

MECHANISMS CONTROLLING THE DISTRIBUTION OF TWO INVASIVE *BROMUS* SPECIES

by

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Abstract

In order to predict future range shifts for invasive species it is important to explore their ability to acclimate to the new environment and understand physiological and reproductive constraints controlling their distribution. My dissertation studied mechanisms by which temperature may affect the distribution of two of the most aggressive plant invaders in North America, *Bromus tectorum* and *Bromus rubens*. While *Bromus tectorum* is dominant in the “cold desert” steppes of the Intermountain region of western North America, *B. rubens* is one of the severe grass invaders in the “hot deserts” of southwestern North America. I first evaluated whether winter freezing tolerance is the mechanism responsible for the distinct northern range limits of *Bromus* species. *Bromus rubens* has a slower rate of freezing acclimation that leads to intolerance of sudden, late-autumn reductions in temperature below -12°C, *Bromus tectorum*, by contrast, cold hardens rapidly and is not impacted by the sudden severe late-autumn cold. Photosynthetic response to temperature does not explain their current range separation. *Bromus* species differ little in their photosynthetic temperature responses and the acclimation pattern of photosynthesis. Both species acclimated to a broad range of temperature through the amelioration of Pi regeneration limitation at sub-optimal

temperatures and improved carboxylation capacity above the thermal optimum which probably resulted from increased thermostability of Rubisco activase. The effect of elevated temperatures during flowering on the seed yield of *Bromus* species demonstrates that neither species produces seed at 36°C and above. These thresholds are close to temperatures encountered during flowering in their natural environment. In summary, climatic changes will cause northward range expansion of *Bromus* species due to less severe autumn and winter, while reproductive failure could cause range contraction at their southern margins.

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Chapter One - Introduction

“We are living in a period of the world’s history when the mingling of thousands of kinds of organisms from different parts of the world is setting up terrific dislocations in nature” Charles Elton, 1958 p.18.

Anthropogenic warming of the global climate has already begun and will have widespread impacts on the earth’s vegetation, particularly for higher latitude and altitude ecosystems where climate change is predicted to be more severe (McGuire *et al.*, 2005). Consequences of warming have been reported on bird, insect and plant populations (Grabherr *et al.*, 1994; Thomas & Lennon, 1999; Parmesan *et al.*, 1999). If global temperatures continue to rise, climatic zones and species geographic range limits will shift dramatically (IPCC, 2007). Among the most ominous consequences of climate change is the expansion of the geographic range of invasive species that have already caused ecological meltdowns in North America. The spread of these species has been considered one of the unpredictable elements of the global climate change (Rimer & Evans, 2006).

The term ‘invasive species’ has a number of definitions in the literature. Here I define it as ‘non-native species that pose a major threat to native ecosystems and biodiversity’ (GAO, 2000). The marked increase in the number of invasive species in North America during the last hundred years has been facilitated by increased international trade and faster transportation. Weedy ‘disturbance-adapted’ species comprise the largest category of North American plant invaders that have had a large impact on native species

and the communities (Policha, 2007). Invasion of weedy vines like *Hedera helix* (English ivy) and *Pueraria lobata* (kudzu) significantly affected native tree populations in North America by gradually turning invaded areas into the ‘ivy wastelands’ and ‘kudzuscapes’ (Pitcher, 2005; Taylor, 2008). As the global climate continues to change, these weedy invaders might expand northward; however, the magnitude of their further range expansion remains an open question (Sasek & Strain, 1990).

To control the invasion process and further spread of exotic species under climate changes it is necessary to understand environmental constraints controlling the distribution of these species. Even closely related invaders can show distinct physiological responses to climate and therefore will react differently to future changes. My dissertation investigates the effect of temperature on the distribution and invasive potential of catastrophic plant invaders. This work is focused on two invasive grass species from genus *Bromus*, *Bromus tectorum* and *Bromus rubens*, their current distribution, physiological responses to temperature and possible effects of global climate changes on their invasive potential. I also investigate the effect of elevated temperatures on the reproductive performance *Bromus* species and their range formation.

1. Invasion process

An invasion process is comprised out of four major stages: ‘import’, ‘introduction’, ‘colonization’ and ‘pest’ (Williamson & Fitter, 1996). Once an alien species is brought into the country and introduced it has to pass the reproduction threshold and go through the colonization process, which requires an ability to overcome different biotic and abiotic

barriers (Richardson *et al.*, 2000; Jackson *et al.*, 2009). It also has to acclimate to the new environment and adjust to the community changes that occur during the process of invasion (Müller *et al.*, 2010). As a result, not every species brought into the region develops into a pest. The “Tens rule” suggests that only ten percent of species imported to the area become introduced, ten percent of introduced are established and ten percent of those established become invaders (Williamson & Fitter, 1996). This rule works well for a large number of species and habitats and was supported with the data on angiosperms, gymnosperms, Australian pasture plants and high impact invaders (Williamson, 1993; Williamson & Fitter, 1996).

A successful invader usually has broad tolerance ranges, good dispersal capacity and is able to survive and reproduce under extreme climatic conditions (Walther *et al.*, 2009; Alexander *et al.*, 2011). In addition, it is often characterized by fast and unspecialized germination as well as small seed size (Whitney & Gabler, 2008). Its introduction brings novel traits into the ecosystem and changes its function once the species reaches the stage of a ‘pest’ (Wardle *et al.*, 2011). The greater the difference between the native and introduced traits, the stronger is the invasive impact on the ecosystem (Wardle *et al.*, 2011). This effect is also biomass-dependent and is amplified with increasing proportion of exotic biomass (Wardle *et al.*, 2011). It was suggested that exotic species usually have distinct leaf traits which ensure faster growth rates and accelerated biomass accumulation. The comparison of 75 native and 90 coexisting invasive species showed that invaders had higher nitrogen and phosphorus foliar content (28%), higher leaf area ratio and elevated photosynthetic rates (8%; Leishman *et al.*, 2007). Moreover, bioinvaders also exhibit higher specific leaf area and water-use efficiency (Rejmanek *et al.*, 2005; Vitousek, 2007).

2. *Bromus tectorum* and *Bromus rubens*

2.1. Botanical and ecological characteristics

Bromus tectorum (cheatgrass) and *Bromus rubens* (red brome) are two annual C₃ grasses from Europe that are among the most destructive bioinvaders of North America (Mack, 1981). The genus *Bromus*, (*Poaceae*), contains approximately 150 species, 29 of which are native to North America, while 24 are introduced to this continent (Armstrong, 1991; Clayton & Renvoize, 1986; Watson & Dallwitz, 1992.). *Bromus tectorum* and *Bromus rubens* belong to the same section (*Genea*) and phylogenetic clade (Fortune *et al.*, 2008). The analysis of the waxy gene performed by Fortune *et al.* (2008) demonstrated that *B. rubens* originated as a result of natural hybridization of *B. fasciculatus* (maternal lineage) and *B. tectorum* (paternal). Morphologically, *B. tectorum* and *B. rubens* are very similar, yet have distinct inflorescence structures. While in *B. rubens* the inflorescence is compact with branches shorter than spikelets, in *B. tectorum* the inflorescence sparsely contracted (similar to *B. fasciculatus*) (Pavlick, 1995).

Bromus tectorum and *Bromus rubens* are self-pollinated (Hulbert, 1955; Rice & Mack, 1991). Their high phenotypic and germination plasticity, as well as the ability for rapid establishment on disturbed sites allowed them to occupy more than 400,000 km² in the western USA (Mack, 1981; Novak *et al.*, 1991; Novak *et al.*, 1993; Smith *et al.*, 1997). This ecological success has been attributed to their capacity to germinate in the autumn and to remain active in the winter when native species are dormant (Rogehr & Bazzaz, 1976; Aguirre & Johnson, 1991; Chambers *et al.*, 2007). Overwintering *Bromus* species are the first grasses that resume growth in the spring. Once the temperature rises above zero they

invest their resources to produce a large leaf canopy, and begin utilizing soil moisture before native species can break dormancy (Bradley & Mustard, 2006). High growth rates lead to large seed production before water availability becomes a limiting factor (Bradley & Mustard, 2006). Once the seeds are formed, *B. tectorum* and *B. rubens* senesce, and by late-spring form a tinder dry biomass on the landscape (Bradley & Mustard, 2006). This dry biomass promotes destructive wildfires and reduces the fire-return interval from decades to less than 10 years (Klemmedson & Smith, 1964; Link *et al.*, 1995; Bradley *et al.*, 2006). In addition to direct competition, the increase in fire frequency and intensity leads to massive decline in local biodiversity, soil carbon and ecosystem integrity (D'Antonio & Vitousek, 1992, Bradley *et al.*, 2006).

Unlike representatives of section *Bromopsis* (*B. inermis*, *B. pumpellianus*, *B. richardsonii*) *B. tectorum* and *B. rubens* are not heavily grazed by the livestock which makes their distribution less likely to be affected by biotic factors in North America (Pavlick, 1995). This is mainly due to their low nutritional value as well as stiff awns that irritate livestock digestive tracts (Cook & Harris, 1952; Crampton, 1974). In spring, both grasses remain green for a short period of time and become unpalatable as soon as they enter the reproductive stage (Cook & Harris, 1952). Dry matter of *B. tectorum* lacks vitamin A which results in severe livestock vitamin A deficiencies (Hart *et al.*, 1933). Moreover, as the spring progresses, the digestibility of *B. tectorum* decreases (Cook & Harris, 1952). While at the beginning of the growing season its total protein content is above 15%, at the onset of the seed shattering stage it falls below 7%. In a landscape stocked with cattle or sheep, the low consumption of *B. tectorum* and *B. rubens* allows them

to gain competitive edge, and rapidly spread. In North America, this happened in the first half of the 20th century.

2.2. Distribution of *Bromus tectorum* and *Bromus rubens*

Bromus tectorum is native to most of Europe, northern Africa, Asia, as well as Tibet, Kashmir, and southern Pakistan (Pierson & Mack, 1990; Novak & Mack, 2001). Its introduced range includes the United States (exp. Florida), southern Canada, the UK, New Zealand, Hawaii, Australia and Japan (Hitchcock & Chase, 1971; Upadhyaya *et al.*, 1986; Stace 1997; Novak & Mack, 2001). The first ‘North American specimen’ was collected in Pennsylvania in 1859 (Novak & Mack, 2001). In North America, the *B. tectorum* population originated from at least 14 distinct introductions from European ancestors (Mack, 1981; Novak *et al.*, 1993; Novak & Mack, 2001).

Unlike *B. tectorum*, which is found throughout Europe, *B. rubens* is native only to its southern regions (France, Greece, Italy, Spain and Portugal). Besides it also widespread in south-western Asia (e.g. Afghanistan, Iran, Iraq, Israel, Jordan, Turkey) and northern Africa (e.g. Egypt, Libya, Morocco) (Winkler, 1987; Brooks, 2000, Newman, 1992, Burgess *et al.*, 1991, GRIN, 2000) where it grows on sandy or rocky sites from 0m to 1300m elevation (Brooks, 2000). The principle invasive range of *B. rubens* is the Mojave desert region of south-western North America where it was first detected at the beginning of the 20th century (Tucson, Arizona) (Burgess *et al.*, 1991). Although *Bromus rubens* is reported to grow on disturbed soils (Brooks, 2000), it occupies large areas of Mojave desert landscape (Chapter 3). Unlike *B. tectorum*, there is a limited amount of information on the origin of *B. rubens*

populations in the US. Moreover, it is unknown whether these *B. rubens* populations were introduced from different sources or had a solitary European origin.

Despite morphological and life-history similarities, *Bromus* species tend to occupy distinct thermal habitats. In North America, *B. tectorum* and *B. rubens* can occur at the same site but are usually geographically separated along latitudinal and altitudinal gradients (Beatley, 1966). While *B. tectorum* grows primarily at high elevation sites, above 1,500m, *B. rubens* is present at low and mid elevations, below 1,500m (Beatley, 1966). Moreover, while the northern limit of *B. tectorum* distribution is 65° N (Valliant *et al.*, 2007), *B. rubens* is mainly found only up to the 48°N latitude (Kartesz & BONAP, 2011). Dense stands of these species are also separated. *Bromus rubens* is a severe invader in the Mojave desert region of south-western North America; its sister species, *B. tectorum* is dominant in the colder Intermountain region of western North America (Salo, 2004, 2005; Pavlick & Anderton, 2007).

Current range separation and distinct thermal habitats of *B. tectorum* and *B. rubens* lead to the assumption that temperature is an important control over their geographic distribution. Existing ecological reports conclude temperature somehow influences the distribution of *Bromus* species, possibly due to a physiological mechanism (Hulbert, 1955; Chambers *et al.*, 2007; Leger *et al.*, 2009). *Bromus tectorum*, for instance, exhibits low establishment and winter survival rates at its upper range limit, yet the mechanism responsible for the observed mortality is unknown (Evans & Young, 1972; Chambers *et al.*, 2007; Leger *et al.*, 2009). In 1955 Hulbert compared winter survival rates of *B. tectorum* and *B. rubens* in Washington state and Minnesota and concluded that *B. tectorum* was able to survive winter conditions that killed *B. rubens* (Hulbert, 1955); however, no work has

identified whether these species actually differ in their physiological tolerance of cold. The author showed that minimum winter temperatures during the experimental period went down to -23°C , but he was unable to identify the exact time when *B. rubens* plants were winter killed. Hulbert (1955) only examined survival in plots established in the fall and measured the following spring, with temperatures being recorded using a max-min thermometer set upon the ground. This procedure does not evaluate when mortality occurred, which process is associated with it and how *B. tectorum* and *B. rubens* are going to respond to future winter temperature alternations.

3. Climate change and migration

Global temperatures are predicted to increase by 1.5° to 5.8°C within the next century with high-latitudes and altitudes experiencing the most warming (IPCC, 2002). If this happens, species like *B. tectorum* and *B. rubens* might shift their ranges polewards and to higher latitudes. Climate change has already triggered earlier flowering, changes in the length of the growing season and plant migrations (IPCC, 2002, 2007; Parmesan & Yohe, 2003; Thomas, 2010). An analysis of changes in the range boundaries of 66 herbs and shrubs within the last 80 years indicated that approximately 20% of species have shifted their ranges along with climatic warming (Parmesan & Yohe, 2003). Moreover, 44% out of 461 plant species exhibited significant changes in their phenology (Parmesan & Yohe, 2003). This data was supported by a more recent meta-analysis which also showed a causal link between climate warming and changes in species distributions (Chen *et al.*, 2011). According to Chen *et al.* (2011), animal and plant species are moving towards higher

latitudes at the rate of 17km per decade and uphill at the rate of 11m per decade (Chen *et al.*, 2011). As a result, during the last 100 years species richness increased at high-elevation sites in the Alpine regions (Grabherr *et al.*, 1994). Invasive species, in particular, are moving to higher elevations causing structural changes in upland ecosystems (Daehler, 2005; Pauchard *et al.*, 2009). Pyšek *et al.* (2011) suggested that recent climatic changes triggered an increase in the altitudinal maxima of plant invaders. The authors analyzed 28,288 distributional records for 65 alien species in the Czech Republic and showed a substantial shift in their distributional ranges. Most of these species arrived to 310m elevation and then spread to higher elevation sites (731 ± 261 m) with time (Pyšek *et al.*, 2011). Currently, almost 1,000 alien plant species are found in the mountain regions all over the world (McDougall *et al.*, 2011) with climatic warming constantly increasing this number. In the Rocky Mountain region, for example, a number of non-native herbaceous species, such as *Lactuca serriola*, *Agropyron cristatum*, *Bromus japonicas*, *Poa pratensis*, *Tragopogon dubius* are threatening high-elevation grasslands of Grand Teton National Park, Yellowstone Park and Glacier National Park area in Montana (Weaver *et al.*, 2001). In the Colorado Front Range, *Taraxacum officinale*, the common dandelion, has spread from low (2370m) to high-elevation (3560m) pine stands (Stohlgren *et al.*, 2000).

4. Hutchinsonian niche and species distribution modelling

To project future range shifts it is necessary to understand the environmental constraints limiting current species distribution. This requirement is developed in niche theory (Kearney & Porter, 2009). The ecological niche according to Hutchinson is an ‘n-

dimensional hypervolume, every point in which corresponds to a state of the environment which would permit the species to exist' (Hutchinson, 1977). The ecological niche response follows a bell-shaped curve with one optimum and two survival thresholds which can change depending on the developmental and the life-history stages (Jackson *et al.*, 2009). Ecological niche is also called a fundamental niche and has to be distinguished from its subset – the realized niche, which is rather an actual environmental space occupied by a certain species. The realized niche is usually narrower than the fundamental since it takes into account various biological interactions (Mueller, 2004). The realized niche can vary from region to region and equal the fundamental niche if predators and competitors are not present, as may occur with invasive species. In Britain, for instance, the native range of winter annual grass, *Vulpia fasciculata*, is very narrow and restricted primarily to the sand dunes ecotones (Watkinson, 1978). In Australia, by contrast, this species is a severe invader and is distributed over various habitat types (McIntyre & Whalley, 1990). Another invader, *Centaurea maculosa*, also shows differences in its native and invasive ranges (Broennimann *et al.*, 2007). A climatic niche shift following the invasion of this species in western North America has been recently demonstrated by Broennimann *et al.* (2007).

The ecological niche in its Hutchinsonian interpretation is the central concept in species distribution modelling widely used to predict species range shifts in response to climatic changes (Kearney & Porter, 2009). The most extensively implemented type of species distribution models (SDMs) are correlative which produce statistical relationships between climate and current geographical ranges (Kearney & Porter, 2009). Correlative SDMs do not require a detailed analysis of species physiology and reproduction can be applied to every species and produce detailed probability maps which later can be converted

into the species range maps (Hannah, 2010). These models require just the distributional data which is available for most species. As a result, they are less likely to overestimate distributional changes since they look at the realized rather than fundamental niches (Morin & Lechowicz, 2008). Correlative SDMs assume that climate controls the distribution of plants and suggests that current bioclimatic envelopes are the projection of plants' physiological responses to climate (Kutner & Morse, 1996). They also assume that all species are currently in equilibrium with climate, which probably is not the case for many plant species and particularly aggressive invaders (Kutner & Morse, 1996). Climatic niche shifts of invasive species show low predictive capacity from correlative models (Broennimann *et al.*, 2007). Statistical relationships built entirely on the current climatic variables and distribution ranges do not take into the account acclimation capacity of each species, nor other ecological factors such as dispersal limitation, stochastic events or human activity. Correlative models do not take into account future adaptations, populational differences, human activity, and therefore are criticized for their low predictability (Kelly *et al.*, 2012). Agricultural production, for example, can destroy large areas of habitats and shrink the realized range. An alternative to correlative SDM are their mechanistic counterparts that link species functional traits to the spatial data (Kearney & Porter, 2009). These models permit more precise assessment of biodiversity responses to climatic changes, yet require a detailed knowledge on species morphology, physiology and behaviour (Kearney & Porter, 2009). The mechanistic SDMs also allow incorporating evolutionary responses and may be used in other models. The challenge to the success of the mechanistic SDMs is therefore the ability to gather necessary mechanistic data to properly parameterize the model.

To evaluate how distribution of *Bromus* species will change in coming decades, it is necessary to develop species distribution models that incorporate their major functional traits. Currently, SD modellers are not accounting for the possibility that the distribution of *B. tectorum* and *B. rubens* might be temperature-limited. Correlative SDMs of Bradley (2009) and Bradley *et al.* (2009) suggest that increasing aridity will be the leading cause of range expansion by *B. tectorum* in future climates. These models are looking at the distribution of dense infestations of *B. tectorum* in the Intermountain region of the Western USA, as determined by remote sensing. Therefore they are not set up to predict distribution of the species, but rather, occurrence of dense infestations observable through remote sensing. Moreover, the lack of physiological basis makes these projections somewhat uncertain.

5. Species distribution and temperature

5.1. Temperature isoclines and species range limits

It is widely accepted that species distribution is determined by environmental factors that restrict survival and reproduction (Cox & Moore, 2005). Good's Theory of Tolerance suggests that 'each and every species is able to exist and reproduce successfully only within a defined range of climatic and edaphic conditions' and that 'this range represents the tolerance of species to external condition' (Good, 1931). Since the beginning of the 20th century much attention of plant ecologists and biogeographers addressed the relationship between temperature and plant distribution (e.g. Dansereau 1957; Sasek & Strain, 1990; Table 1.1). However, processes underlying these relationships are not fully understood in

most cases. Many species distribution limits coincide with temperature isoclines. As early as 1920 Uphof (1920) implied that upper altitudinal distribution of *Carnegiea gigantea*, the Saguaro cactus, in Arizona is approximated by the -10°C isocline of minimum winter temperature. Salisbury (1926) showed that the northern range limit of *Rubia pergrina* in Europe coincided with the mean 4.5°C January isocline. January is a period of new shoot formation in *Rubia pergrina* and exposure to chilling temperatures during this period negatively affects its growth rates (Salisbury, 1926; George & Burke, 1986). The northern range of hair-grass (*Corynephorus canescens*) in Western Europe, by contrast, correlates with the 15°C July mean isocline and is related to delays in flowering of this species and a consequent inability to germinate later in the season (Marshall, 1968). The northern distribution of *Acer saccharophorum* (sugar maple) in North America is limited by the -40°C mean minimum isocline, whereas its southern range coincides with the -20°C isocline (Dansereau, 1957).

