased plant growth of *G. alypum* in Spain (29.5% relative to control treatment) and had no significant effect on *E. multiflora* or on the species of Welsh site (Fig. 3D).

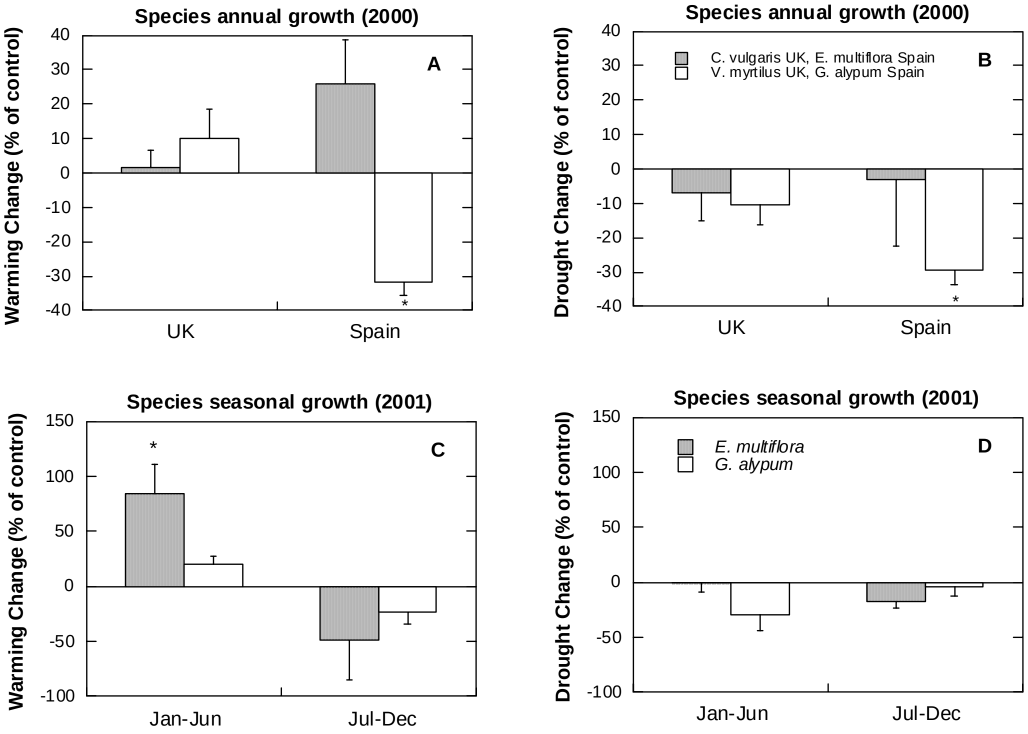


Fig. 3. Changes produced by the warming (A) and drought (B) treatments in the annual growth of dominant plant species. Growth was measured as increases in shoot length in the UK and in basal diameter in Spain. Measurements were conducted in the second year of experimentation (2000). Seasonal changes in the following year (2001) are also depicted in the lower panels (C for warming and D for drought). Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * $p<0.05$ in the one way ANOVA conducted with actual shoot length or basal diameter (mm) values per plot.

Plant cover and biomass

Plant biomass (cover) calculated from allometric relationships between pin-point measurements and biomass was significantly greater in the UK site than in the other ones. The Spanish site was the one with smallest total plant biomass (Fig. 1B). The warming treatment increased the total plant biomass only in the northern sites (up to 15% in the UK site) but not in the southern Mediterranean site (Fig. 4A). Drought treatment significantly reduced it (14%) only in the Spanish site (Fig. 4B).

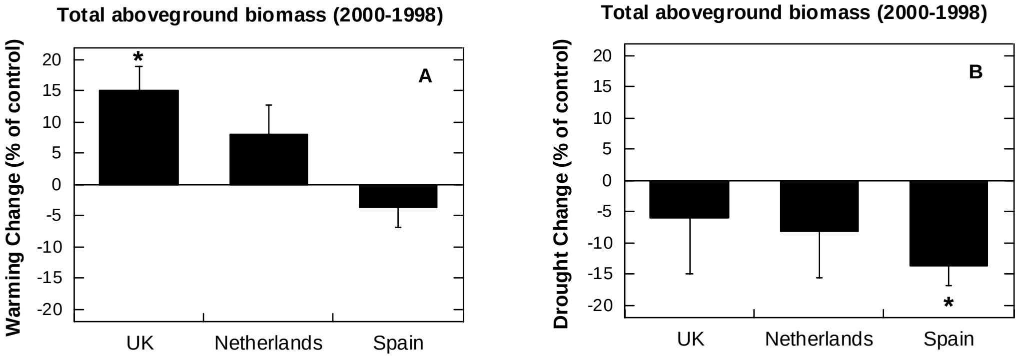


Fig. 4. Changes produced by the warming (A) and drought (B) treatments in the total plant aboveground biomass after two growing seasons of experimentation. They were measured as differences in the number of hits between summer 2000 and summer 1998. Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * (p<0.05) in the one way ANOVA conducted with actual hit differences in each plot.

Flowering (Reproductive effort)

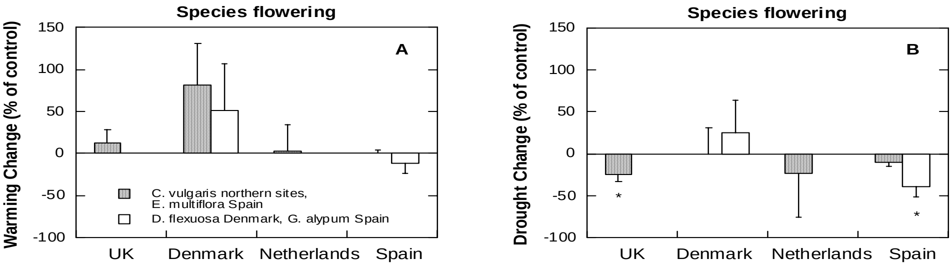


Fig. 5. Changes in species flowering resulting from the warming (A) and drought (B) treatments after two growing seasons of experimentation (data from year 2000). Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * p<0.05 in the one way ANOVA conducted with actual flowering percentages per plot.

The warming treatments did not significantly affect plant flowering in any of the studied sites (Fig. 5A). However, in the Spanish site the warming treatment increased flowering of *G. alypum* at the end of autumn flowering peak whereas it decreased it at the end of summer flowering peak (Fig. 6). The drought treatments decreased flowering of *C. vulgaris* at the UK site (24%) and of *G. alypum* at the Spain site (40%) (Fig. 5B). In the

Spain site, when comparing seasons, it was found that drought treatment decreased flowering in summer (Fig. 6).

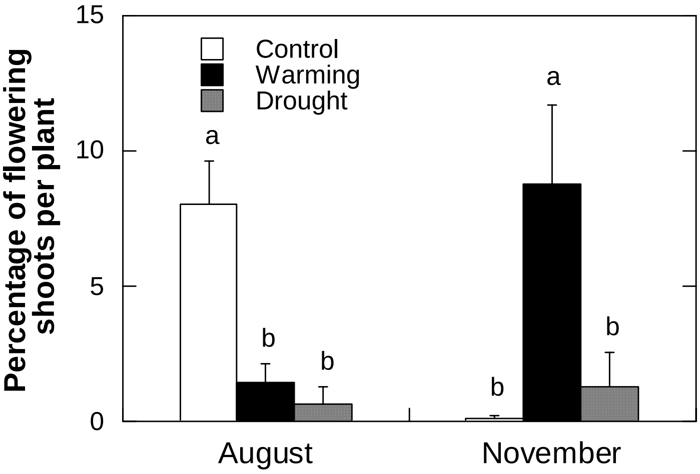


Fig. 6. Changes produced by the warming and drought treatments on flowering of *G. alypum* at the end of summer flowering peak and at the end of autumn flowering peak (data from year 2000). Bars indicate the standard errors of the mean (n=3). Different letters indicate significantly ($p<0.05$) different means (Tuckey post-hoc test of the ANOVA).

Litterfall

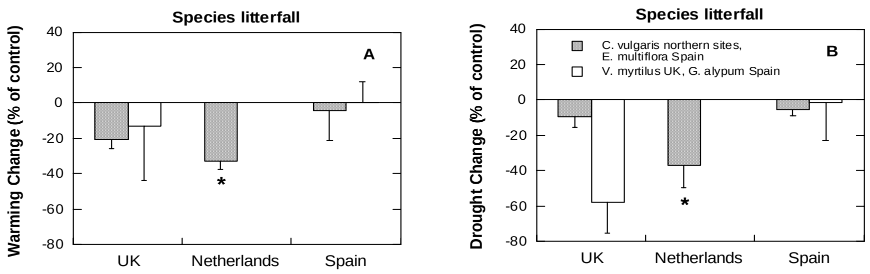


Fig. 7. Changes produced by the warming (A) and drought (B) treatments on species litterfall amount (data from year 2000). Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * $p<0.05$ in the one way ANOVA conducted with actual annual litterfall amounts in g m^{-2} per plot.

Plant litterfall amounts were smaller at the Spanish site compared to the other sites (Fig. 1D) in consonance with the smaller plant biomass. The warming treatment significantly decreased (33%) the amount of litterfall only in the Netherlands site (no data available for Denmark) (Fig. 7A). Similarly, the drought treatment significantly decreased it (37%) also only in the Netherlands site (Fig. 7B).