In general, minimum winter temperatures set the northern range limits of a large number of species (Table 1.1). Sakai (1978) demonstrated a strong correlation between the plants freezing resistance and minimum winter temperature isoclines at their northern boundaries and suggested that changes in vegetation are dictated by species freezing temperature thresholds. The author evaluated the freezing tolerance of dormant one-year-old twigs collected from the mature trees of 110 species in Japan and concluded that trees within the same climatic region exhibit similar sensitivities to freezing temperatures (Sakai, 1978). Tropical trees, for instance, (e.g *Kandella candel*, *Burgulera conjugata*, *Rhizophora stylosa*, *Thespesia populnea*) which are unable to tolerate temperatures below 0°C , grow only in the frost-free zones, such as Ryuku and Yaku and Tanega islands in Japan (Sakai,

1978). The evergreen broad-leaved trees (*Actinodaphne lancifolia*, *Litsea japonica*, *Prunus zippeliana*, *Eleocarpus sylvestris*), by contrast, are hardy to -8°C -10°C and therefore dominate cooler regions where temperatures go down to -10°C (Sakai, 1978). Unlike tropical and evergreen broad-leaved trees, the cool-temperate deciduous trees (e.g. *Acer rufinerve*, *Carpinus tschonoski* and *Gagus crenata*) from the northern Japan show an ability to survive prolonged freezing at much lower temperatures. Their northernmost populations are limited by the -30°C isocline, which corresponds to the freezing tolerance of their buds, xylem and cortex (Sakai, 1978).

In some species, the minimum winter isoclines controlling their northern ranges coincide with the frost tolerance of their vegetative and flower buds. The northern range of *Prunus persia* in North America, for example, is associated with the -23°C isocline of average annual minimum temperature and is related to the cold hardiness of its leaf buds (Quamme *et al.*, 1982). While the xylem of this species survives temperatures down to -35°C, its leaf buds are killed at -24°C. The leaf buds of *P. salicina*, by contrast, withstand freezing down to -30°C, which explains the ability of this species to grow up to the -29°C isocline (Quamme *et al.*, 1982).

In addition to the direct effects of freezing on the vegetative and reproductive tissues, minimum winter temperatures limit species distribution through the effect on water transport (Pockman & Sperry, 1997). The northern limit of *Larrea tridentata*, an evergreen shrub, in Mohave and Sanoran deserts coincides with the -20°C isocline of the minimum winter temperature and is controlled by freezing-induced xylem cavitation (Pockman & Sperry, 1997). In the laboratory experiments, Pockman & Sperry (1997) found that exposure to -16°C to -20°C triggers complete xylem embolism in *Larrea tridentata* which

restricts its distribution to North American warm deserts. Exposure to temperatures below -5°C promotes the freezing of the xylem sap, air bubble formation and induces a subsequent xylem cavitation. The percentage embolism in *Larrea tridentata* increases with decreasing air temperature and reaches 100% at -20°C.

Freezing temperatures also limit the distribution of North American succulents. Frost injury of the apical meristem was demonstrated in the northernmost and uppermost populations of agaves and columnar cacti, such as *C. gigantea*, *L. thurberi* and *L. schottii* (Turnage & Hinckley, 1938; Nobel, 1980). The northern ranges of these species are controlled by their cold hardiness and specific morphological adaptations that increase the minimum temperature of their most susceptible tissues (Nobel, 1980; Nobel & Smith, 1983). Greater pubescence depth in cacti protects their apical meristems and reduces freezing sensitivity. Smaller rosette size increases the minimum leaf temperature in agaves and contributes to their northward expansion (Nobel & Smith, 1983). In addition, most agaves are usually able to acclimate to low temperatures and adjust their freezing tolerance thresholds. The further north the agave can survive, the higher is its acclimation capacity. The initial freezing tolerance of *A. utahensis* and *A. parryi*, for instance, grown under 10°/0°C is around -18° and -20°C. Yet, growth under lower temperatures increases their low temperature tolerance and allows them to survive freezing down to -25°C at their northern margins (Nobel & Smith, 1983).

Although the minimum winter temperature is often described as the major predictor of species distribution limits, the maximum summer temperature can also play a role in species range formation. The southern and upper ranges of alpine plants in Scandinavia regularly coincide with the maximum summer temperature isoclines (Dahl, 1951). The

analysis of the distribution of over two hundred alpine species in this region showed that at least 150 of them follow the same correlative pattern of their distribution (Dahl, 1951). The southern range of *Poa flexuosa*, for instance, is close to the 24°C isocline of the maximum summer temperature, whereas the distribution of *Saussurea alpina* is associated with the 29°C isocline (Dahl, 1951). The mechanism underlying this correlation is unknown; nonetheless, the author suggested that it might be a detrimental effect of elevated temperatures on the protoplasm or reductions in net CO₂ assimilation rates at elevated temperatures (Dahl, 1951). Moreover, elevated temperatures might have an effect on the reproductive capacity of alpine plants, yet as far as none of these factors was experimentally tested, it is impossible to draw any conclusions.

Climatic conditions endured by a plant should allow it to produce an adequate number of offspring (Salisbury, 1926). While vegetative tolerance determines survival and existence during adult and juvenile stages, the reproductive tolerance defines the ability of mature individuals to produce offspring and maintain the community structure. Both vegetative and reproductive phenophases are sensitive to environmental conditions and therefore can shape the niche. Grubb (1977) noted that viable seed production is one of the central components of the regeneration niche, which is a type of fundamental niche that represents plants requirements for a successful replacement of one mature individual by another (Grubb, 1977). The regeneration niche includes all processes which lead to seed production, germination and seedling survival.

In my thesis I have decided to focus on reproductive as well physiological processes in *Bromus* species in order to understand major constraints limiting their distribution. I recognize that there are more than several factors limiting the distribution of the plants, yet,

here I would like to focus on temperature as one of the major climatic factors. I will look at temperature as an abiotic factor that affects species northern and southern range limits as well as their abundance. Several authors emphasized the importance of multidirectional analysis of species range limits (Kirkpatrick & Barton, 1997; Gross & Price, 2000); yet, no one has looked at the distribution of plant invaders from the perspective of synthesis of reproductive and physiological approaches. I will examine in details the photosynthetic performance and limitations of *Bromus* species grown under various temperature regimes, their upper reproductive limits and lower survival thresholds.

5.2. Freezing temperatures and plants winter survival

5.2.1. Effect of freezing temperatures on plasma membrane and PSII

The low temperature threshold reflects the absolute survival temperature where the major physiological processes cease. This usually represents the temperature that induces plasma membrane instability. The plasma membrane is a primary site of cell injury in freezing and chilling (Stout *et al.*, 1980). At harmful freezing temperatures, the plasma membrane can be either mechanically damaged by ‘expansion-induced lysis’, or undergo structural changes that weaken it (Steponkus, 1984; Herman *et al.*, 2006). Nevertheless, many plant species at low temperatures are able to improve their membrane properties and enhance their freezing tolerance. The plasma membrane of cold acclimated species has less closely appressed lipid bilayers due to the presence of sugars (Steponkus, 1993). Cold-tolerant membranes contain higher proportion of unsaturated fatty acids that enhance their fluidity (Fujikawa *et al.*, 1999; Skinner *et al.*, 2005). Fifteen minute exposure to low, non-freezing temperatures

induces expression of the C-repeat binding factor (CBF) genes promoted by the cold-responsive inducer of CBF expression (ICE) (Gilmour *et al.*, 1988; Zarka *et al.*, 2003). CBF genes activate sugar synthesis and lipid unsaturation which then improves cryoprotection of the plasma membrane through a decrease in its melting temperature (Fukuchi-Mizutani *et al.*, 1998; Dong *et al.*, 2006; Zhu *et al.*, 2007).

In addition to membrane damage, exposure to low temperature stress inactivates PSII in the light. Cold impairs the turnover of D1 polypeptide, a key protein of PSII responsible for plastoquinone reduction and water oxidation. This leads to chronic photoinhibition (Nixon *et al.*, 1992; Bertamini *et al.*, 2004). The mechanism for dealing with cold effects on PSII varies with species. Chilling-sensitive plants tend to increase the rate of D1 protein repair in order to compensate for the photodamage, while species like winter wheat and barley increase the capacity of photochemical quenching (qP) through the improvement of RuBP regeneration capacity and an enhancement of CO₂ assimilation rates (Nishida & Murata, 1996; Krause & Jahns, 2003; Ivanov *et al.*, 2008). The primary electron-accepting quinone in the PSII reaction centres (Q_A) of these species stays oxidized, allowing the PSII centers to remain open (Humer *et al.*, 1998; Ivanov *et al.*, 2008). In addition, non-photochemical quenching (NPQ) is used by plants as a mechanism to dissipate the excess excitation energy (Ivanov *et al.*, 2008). This process is closely related to the accumulation of the xanthophyll pigment zeaxanthin (Z) produced by violaxanthin (V) oxidation and is considered to be the most important PSII photoprotective strategy (Demming *et al.*, 1987; Ort, 2001; Ivanov *et al.*, 2008). An increase in the zeaxanthin content induces the dissipation of excitation energy in the light harvesting antenna complexes (LHC).

Chlorophyll fluorescence measurements may be used to investigate the influence of temperature on the effective quantum yield of PSII (F_v'/F_m') and to detect changes in maximum quantum yield of PSII (F_v/F_m). While F_v/F_m values are measured on dark-adapted material and reflect the potential quantum efficiency of PSII, F_v'/F_m' values are measured on light-adapted samples and reflect changes in the efficiency of NPQ (Maxwell & Johnson, 2000). For most C3 plant species, an F_v/F_m value of healthy plants is above 0.83 and the decrease in this value below 0.8 is a good indicator of photoinhibition (Baker, 2008).

5.2.2. Development of freezing resistance

Gusta *et al.* (2005) suggested that cold acclimation in plants proceeds in one of three stages. The first refers to the primary acclimation (changes in day length, exposure to sub-optimal temperatures), the second – moderate acclimation that involves the production of cryoprotective compounds (exposure to temperatures approaching 0°C), and the third - extreme freezing tolerance that requires exposure to subzero temperatures (Gusta *et al.*, 2005). Development of subzero tolerance during stage 2 or even 3 is a key element of winter survival and can be achieved through two general strategies: freezing avoidance and freezing tolerance (Hurry *et al.*, 1995; Strand *et al.* 1997; Gusta *et al.*, 2005). Freezing avoidance includes supercooling and osmotic adjustments that prevent ice formation. Freezing-tolerance, by contrast, safely allows extracellular ice formation and leads to protoplast dehydration (Link *et al.*, 2010). While freezing tolerance is the most effective mechanism in coping with prolonged and severe frost events, freezing avoidance is usually effective over the short-term (hours to days of freezing) (Goldstein *et al.*, 1985; Rada *et al.*,

1987; Sierra-Almeida *et al.*, 2009). Some species are able to switch between strategies during their life-time. Sierra-Almeida *et al.* (2009) demonstrated that low-elevation Andean herbaceous species change their freezing resistance strategies during the growth season. Twenty-five percent of all studied low-elevation species (e.g. *Acaena splendens*, *Taraxacum officinale*) exhibit a freezing tolerance strategy at the early stage of their growth and then switch to freezing-avoidance as the growing season progresses (Sierra-Almeida *et al.*, 2009). High-elevation species, by contrast, use a freezing-tolerance strategy throughout the growing season and survive long lasting frosts (Sierra-Almeida *et al.*, 2009). Changes in freezing resistance mechanisms, according to Sierra-Almeida *et al.* (2009) were linked to changes in plant water status that altered plants' ice nucleation temperatures.

The maximum level of freezing tolerance and acclimation are not static during the life-cycle of the plant. They can vary depending on the age of the plant as well as its' physiological condition. As plants age, the leaf morphology as well as leaf membrane composition changes. These alternations may affect membrane susceptibility for freezing injury and trigger changes in freezing tolerance of the plant. Leaf shape, colour, antioxidant capacity, leaf membrane composition and pigment content may change during the aging process (Irving, 1969; Strong & Ray, 1975; Brown & Bixby, 1976; Lim *et al.*, 1999; Fadzly *et al.*, 2009). For instance, young leaves of tropical forest trees, such as *Swietenia macrophylla*, *Castilla elastic*, *Ficus insipida* and *Antirrhoea trichantha* contain 50% less chlorophyll per unit leaf area and possess larger pools of zeaxanthin than older leaves (Krauze *et al.*, 1995). Moreover, plasma membranes polypeptides undergo changes during the leaf development in tobacco which might result in distinct freezing tolerance of its young and adult leaves (Logan *et al.*, 1997). In general, seedlings are more susceptible to

freezing temperatures than adult plants (Irving & Lanphear, 1968). In *Phellodendron sachalinense*, the 10- and 22-month-old seedlings were not able to withstand temperatures below -4°C , while 35-year-old trees were cold tolerant down to -16°C (McNamara & Pellett, 2000). Freezing-tolerance in juvenile seedlings of *Rhododendron maximum* is near -38°C , yet in adult plants it drops to -50°C (Lim *et al.*, 1998).

5.3. Temperature and photosynthesis

Photosynthesis involves dozens of reactions that are individually affected by temperature (Sage & Reid, 1994). In general, there are three limiting categories for light-saturated photosynthesis in C_3 species (e.g. *B. tectorum*, *B. rubens*): 1) the rate of Rubisco-catalyzed consumption of RuBP (Rubisco limitation); 2) the capacity to regenerate RuBP (RuBP regeneration limitation); and 3) the capacity of starch and sucrose synthesis to regenerate Pi for ATP synthesis via triose-phosphate utilization (Pi regeneration limitation) (von Caemmerer & Farquhar, 1981; Sage & Kubien, 2007).

Rubisco limitation arises when the ability of Rubisco to fix CO_2 to RuBP is inadequate for the RuBP pool size (Sage & Reid, 1994; Sage *et al.*, 2008). This type of limitation in C_3 species predominates at low CO_2 concentrations (below $300\mu\text{mol mol}^{-1}$) (Sage, 2002). Cold acclimation of C_3 plants can be accompanied by an increased content and/or synthesis of new forms of Rubisco and Rubisco activase, an enzyme which maintains the catalytic activity of Rubisco (Vu *et al.*, 1995; Goulas *et al.*, 2006). Cold hardening of citrus, for example, was associated with changes in Rubisco protein levels (Vu *et al.*, 1995). The large subunit content of Rubisco in cold acclimated citrus was significantly higher than

in non-acclimated citrus (Vu *et al.*, 1995). Hikosaka *et al.* (2006) suggested that changes in the subunit combinations result in changes in Rubisco temperature responses. Altered balance between large and small subunit complexes of Rubisco affects the activation energy of V_{cmax} (Hikosaka *et al.*, 2006). Modified Rubisco might possess different temperature responses, kinetics properties and have altered Rubisco activase preferences/specificity (Yamori *et al.*, 2006; Portis *et al.*, 2008).

The second limitation is RuBP regeneration limitation which arises when the capacity of light harvesting, electron transport or the Calvin cycle to regenerate RuBP limits CO_2 assimilation (Sage & Reid, 1994). Regeneration of RuBP depends upon ATP and NADPH production and therefore is dependent on the electron transport process, which, in turn, is highly sensitive to growth temperature (Sage & Reid, 1994; Yamasaki *et al.*, 2002). Although short-term exposure to sub-optimal temperatures decreases RuBP regeneration capacity in some species, acclimation to low temperatures can ameliorate this limitation through the increased ratio of electron transport capacity to RuBP carboxylation capacity (J_{max}/V_{cmax}) (Hikosaka *et al.*, 1999).

Finally, inorganic phosphate regeneration (Pi regeneration) limitation is caused by limitation in the rate of (Pi) recycling by carbohydrate metabolism or when the cytoplasmic (Pi) level is below the optimum (Leegood & Edwards, 1996). When the capacity of starch and sucrose synthesis to metabolize triose-phosphates (TP) is insufficient, (Pi) levels in the chloroplast decline and limit synthesis of ATP. In C_3 plants, this process is often a major limiting process at sub-optimal temperatures, particularly at elevated CO_2 concentrations (Sage, 2002). Nevertheless, cool acclimation partially removes a limitation in (TPU)

capacity, either by increased expression of enzymes of starch and sucrose synthesis or though an elevated concentration of cytoplasmic (Pi) (Sage & Kubien, 2007).

The thermal optimum of C_3 photosynthesis is usually between 20°C and 30°C (Sage *et al.*, 2008). At supra-optimal temperatures the decline in the assimilation rate could be due to the stomatal and mesophyll conductance limitations, declines in electron transport capacity, decreased Rubisco activity and an increased photorespiration rate (Cen & Sage 2005; Norisada & Kojima, 2007). An increase in temperature causes a decrease in the CO_2/O_2 specificity of Rubisco (Jordan & Ogren, 1984). Moreover, as temperature increases the solubility of CO_2 declines more strongly than O_2 solubility, enhancing Rubisco oxygenation and the significance of photorespiration (Jordan & Ogren, 1984). Deactivation of Rubisco is considered to be the first biochemical response of photosynthetic apparatus to high temperature stress (Weis, 1981). Usually, from 15°C to 30°C the maximum rate of carboxylation activity (V_{cmax}) increases exponentially; however, above 30°C Rubisco catalytic sites deactivate with increasing temperature (Salvucci & Crafts-Brandner, 2004; Hikosaka *et al.*, 2006). This could be a feedback from Pi regeneration or RuBP regeneration limitations or a result of a decreased activity of Rubisco activase (Ruuska *et al.*, 1998; Salvucci & Crafts-Brandner, 2004; Cen & Sage, 2005; Makino & Sage, 2007). In order to catalyze carboxylation/oxygenation reactions, the Rubisco enzyme has to be activated. Catalytically inactive sites of Rubisco become activated through the carbamylation process which requires activity of Rubisco activase (Portis, 2003).

Deactivation of Rubisco above the thermal optimum was first demonstrated in spinach grown at 20°/15°C (Weis, 1981). This finding was supported by a number of studies that showed a decline in Rubisco activation state at supra-optimal temperatures (e.g.

Kobza & Edward, 1987; Salvucci & Crafts-Brandner, 2004). Nevertheless, whether the observed declines were a result of Rubisco deactivation or a regulatory response to Pi or RuBP regeneration limitation, remains under the debate (Sage *et al.*, 1988; Craft-Brandner & Salvucci, 2000; Salvucci & Crafts-Brandner, 2004). Rubisco activase is highly sensitive to ATP/ADP ratio, therefore Pi and RuBP limitations that inhibit ATP synthesis are able to decrease the ability of Rubisco activase to remove bound RuBP (Leegood, 1996, Jensen, 2000). Suppressed carboxylation rate, in its turn, reduces the number of Rubisco active sites (Eckardt & Portis, 1997; Salvucci *et al.*, 2006). Experiments with transgenic rice plants with reduced Rubisco content (Antisense' *rbcS*), for instance, demonstrated that a decline in Rubisco activation state above 20°C was rather a regulatory response to electron transport limitation rather than a result of a deactivation process (Makino & Sage, 2007). Exposure to moderate and high temperatures increases the rate of cyclic electron flow around PSI. This suppresses the rate of linear electron transport, decreases NADPH production and inhibits RuBP regeneration (Sayed *et al.*, 1994; Medlyn *et al.*, 2002; Sharkey & Schrader, 2006; Sage & Kubien, 2007; Yamori *et al.*, 2008).

Acclimation to supra-optimal temperatures also induces synthesis of new and less heat sensitive isoforms of Rubisco activase in wheat, maize and cotton (Sanchez de Jimenez *et al.*, 1995; Law & Crafts-Brandner, 2001; Law *et al.*, 2001; Crafts-Brandner & Salvucci, 2002; Stasik & Jones, 2007). Growth under 41°C, for instance, induced of a new form of activase in cotton leaves (*Gossypium hirsutum*) through the alternative splicing of the mRNA (Law *et al.*, 2001, Salvucchi *et al.*, 2003 Yamori *et al.*, 2005; Hikosaka *et al.*, 2006). The isoforms (α and β) detected in cotton had distinct thermostolerance levels and distinct specific activity for ATP hydrolysis (Salvucchi *et al.*, 2003). The specific activity for ATP

hydrolysis in β -form activases was more than twice lower than in α -form activases (Salvucchi *et al.*, 2003). Moreover, the initial rate of Rubisco activation was lower in β -form activases (Salvucchi *et al.*, 2003). The presence of two isoforms might improve the assimilation rates at elevated temperatures and allow growth under extreme heat projected to increase in the next 100 years (IPCC, 2001).