Foliar N, P and condensed tannin concentrations

Foliar N and P concentrations were significantly smaller at the Spanish site than at the other sites (Fig. 8A,B). N/P ratio was greater in the Netherlands (ca 21) and Spain (15-20) compared to Wales (12-13) and Denmark (8-10). The warming treatment had no effect on the foliar N concentrations in any site except in the Danish site attacked by heather beetle, where warming produced a significant increase in *C. vulgaris* (11%) (Fig. 9A). The drought treatment did not produce any significant effect in any site (Fig. 9B).

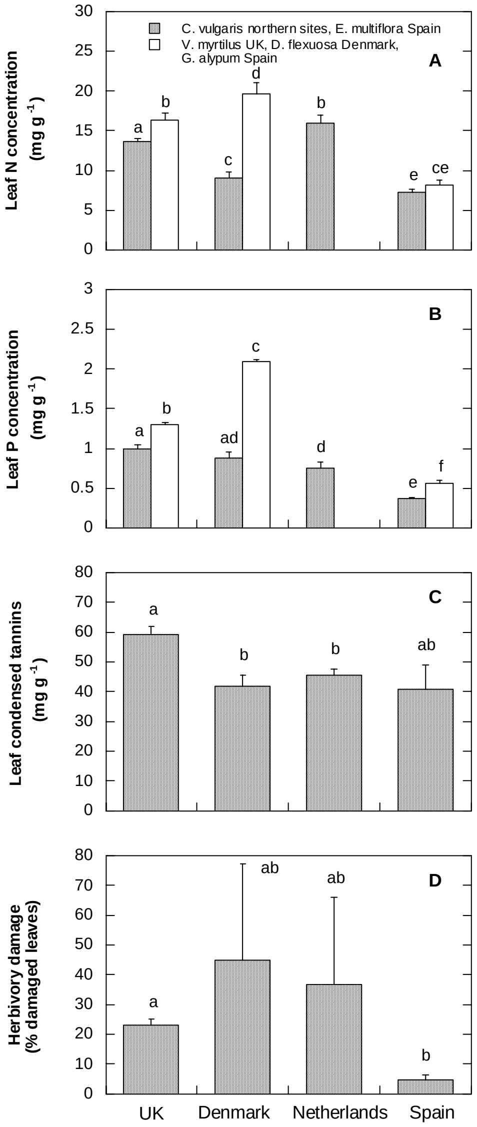


Fig. 8. Plant variables related to plant chemistry: foliar N (panel A), P (panel B) and condensed tannin (panel C) concentrations, and herbivory damage (panel D) in the control plots of the UK, Denmark, the Netherlands and Spain sites. Bars indicate the standard errors of the mean (n=3). Data from year 2000 (except for Denmark where we used 1999 data since the heather beetles wholly ate *C.vulgaris* plants in 2000). Different letters indicate significantly different means (Tuckey post-hoc test of the ANOVA).

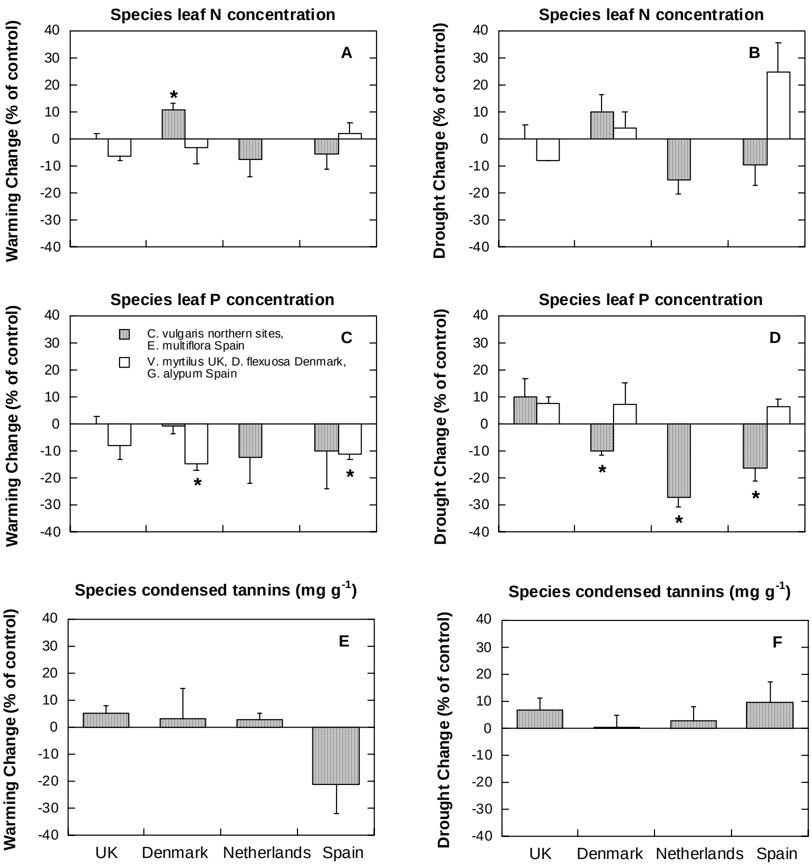


Fig. 9. Changes in species leaf N (panels A and B), P (panels C and D) and condensed tannin (panels E and F) concentrations resulting from the warming (A,C,E) and drought (B,D,F) treatments after two growing seasons of experimentation (data from year 2000 (except for Denmark where we used 1999 data since the heather beetles wholly ate *C.vulgaris* plants in 2000). Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * $p<0.05$ in the one way ANOVA conducted with actual leaf N, P and condensed tannin concentration values (mg g^{-1}) per plot.

The warming treatment decreased the foliar P concentrations of *D. flexuosa* in Denmark (15%) and *G. alypum* in Spain (11%) (Fig. 9C). The drought treatment significantly decreased the leaf P concentration of *C. vulgaris* in Denmark (10%) and in the Netherlands (27%), and of *E. multiflora* in Spain (16%) (Fig. 9D). As a consequence of these warming and drought effects, the N/P ratios tended to increase in the warming treatments for all species and sites (ca 5-15%) and in the drought treatments for all sites (10-22%) except for Wales where the ratio decreased 5-10% depending on the species.

The tissue condensed tannin concentration did not significantly change with warming or drought in any site (Fig. 9E,F).

Herbivory

Herbivory damage was significantly smaller at the Spanish site (less than 5%) than at the other sites (20-50%) (Fig. 8D). The warming treatment increased the herbivory foliar damage in the UK 45% relative to control, but had no significant effect in the other sites (Fig. 10A). The drought treatment had no significant effect in any site (Fig. 10B).

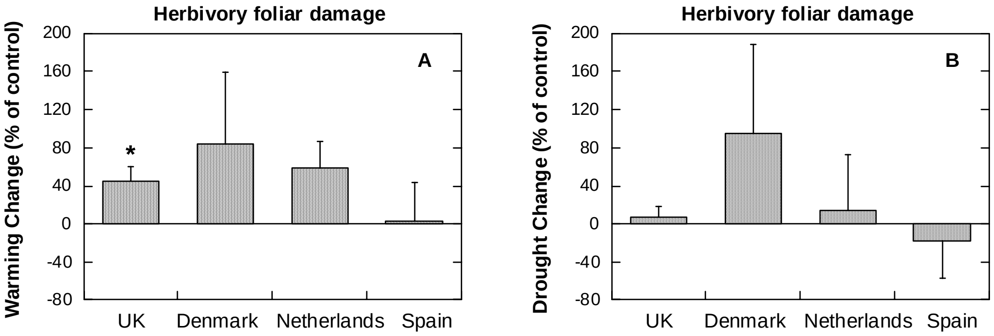


Fig. 10. Changes in herbivory damage resulting from the warming (A) and drought (B) treatments after two growing seasons of experimentation (data from year 2000 except for Denmark: year 1999). Changes are expressed relative to the control treatment. Bars indicate the standard errors of the mean (n=3). * p<0.05 in the one way ANOVA conducted with actual herbivory damage values (percentage of damaged leaves) per plot.

Relationship with site temperature and precipitation

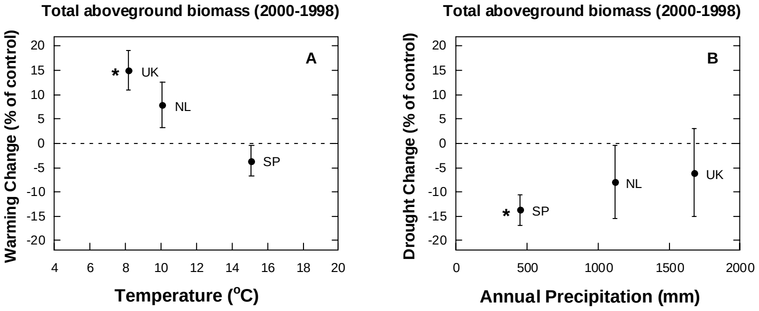


Fig. 11. Changes produced by the warming (A) and drought (B) treatments in the total plant aboveground biomass plotted versus annual precipitation and mean annual temperature for the three sites for which we had complete data. Bars indicate the standard errors of the mean (n=3 variables). * (p<0.05) in the one way ANOVA comparing treatment and control.