At higher temperatures ($>40^{\circ}$ - 45°C), the decline in the CO_2 assimilation rate is driven primarily by the damage to Photosystem II (PSII) (Sharkey & Schrader, 2006). Unlike Photosystem I (PSI), which is damaged by temperatures above 70°C , PSII is disrupted around 40°C (Percy *et al.*, 1977; Sayed *et al.*, 1989; Camejo *et al.*, 2005). Such exposure interrupts the electron flow from PSII to PSI, increases fluidity of thylakoid membranes and causes denaturation of PSII membrane protein complex (Terzaghi *et al.*, 1989; Thompson *et al.*, 1989; De Ronde *et al.*, 2005). Heat stress first blocks the PSII reaction centers and then triggers the dissociation of antenna pigment-protein complexes (Georgieva, 1999). The denaturation of PSII is in the range of 43° - 51°C for most of the plant species (Downton *et al.*, 1983; Terzaghi *et al.* 1989; Hu *et al.*, 2004). In soybean, for instance, inactivation of PSII was found to be irreversible above 45°C (Nishiyama *et al.*, 2006). Nonetheless, this breakpoint might increase in response to elevated growth temperature (Yamasaki *et al.*, 2002). It was demonstrated that the upward shift in the growth temperature from 25°C to 35°C resulted in enhancement of thermostability of PSII in soybeans by 3°C (Nishiyama *et al.*, 2006). In *Atriplex hymenelytra*, a desert perennial, the critical temperature of PSII increased from 43° to 47°C when growth temperature was raised from 20° to 35°C (Schreiber & Berry, 1977). Modulation of plastoquinone and water-oxidation complexes could be the major heat acclimation response of PSII

responsible for this shift (Yamasaki *et al.*, 2002). Growth under supra-optimal temperatures induces an upward shift in the temperature response of the slow decay component of Q_A oxidation and causes a consequent shift in the optimum temperature for PSII electron transport (Yamasaki *et al.*, 2002). Yet, structural changes in thylakoid membranes such as adjustments in fatty acid composition might also occur (Sharkey, 2005; Ladjal *et al.*, 2000; Marakami *et al.*, 2000; Yordanov, 1992).

In addition to changes in thylakoid membranes and Rubisco activase properties, growth under warmer temperatures might cause substantial respiratory adjustments (Pearcy, 1977; Tjoelker *et al.*, 1999; Atkin & Tjoelker, 2003). The respiration rate (R) usually increases with increasing measurement temperature; however, the upward shift in the growth temperature might reduce the Q_{10} of R and decrease R at elevated temperatures (Type I acclimation) (Atkin & Tjoelker, 2003). During this first type of respiratory adjustment, the respiration rate remains unchanged at low temperatures. Alternatively, respiratory acclimation could result in changes of R at all measured temperatures and is often associated with reductions in leaf nitrogen content (Type II acclimation) (Tjoelker *et al.*, 1999; Atkin & Tjoelker, 2003).

5.4. Temperature and reproduction (parts of this section were submitted as:

Bykova O, Chuine I, Morin X, Higgins S (2012) The Importance of reproduction niche for plant species distributions. *Journal of Biogeography*, under revision)

Sexual reproduction affects species distribution and abundance through the response of its major processes to biotic and abiotic factors (Bykova *et al.*, 2012, under revision). Seed

production is a part of regeneration niche which plays a central role in biodiversity maintenance (Grubb, 1977). Failure to produce seeds in back-to-back years may result in species range contraction and affect their competitive advantage. Unfortunately, most of the studies examining plant reproductive success in a distributional context have been limited in their depth (e.g. Garcia *et al.*, 2000; Jump & Woodward, 2003). Very few studies have combined detailed experimental and observational data and show a direct impact of sexual reproduction processes on species distribution. The most comprehensive work was conducted on *Tilia cordata* (small leaved lime) which is characterized by low seed-fertility near its northern limit in England and Finland (Pigott, 1980, 1981, 1989, 1992; Pigott & Huntley, 1981). The northern range limit of *T. cordata* in England coincides with the 18°C isocline of the average maximum August temperature (Pigott, 1992). August is the flowering period of *T. cordata*. Flowering is controlled by the temperature sensitivity of two pre-zygotic processes: pollen germination and pollen tube growth (Pigott & Huntley, 1981; Pigott, 1992). Pollen grains of *T. cordata* fail to germinate below 15°C and do not develop pollen tubes at temperatures below 19°C (Pigott & Huntley, 1981). These processes restrict the distribution of *T. cordata* in the British Isles, yet have no effect on its distribution in Finland where the temperatures during flowering of *T. cordata* are milder, with August temperatures averaging >19°C (Pigott, 1981). In Finland, seed sterility in the northern-most populations of *T. cordata* is caused by low autumn temperatures during seed maturation that prevent embryo development, thereby preventing movement further north (Pigott, 1981).

Male reproductive development is highly sensitive to temperature changes. When stored, mature pollen is tolerant of temperature stress and can survive extreme temperatures

(from 60° to -20°C) without functional impairment (Brown & Dyer, 1991; Rao *et al.*, 1992; Lardon & Triboui-Blondel, 1994). By contrast, the undeveloped pollen is extremely sensitive to temperature stress (Lardon & Triboui-Blondel, 1994). Temperature stress during the critical stages of meiotic and mitotic division can alter pollen viability and lead to a 100% pollen sterility (Mamun *et al.*, 2006; Endo *et al.*, 2009). Male sterility at temperatures above 30°C has been demonstrated in sorghum, tomatoes, rice, wheat, peanut, beans, cowpea, groundnut and barley (Satake, 1976; Heenan, 1984; Ahmed *et al.*, 1992; Lalonde *et al.*, 1997; Peet *et al.*, 1998; Vara Prasad *et al.*, 1999; Sakata *et al.*, 2000; Suzuki *et al.*, 2001; Matsui & Omasa, 2002). Reproductive changes in rice, winter rape, wheat, barley and bean that occur as a result of temperature stress include failure of meiosis, disruptions in mitosis, and reductions in the anther size (Jenner *et al.*, 1993; Lardon & Triboui-Blondel, 1994; Dorion *et al.*, 1996; Saini, 1997; Lalonde *et al.*, 1997; Ahmed *et al.*, 1998; Wallwork *et al.*, 1998; Sakata *et al.*, 2000; Suzuki *et al.*, 2001; Oliver *et al.* 2005; Mamun *et al.*, 2006; Endo *et al.*, 2009).

Possible bases of pollen meiotic arrest induced under high and low temperature stress are: 1) water deficits that disrupt carbohydrate availability; 2) dislocation of sugar flow to the developing gametophyte and 3) altered activity of starch biosynthetic (heat stress) and degradative enzymes (chilling stress) (Hotta *et al.*, 1985; 1888; Jenner *et al.*, 1993; Dorion *et al.*, 1996; Saini, 1997; Lalonde *et al.*, 1997; Wallwork *et al.*, 1998; Sakata *et al.*, 2000, Oliver *et al.* 2005; Mamun *et al.*, 2006). If meiosis is successful, high and low temperature stress during the subsequent mitotic division causes male sterility through the disruptions at the first step of androgenesis, abnormal tapetum development and degeneration and disrupted tapetum functions (Lardon & Triboui-Blondel, 1994; Ahmed *et al.*, 1998; Mamun

et al., 2006; Suzuki *et al.*, 2001; Endo *et al.*, 2009). Overall, thermal limits of male reproductive development are species-specific. For instance, while the lower temperature threshold for male reproductive development in rice is 15°-19°C (Nishiyama, 1984), in mango it is around 10°C (Issarakraisila & Considine, 1994). The upper threshold temperature in tomatoes is around 25°C, whereas in rice it is 30°C (Peet *et al.*, 1998; He *et al.*, 2006).

6. Thesis objectives

The main objective of my research is to assess physiological and reproductive responses of *B. tectorum* and *B. rubens* to temperature variation in order to understand how climate warming will affect distribution and ecological success of these species. I have focused on the freezing tolerance, male reproductive development and photosynthetic acclimation of *Bromus* species and explored the effect of growth under distinct temperature regimes on their photosynthetic limitations. My specific objectives are to examine possible differences in *B. tectorum* and *B. rubens* physiological response to temperature and evaluate the role of temperature in their distribution. Two closely related *Bromus* species are chosen as the study organisms due to their high invasive potential as well as current range separation along latitude and altitude gradients. As *B. tectorum* and *B. rubens* usually grow in distinct thermal habitats, I was interested if they have different physiological responses to temperature. Physiological results from this study could be used to better parameterize vegetation climate models that predict when and where climate will favour the *Bromus* invasions.

In chapter two, I look at the development of cold tolerance, frost resistance and minimum cold tolerance levels in *Bromus* species. In this study I examine distinct northern range limits of *B. tectorum* and *B. rubens* and investigate possible physiological mechanisms responsible for their range separation. I also explore the capacity of each species to acclimate to rapid freezing events at the beginning of the cold season and examine differences in their responses at young and mature developmental stages. Identification of any winter cold dependence controlling northern range limits of *Bromus* species can provide the insights towards their future invasive potential in higher latitudes and elevations.

Reproductive failure is known to limit species distribution. The effect of elevated temperatures on reproductive development as well as the current distribution of *B. tectorum* and *B. rubens* along the elevation gradients is addressed in chapter three. In this chapter, I evaluate possible reasons for distinct southern ranges of *Bromus* species and compare responses of their male reproductive development and tillering. Chapter three is the first study on the effect of climate change on the reproductive development of invasive grasses. It is also the first to look at heat induced male sterility in plants as an important contributor to species distributions. The experimental manipulations use microscopic observations to evaluate changes in anther development, pollen production and cell division. By comparing closely related species that segregate along a thermal cline, I also evaluate natural variability in the sterility threshold.

The fourth chapter examines the effect of distinct growth temperatures on the photosynthetic performance of *B. tectorum* and *B. rubens*. It combines experimental gas-exchange measurements with biochemical modelling in order to understand major processes

limiting photosynthesis over the entire temperature range and to examine the effect of growth temperatures on photosynthetic performance. Growing *B. tectorum* and *B. rubens* under four temperature regimes provides one of the most comprehensive pictures of photosynthetic acclimation over growing season.

Finally, in chapter five, which is a general discussion, I synthesize the results obtained in chapters 2, 3, and 4 and give their possible implications in the context of climate changes and range separation of two closely related species.

Table 1.1 - Studies demonstrating the relationships between temperature and species
geographic range limits

Species	Region	Limiting variable	Reference
<i>Carnegie gigantea</i>	Arizona, USA	min winter temp	Uphof, 1920
<i>Ruscus aculeatus</i>	Europe (UK)	mean ma March temp	Salisbury, 1926
<i>Rubia peregrina</i>	Europe	mean January temp	Salisbury, 1926
<i>Lemaireocereus schottii</i>	North America	min winter temp	Turnage & Hinckley, 1938
Alpine plants (e.g. <i>Latuca alpine</i> , <i>Saxifraga foliolosa</i>)	Europe (Scandinavia and Denmark)	max summer temp	Dahl, 1951
<i>Acer saccharophorum</i>	North America	yearly mean min temp	Dansereau, 1957
<i>Betula alleghaniensis</i>	North America	average annual temp (north), yearly max temp(south)	Dansereau & Pageau, 1966; Bourque <i>et al.</i> , 2005
<i>Corynephorus canescens</i>	Western Europe	mean July temp	Marshall, 1968
C4 grasses	North America	min July temp	Teeri & Stowe, 1976
<i>Opuntia versicolor</i>	North America	mean January temp	Kinraide, 1978

Species	Region	Limiting variable	Reference
Evergreen broad-leaved trees, warm-temperate broad-leaved trees, deciduous trees	Japan	min winter temp	Sakai, 1978
<i>Ilex</i> , <i>Viburnum</i> and <i>Quercus</i> species	Japan, Russia	min winter temp	Kaku & Iwaya, 1979
<i>Carnegia gigantea</i> , <i>Lemaireocereus thurberi</i> , <i>Lophocereus schottii</i>	North America	min winter temp	Nobel, 1980
<i>Coryphantha vivipara</i>	North America (Southern Nevada)	min winter temp	Nobel, 1981
<i>Prunus</i> species and hybrids	North America	average annual min temp	Quamme <i>et al.</i> , 1982
Agaves (14species)	North America	min winter temp	Nobel & Smith, 1983
<i>Tilia cordata</i>	Europe (UK and Finland)	max August and min autumn temp	Pigott, 1981, 1989, 1992; Pigott & Huntley 1980, 1981
<i>Bromus sterilis</i>	North America	heat sum of 2000 day-degree	Woodward, 1988
<i>Fagus</i> species	Europe and North America	mean January and July temp	Huntley <i>et al.</i> , 1989

Species	Region	Limiting variable	Reference
<i>Pueraria lobata</i>	North America	min winter temp	Sasek & Strain, 1990; Coiner <i>et al.</i> , 2011
<i>Fallopia japonica</i> and <i>Impatiens glandulifera</i>	North-western Europe	absolute min temp	Beerling, 1993
<i>Larrea tridentata</i>	North America	yearly min temp	Pockman & Sperry, 1997; Martinez-Vilalta & Pockman, 2002
<i>Rhododendron arboreum</i> , <i>R. campanulatum</i> , <i>R. barbatum</i> and <i>R. wallichii</i>	Central Himalayas	mean annual temp	Vetaas, 2002
<i>Pinus ponderosa</i>	South-western USA	max July temp	Humphries & Bourgeron, 2003
<i>Fagus</i> species (beech)	Japan and North America	summer temp (min, mean, max)	Fang & Lechowicz, 2006
Mangrove trees	Australia and US (Florida)	yearly min temp	Stuart <i>et al.</i> , 2007
159 tropical plant families	Neotropics	non-specified	Punyasena <i>et al.</i> , 2008
<i>Fagus sylvatica</i>	Europe (France)	mean May temp	Seynave <i>et al.</i> , 2008

Chapter Two - Freezing tolerance explains the different range limits of *Bromus tectorum* and *Bromus rubens*, two severe invasive species in North America.

Abstract

The winter freezing tolerance of *Bromus tectorum* and *Bromus rubens* was measured to evaluate climatic thresholds controlling their distribution and range separation. Freezing tolerance thresholds were determined using electrolyte leakage, leaf fluorescence and whole-plant mortality. Plants of each species responded the same to the winter conditions and artificial freezing treatments in 2007-2008 and 2009-2010. Both species adjusted their freezing tolerance limits downward, from -10°C at a daily minimum of 10°C, to -30°C at a daily minimum near -10°C. Both survived temperatures down to -31°C when fully acclimated to sub-zero temperatures. In the winter of 2010-2011, a sudden severe cold event on December 9 killed all *B. rubens* populations, while *B. tectorum* was not harmed. Controlled acclimation experiments revealed that two-month old plants of *B. rubens* had a slower acclimation rate to sub-zero temperatures and could not survive a sudden temperature drop from 1°C to -14°C. Older, four-month old *B. rubens* populations were as cold tolerant as *B. tectorum*. Our results demonstrate that different responses to rapid freeze events at the onset of winter explain, at least in part, the northern and elevational range separation of *B. tectorum* and *B. rubens* in North America.

Introduction

Physiological tolerance to climate can limit species distribution and explain variation in species range limits (Good, 1931, 1974). According to Good's Theory of Tolerance (Good, 1931), when two closely related species possess different levels of tolerance to environmental stress, they tend to occupy distinct habitats. Determinants of range limits are usually not well understood, yet the existing evidence indicates that temperature sensitivity of major physiological processes plays an important role (Kearney & Porter, 2009). This is particularly crucial for invasive species because they often escape biotic controls and are more likely to exhibit a distribution that reflects their physiological tolerances (Blaney & Kotanen, 2001).

In this study we evaluate whether cold tolerance explains the different geographic distribution of *Bromus tectorum* and *Bromus rubens*, two closely-related winter annual C₃ grasses that are among the most destructive invaders in North America (Mack, 1986; Vinton & Goergen, 2006). Both species belong to the *Genea* section and branch in sister positions on the *Bromus* phylogeny (Fortune *et al.*, 2008). Both have similar morphological characteristics, yet their dominance zones and range limits are geographically distinct along altitude and longitude gradients. *Bromus tectorum* is considered to be the more cold-tolerant of the two species, as it occurs at higher latitude than *B. rubens* and has been shown in field trials to survive winter conditions that are lethal to *B. rubens* (Hulbert, 1955). *Bromus tectorum* is the dominant invasive grass in the "cold desert" steppes of the Intermountain region of western North America, while *B. rubens* is one of the severe grass invaders in the "hot deserts" of southwestern North America (Mack, 1981; Salo, 2004,

2005). *Bromus* species have also distinct distributions along elevational gradients (Beatley, 1966; Brown & Rowe, 2004). Whereas *B. rubens* is present in low and mid elevation habitats (below 5,000 ft.), *B. tectorum* is persistent mainly at high-elevations with correspondingly lower mean temperatures (5,000-7,500 ft) (Beatley, 1966; Brown & Rowe, 2004).

The current distribution of *B. tectorum* in North America lies between 29° and 65° N latitude (Valliant *et al.*, 2007), and its northern limit in western North America coincides with the -30°C isocline for minimum winter temperature in western Canada (Mack, 1981; Valliant *et al.*, 2007). It is unknown whether this distribution limit is determined by a physiological limitation at extreme temperature. Numerous ecological reports conclude temperature somehow influences its distribution limit, possibly due to a physiological mechanism (Hulbert, 1955; Chambers *et al.*, 2007; Leger *et al.*, 2009). At high elevation near the upper range limit of *B. tectorum*, seedling growth and establishment is poor, winter survivorship low, and seed germination reduced (Evans & Young, 1972; Chambers *et al.*, 2007; Leger *et al.*, 2009). As a result, high elevation *B. tectorum* plants have low biomass and fecundity (Chambers *et al.*, 2007). It is unknown which aspect of the thermal regime is responsible for the poor performance at the upper range limit. One possibility is freezing injury of exposed tissues, since *B. tectorum* leaves are exposed to winter cold following their establishment the previous autumn.

The invasion of North America by *B. rubens* is restricted to landscapes south of 46°N latitude in western North America, and has become a problem species only in the “hot deserts” of the American southwest and the interior valleys of California. Wildfires that were not a major phenomenon in the “hot desert” ecosystems have become common, in part

due to *B. rubens* infestation (Smith *et al.*, 1997). As a result, the ecosystem function of many “hot desert” communities has been degraded by greater fire frequency and the associated loss of shrubs and stem succulents (Janzen *et al.*, 1997; Brooks, 2000; Bradley *et al.*, 2006). The restriction of *B. rubens* to lower latitudes and elevations than *B. tectorum* indicates it is less tolerant of winter cold than *B. tectorum*. This hypothesis is supported by an early study that demonstrated *B. tectorum* was able to survive winter conditions that killed *B. rubens* (Hulbert, 1955); however, no work has identified whether these species actually differ in their physiological tolerance of cold, and thus explanations of their different distributions remain conjecture.

Explanation of a species distribution and limits requires an understanding of the physiological, life history and environmental features that determine its fundamental and realized niche (Kearney & Porter, 2009). In this study, we evaluated the freezing tolerance of *B. tectorum* and *B. rubens* at the whole-plant and leaf tissue level as a first step towards understanding the mechanisms controlling the distribution of these species. We hypothesized that freezing tolerance determines the northern distribution limit of *B. tectorum* and *B. rubens* and that *B. rubens* is more cold sensitive than *B. tectorum*. To evaluate the low temperature threshold for injury, we used the electrolyte leakage method which assesses the membrane integrity of *B. tectorum* and *B. rubens* leaves at subzero temperatures. At harmful temperature, the cell membrane experiences ‘expansion-induced lysis’ or undergoes structural changes that weaken it (Steponkus, 1984). Cell membranes with altered structure become permeable to electrolytes, which are then detected in a bathing solution using an electrolyte leakage test (Stout *et al.*, 1980). Also, chlorophyll fluorescence measurements were used to investigate temperature effects on the effective

quantum yield of PSII in the two *Bromus* species, as well as to detect damage to the PSII complex via dark-acclimated *Fv/Fm* at low temperatures (Örlander, 1993). We also determined the effect of cold acclimation on the ice nucleation temperature of *B. tectorum* and *B. rubens* and used this to investigate changes in the freezing resistance mechanisms following prolonged exposure to sub-zero cold.

Materials and methods

Plant material

Bromus tectorum and *Bromus rubens* seeds were collected in 11 locations in 2006, 2009 and 2010 in Toronto, Canada and the western parts of the USA (Table 2.1). In October 2007, eight plants from accession T1 of *B. tectorum* and R1 of *Bromus rubens* were established in 10 L pots in the greenhouse and moved outside onto the roof of the Earth Sciences building, University of Toronto, where they were grown until May 2008. The same procedure was repeated in October 2009 with the seedlings of T1 and T5 *B. tectorum* and R1 *B. rubens*, and in October 2010 with seedlings of T1, T2, T3, T4, T5 *B. tectorum* and R1, R2, R3, R4, R5 *B. rubens* using ten plants per population (Table 2.1). Plants were watered to avoid drought stress, and fertilized weekly with 50:50 blend of Miracle Grow (Scotts Canada Ltd, Mississauga Canada) and an evergreen and shrub fertilizer (Plant Products Co. Ltd, Brantford, Canada). Air temperature, precipitation and diurnal light fluctuations were monitored using a weather station (Priva, Ontario, Canada) at the same location. Leaf temperatures were recorded with 36 gauge copper-constantan thermocouples attached to a

data logger (model SP-1700, Veriteq Instruments, Richmond, BC, Canada). These plants were used for assessment of cold injury, photoinhibition and ice nucleation.