Net carbon uptake, biomass accumulation, flowering, litterfall, leaf N and P concentrations, and herbivory responses to warming and drought followed a gradient from wet-cold to dry-warm climatic environment. Fig. 11 highlights it by showing the net aboveground biomass growth in relationship to the temperature and rainfall of each site. The responses to warming were more positive the colder was the site (Fig. 11A), and the responses to drought were less negative the wetter was the site (Fig. 11B), i.e. the positive response to warming was larger in colder systems with higher precipitation and the negative response to drought was larger in warmer systems with lower precipitation.

Discussion

Plant biomass accumulation, litterfall and flowering

The two years of ca. 1 ºC experimental warming increase 15% plant growth in the UK site. This increase is close to the 19% increased plant productivity found in response to a meta-analysis of 20 studies of effects of warming (0.3-6 ºC) in arctic and temperate ecosystems (Rustad et al. 2001). This increase might be a direct effect of increased rates of photosynthesis (Körner and Larcher 1988), which however does not seem to be the case here according to Llorens et al (2003). A direct effect of longer growth seasons seems more likely. Northern sites presented larger increases in growing degree-days; and the largest was that of the UK site (see Table 5 in Beier et al. 2003). An indirect effect of increased nutrient availability resulting from increased rates of litter decomposition and N mineralization (Robinson et al 1997, and see data from Emmett et al. 2003 and Schmidt et al. 2003) may also contribute. These two factors have been described in studies of plant growth and productivity in arctic and subarctic ecosystems which are generally limited by low availability of N and/or P (Jonasson et al. 1999b, Shaver and Chapin 1995) and where plant growth is affected by short-term temperature enhancement, partly because of direct effects of warming and partly because of indirect effects of increasing nutrient mineralization in the warmed soils (Callaghan and Jonasson 1995). These results also show that there were no overall significant warming negative effects on plant growth through increased frost damage in early phenological stages as suggested in other studies (Ögren 1996) since warming increased total aboveground biomass (Fig. 4A).

In the water-stressed southern site, there was no general increase in plant productivity as result of the warming treatment as it had been hypothesized since warming increases water loss (see data from Spain and the Netherlands in Fig. 9 of

Beier et al. 2003), and temperatures in those ecosystems are already close to the optimum for photosynthesis and growth (Shaver et al 2000, Larcher 2000). Photosynthetic rates may even decrease due to overheating in the warmest seasons or microenvironments (Shaver et al 2000, Peñuelas and Llusià 2002, Llorens et al. 2003). The other most often cited explanations for the lack of response or negative plant response to increasing temperature are the concomitant increases in evapotranspiration and decline in soil moisture often associated with warming, and also the decreased availability of nutrient resources such as N and P (Rustad et al 2000). Both fit for the studied Mediterranean shrubland (Fig. 9 in Beier et al. 2003 and Fig 9A,C).

The southern site presented instead the most negative response to drought. In fact, in most southern semiarid areas drought is already limiting plant productivity (Mitrakos 1980, Peñuelas 2000, Larcher 2000, Peñuelas et al. 2001b, Terradas 2001) as already shown by the low ^{14}C recoveries (Fig. 1A), photosynthetic rates (Llorens et al. 2003), and standing plant cover and biomass of this Mediterranean shrubland (Fig. 1B). The dry and warm conditions force the plants to close their stomata to control transpiration, causing an accompanying reduction in photosynthesis (Llorens et al 2003) and in nutrient uptake by roots, which is under soil water control (Chapin 1980). Both direct effects through decreased photosynthetic rates and indirect effects through decreased nutrient availability accompanying drought treatment would go in the same direction of decreased plant productivity.

The increases in plant productivity in response to warming were expected to result in increased flux of leaf litter to soils (e.g. Yoneda et al. 2000), but instead there was a decrease in litterfall in the Netherlands site (Fig. 5A). Leaves may have remained on the plants for a longer period of time in response to higher temperatures in this northern site. Further monitoring during the following years is thus warranted. In the drought treatment of the Netherlands site there was also a decrease in the litterfall amount in consonance with the decrease in biomass growth and litter supply (Huntingford et al. 2000). Litter production may also be more affected by precipitation than by temperature, with lower litter production, the lower the precipitations (Simmons et al. 1996). No effects of warming or drought were found in the Spanish site, which was also unexpected since drought was expected to enhance leaf dropping (Harley et al. 1987, Oliveira and Peñuelas 2002) and warming to decrease leaf life span (Gracia et al. 2000). Further monitoring during the following years is needed to draw reliable conclusions at this point.

The flowering responses to warming and drought showed that the sexual reproduction effort, although was not significantly affected by the experimental warming, tended to be increased in the northern sites. Flowering was instead decreased by drought both in the northern and southern sites in consonance with the effects on carbon fixation and biomass accumulation. The responses of the southern site also fitted the trend when looked at the seasonal detail: whereas warming decreased flowering in the warm August, warming enhanced flowering in the cold November.

Plant chemistry and herbivory

The tissue P concentrations generally decreased with warming (the only significant change for N concentration was an increase in Denmark, likely linked to a beetle attack strongly reducing plant biomass). As the concentration change depends on the balance between nutrient and biomass change, the decreases in leaf concentrations indicate a relatively larger increase in biomass than in P uptake. These results agree with those of Jonasson et al (1999a) who also found a decline in P (and N) concentration in response to warming in many cases in their Northern latitude study because the P (and the N) generally were diluted by the increased growth. In fact, nutrient limitation appears as a major constraint to increased carbon accumulation by the vegetation in response to warming (Chapin et al. 1995). There were no significant responses of N concentrations to drought (Fig. 9B), in disagreement with the hypothesized lower nutrient availability and lower nutrient concentration. It again seems a consequence of the balance between nutrient and biomass changes. Since leaf P concentrations also decreased in response to drought in all sites except in the British one (Fig. 9D), the increases in the ratio N/P in almost all sites and treatments highlight a progressive importance of P limitation as a consequence of warming and drought.

There was no significant change in the tissue tannin concentration in response to warming or drought. Therefore, tannin concentrations did not respond following the carbon-nutrient balance hypothesis (Bryant et al. 1983, Peñuelas and Estiarte 1998) that predicted higher condensed tannin concentrations in response to the lower nutrient concentrations.

The warming and consequent extension of the growing season and winter survival may have enhanced herbivore development, abundance and activity in the Welsh and the other northern sites where temperature is more limiting for these processes (Bale et al. 2002). Drought was also expected to enhance the relative herbivory damage to the plants since less biomass was expected to be available for herbivores. However, drought did not significantly enhance herbivory in any site. It has to be noted, however, that herbivory studies in small 20 m^2 plots may be associated with potential artifacts as the herbivores can move among plots and controls.

Geographical, seasonal, and species-specific response to warming and drought

Overall, and in spite of the low potential of the tests (with only three replicate-plots per treatment), the data supported the original hypothesis that the northern sites would be more sensitive to warming and the southern site more sensitive to drought. This was the case for all variables related to plant biomass. For chemical concentrations there were no clear site differences in the effects of warming and drought. The biomass changes may have buffered chemical changes.

When considering the three sites for which we had more complete data, there was a gradient of overall positive response to warming treatment: the UK>the Netherlands>Spain and a gradient of negative responses to drought: the UK <the Netherlands<Spain, i.e. from the coldest-wettest to the warmest-driest site (Fig. 11). In the meta-analysis of ecosystem warming data conducted recently by Rustad *et al*. (2001) with data from 20 research mostly arctic and temperate sites, the positive response of aboveground plant productivity to warming was also generally larger in colder systems. However, we did not find a larger response in systems with lower precipitation. In our study we dealt with a wider range of precipitations that included warmer drier ecosystems such as the Mediterranean ones, and found that the warming effect was larger in the colder sites with larger precipitation.

The results not only show different responses to warming and drought among sites depending on their environmental conditions and constraints, but also among the different seasons and species. Plant responses to warming were stronger in the colder season, in winter, in the Spanish site (positive effects in the cold season and negative effects in the warm season – Figs. 3C and 6) reflecting the pattern observed across the sites. These results reinforce the general hypothesis of greater responses to warming in the colder environments, which are more limited by temperature. These results also provide further evidence of the existence of a cold temperature limitation for plants in the Mediterranean winter (Mitrakos 1980, Oliveira and Peñuelas 2000). The different responses found for the different species must also be stressed. In the UK, *C. vulgaris* was less responsive to warming than *V. myrtillus*, and in Spain warming enhanced growth of *E. multiflora* but decreased growth of *G. alypum* (Fig. 3A). Similarly, the effect of warming on flowering was more negative in the latter species. Although weaker, there were also species-specific differences in the response to the drought treatment (Fig. 3B, 6, and 9B,D,F)

Ecosystem and community level responses

As a result of these plant responses, ecosystem and community level consequences may be expected and some have already been found. For example, in a study of the seedling recruitment in the Spanish site (Lloret et al. in preparation) a decrease in seedling diversity in the warming treatment was observed, with an even greater negative response recorded in the drought treatment. In the northern sites, especially in the Danish one, we found an interesting interaction of warming and drought with herbivory attack. Both treatments, especially warming, tended to enhance herbivory (heather beetle, *Lochmaea suturalis* larvae) effects (Fig. 10A,B), either by enhancing insect metabolism (directly by warming, and indirectly by decreased plant nutritional value likely obliging to larger consumption) or by producing growth collapse (drought). The increased grazing on *Calluna vulgaris* may finally shift the ecosystem balance towards a greater dominance by grasses.