Five plants of the T1 *B. tectorum* and R1 *B. rubens* were also grown in 10L pots in a “cool” greenhouse set at 12°C day and 10°C night. Leaf temperatures were within 2°C of this set point. These plants were used in the experiment on ice nucleation temperature. Ten plants of each *B. tectorum* (T1-T5) and *B. rubens* (R1-R5) population were also grown in the “cool” greenhouse at 12°/10°C day/night temperatures for the assessment of their absolute minimum cold tolerance limits. Eight plants of the T1 *B. tectorum* and R1 *B. rubens* were grown in a “warm” greenhouse at approximately 26°C day, 20°C night temperature and assessed for initial freezing tolerance.

Freezing injury determination

Freezing injury of detached leaves was assessed using the electrolyte leakage method between October 2007 and April 2008, between October 2009 and February 2010, and between October 2010 and March 2011 (Ritchie & Landis, 2003). One 3.5 cm leaf segment was cut from the middle of a leaf from each plant, rinsed in double-distilled water, blotted dry, and put into 7-ml glass vials. Five µl of distilled water was added to each vial to prevent supercooling and allow for uniform freezing (Clement & Van Hasselt, 1996; Appendix I, Fig. A.1). Tubes were placed into a programmable freezer (S-1.2C-B, Thermotron, Holland, MI, USA) and cooled to +5°C, -5, -10, -15, -20, -25, -30, -35, and -40°C. Samples spent 4 hr at the target temperature. Cooling and warming rates were

4.5°C/hour and 9°C/hour respectively, and were selected after testing different rates (Appendix I; Fig. A.2).

After the freezing cycle, the Fv/Fm of dark-adapted samples was immediately measured using a pulse-amplitude-modulated fluorometer (PAM 2100, Walz, Effeltrich, Germany) (Naidoo 2006). Five ml of double-distilled water was added to each vial, and after 24-hr electrolyte leakage was measured with a conductivity meter (Ultrameter 4P, Myron L, Carlsbad, CA, USA). Samples were then placed into boiling water for 30 minutes, cooled to room temperature and electrolyte leakage remeasured. Electrolyte leakage was indexed as: $RC = C_{\text{treatment}} / C_{\text{total}}$, where RC is relative conductivity, $C_{\text{treatment}}$ is conductivity after a 24-hour incubation period, and C_{total} is conductivity after boiling. To calculate the temperature causing 50% leakage of electrolytes (TEL₅₀), a nonlinear regression analysis was used (Rapacz, 2002). TEL₅₀ is commonly used as an indicator of tissue freezing injury (Rapacz, 2002; Pennycooke *et al.*, 2003).

To evaluate whether electrolyte leakage coincides with plant mortality, six plants each of T1 *B. tectorum* and R1 *B. rubens* were moved in January 2010 from outdoors to large, 16ft² programmable freezers (Thermotron S-16-8200, Thermotron, Holland, MI, USA) and pre-exposed to one of four target temperatures (-20°, -25°, -30° and -35°C) for 4 hours with cooling and warming rates of 4.5°C/hour and 9°C/hour respectively. An electrolyte leakage test was then performed on one leaf segment per plant, and plants were moved to a glasshouse where the number of surviving plants was assessed one week later.

Absolute minimum cold tolerance limit

To evaluate the lowest possible survival temperature of fully hardened plants (the absolute minimum cold tolerance limit), five plants of each population of *B. tectorum* (T1-T5) and *B. rubens* (R1-R5) were grown in the cool greenhouse (12°/10°C) and acclimated for 3 days to 0°C in the Thermotrons. The plants were then subjected to seven additional acclimation cycles with a one-day “rest” outside on the roof between each cycle during winter condition of January 2010. Acclimation treatments consisted of lowering the temperature during the dark in programmable freezers from 0°C to one of seven target temperatures (treatment 1: -5°C; treatment 2: -10°C; treatment 3: -15°C; treatment 4: -20°C, and treatment 5: -25°C; treatment 6: -30°C, and treatment 7: -35°C) at a cooling rate of 2°C/hour. The plants were kept at the target temperature for 4 hours then the temperature was increased to -2°C at 2°C/hour. Each temperature cycle was repeated three times during a three- to four-day period. At the end of each acclimation treatment the plants were sampled for electrolyte leakage. After the third cycle (target temperature of -35°C) which caused >80% loss of tissue electrolytes, plants were moved to the green house and the percentage of surviving plants evaluated after two weeks.

Freezing tolerance after distinct frost hardening periods

To evaluate differences in freezing tolerance of non-hardened plants subjected to defined frost hardening periods, four young (two-month old) or old (four-month old) plants of each *B. tectorum* (T1, T2, T3) and *B. rubens* (R1, R2, R3) populations were grown in the “cool”

greenhouse (10°/12°C), tested for the initial TEL₅₀ and separated into two groups (A and B) and exposed to (A) -5°C for 4 days and (B) -5°C for 7 days in Thermotrons (without a pre-acclimation at 0°C). The temperature during the hardening period was decreased from +10° to -5°C, kept at this temperature for 4 days (group A) or 7 days (group B) and then increased to 0°C. Cooling and warming rates were 2°C/hour. After this treatment the plants were tested for freezing tolerance (TEL₅₀) and then subjected to freezing at -14°C for 4 hours with cooling and warming rates of 4.5°C/hour and 9°C/hour, respectively. After the freezing cycle, plants were moved to the green house and the percentage of surviving plants evaluated after two weeks. The experiment was conducted four times, twice using 2-month old plants, and twice with 4-month old plants.

Ice nucleation temperatures

To evaluate the supercooling capacity of *B. tectorum* and *B. rubens*, one leaf segment from each of five plants grown outdoors or in a cool greenhouse was harvested in January 2010, placed in a vial and put into a Thermotron freezer. Air and leaf temperatures were monitored at 10-s intervals with fine-wire thermocouples and a data logger (SP-1700, Veriteq Instruments, Richmond, BC, Canada). Air temperature was decreased from +4 to -35°C at 2°C/hour. Thermocouples attached to the leaf segments recorded a sudden temperature rise (exotherm) caused by the heat release during the extracellular freezing. The lowest temperature before the exotherm indicated the ice nucleation point (INP) (Sierra-Almeida *et al.* 2009). To compare the effect of the cooling rate on the INP, the experiment was repeated at a faster rate of 4.5°C/hour (Appendix I; Table S1).

Photoinhibition of outside grown plants

Photoinhibition in *B. tectorum* and *B. rubens* was evaluated in November to mid-December 2007 on eight plants grown outdoors. Effective quantum yield of PSII (F_v'/F_m') was measured at noon at light intensities $>1300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ using a PAM 2100 fluorometer (Walz, Effeltrich, Germany). These leaves were then detached, kept in the dark for 30 minutes at 20° to 22°C and F_v/F_m measured. The temperatures of six leaves were measured with fine-wire thermocouples and a datalogger (SP-1700, Veriteq Instruments, Richmond, BC, Canada) throughout the experiment.

Statistical analysis

Data were analyzed using SIGMASTAT v.3.0.1 (SPSS, Chicago, IL, USA). Differences in TEL_{50} and F_v/F_m were assessed with a three-way ANOVA followed by Holm-Sidak post-hoc test with testing temperature, growth temperature and population as variables. To evaluate the freezing resistance mechanism, we assessed the differences in ice nucleation temperature and TEL_{50} values using a Bonferroni *t*-test. The difference in F_v'/F_m' and F_v/F_m values was evaluated using one-way ANOVA with air temperature as a variable.

Results

Freezing injury

All outdoor plants survived the winter of 2007-2008 in good condition. On November 2, 2007 (day 306), TEL₅₀ of both species was near -12°C when minimum air temperature was above freezing (Fig. 2.1a, b). This TEL₅₀ did not differ from the values of plants grown in a warm greenhouse where the temperatures were approximately 26°C/20°C (day/night). On December 9, 2007 (day 343), when the minimum temperature was below 0°C, the TEL₅₀ of *B. tectorum* was -15°C and that of *B. rubens* -22°C (Fig. 2.1a, b). At the beginning of January 2008, the minimum air temperature rose from -14°C to daily highs near 14°C for almost a week (Fig. 2.1a) and the corresponding TEL₅₀ measured on January 10, 2008 (day 10) was -17°C in both species (Fig. 2.1b). In February 2008, air temperature was generally below 0°C and the corresponding TEL₅₀ of *B. tectorum* and *B. rubens* on February 10, 2008 (day 43) was near -22°C. The TEL₅₀ of both species remained below -20°C in March (day 73), but rose to near -12°C in mid-April (day 105) with the advent of warmer temperatures (Fig. 2.1a, b). During the course of the experiment the temperature causing 50% reduction in Fv/Fm coincided with the TEL₅₀ values, in that it was near -12°C in mid-Autumn and Spring, and declined to near -20°C in February when subzero temperatures were common (Fig. 2.1c). The Fv/Fm differed little between the species, with the exception of day 343 (Dec. 9, 2007), when *B. rubens* showed a 9°C reduction in the exposure temperature required to reduce Fv/Fm by 50%, while *B. tectorum* showed a modest decline in this parameter relative to the day 306 values. According to Fig 2.1c, the temperatures required

to reduce Fv/Fm by 50% were unchanged by the January warm spell in *B. tectorum* and rose to -17°C in *B. rubens*.

All outdoor plants also survived the winter of 2009-2010. There was no difference in TEL₅₀ between species and populations, although there was a significant seasonal effect ($P < 0.001$). On November 1, 2009 (day 305), the TEL₅₀ of *B. rubens* (R1) and *B. tectorum* populations /T1 and T5/ was -12°C (Fig. 2.2a, b). In January, when air temperature was below -10°C, the TEL₅₀ was near -30°C which was 8°C lower than in 2008 (Fig. 2.2b). Moderate warming with air temperatures above 0°C occurred at end of January 2010 and on February 9, 2010 (day 40), the TEL₅₀ of both species rose to near -25°C. The temperature of 50% reduction in Fv/Fm measured in 2009-2010 coincided with the TEL₅₀ values (Fig. 2.2c).

All outdoor plants from every tested *B. tectorum* population (T1-T5) survived the winter of 2010-2011. By contrast, all plants from every *B. rubens* population (R1-R5) were dead on the 9th of December, 2010 after the air temperature went down to -14°C for 2 hours. On December 4, 2010 (day 338) the TEL₅₀ values of all *B. tectorum* and *B. rubens* populations were around -12°C. All *B. tectorum* populations (T1-T5) showed the TEL₅₀ between -22° and -25°C on January 8, 2011 (Fig. 2.3).

The response of whole-plant mortality mirrored the temperature response of relative conductivity in plants growing outdoors in January 2010 (Fig. 2.4). Temperatures that caused 50% and >80% loss of tissue electrolytes (TEL₅₀, TEL₁₀₀ respectively) and resulted in 50% and 100% mortality (LT₅₀, LT₁₀₀) were near -30°C for both species. TEL₅₀ is thus a robust index of the thermal threshold for survival in these species.

Absolute minimum limit for cold tolerance

An ability to adjust the low temperature tolerance during thermal acclimation complicates estimates of the lowest possible tolerance temperatures, since the results depend on the minimum air temperatures encountered in any given year. To overcome this limitation, we first acclimated plants to 0°C in the controllable freezers, and examined their TEL₅₀ and mortality. We then followed this with acclimation cycles to -5°C, -10°C, -15°C, -20°C, -25°C, -30°C and -35°C, determining the TEL₅₀ of acclimated leaves from *B. tectorum* (T1-T5) and *B. rubens* (R1-R5) at the end of each cycle. This procedure allowed the plants to acclimate their freezing tolerance thresholds to the absolute lower limit. In plants artificially acclimated to -20°C, the TEL₅₀ was around -30.5°C in both species and populations, which was the same as that of the plants grown outdoors on December 5, 2009 (Fig. 2.5; P=0.694). The minimum temperature in the 48 hr prior to sampling the outdoor-grown plants (2009) was -10.6°C. In figure 2.6, we present the relationship between the minimum exposure temperature observed 48 hours prior to tissue sampling, and the TEL₅₀ of all measurements made outdoors in 2007-2008 and 2009-2010. The measurements were conducted on frost-hardened material grown outdoors and on the plants experimentally acclimated to below -20° in the Thermotron freezers. Above -10°C, both frost-hardened *Bromus* species adjusted TEL₅₀ such that it was 20°C below the minimum exposure temperature observed the 48 hour period prior to sampling. Below exposure temperature of -10°C, however, there was no further reduction in TEL₅₀, which remained close to -30°C despite acclimation to temperatures between -20°C and -35°C.

Freezing tolerance after distinct frost hardening periods

The TEL₅₀ of the non-hardening plants grown in the “cool” greenhouse was -12°C to -13°C in all populations of both species. A 4-day exposure to -5°C decreased the TEL₅₀ to -23°C in 2- and 4 month-old *B. tectorum* plants and 4 month-old *B. rubens* plants. By contrast, the TEL₅₀ of 2 month-old *B. rubens* plants exposed to -5°C for 4 days remained unchanged at -12°C in all populations (R1, R2, R3). A 7-day exposure to -5°C decreased the TEL₅₀ of both species and populations to -23°C, independent on the plant age (Table 2.2). Exposure of whole plants from group A and B to -14°C, resulted in almost 100% mortality of 2 month-old *B. rubens*, but did not have an effect on the survival of 2- and 4 month-old *B. tectorum* and 4 month-old *B. rubens* plants (Table 2.2).

Ice nucleation temperatures

A representative freezing curve showing a distinct exotherm is presented in Fig. 2.7. Plants grown in a greenhouse at chilling temperatures (12°/10°C, day/night) had lower ice nucleation temperatures (INP) than outdoor-grown plants acclimated to low mid-winter temperatures ($P < 0.001$). The INP of outdoor grown *B. tectorum* and *B. rubens* was around -8.5°C in January, 2010, while the TEL₅₀ of the same plant material was about -30°C (Table 2.3). By contrast, INP of plants grown in a “cool” greenhouse was near -12°C, which was close to the TEL₅₀ of these species (Table 2.1). There was no observed effect of the cooling rate on the exotherm temperature (Appendix I; Table A.1).

Photoinhibition of outside grown material

Dark-adapted F_v/F_m values of *B. tectorum* and *B. rubens* were around 0.78 on the first day of sampling, on November 11, 2007, when the leaf temperature was $> 0^{\circ}\text{C}$ (Fig. 2.8). When the temperature went below -9°C on November 24 and December 2, 2007, both species showed a reduction in F_v/F_m to approximately 0.6 and full recovery in January following a brief warming. Fluorescence measurements made during the same period at noon under a photon flux density of $1350 \mu\text{mol m}^{-2} \text{s}^{-1}$ showed F_v'/F_m' below 0°C was near 0.05 in both species (Appendix I; Fig. A.3). Above 0°C , F_v'/F_m' rose from about 0.2 at 5°C to 0.45 at 15°C (Appendix I; Fig. A.3).

Table 2.1 - The description and location of *Bromus tectorum* and *Bromus rubens* seed collection sites. Seeds were collected July-August 2007, 2009 and 2010.

Species	Population	Location	Elevation (m)
<i>Bromus tectorum</i>	T1	Hidden Valley Drive, Reno, Nevada (39°29' N, 119°42' W)	1370
	T2	Mount Charleston town, Nevada, highway 157, (36°15' N, 115°37' W)	2300
	T3	Picnic area, Santa Catalina Mountains, Arizona (32°27'N, 110°42'W)	1800
	T4	Highway 157, Spring Mountains, Nevada (36°25' N, 115°24' W)	1050
	T5	Toronto, Ontario (43° 40' N; 79° 24' W)	76
<i>Bromus rubens</i>	R1	National Desert Wildlife Refuge, Nevada (36°20' N, 115°08' W)	905
	R2	Roadside Wikieup, Arizona (113°36'W, 34° 42'N)	580
	R3	Picnic area, Santa Catalina Mountains, Arizona (32°27'N, 110°42'W)	1800
	R4	Highway 157, Spring Mountains, Nevada (36°25' N, 115°24' W)	1670
	R5	Highway 157, Spring Mountains, Nevada (36°17' N, 115°39' W)	1050

Table 2.2 - The ice nucleation temperature (INP), and the temperature at which 50% of electrolytes are leached out of leaf segments (TEL₅₀) of *Bromus tectorum* (T1), and *Bromus rubens* (R1) grown under chilling (10°to 12°C) or sub-zero temperatures (<°0).

	INP (°C)	TEL ₅₀ (°C)	Thermal Difference	Cold Tolerance Mechanism
<i>B. tectorum</i> grown in a greenhouse at 12°/10°C	-13.8 ± 1.9	-13.4 ± 1.3	-0.5	FA
<i>B. tectorum</i> grown outdoors and exposed to minimum temperatures below -5°C	-8.6 ± 0.6 ^{***}	-30.9 ± 0.7 ^{***}	22.3 ^a	FT
<i>B. rubens</i> grown in a greenhouse at 12°/10°C	-12.4 ± 1.5	-12.0 ± 1.9	-0.4	FA
<i>B. rubens</i> grown outdoors and exposed to minimum temperatures below -5°C	-8.7 ± 2.4 ^{***}	-30.0 ± 1.9 ^{***}	21.3 ^a	FT

Means ± SD, asterisks indicate significant treatment effect within the species***P<0.001 (n=8) (two-way Anova); superscript letters indicate differences between INP and TEL₅₀, ^a P<0.001 (n=8), (Benferroni *t*-test). No difference between INP and TEL₅₀ indicated the presence of freezing avoidance strategy (FA), while a significant difference between INP and TEL₅₀ indicated that plants were freezing tolerant (FT)

Table 2.3 - The effect of two frost hardening periods on the (TEL₅₀) of *Bromus tectorum* and *Bromus rubens*

Plant age	Species	Population	Initial TEL ₅₀	TEL ₅₀ after frost hardening under -5°C		Survival of the frost hardened plants after exposure to -14°C, %	
				for 4 days	for 7 days	for 4 days	for 7 days
2-month	<i>B. tectorum</i>	T1	-12.1±1.1 ^a	-22.9±2.7 ^b	-22.1±3.8 ^b	88±12	88±12
		T2	-13.0±1.0 ^a	-23.3±2.6 ^b	-24.3±3.7 ^b	88±12	100±0
		T3	-11.8±1.7 ^a	-23.3±2.6 ^b	-24.3±3.7 ^b	100±0	100±0
	<i>B. rubens</i>	R1	-12.3±2.2 ^a	-11.9±1.3 ^a	-23.8±2.7 ^b	13±13	100±0
		R2	-12.6±1.9 ^a	-12.4±1.8 ^a	-22.9±3.1 ^b	0±0	88±12
		R3	-12.1±2.4 ^a	-13.3±2.4 ^a	-24.4±2.4 ^b	0±0	100±0
4-month	<i>B. tectorum</i>	T1	-12.2±1.6 ^a	-22.5±2.7 ^b	-23.7±2.3 ^b	100±0	100±0
		T2	-11.8±1.3 ^a	-24.1±3.7 ^b	-24.0±2.7 ^b	100±0	88±12
		T3	-12.7±1.6 ^a	-22.9±2.7 ^b	-23.7±2.9 ^{ab}	100±0	100±0
	<i>B. rubens</i>	R1	-11.5±3.0 ^a	-19.3±4.6 ^c	-21.4±3.4 ^b	88±12	100±0
		R2	-12.9±1.9 ^a	-23.0±1.9 ^b	-22.9±3.3 ^b	100±0	100±0
		R3	-13.0±2.6 ^a	-21.5±4.3 ^b	-22.3±3.2 ^b	100±0	100±0

Means ± SD (for TEL₅₀; n=8); different superscript letters indicate significant differences at P<0.05 level, (two-way Anova, Holm-Sidak); Means ± range (for survival data, n=2 replicates)

Table 2.4 - The number of early frost events (November 10 – December 10) with temperatures of $\leq -13^{\circ}\text{C}$ that occurred during the 40-year period (1970-2010) within *B. rubens* and *B. tectorum* distribution ranges

# Pair	Site location	Species distribution range	Number of early frost events of $\leq -13^{\circ}\text{C}$ (November, 10 – December, 10) occurred during the 40-year period (1970-2010)
1	Las Vegas, Nevada ($36^{\circ}10' \text{ N}$, $115^{\circ}08' \text{ W}$)	<i>B. rubens</i> present	0
	Caliente, Nevada ($37^{\circ}36' \text{ N}$, $114^{\circ}30' \text{ W}$)	<i>B. rubens</i> absent	9
2	Kingman, Arizona ($35^{\circ}11' \text{ N}$, $114^{\circ}3' \text{ W}$)	<i>B. rubens</i> present	0
	Prescott, Arizona ($34^{\circ}34' \text{ N}$, $112^{\circ}27' \text{ W}$)	<i>B. rubens</i> absent	7
3	Palmdale, California ($34^{\circ}34' \text{ N}$, $118^{\circ}6' \text{ W}$)	<i>B. rubens</i> present	0
	Reno, Nevada ($39^{\circ}31' \text{ N}$, $119^{\circ}49' \text{ W}$)	<i>B. rubens</i> absent	10

The climatologic data was retrieved from the Weather Underground (<http://www.wunderground.com/>), Tu Tiempo (<http://www.tutiempo.net>), Old Farmer's Almanac (<http://www.almanac.com>) and The Community Environmental Monitoring Program (<http://www.cemp.dri.edu>). Site locations for each pair were chosen such as one member of the pair is in the northern *B. rubens* range, and the other is the closest climate station beyond the *B. rubens* range along a latitude and elevation cline.