If these changes in plant species (and even functional types) composition of the community become established, they will in turn affect the ecosystem response to warming and drought (Cornelissen et al. 1999) since species composition affects ecosystem properties such as plant growth rates or litter quantity and quality (and therefore microbial dynamics and litter decomposition), frost hardiness, drought resistance, among many others,… As most of these changes may occur over many years or decades, it is clear that we need to conduct longer-term monitoring of these treatments, especially if it happens as reported in other studies (Arft et al 1999) that process changes such as the measurable increase in vegetative growth after warming occur only in the early years of the experiment. Thereafter there may be time enough for manifestation of the complex interactions between environmental factors that limit plant growth.

Finally, as a summary, we can conclude that the magnitude of the response to warming and drought was very sensitive to differences among sites, seasons and species. The differences reported here, those reported in the literature (Shaver et al. 2000, Rustad et al 2001), and the fact that plant responses may also be transient and change over time (Arft et al 1999) illustrate on the multiple responses to warming and drought and the complex interactions with other environmental factors that condition plant performance. So the net response may be difficult to predict. The prolongation of this study in the following years will help to clarify some of these interactions and longer-term effects.

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Garraf site

- Under the present climate conditions, *G. alypum* is able to take a greater advantage of periods with high water availability than *E. multiflora*, showing a greater carbon gain and a better photosynthetic performance, which is translated into a higher annual growth. Moreover, *G. alypum* can present a unimodal or a bimodal flowering pattern depending on the year, whereas *E. multiflora* shows a strong and highly synchronous between years peak of functional flowers.

- Water availability is a key factor in the regulation of the photosynthetic rates, growth and flowering of *E. multiflora* and *G. alypum*. Drier conditions decreased the shoot water potentials and leaf gas exchange rates of both species (although decreases in leaf net photosynthetic rates were not significant for *G. alypum*), reducing their stem growth and their estimated total stem biomass production. An extension of the drought period delayed most of the flowering phenophases of these two species, changing the length of their flowering periods. Drier conditions reduced the production of functional flowers (thus, floral construction costs), but increased flower life span (thus, floral maintenance costs), in both species.

- Despite physiological stress levels were higher during summer, cold temperatures in winter also constrained the photosynthetic performance of *E. multiflora* and *G. alypum*. Enhanced temperatures increased the potential photochemical efficiency of photosystem II in both species (but especially of *G. alypum*) mainly in autumn and winter, indicating that near future warming could alleviate low temperature constraints on the photosynthetic performance of these species during the colder seasons. During summer, temperature increases of *ca* 1ºC did not alter significantly the already depressed photosynthetic activity and performance of such Mediterranean shrubs.

- Despite warming treatment did not affect instantaneous measurements, such as leaf gas exchange rates, it altered more integrated processes such as growth or phenology. Indeed, warmer conditions increased the stem basal diameter growth of *E. multiflora*, raising its estimated stem biomass production. Conversely, warming decreased the stem basal diameter growth of *G. alypum*, but this reduction did not affect significantly its estimated stem biomass production, at least in the short-term. A *ca* 1ºC increase in temperature did not affect the flowering phenology of *E. multiflora* (the only effect of warming on *E. multiflora* flowering traits was a reduction in flower size), whereas warmer conditions delayed and decreased spring-bud flowering and advanced and increased autumn-bud flowering of *G. alypum* in certain years. Hence, future warming could especially beneficiate those species whose flower bud production depends on late autumn or winter growth. Conversely, warming could be detrimental for those species whose flower buds are developed in spring but they do not open them until the end of summer or autumn.

- The observed differential sensitivities and responses to drought and warming of the two studied Mediterranean species point to changes in their competitive ability that could modify their relative abundances in the coastal Mediterranean shrublands. Moreover, the observed phenological adjustments in response to drought and warming might alter their interactions with other organisms (e.g., pollinators, herbivores). Altogether could lead to a change in the species composition and structure of the ecosystem in the long term.

European transect

- Leaf net photosynthetic rates of the two studied Ericaceous shrubs clearly followed a gradient from northern to southern countries in agreement with the geographical gradient in water availability. Reductions in water availability during the growing season decreased leaf gas exchange rates of the studied species in the four European sites. However, plants were able to maintain higher leaf net photosynthetic rates in relation to the accumulated rainfall over the previous months under drier conditions.

- The amount of precipitation during the growing season was also an important factor controlling the differences in the potential photochemical efficiency (Fv/Fm) at predawn of *C. vulgaris* plants across the three northern sites. Lower amounts of rainfall were correlated with higher Fv/Fm values. Plants under drier conditions also showed higher Fv/Fm values than controls at midday in all sites. Thus, drier conditions might confer an enhanced ability to cope with superimposed constraints.