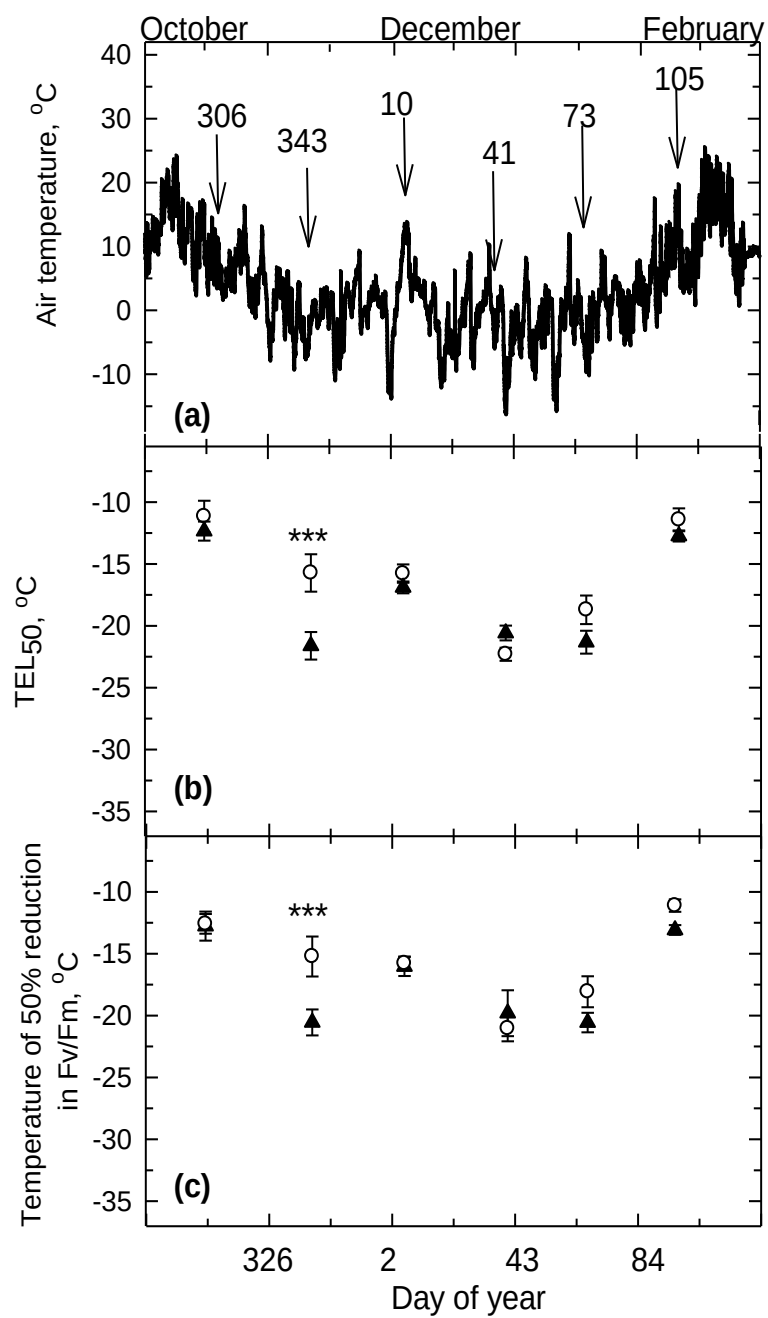


Figure 2.1.

Figure 2.1 - Results of the freezing tests of 2007–2008, as a function of the day of year for *Bromus tectorum*, T1 (open circles) and *Bromus rubens* R1 (filled triangles). **(a)** Air temperature during the evaluation of cold hardiness in outdoor-grown plants. Arrows indicate the day of the sampling for the electrolyte leakage test. **(b)** The temperature causing 50% electrolyte leakage, TEL₅₀; **(c)** the relationship between the temperature causing 50% reduction in Fv/Fm 24 hours after the freezing cycle. Means $\pm$ SD, N = 8 plants per species. Asterisks indicate significant differences in species response, *** P < 0.001 (three-way ANOVA followed by Holm-Sidak post-hoc test)

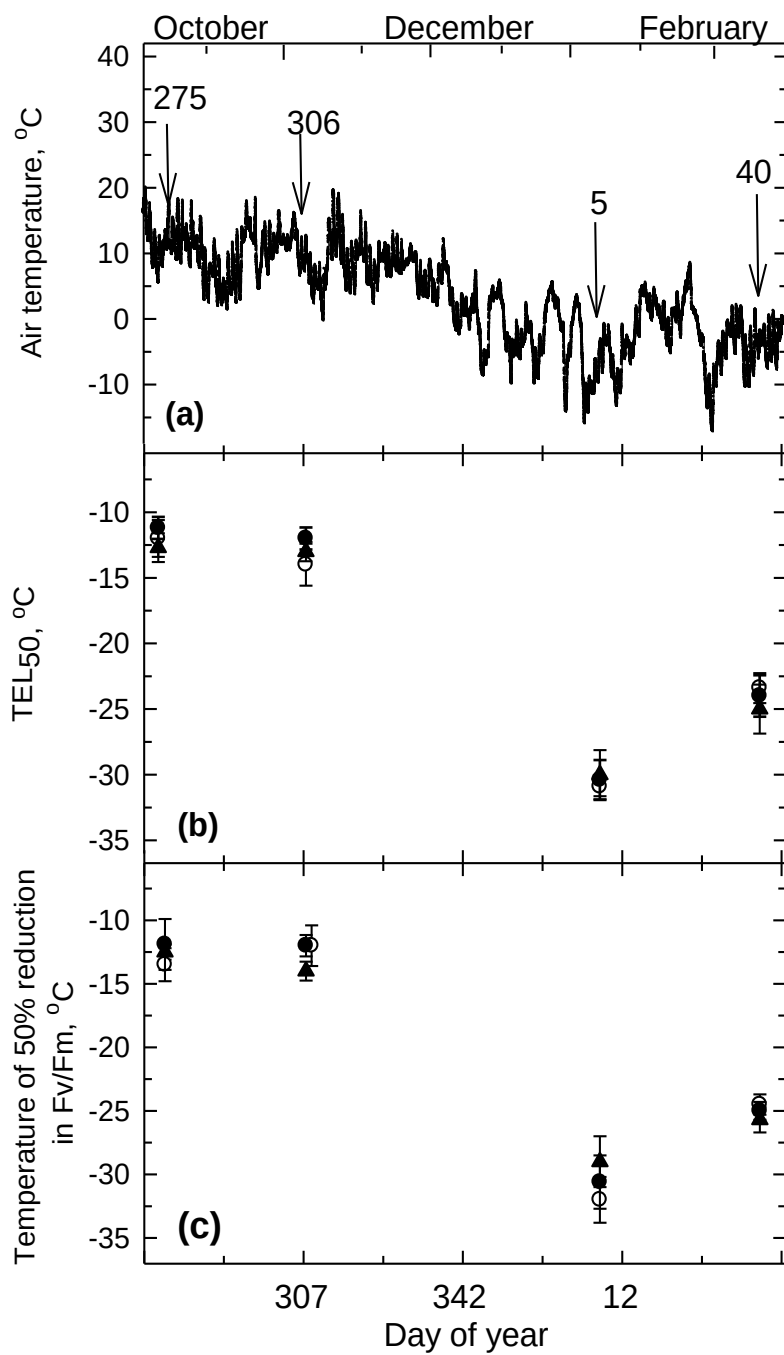


Figure 2.2.

Figure 2.2 - Results of the freezing tests of 2009–2010, as a function of the day of year for *Bromus tectorum*, T1 (open circles), *Bromus tectorum*, T5 (filled circles) and *Bromus rubens* R1 (filled triangles). **(a)** Air temperature during the evaluation of cold hardiness in outdoor-grown plants. Arrows indicate the day of the sampling for the electrolyte leakage test. **(b)** The temperature causing 50% electrolyte leakage; **(c)** the relationship between the temperature causing 50% reduction in Fv/Fm 24 hours after the freezing cycle. Means $\pm$ SD, N = 5 plants per population.

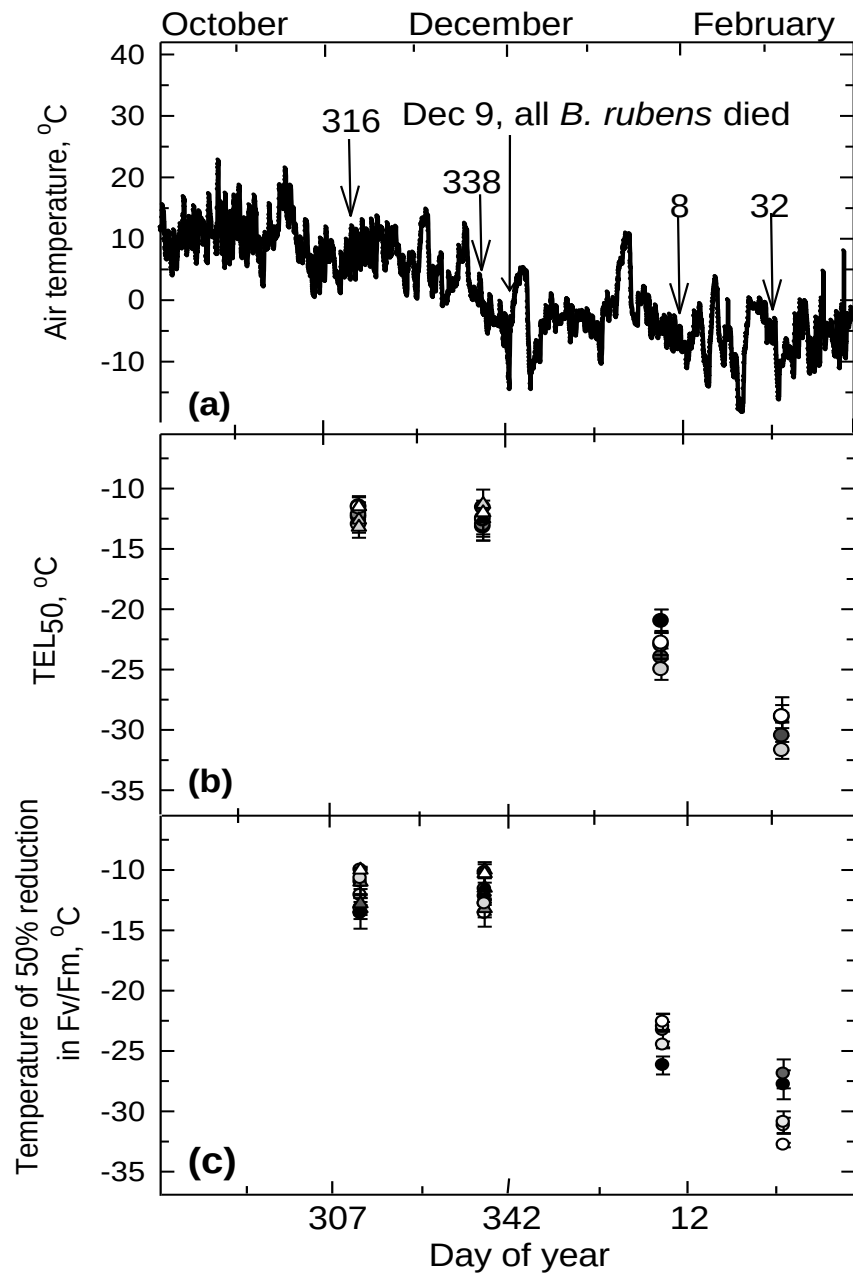


Figure 2. 3

Figure 2. 3 - Results of the freezing tests of 2010–2011, as a function of the day of year for *Bromus tectorum* (T1-T5), (circles), and *Bromus rubens* (R1-R5) (triangles). **(a)** Air temperature during the evaluation of cold hardiness in outdoor-grown plants. Arrows indicate the day of the sampling for the electrolyte leakage test. **(b)** The temperature causing 50% electrolyte leakage; **(c)** the relationship between the temperature causing 50% reduction in F_v/F_m 24 hours after the freezing cycle. Means $\pm$ SD, N = 10 plants per population.

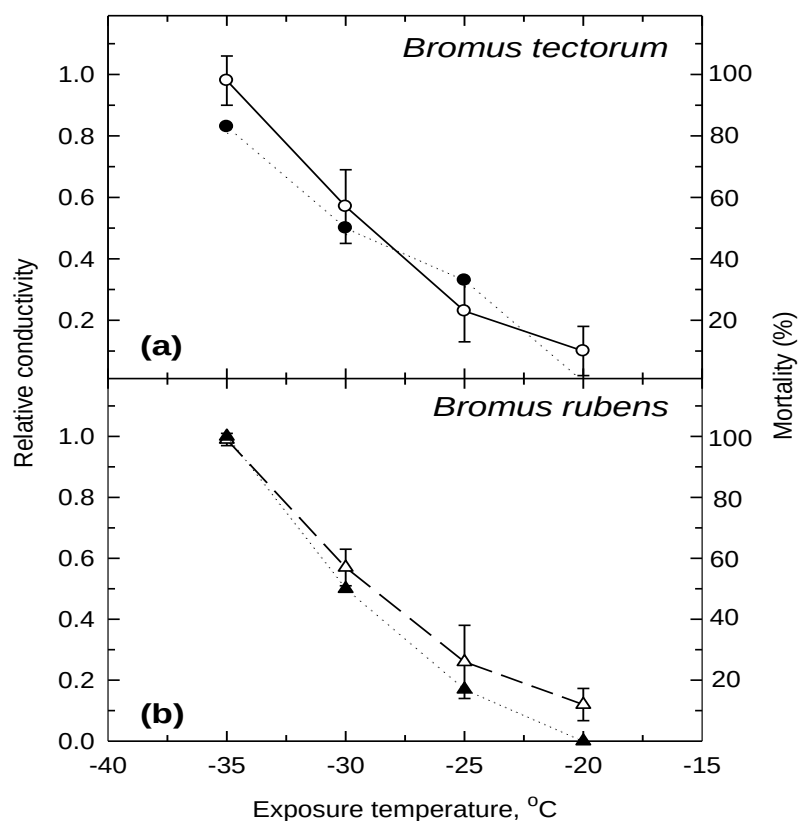


Figure 2. 4 - The effect of exposure temperature on whole-plant mortality and electrolyte leakage of leaves. **(a)** *Bromus tectorum*, where the dotted line with closed circles indicates mortality and the solid line with opened circles indicates relative conductivity; **(b)** *Bromus rubens*, where the solid line with filled triangles indicates mortality and the dashed line with opened triangles indicates relative conductivity. Plants used in this experiment were growing outdoors and sampled in January 2010. Means $\pm$ SD, N = 6 plants per species.

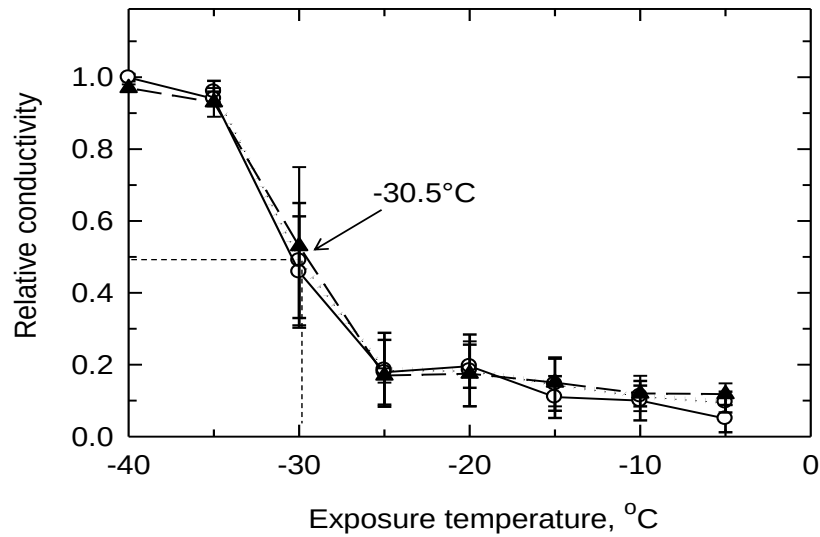


Figure 2.5 - The response of electrolyte leakage to low temperature in leaf samples of *Bromus tectorum*, Reno population T1 (solid line with open circles), *Bromus tectorum*, Toronto T5 (dotted line with filled circles) and *Bromus rubens* R1 (dashed line with filled triangles) artificially acclimated in the programmable freezers to -20°C . Measurements were conducted in January 2010. Means $\pm$ SD, $N = 5$ plants per species. Arrows indicate the TEL_{50} values.

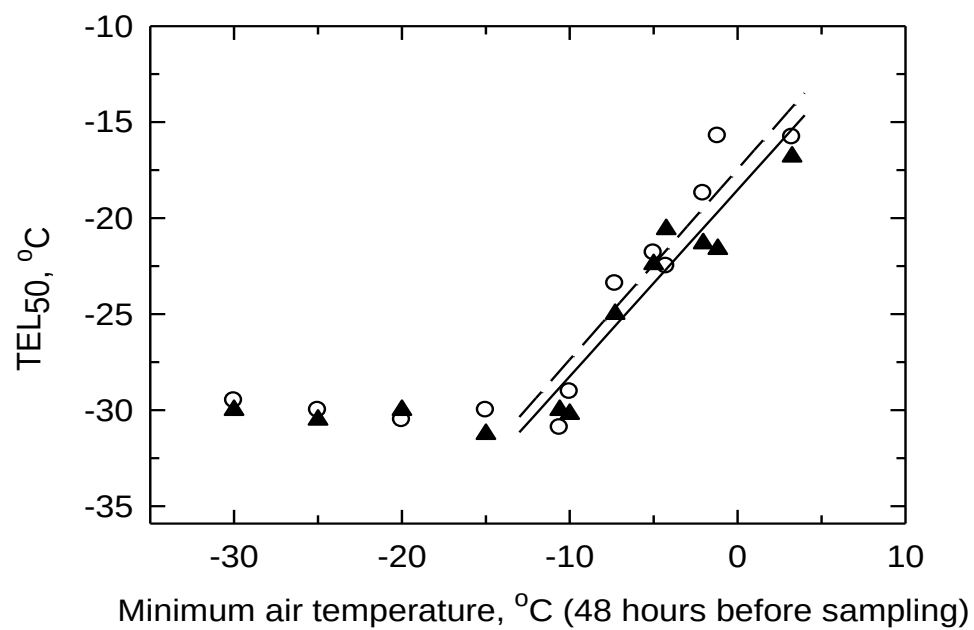


Figure 2.6

Figure 2.6 - The effect of average minimum air temperature 48 hours before sampling on TEL₅₀ of *Bromus tectorum* (open circles) and *Bromus rubens* (filled triangles) plants that were frost hardened naturally outdoors or artificially during the assessment of the absolute cold tolerance limit. N = 8 plants per species (2007-2008) and five plants per species (2009-2010). The regressions between 10°C and -10°C are $y = 0.9922x - 17.453$ ($r^2 = 0.86$) for *Bromus tectorum* (solid line) and $y = 0.9713x - 18.526$ ($r^2 = 0.91$) for *Bromus rubens* (dashed line). The average minimum air temperature 48 hours before sampling was computed as the average of temperatures during the coldest hour in the 48 hour period before sampling.

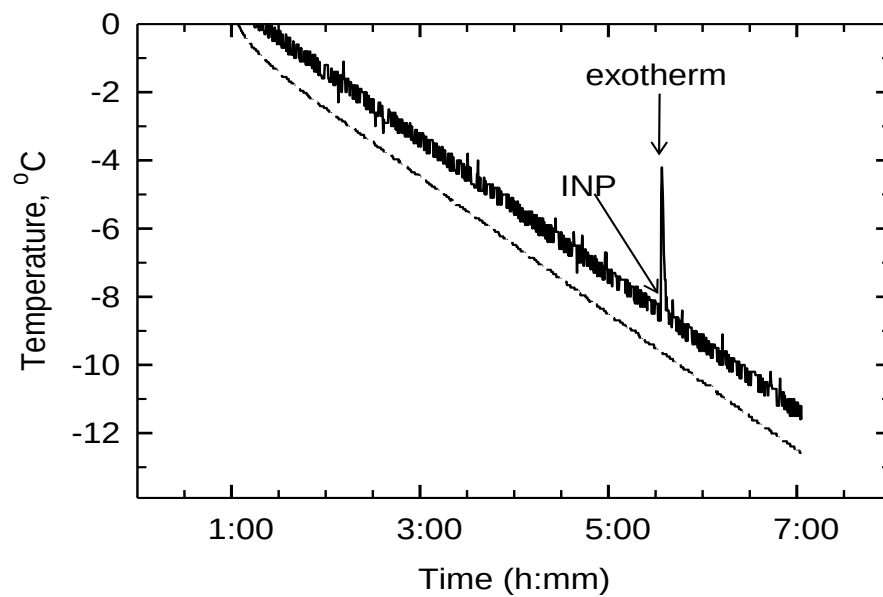


Figure 2.7 - A representative freezing curve obtained on a sub-zero hardened *Bromus tectorum*. The solid line represents the leaf temperature; the dotted line represents the temperature of a reference thermocouple recorded during the freezing time course. The lowest temperature of the exotherm is the ice nucleation point (INP).

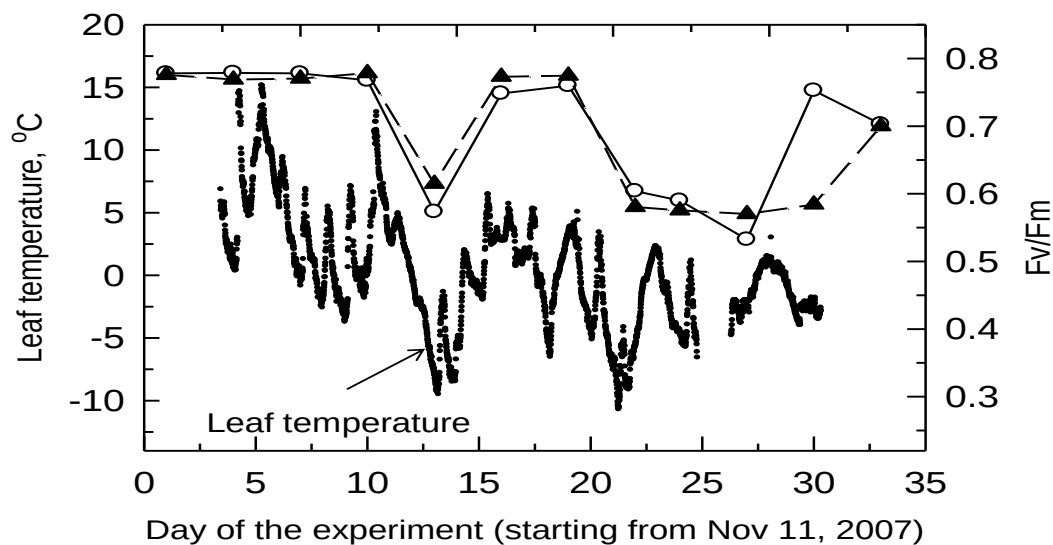


Figure 2.8 - Leaf temperature and the dark-adapted photochemical efficiency (Fv/Fm) of *Bromus tectorum*, Reno, T1 population (solid line with open circles) and *Bromus rubens*, R1 population (dotted line with filled triangles). Fv/Fm was measured on dark-adapted samples 30 min after excision from outdoor-grown plants exposed to bright sunshine on the days indicated by the symbols. Leaf temperature was logged every 10 minutes on 6 leaves per species with thermocouples during the period of experiment using the data loggers (SP-1700, Veriteq Instruments, Richmond, BC, Canada).

Discussion

Bromus tectorum and *B. rubens* are two of the most pernicious invaders in western North America. *Bromus tectorum* has become dominant throughout the Great Basin and Columbia plateau (Smith *et al.*, 1997), and is common above 1400m in the Mojave desert (Bykova *et al.*, unpublished). *Bromus rubens* dominates low-to-mid elevations (500-1600m) in the Mojave desert region of California, Nevada and Arizona (Beatley 1966). Because as invaders they have largely escaped biotic controls (Blaney & Kotanen, 2001), we hypothesize that their range distribution reflects abiotic controls over their physiology and performance; specifically, their northern and upper range limits reflect a mechanism controlling tolerance of winter cold. Initial experiments did not support the hypothesis, as plants of each species growing outside during the winters of 2007-2008 and 2009-2010 responded the same to the winter conditions and artificial freezing treatments. Both species were able to survive to -31°C when fully acclimated to below -10°C. During a third outdoor trial in the winter of 2010-2011, a sudden severe cold event on December 8-9, 2011 killed all *B. rubens* accessions while *B. tectorum* was not harmed. At the time, the *B. rubens* and *B. tectorum* plants were 7-8 weeks old. To investigate this result further, we experimentally exposed young (2 month old) and mature (4 month old) plants of each species to mild freezing events lasting 4 to 7 days. At the start of these experiments, neither species was in a hardened state, having spent their lives in a “cool” greenhouse at 12°/10°C day/night temperatures. Both immature and mature population of *B. tectorum*, and the mature populations of *B. rubens*, were able to reduce the TEL₅₀ to < -20°C and survive a -14°C treatment. This reduction in TEL₅₀ reflected the acquisition of freezing tolerance of tissues, as indicated by a rise in the INP to -8° to -9°C from near -13°C. However, young *B. rubens*

plants maintained the TEL₅₀ to near -12°C after 4 days of -5°C exposure and were killed by an experimental reduction in temperature to -14°C. These results demonstrate a slower acquisition of freezing tolerance in young *B. rubens* plants. This would establish a vulnerability window in the autumn when non-hardened plants might experience a sudden severe freeze, as occurred on December 8-9, 2011. Not all years would have the rapid change in conditions from mild to severe cold, and so not all years would be lethal. Gradual reduction in autumn lows, or an extended period between 0° and -10°C would cold-harden *B. rubens*, such that its cold tolerance rivals that of *B. tectorum*. This would explain the 2007-2009 survival, where the onset of winter was punctuated by a series of sublethal freezing days with minimum air temperatures above -10°C. By contrast, autumn cold only fell to between 0° and -5°C for 4 days prior to the sudden drop to -14°C on December 9, 2011.

We therefore hypothesize that the slower acclimation rate of *B. rubens* explains the difference in range distribution of the two species. In the lower elevations of Mojave desert, *B. rubens* does not normally encounter rapid onset of autumn chill to $\leq -13^{\circ}\text{C}$, whereas such events might be sufficiently common at higher elevations and in the Great Basin further north to reduce the survival of *B. rubens* (Table 4). If the sudden lethal freeze events are frequent enough, then *B. rubens* could not maintain viable populations, particularly since the seed bank of *B. rubens* and *B. tectorum* is short-lived (Wu & Jain, 1978; Smith *et al.*, 2008). To test this hypothesis, we selected three pairs of climate stations and examined them for the occurrence of severe frost events ($\leq -13^{\circ}\text{C}$) for the period November 10 to December 10. The climate stations were selected so that one station was in the northern *B. rubens* range and the other was the closest climate station above the *B. rubens* range along

an elevation or latitude cline. The analysis of climatic data showed that at the stations located along the northern edge of the *B. rubens* distribution, air temperatures never fell below -13°C between November 10 and December 10 during the past 40 years. By contrast, at the sites beyond the *B. rubens* range, severe frost events occurred 7 to 10 times between November 10 and December 10 during the last 40 years, which is approximately once in five years (Table 4).

Our observations could also explain results of Hulbert (1955), the only previous comparison of winter survival in *B. tectorum* and *B. rubens*. Hulbert (1955) demonstrated that *B. rubens* did not survive winter temperatures in Washington state, USA and Minnesota, USA that fell to -23°C , while *B. tectorum* showed high survivorship. When the cold occurred at his field sites is unclear, although climate records indicate that young *B. rubens* plants likely experienced lethal cold at both field sites in the autumn. The air temperature in November 1950 and the first week of December 1953 at the weather stations near both field sites fell to -10°C (Hulbert, 1955; Minnesota Climatology Working Group, 2011). With boundary layer cooling on clear nights, leaf temperatures could have fallen below air temperature by $2\text{--}3^{\circ}\text{C}$, which according to our TEL_{50} data would exceed the lethal threshold.

Cold acclimation in plants proceeds step-wise in approximately three stages (Greer *et al.*, 2000; Gusta *et al.*, 2005). The first stage is the primary acclimation, characterized by transcription factor upregulation (e.g. dehydrin transcription); the second is moderate acclimation that involves the production of cryoprotective compounds, and the third stage represents acclimation that results in the achievement of extreme freezing tolerance and requires exposure to freezing temperatures (Gusta *et al.*, 2005). Development of freezing

tolerance during stages 2 and 3 is a key element of survival for winter annuals (Hurry *et al.*, 1995; Strand *et al.*, 1997; Gusta *et al.*, 2005). Nonetheless, because the rate of frost hardening varies between species and within the acclimation stages, species with the same absolute tolerance limits might correspond to different winter survival rates (Harrison *et al.* 1978; Greer 1983). Frost-resistant cultivars of wheat and barley, for instance, usually harden faster than frost-sensitive cultivars which results in variation in their rates of winter survival (Stanca *et al.*, 1996; Saulescu & Braun, 2001). A lag phase between the acclimation stages and different induction temperatures could account for these discrepancies (Fuchigami *et al.*, 1971; Stanca *et al.*, 1996; Greer & Warrington, 1982). In frost-resistant cultivars of barley, for example, cold-induced proteins (COR14) have higher induction temperatures than those of in frost-sensitive cultivars (Stanca *et al.*, 1996).

Absolute minimum tolerance limits

Once fully hardened, *Bromus tectorum* and *Bromus rubens* are identical in their minimum tolerance limits. They express the same degree of maximum cold hardiness as high-latitude grasses such as annual bluegrass (*Poa annua* L., $LT_{50} = -31.5^{\circ}\text{C}$, Dionne *et al.*, 2001) and Antarctic hair grass (*Deschampsia Antarctica*, $LT_{50} = -27^{\circ}\text{C}$, Bravo *et al.*, 2001) as well as some frost-resistant cultivars of winter wheat (*Triticum aestivum*, Frontier and Puma, minimum survival temperature = -33°C , Fowler, 1992). Once hardened, the freezing tolerance threshold of both *Bromus* species tracked changes in air temperature above -10°C . For example, on January 5, 2010 (day 5), when the TEL_{50} was near -30°C , minimum air temperature averaged -10°C in the 48 hours prior to sampling. Average minimum air

temperatures 48 h prior to the February 2010 sampling date was -4°C , and TEL50 rose to near -22°C . When air temperature rose above 0°C , as it did during January 2008, we observed an increase in the minimum tolerance temperature of both *Bromus* to approximately -15°C . This shifting of the minimum tolerance temperature indicates the absolute hardened state for *B. tectorum* and mature *B. rubens* is relaxed as temperatures rise above the point of tissue freezing, which is near -9°C in winter-hardened leaves. During milder days, as temperatures rise above 0°C , relaxation of the minimum tolerance threshold may facilitate the re-establishment of photosynthetic activity and growth. Both *Bromus tectorum* and *B. rubens* have the ability to re-engage photosynthesis on milder winter days as indicated by the rapid recovery of dark-adapted Fv/Fm following freezing events (Fig. 8). Reactivation of photosynthesis in winter would enhance carbon gain when much of the competing vegetation is dormant. As a consequence, both bromes could establish a dense root system before the native species. Competition for soil water in springtime is an important means by which *B. tectorum* is able to suppress native vegetation and dominate a site (Smith *et al.*, 1997).

Freezing-resistance mechanisms and photoprotection

We examined the freezing-resistance mechanism and photoprotection capacity of *B. tectorum* and *B. rubens* in order to determine if there are any species-specific differences in winter survival strategies. In general, plants survive sub-zero temperatures by two strategies: freezing avoidance and freezing tolerance (Sierra-Almeida *et al.*, 2009). Freeze-tolerant species avoid crystallization of cell water by promoting extracellular ice formation,

while the protoplast experiences dehydration. Freezing-avoiding species prevent ice formation through osmotic adjustment or supercooling (Link *et al.*, 2010). *Bromus tectorum* and *Bromus rubens* exhibit both strategies during their lifecycle. The absence of thermal difference between INP and TEL₅₀ of non-hardened *B. tectorum* and *B. rubens* plants growing in a "cool" greenhouse at 12°/10°C day/night temperatures indicates they exploit a freezing-avoidance strategy at the beginning of the cold season, before the frost-hardening period. By contrast, differences between INP and TEL₅₀ in *B. tectorum* and *B. rubens* plants exposed to temperatures below -5°C show that with the onset of prolonged freezing, both *Bromus* species switch to the freezing-tolerance strategy, which allows them to survive below -13°C without injury. This is accompanied by an increase in the INP from -12° to -8°C. However, young *B. rubens* plants are delayed in their ability to switch cold tolerance strategies. A similar change in freezing resistance mechanism during the onset of winter has also been demonstrated in low- to mid-elevation species in the Andes (*Acaena splendens* and *Taraxacum officinale*; Sierra –Almeida *et al.*, 2009).

Avoidance of ice formation via supercooling is generally a prerequisite for active growth of cold-adapted plants (Sakai & Larcher, 1987; Reyes-Diaz *et al.*, 2006). During the autumn, active growth of *B. tectorum* and *B. rubens* is required for plant establishment prior to the onset of winter (Regehr & Bazzaz, 1976; Aguirre & Johnson, 1991; Chambers *et al.*, 2007). Supercooling allows survival of mild freezes and is compatible with active growth (Sakai & Larcher, 1987; Reyes-Diaz *et al.*, 2006). As indicated by plants grown at 12°/10°C in a greenhouse, the freezing avoidance strategy of both *Bromus* species will protect leaves to -12°C, which is sufficient for most autumn and early spring conditions. The switch to a freezing tolerance strategy would restrict growth because it requires cellular

dehydration (Nilsen & Orcutt, 1996; Link *et al.*, 2010). The failure of young *B. rubens* plants to switch strategies could enable them to maintain active growth longer during the autumn and early winter, which may allow them to competitively suppress *B. tectorum* in the Mojave desert region.

Along with freezing avoidance, photoprotection is another mechanism that supports active growth of *B. tectorum* and *B. rubens* during the spring and autumn period. When plants are exposed to chilling (0° to 12°C) and freezing temperatures (<0°C) they become susceptible to photoinhibition due to slowing of both carbon gain and repair of the photosystems in the thylakoid membrane (Demming-Adams & Adams, 1992; Huner *et al.*, 1993; Terao & Katoh, 1996; Busch *et al.*, 2007). The development of effective photoprotection allows plants to avoid photooxidative damage and maintain growth (Demmig-Adams *et al.*, 1989). In *Bromus tectorum* and *Bromus rubens*, high-light conditions and temperatures below -12°C reduced the efficiency of open PSII centers (F_v'/F_m'); however no irreversible damage of PSII occurred as shown by the recovery of F_v/F_m following darkening for 30 min. Thus, both species appear equally capable of protecting their photosystems during winter cold exposure.

Winter temperatures and northern limits of *Bromus tectorum* and *Bromus rubens*

Our results demonstrate that distinct responses to rapid freeze events at the onset of the winter season explain in part, the northern and altitude range separation of *B. tectorum* and

B. rubens in North America. While in *B. rubens*, the northern range limit is partially controlled by its ability to frost harden rapidly at the beginning of the cold season, in *B. tectorum* it appears to be limited by its absolute freezing tolerance limit. The northern distribution limit of *B. tectorum* in western North America corresponds to the minimum winter temperature isoclines of -31°C (Barkworth *et al.*, 2000; USDA, 2002; Environment Canada, 2007; AKEPIC, 2011). In *B. rubens*, by contrast, the northern range limit of 46°N is approximated by the minimum autumn temperature isocline of -10°C which is roughly equivalent to the tolerance limit of young plants (Barkworth *et al.*, 2000; USDA, 2002; Environment Canada, 2007). If differences in acclimation capacity are the predominant factor affecting the respective range distributions, then tolerance thresholds for each species provide critical information for building physiological-based models of range distribution and range shifts following climate change. Northward expansion of both of these species appears probable with continued climate warming. With climate change, range expansion of *B. tectorum* and *B. rubens* should track projected moderation of minimum winter and autumn temperatures (Environment Canada, 2007). If this happens, *B. tectorum* could impact boreal and tundra ecosystems in western North America. Predicted increases in fire frequency in the boreal zone (Stocks *et al.*, 1998) should disrupt the life cycles of native species and promote aggressive establishment of fire adapted grasses such as *B. tectorum*, with similar consequences as currently observed in the Great Basin region. With the moderation of late-autumn freezing events, *Bromus rubens* should be able to spread into regions now dominated by *B. tectorum*, potentially replacing one noxious invader with another (Bradley *et al.* 2009).

Chapter Three - Heat sterility of reproduction in a changing global climate: Implications for the pernicious invaders *Bromus tectorum* and *Bromus rubens* in North America

(This chapter was contributed by Tammy L. Sage and Rowan F. Sage and who conducted the field survey and Jeffrey Harsant who performed the cell counting for Table 3.4 and GIS mapping)

Abstract

The importance of heat induced sterility in biodiversity and species distribution could increase following climate warming. To evaluate this possibility, we examined the effect of moderate (32°/22°C) and elevated growth temperatures (36°/24°C) on reproductive yield in two severe grass invaders *Bromus tectorum* and *Bromus rubens*. Field observations demonstrated that *B. tectorum* and *B. rubens* tend to segregate by altitude, with *B. rubens* forming dense stands at lower elevations (750-1500m) and *B. tectorum* dominating at higher elevations (1500-2500m). Exposure to 32°C during reproductive development under controlled environmental conditions resulted in up to a 35% decline in *Bromus tectorum* seed yield and 8-14% decline in *Bromus rubens* seed yield. At 36°C, by contrast, seed production was absent in both species. Seed yield reductions in both *Bromus* species at

32°C were associated with the reductions in spikelet numbers, whereas at 36°C the elimination of seed production was associated with near total pollen abortion. In the field, both species flower when inflorescence temperature approach values where reproductive failure can become a problem. Reproductive failure could be a contributing factor to the reduced abundance of *Bromus tectorum* and *Bromus rubens* at lower elevations in the southern regions of the respective species range. With climate warming, increased incidents of reproductive failure will likely contribute to a range shift in both species away from the warm distribution limit.

Introduction

Reproduction is one of the most sensitive phases of a plant's life cycle to elevated temperature (Sharkey & Shrader, 2006). Above 30°C, reproductive output declines in numerous plants of economic importance (Wardlaw *et al.*, 1989; Jagadish *et al.*, 2007, 2010; Endo *et al.*, 2009; Mason *et al.*, 2010). In wheat, maize and barley, temperatures above 30°C decrease seed production by reducing the number of tillers and spikelets per plant and the number of florets per spike (Frank *et al.*, 1992; Ramadoss *et al.*, 2004; Alam *et al.*, 2007; Mitra & Bhatia, 2008; Modarresi *et al.*, 2010; Foulkes *et al.*, 2010). In addition, exposure to 34°C to 36°C during anthesis decreases pollen viability in numerous agronomic species, such as wheat, rice and sorghum, thereby leading to floret sterility (Saini & Aspinall, 1982; Sakata *et al.*, 2000; Vara Prasad *et al.*, 2006; Jagadish *et al.*, 2010). In contrast to agronomic species, the occurrence of heat-induced sterility in wild species is poorly studied, with *Arabidopsis*, which becomes sterile at temperatures above 31°C, being

the principle exception (e.g. Kim *et al.*, 2001, Sakata *et al.*, 2010). For species where fecundity loss occurs from heat events, it is possible that the distribution limit at the warmer end of the species range is influenced by heat-induced sterility. In such cases, the expected increase in heat events following climate change could reduce population size and constrict species distribution. This could have major consequences for species diversity, biogeochemical cycling and ecosystem function.

An important aspect of heat effects on reproduction is the variation in the temperature threshold for heat-induced sterility. In crops, heat sterility occurs over a relatively narrow range in most genotypes. For instance, rice grain yield is significantly reduced within the critical range of 32°-36°C (Ziska & Manalo, 1996; Peng *et al.* 2004). Crops are highly bred and hence may have narrower ranges of heat sterility than natural populations. Because enzyme systems, phenological development, and membrane structure show adaptation to different thermal regimes (Hochachka & Somero, 2002; Hall, 2004), it is logical to hypothesize that thresholds for heat sterility should also vary between populations from different climate zones, and thus could potentially allow for adaptive evolution as climates change. Such variation, however, has not been examined in wild populations, and hence remains unknown. One approach to addressing this issue is to examine heat effects on reproductive processes of closely related species that are similar in terms of life form and ecological requirements, but differ in their thermal habitat. In western North America, two invasive *Bromus* grasses are largely separated by local climate. One species, *Bromus tectorum* (cheatgrass), forms dense swards in the “cold desert” region of the Great Basin, the Rockies Mountains and the Columbia-Snake river plateau (Pavlick & Anderton, 2007). The other species, *Bromus rubens*, forms concentrated stands in the hot deserts of the

American southwest, particularly in the Mojave and Sonoran Desert regions (Salo, 2004, 2005). These regions where *B. rubens* and *B. tectorum* form dense, dominating grasslands have been termed the invasive range of the species, to delineate the areas where the species are pernicious, weedy invaders from the larger region where the species are present but not considered to be severe weeds (Bradley, 2009). In regions where *B. tectorum* and *B. rubens* co-occur, they tend to segregate by altitude, with *B. rubens* forming populations' stands at lower elevations and *B. tectorum* dominating at higher elevations (Beatley, 1966). These differences in distribution indicate there may be inherent differences in thermal tolerance, possibly associated with reproduction. Moreover, because they are invasive species with high dispersal potential, they have escaped biotic controls and hence their distribution limits more likely reflect a physiological tolerance limit (Blaney & Kotanen, 2001). By studying the thermal sensitivity of reproduction in these congeneric invasive species, it is possible to evaluate the variation in heat sensitivity of reproduction and whether it can influence their geographic range limits.