- According to the results obtained in the Garraf site, warming did not affect significantly instantaneous ecophysiological variables, such as leaf gas exchange rates and clorophyll fluorescence, in any of the four European sites. However, warming affected integrated variables such as growth and phenology, being the northern sites more sensitive to the enhanced temperatures than the southern site.

- A longer and stronger drought period increased the physiological stress during leaf development of *V. myrtillus* in the Welsh site. Conversely, enhanced temperatures decreased the physiological stress during leaf development of this shrub in the colder year.

- The tissue P concentrations generally decreased and the ratio N/P increased with warming and drought (except in the UK site), indicating a progressive importance of P limitation under warmer and drier conditions.

- In general, the magnitude of the response to warming and drought was different depending on the sites (cold-wet northern sites were more sensitive to warming and the warm-dry southern site was more sensitive to drought), seasons (plant processes were more sensitive to warming during the winter than during the summer), and species (for instance, *V. myrtillus* was more sensitive to warming than *C. vulgaris*).

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En començar a escriure aquestes ratlles sento una terrible responsabilitat ... sóc conscient de que probablement estic començant a redactar la part de la tesi que més gent es llegirà ... Fer una tesi implica recórrer un llarg camí. En fer aquest camí, una sèrie de gent m’ha acompanyat, ja sigui durant tot el recorregut o només durant una part. Entre la gent que em va ajudar a trobar el meu camí voldria agrair a la Isabel Fleck, en Xavier Aranda i en Quim Azcón-Bieto tot el seu suport i confiança, així com les hores passades discutint temes científics i d’altres no tan científics. Si ells em van ajudar a trobar el meu camí, la persona que va fer que aquest camí fos transitable és en Josep Peñuelas, qui en tot moment m´ha guiat per a que seguís la direcció correcta i no em perdés enmig de l’embolic de camins i caminets en què de vegades es converteix una tesi. Una altra persona que ha recorregut amb mi tot el camí és en Marc Estiarte, amb qui he passat dies i dies al camp, compartint els bons moments que té la feina de camp, però també el fred, la calor, les mosques i algun que d’altre ensurt ... gràcies Marc per la teva companyia i per tota la teva ajuda. A continuació voldria donar les gràcies a la gent que ha compartit amb mi el pessebre durant aquests anys, ja que tots ells han sigut uns companys i amics excel·lents, gràcies especialment a la Marta, al Jordi, a la Isabel, a la Maria i a la Graça. Gràcies per la vostra ajuda, la vostra amistat i el “bon rotllo”. També moltes gràcies a d’altres tricicleros per estar allà en els bons i en els no tan bons moments, gràcies Romà, Angi, Poto, Roger, Eduards, Pilar, Nacima, Imma... I també vull agrair a la penya que va a dinar a la 1h (Carles, Isabel, Joan, Teresa, Marta, Jordi, Montse, Lluís, Santi ... i tots aquells que s’hi apunten de tant en tant) l’haver fet possible que durant una estoneta cada dia desconnectés de la feina i em submergís en la complexitat del món que ens envolta. A la Marta Barceló, en Joan Masó i en Carles

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Fóra del context del CREAF, hi ha hagut tota una sèrie de gent que ha compartit amb mi aquesta tesi, però també els bons i no tan bons moments de la meva vida durant aquests anys; m'estic referint a la Bea, l'Anna Ruiz, la Sara, la Beatriz, la Caro, l'Anna Claramunt, l'Andreu, el Jordi, el Marià, el Fausto, el Marc, l'Alfonso i la Clelia. Sóu uns amics fantàstics. I a la penya d'squash (Carles, Marina, Xavi, Marta, Joan i JJ) els he d'agrair uns sopars d'allò més entretinguts ... la cambrera de la Bodega del Rosal encara deixa el cigarret en el nas del porc senglar dissecat els dijous en espera de que hi anem a sopar?? A la gent relacionada amb el Departament de Biologia Animal (Vertebrats): Santi, Vitto, Bet, Teresa, Cristina, Ignasi, Miguel Ángel, Victor, Eduardo, Mila, Xavier, Joan Carles, ... vosaltres sí que sabeu el que és una festa !! ... I a la Dulce, la Fátima, la Coralie, en Dimitri, en Javier i l'Eli, "with you Sheffield is a more charming place". A en David Beerling, "many thanks for giving me the opportunity of joining your group".

A la meva família, sobretot als meus pares i als meus avis, els he d'agrair TOT. No tinc paraules per definir aquest TOT, perquè és inabastable, no té límits, va més enllà d'allò que es pot descriure. Al Jacob, li he d'agrair TOT i MÉS.