Bromus tectorum and *Bromus rubens*, are among the most destructive invaders in North America (Mack, 1981). High phenotypic and germination plasticity, and an ability to rapidly colonize disturbed sites have enabled these natives of southern Europe and North Africa to occupy more than 400,000 km² in the Intermountain West (Mack, 1981; Novak *et al.*, 1991, 1993; Smith *et al.*, 1997). Both species are winter annuals, typically germinating in mid-autumn with the onset of winter rains. Following winter dormancy, *B. tectorum* and *B. rubens* begin active growth as soon as soils thaw and air temperature rises above 0°C. *Bromus rubens* begins flowering in March at the southern end of its geographic range, while the southern-most populations of *B. tectorum* initiate flowering in April (Hulbert, 1955,

Munz & Keck, 1968; Cronquist *et al.*, 1977; Powell, 1994; Baldwin *et al.*, 2002). Once seeds are formed, *B. tectorum* and *B. rubens* senesce, leaving an abundance of dry biomass on the landscape that promotes summer wildfire (Smith *et al.*, 1997; Bradley & Mustard, 2006). As a result, fire return intervals in the region have been reduced from decades to a few years. Both *B. tectorum* and *B. rubens* rapidly exploit soil resources in the subsequent cool season following a fire, typically forming dense stands that suppress native species, and by doing so reduce biodiversity and soil carbon stocks, and alter biogeochemical cycling (Evans *et al.*, 2001; Brearley *et al.*, 2003). Because of their severe impact on ecosystems, understanding how climate will affect the future success of these species is important for predicting global change impacts in western North America, and potentially in Europe and central Asia, where these species may become aggressive weeds outside of their current range

The main objective of the present investigation is to examine the effect of moderate (32° day/22°C night) and elevated (36° day/24°C night) thermal regimes on seed production in *B. tectorum* and *B. rubens*. These temperature regimes were chosen to approximate the maximum temperatures experienced by *B. tectorum* (32°/22°C) and *B. rubens* (36°/24°C) at the southern end of their invasive range during the months of March and April when anthesis begins (Hulbert, 1955, Munz & Keck, 1968; Cronquist *et al.*, 1977; Powell, 1994; Baldwin *et al.*, 2002; Weather Underground weather service, 2011). Three specific questions were addressed: (1) Do high temperatures reduce reproductive output in *B. tectorum* and *B. rubens*? (2) If high temperatures result in less seed, which morphological yield components (tillers, spikes, spikelets, florets) are affected? (3) Does high temperature

reduce viable pollen numbers? The implication of these results for the future distribution of these species is subsequently discussed.

Materials and methods

Field observations

Abundance, flowering behavior and floret temperatures of *B. tectorum* and *B. rubens* in the Mojave Desert region were evaluated between April 15 and 20, 2011. Abundance was measured along a series of elevation transects in the northern Mojave Desert region of Nevada, California and Arizona, USA. Transects were sited along highways that spanned an elevation change of over 500 m. Assessments of plant abundance were recorded in the highway margins where disturbance was common, and over 30 m away from the highway where recent disturbance was minimal. To allow for many assessments in the limited time available, abundance was classified according to the following numerical classes: 0 – absent; 1 – rare, <1 plant per m²; 2 – scattered, 1 to 10 plants per 100 m²; 3 – common, 1 to 10 plants per 10 m²; 4 – abundant, 1 to 10 plants per m²; 5 – dense, >10 plants per m². Numbers at each elevation site were estimated along a transect of approximately 100 m². Due to the clumped nature of the distributions at many sites, the numbers are considered as rough approximations. Roads used for the elevation transects were: 1) Nevada – the entire length of routes 156, 157, 164, 165; 2) California – I-15 (Nevada line to Victorville), US 14 (US 395 junction to Mojave), US 395 (I-15 to Ridgecrest), state route 178 (Trona Pinnacles to Walker pass); 3) Arizona – I-40 (Ash Fork to Kingman), US 93 (Kingman to Hoover Dam), state route 89 (Wickenburg to Ash Fork).

At 2/3 of the elevation stops in the transects used to measure abundance, flowering phenology was estimated for 10 to 12 plants selected at random from the local population. Developmental stages of the florets were classified as follows: 1 – no flowers, vegetative; 2 – green anthers; 3 – yellow anthers, pre-dehiscent; 4 – yellow anthers, dehiscent; 5 – green, elongating seed, less than 75% of final size; 6 – elongated seed, soft, green in color; 7 – mature hard seed; 8 – mature seed abscised or abscising. Floret temperatures were measured in plants growing along a drainage at 1600 m elevation in Kyle canyon, Nevada about 30 m south of highway 157 and 20 km west of US highway 95 (about 30 km northwest of Las Vegas, Nevada). Floret temperature was measured with 36 gauge thermocouples slipped inside the palea of green florets (development stage 2 to 3) of three plants of each species growing beside each other. Thermocouple outputs were recorded with a Veriteq datalogger (model VL-1700, Veriteq, Richmond British Columbia) in a weatherproof housing.

Experimental manipulations

Seeds were collected from four populations of *B. tectorum* and four populations of *B. rubens* in two Western States: Nevada and Arizona, USA (Table 3.1). Two geographically distinct populations (T1 and R1) were collected in 2006 and 2008. Subsequently, populations from similar geographic regions but different elevations (T2-T4; R2-R4) were collected in 2010. Within these eight elevationally distinct populations, two were from sympatric zones (T3, T4 in *B. tectorum* and R3 and R4 in *B. rubens*; Table 1) and at least one from each species' invasive zone (T1 in *B. tectorum* and R1 and R2 in *B. rubens*; Table

3.1). Seeds were germinated in 5 liter pots in a greenhouse set to approximately 26°C day, 20°C night temperature. Plants were grown in the greenhouse for several months and then transferred to growth chambers at the onset of their reproductive phase. All reproductive parts developed in the growth chambers, under the controlled environmental conditions. The seeds from each tiller were counted and summed to give seed yield.

To evaluate the effect of high temperatures on the development of yield components and male reproduction, three plants of each species from the invasive zone were grown in plant growth chambers (model GC-20; Enconair Ecological Chambers, Winnipeg, Manitoba, Canada) at 22°/14°C, 32°/22°C, and 36°/24°C day/night temperatures. Each chamber contained 6 plants in total. Light was provided for 14 hours daily; with light intensity of 250 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at canopy height during the first and the last hour of the light period, and 550 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during the remaining 12 hours. The experiment was replicated twice for the quantification of the impact of temperature on male reproduction, and three times for the quantification of the impact of temperature on the morphological yield components, cell division and length in tiller pedicels. During each replicate a different chamber was used per treatment. The tiller temperature was monitored once a week with an infrared thermometer and was within 2°C of the chamber set-point temperature.

The impact of high temperatures on the number of tillers, spikes, spikelets, florets and seed was determined after grain maturation. Tiller node number, spike and tiller length, the number of spikelets per tiller and the number of florets per spikelet were measured on three tillers per plant. To determine cell length and number, one 3-cm segment from the middle of each uppermost internode (peduncle) was sampled (1 segment per tillers, 3 tillers

per plant). The segments were stored in 95% ethanol, mounted on the microscopic slide and photographed with a light microscope (Zeiss Axioplan, Oberkochen, Germany), then assessed using Image J software (National Institutes of Health, Bethesda, MD, USA) (30-75 cells per segment). Cell number was calculated by dividing the length of uppermost internode by cell length.

The impact of high temperature on pollen development was determined by sampling 27 anthers from each plant (3 anthers per floret, 3 florets per tiller, and 3 tillers per plant) just prior to dehiscence and staining with Alexander's triple stain (Alexander, 1969). Alexander's triple stain colors the pollen wall blue-green and the cytoplasm purple-red. Aborted pollen grains lack cytoplasm and therefore show little, if any, red staining. The total number of pollen grains/locule and number of aborted and nonaborted grains/locule were quantified. Anther area and length was assessed using Image J software (National Institutes of Health, Bethesda, MD, USA) to address whether or not potential declines in total pollen grain number at higher temperatures were related to declines in anther size and cell division.

Statistical analysis

Data were analyzed using SIGMASTAT (v.3.0.1. SPSS). Treatment and species effects on seed yield, number of seeds per tiller, tiller length, spike area, cell number, cell length, number of nodes, tillers, anther area, anther length and % of pollen abortion were assessed with two-way ANOVA followed by Holm-Sidak post-hoc tests. The proportion of non-viable pollen grains was first transformed using the arcsine method and then analyzed with a

two-way ANOVA. An additional two-way ANOVA test was used to examine the effect of population and growth temperature on the seed yield. A Chi-square test was used to analyze the effect of species and treatment on the number of pollen grains produced by both species.

Results

Field observations

Abundance along a series of elevation transects

The two *Bromus* species showed distinct altitude distributions with *Bromus rubens* occurring predominately at low and intermediate elevations, while *B. tectorum* was more common at high-elevations (Fig. 3.1). In the Mojave Desert regions, we mainly observed *B. tectorum* in disturbed sites along highways, in overgrazed fields or near overturned earth. It was much less abundant away from disturbed areas. In disturbed sites, *B. tectorum* was rare or scattered below 1400m, but abundant at 1400 to 1600m and scattered between 1600 and 2300m. In the undisturbed sites *B. tectorum* was mainly present between 1400 to 1700m and was rare to absent below 1400m. In contrast to *B. tectorum*, *B. rubens* formed dense stands across the landscape between 1000m and 1400m in both undisturbed and disturbed locations (Fig. 3.1). It was abundant between 600 and 1000m elevations, but became scattered to rare below 500m. At the lowest elevations where it occurred, *B. rubens* was found exclusively on north-facing slopes or at the base of north-facing bluffs, where solar exposure was reduced during the October to April growing season. Above 1500m, *B. rubens* abundance rapidly decreased and it was not observed above 1800m.

Inflorescence temperature and stage of floret development

Inflorescence temperatures were recorded on *B. tectorum* and *B. rubens* plants growing 0.5m apart in identical microsites at 1600m elevation. Both species had indehiscent anthers on April 13, 2001 when the temperature loggers were set up, and dehiscent anthers one week later. The inflorescence temperatures of *B. tectorum* and *B. rubens* fluctuated between 0° and 18°C during the night and went up to near 35° to 40°C at noon (Fig. 3.2). The maximum inflorescence temperature recorded in *B. tectorum* was around 35°C and in *B. rubens* it approached 40°C. Air temperature at the closest weather station was between 21° and 34°C (Weather Underground weather service, 2011). With an elevation correction, we estimate air temperature varied between 16° and 29°C at the thermocouple site during midday of April 14-19 (Fig. 3.2).

At the time of our field observations (mid-April), we found no difference in the reproductive development class of *B. tectorum* and *B. rubens* (Fig. 3.3). Both species had the similar phenology of flowering and seed set along an elevational gradient.

Experimental manipulations

High temperature effects on seed production in four populations each of B. tectorum and B. rubens

The seed yield of plants grown at 22°/14°C averaged 620 to 730 seeds/plant in *B. tectorum* populations and 814 to 1020 seeds/plant in *B. rubens* populations (Table 3.2). The number

of seeds per tiller at 22°/14°C averaged 81 to 92 in populations of *B. tectorum* and 95 to 130 in *B. rubens* (Table 3.2). The number of seeds per tiller was 20% to 40% higher in *B. rubens* than in *B. tectorum*. The exposure to moderate heat stress (32°/22°C) reduced seed yield by 8 to 14% in all populations of *B. rubens* and 28 to 35% in the populations of *B. tectorum* ($P < 0.001$). No population of either species produced seed when grown at 36°/24°C.

High temperature and the development of tillers, spikes and florets

To examine in detail the impact of temperature on morphological and anatomical parameters affecting yield, we focused on two populations from the invasive zones of each species (population T1 from Reno, Nevada for *B. tectorum* and population R1 from the National Desert Wildlife refuge, Nevada for *B. rubens*). This decision was necessitated by seed availability. Since seed yield parameters did not differ between populations (Table 3.2), we concluded that comparison of T1 and R1 should not differ from other populations.

The tillers of *B. tectorum* and *B. rubens* are similar to other grasses in morphology (Bell, 2008). A tiller consists of one main stem divided into internodes, separated by nodes, and a branched spike that develops spikelets on which florets arise. In all temperature treatments, the average number of reproductive tillers was approximately 8 in both species and each tiller had 4 nodes (Table 3.3). Although temperature had no impact on the number of tillers/plant, a significant reduction in spikelets/tiller was observed in both species when grown at higher temperatures (Table 3.3). The number of spikelets/tiller in *B. tectorum* was 23% to 27% lower when grown under 32°/22°C and 36°/24°C, respectively, and 5% to 12%

lower in *B. rubens* (Table 3.3). Temperature had no impact on floret production within a spikelet (Table 3). *Bromus rubens* spike length was significantly shorter than in *B. tectorum* when grown at 22°/14°C. Reductions in spike length at 32°/22°C and 36°/24°C were 57% to 59% in *B. tectorum* and 11 and 31% in *B. rubens*. Exposure to 32°/22°C and 36°/24°C reduced total tiller length by 53% in *B. tectorum* and 32% to 37% in *B. rubens* (Table 3.3). Higher temperatures resulted in reductions in cell length in tillers for each species, and a decline in tiller cell number in *B. tectorum* (Table 3.4). By contrast, tiller cell number increased in *B. rubens* following growth at higher temperatures (Table 3.4).

High temperature and the development of anthers and pollen

Anther locules from *B. rubens* plants grown at 22°/14°C contained significantly more pollen than locules of *B. tectorum* ($P < 0.001$; Table 5); there was no aborted pollen in the locules of either species (Fig. 3.4). Growth at 32°/22°C moderately reduced pollen grains/locule in *B. tectorum* by 20% but had no effect on *B. rubens* pollen production (Table 3.5). Pollen abortion at 32°/22°C was 26% to 30% in both species (Fig. 3.4). Anthers produced at 36°/24°C were effectively sterile. At 36°/24°C anthers had 35% to 40% fewer pollen grains in both species; however, the pollen present was largely non-viable as the mean percentage of pollen abortion was 90 to 98% (Fig. 3.4). In addition, in approximately 15% of all sampled locules from both species meiosis did not occur at 36°/24°C. When plants were grown at 32°/22°C, anther area declined by 16% in both species. At 36°/24°C growth temperatures, anther area was reduced by 35% in *B. tectorum* compared to 22°/14°C, while no further reductions were observed in *B. rubens* anther area (Table 3.5). Exposure to

32°/22°C significantly decreased the anther length by 18% and 7% in *B. tectorum* and *B. rubens*, respectively, relative to 22°/14°C grown plants ($P < 0.001$). While in *B. tectorum* the anther length did not change under 36°/24°C conditions, anthers from *B. rubens* that developed at 36°/24°C exhibited an 11% decrease in length (Table 3.5). Overall, the decline in the anther size in *B. tectorum* in warmer treatments was mainly due to the reductions in the anther width, while in *B. rubens* it was both length and width.

Table 3.1 - Collection information for *Bromus tectorum* and *Bromus rubens* seed populations used in this study. Seeds were collected July-August 2007, 2009 and 2010.

Species	Population	Location	Elevation (m)	Site description
<i>Bromus tectorum</i>	T1	Hidden Valley Drive, Reno, Nevada (39°29' N, 119°42' W)	1370	Invasive zone
	T2	Mt. Charleston town, Highway 157, Nevada (36°15' N, 115°37' W)	2300	High elevation site
	T3	Highway 157, Kyle Canyon, Nevada (36°25' N, 115°24' W)	1670	Sympatric zone
	T4	Picnic area, Santa Catalina Mountains, Arizona (32°27' N, 110°42' W)	1800	Sympatric zone
<i>Bromus rubens</i>	R1	National Desert Wildlife Refuge, Nevada (36°20' N, 115°08' W)	905	Invasive zone
	R2	Wikieup, Arizona (113°36' W, 34° 42' N)	580	Invasive zone
	R3	Highway 157, Kyle Canyon, Nevada (36°25' N, 115°24' W)	1670	Sympatric zone
	R4	Picnic area, Santa Catalina Mountains, Arizona (32°27' N, 110°42' W)	1800	Sympatric zone

Table 3.2. - The effect of growth temperatures during development on seed yield of *B. tectorum* and *B. rubens*

Species	Population	Seeds per tiller			Seeds per plant			
		22°/14°C	32°/22°C	36°/24°C	22°/14°C	32°/22°C	% reduction	36°/24°C
<i>Bromus tectorum</i>	T1	83 ± 27	60 ± 19*	no seeds	620 ± 100	450 ± 52*	28	no seeds
	T2	78 ± 22	52 ± 12*	no seeds	664 ± 110	425 ± 68*	35	no seeds
	T3	92 ± 23	63 ± 14*	no seeds	733 ± 104	534 ± 55*	29	no seeds
	T4	81 ± 14	53 ± 12*	no seeds	646 ± 69	446 ± 61*	31	no seeds
<i>Bromus rubens</i>	R1	117 ± 46	100 ± 31*	no seeds	869 ± 82	790 ± 66*	10	no seeds
	R2	125 ± 47	112 ± 34*	no seeds	923 ± 82	852 ± 60*	11	no seeds
	R3	95 ± 26	83 ± 26*	no seeds	814 ± 103	704 ± 77*	14	no seeds
	R4	130 ± 46	115 ± 35*	no seeds	1020 ± 94	931 ± 83*	8	no seeds

Means ± SD; asterisks indicate significant difference from the control temperature treatment (22°/14°C) within each species, *P<0.05 (two-way Anova, Holm-Sidak); T1, T2, T3, T4, R1, R2, R3 and R4 indicate the population numbers given in Table 1; N=9 plants.

Table 3.3 - The effect of growth temperatures on morphological yield components in *Bromus tectorum* (T1) and *Bromus rubens* (R1).

Species	Growth treatment	Tillers/plant	Tiller length (mm)	Spike length (mm)	Nodes/ Tiller	Spikelet/ tiller	Floret/ spikelet	Seeds/ spikelet
<i>Bromus tectorum</i> (T1)	22°/14°C	8 ± 1	551 ± 52a	142 ± 23a	4	60±3a	5-7	1-2
	32°/22°C	8 ± 1	256 ± 24b ^{***}	61 ± 5c ^{***}	4	44±5b ^{***}	5-7	1-2
	36°/24°C	8 ± 2	257 ± 19b ^{***}	58 ± 4d ^{***}	4	46±10b ^{***}	5-7	no seeds
<i>Bromus rubens</i> (R1)	22°/14°C	8 ± 1	550 ± 54a	65 ± 8b	4	43±8b	5-7	2-3
	32°/22°C	8 ± 3	375 ± 40c ^{***}	58 ± 7d ^{***}	4	38±9c ^{***}	5-7	2-3
	36°/24°C	9 ± 3	348 ± 21e ^{***}	45 ± 9f ^{***}	4	41±6c ^{***}	5-7	no seeds

Means ± SD; asterisks indicate significant difference from the control temperature treatment (22°/14°C) within each species, ^{***}P<0.001; different letters indicate significant differences at P<0.05 level for each trait independently (two-way Anova, Holm-Sidak); T1 and R1 indicate the population numbers given in Table 1; N=9 plants.

Table 3.4 - Effect of growth temperature on the length of the uppermost tiller internode, its cell number and individual cell length in *Bromus tectorum* (T1) and *Bromus rubens* (R1).

Species	Treatment	Uppermost internode length (mm)	Cell length (mm)	Cell number
<i>Bromus tectorum</i> (T1)	22°/14°C	190 ± 24a	0.23 ± 0.03a	826 ± 162a
	32°/22°C	78 ± 5b ^{***}	0.21 ± 0.04a	363 ± 65b ^{***}
	36°/24°C	68 ± 9b ^{***}	0.24 ± 0.05a	291 ± 77b ^{***}
<i>Bromus rubens</i> (R1)	22°/14°C	183 ± 14a	0.29 ± 0.04b	628 ± 75c
	32°/22°C	149 ± 11c ^{***}	0.20 ± 0.04a ^{***}	760 ± 197d
	36°/24°C	158 ± 7c ^{***}	0.17 ± 0.05c ^{***}	840 ± 118a [*]

Means ± SD, asterisks indicate significant difference from the control temperature treatment (22°/14°C) within each species, ^{***} P<0.001, ^{*} P<0.05 (two-way Anova, Holm-Sidak); different letters indicate significant differences at P<0.05 level, for each trait independently (two-way Anova, Holm-Sidak); T1 and R1 indicate the population numbers given in Table 1; N=9 plants.

Table 3.5 - The effect of growth temperatures on anther area, anther length and the number of pollen grains in the locules in *B. tectorum* (T1) and *B. rubens* (R1).

Species	Treatment	Anther length (mm)	Anther area (mm ²)	Total pollen number/locule
<i>Bromus tectorum</i> (T1)	22°/14°C	0.75 ± 0.11	0.26 ± 0.05	73 ± 16
	32°/22°C	0.62 ± 0.13 ^{***}	0.22 ± 0.09 ^{***}	60 ± 16 ^{***}
	36°/24°C	0.75 ± 0.09	0.16 ± 0.32 ^{***}	44 ± 13 ^{***}
<i>Bromus rubens</i> (R1)	22°/14°C	0.88 ± 0.13	0.31 ± 0.08	88 ± 18
	32°/22°C	0.82 ± 0.11d ^{***}	0.26 ± 0.07 ^{***}	87 ± 18
	36°/24°C	0.78 ± 0.09 ^{***}	0.28 ± 0.07 ^{***}	56 ± 13 ^{***}

Means ± SD, asterisks indicate significant difference from the control temperature treatment (22°/14°C), within each species ^{***}P<0.001 (two-way Anova, Holm-Sidak for anther area and length, and Chi-square for the number of pollen grains); T1 and R1 indicate the population numbers as indicated in Table 1; N=6 plants; N=162 anthers.

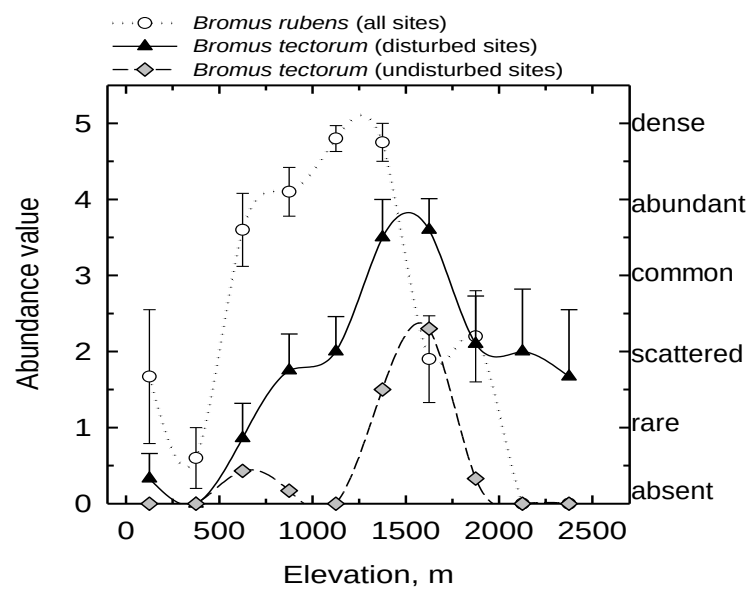


Figure 3.1

Figure 3.1 - The abundance of *Bromus tectorum* and *Bromus rubens* along elevation gradients in the northern Mojave Desert region of Nevada, California and Arizona during April 15 to 20, 2011. Abundance codes are 1) rare – 1 plant per 1000 m²; 2) scattered 1-10 plants per 100m²; 3) common, 1-10 plants per 10 m²; 4) abundant, 1-10 plants per m²; 5) dense, > 10 plants per m². The elevation value shown is the midpoint of a 250m elevation class. For *Bromus tectorum*, values are shown for plants within 30m of the highway or in an obvious disturbance site, and for plants in undisturbed sites > 30m from a highway. Each data point is the mean $\pm$ SE of 2 to 13 locations per elevation class (usually 4 to 12 locations).

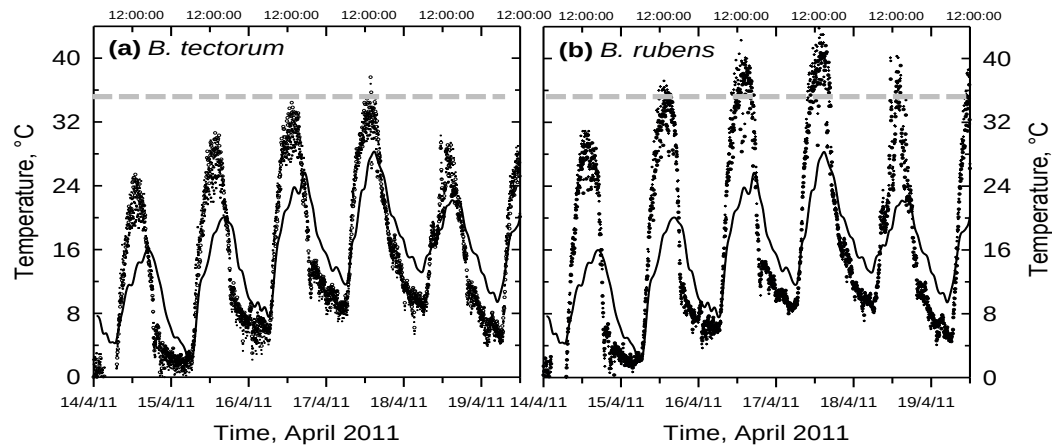


Figure 3.2 - Air temperature (solid line) and floret temperature (scatter plot) of **(a)** *Bromus tectorum* and **(b)** *Bromus rubens*. Floret temperatures were measured with 36 gauge thermocouples slipped inside the palea of green florets (development stage 2 to 3) of three plants growing beside each other. Air temperatures were extracted from the Weather Underground weather service (<http://www.wunderground.com/>) for the Las Vegas region and then corrected for the elevation. Measurements were logged between April 14 and 20, 2011. Grey dashed line represents the temperature of 35°C.

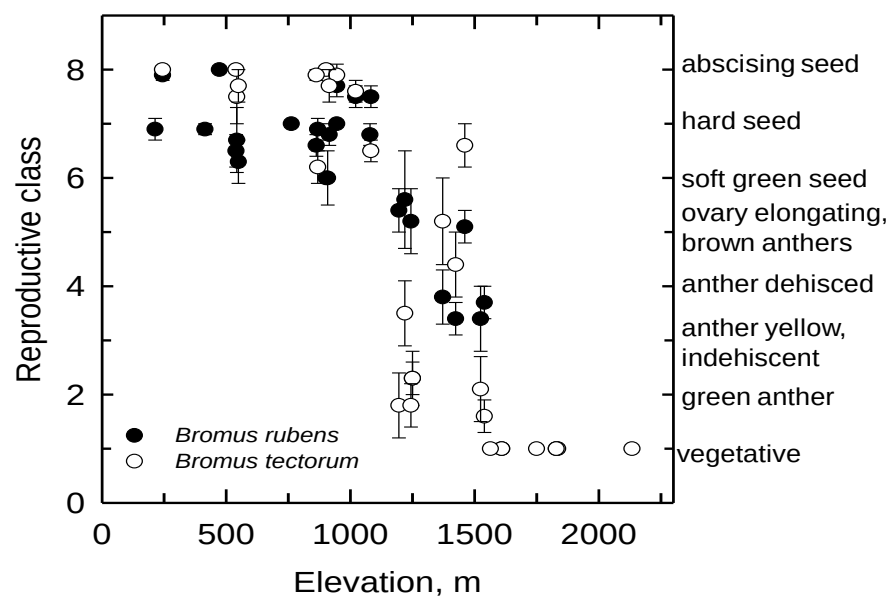


Figure 3.3

Figure 3.3 - The reproductive development class of *Bromus tectorum* and *Bromus rubens* along elevation gradients in the northern Mojave Desert region during April 15 to 20, 2011. Means $\pm$ SE (N=6 to 12). Reproductive classes are: 1 – no flowers, vegetative; 2- green anthers; 3 – yellow anthers, pre-dehiscent; 4 – yellow anthers, dehiscent; 5 – green, elongating seed, typically less than 75% of final size; 6 – elongated seed, soft, green in color; 7 – mature hard seed; 8 – mature seed abscised or abscising. Elevation values for each symbol present are the midpoint of 250 m elevation classes.

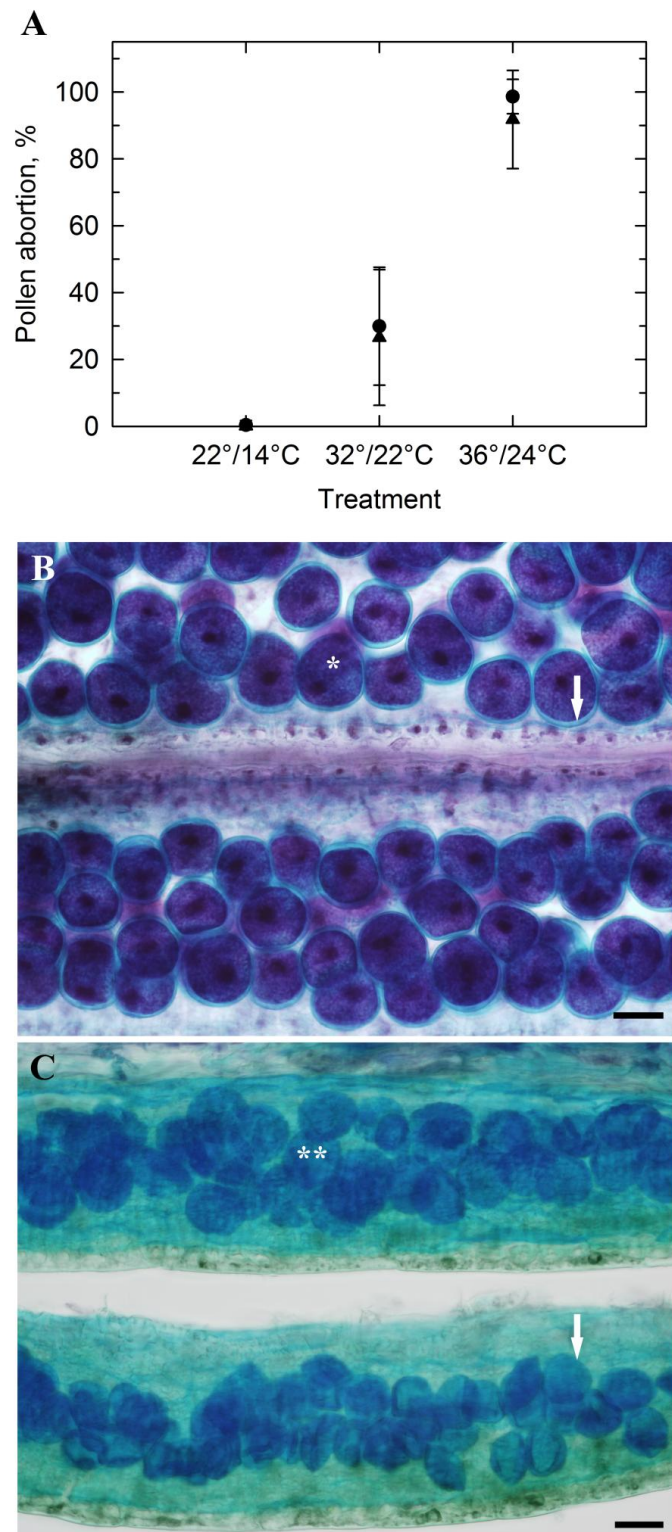


Figure 3.4

Figure 3.4 - The effect of growth temperature on the percent of pollen abortion in *B. tectorum* (closed circles) and *B. rubens* (closed triangles). **(a)** Percent pollen abortion. Temperature had a significant effect on pollen abortion in both species ($P < 0.001$, two-way ANOVA). Means $\pm$ SE; temperature on X-axis refers to day/night growth temperature. **(b)** An anther of *B. rubens* stained with Alexander's triple stain. Pollen wall stains blue-green (arrow). Cytoplasm of nonaborted pollen grain stains red (single asterisk). **(c)** An anther of *B. rubens* stained with Alexander's triple stain. Pollen wall stains blue-green (arrow). Cytoplasm of aborted pollen grain is absent, therefore no red stain is present (double asterisks). Bars = 20 microns.

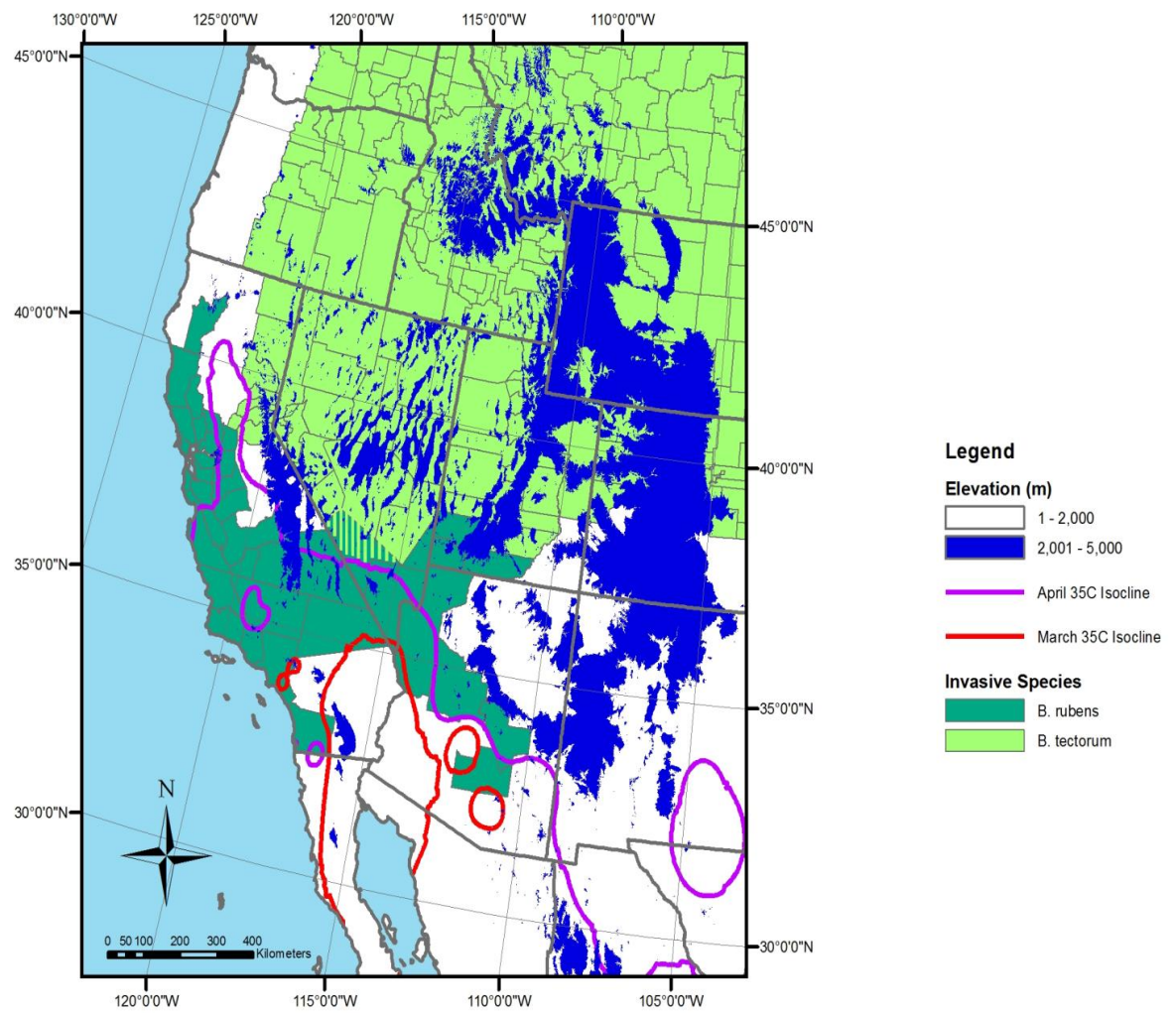


Figure 3.5

Figure 3.5 - The current ranges where *Bromus tectorum* (light green) and *Bromus rubens* (blue-green) are invasive in relation to the isoclines of the 35°C maximum temperature during March (red) and April (purple) in the western US. The temperature data were obtained from the Western Regional Climate Center, <http://www.wrcc.dri.edu/>. Information on *B. tectorum* and *B. rubens* invasion was obtained from Mack (1981) and Salo (2004, 2005), respectively, as well as authors' personal observations. 35°C temperature isoclines were generated in ArcGIS, using maximum temperature data over the ten-year period, 1998-2008, from 212 cities in the Western US, extracted from the 'Local Climate Data Summaries for Western US' (Western Regional Climate Center, <http://www.wrcc.dri.edu>). This data set shows temperature highs (variable: *Extreme Max Temp*) during the last ten-year period based on unedited daily ASOS data. Elevation map layers used for the map construction were obtained from the National Atlas of the United States Raw Data (<http://www.nationalatlas.gov/atlasftp.html>). Blue color indicates areas of higher elevation (>2,000 m). Slashed light green and blue-green lines represent regions where *Bromus tectorum* and *Bromus rubens* co-occur.

Discussion

We demonstrate that *Bromus tectorum* and *Bromus rubens* share the same threshold of heat sterility, which is around 36°C for both species. Exposure to 36°/24°C during flowering triggered complete reproductive failure in all populations of *B. tectorum* and *B. rubens*, as none produced any seeds, due to near complete pollen abortion. Both species exhibited similar phenological patterns of flowering and seed set along an elevational gradient, indicating that timing of flowering with respect to local light and climate conditions do not differ. By exploiting elevation gradients we were able to clarify the distributions of the two species along a climate gradient. *Bromus rubens* is the predominant species between 500m and 1500m elevation, while *B. tectorum* is more abundant between 1500m and 2500m. Both species can occur below 500m; *B. rubens* is typically in refugia from heat (north-facing slopes or in the shadow-of bluffs), while *B. tectorum* is rare and restricted to disturbed sites. These observations indicate that high temperature plays a factor in the success of the species at the warm-end of their distribution range. The exact nature of the limiting factor is unclear, but there does appear to be a lower heat threshold in *B. tectorum*, as it was less abundant than *B. rubens* below 500m and occurred only at low numbers in opportunistic situations established by disturbance. We hypothesized that *B. tectorum* at the lowest elevation sites arise from recurrent introductions and generally fail to establish self-sustaining and expanding populations.

Reproductive failure could be a contributing factor to the reduced abundance of the two *Bromus* species at low elevations, but does not fully explain their different distribution patterns. At a mid-elevation sympatric site (1600m), both species exhibited floret

temperatures close to the sterility threshold, at least for a few hours of the day. These temperatures were elevated with respect to air temperature due to the high solar load and proximity of the florets near the ground, which on sunny day re-radiates much heat. At the time of our field observations (mid-April), floret temperatures at elevation below 1600m would exceed 36°C during anthesis, given the high solar load and warmer air temperature present at lower elevation. Both species at low elevation (<500m) avoid these hot temperatures during reproduction by initiating flowering earlier in the year or avoiding high temperature by occurring in microsites.

With climate warming, fecundity problems should contribute to an upward shift in the distribution range of the two species, although some *B. rubens* may remain in the low elevation sites if heat refugia sufficiently protect it. Given the observation that the plants flower when floret temperatures approach 36°C during the middle of the day, any climate warming will increase the frequency of heat sterility and reduce the fecundity of the populations. How much exposure to 32° to 36°C is required remains to be determined. Our growth chamber experiments demonstrate that ten to twelve hours above 35°C cause complete sterility in *Bromus* species, but the impact of two to six hours of heat exposure during the daytime is uncertain.

Additional evidence that heat sterility could be an important factor at the warm-end of the species range limit is presented in Figure 5. The current distribution of the invasive zone of *B. rubens* is the Mojave Desert region, between about 800m and 1500m elevation. The invasive zone is the area where an invader is abundant on the landscape, as opposed to being merely present (Bradley *et al.*, 2009). For *Bromus tectorum* the invasive zone occurs to the north of the Mojave Desert, in the Intermountain regions of the Great Basin, and the

uplands of the Colorado and Snake River plateaus (Pavlick & Anderton, 2007). When we plot the maximum high temperature isoclines for March and April onto the distribution maps we observe that the 35°C March and April isoclines correspond to the southern-end of the invasive range of *Bromus* species. This demonstrates that exposure to temperatures exceeding 35°C during the peak of the flowering months is probable south of the current invasive range. Exposure >35°C during flowering would not be sufficient to exclude the species, given the presence of heat refugia and disturbed sites, but it could reduce fecundity to the point where the species have reduced invasive potential. Climate warming should shift the spring 35°C isoclines northward, and thus the probability of heat sterility (IPCC, 2001).

Effect of temperature on pollen sterility, seed yield and morphological yield components

Relative to plants grown at 22°/14°C, growth at 32°/22°C caused a slight (8-14%) reduction in seed yield in all populations of *B. rubens*, while the yield reduction in *B. tectorum* was much larger, being on the order of 28% to 35%. This greater loss of fecundity in *B. tectorum* could reduce its competitive ability relative to *B. rubens*, and may contribute to its general restriction to disturbed sites in the 500m to 1300m elevation range, where *B. rubens* forms dense stands. Moreover, a detailed analysis of the morphological components contributing to yield in one population from each species demonstrates that decreases in seed output under two temperature regimes (32°/22°C and 36°/24°C) are attributable to different developmental processes. The reduction in seed output at 32°/22°C was associated

with reductions in spikelet numbers in both species. The elimination of seed production at 36°/24°C in both species was due to near complete pollen abortion after meiosis, although some microsporocytes failed to undergo meiosis. Increased pollen abortion occurred at 32°C relative to 22°C, but this was not severe enough to reduce seed production directly, as there was still an excess of pollen. At 32°C the number of pollen grains per locule was around 50 and 60 in *B. tectorum* and *B. rubens*, respectively. *Bromus* species are self-pollinating (Hulbert, 1955; Rice & Mack, 1991) and thus do not require high levels of airborne pollen to ensure reproductive success. By contrast, the pollen failure at 36°C leaves the plants with no options and total reproductive failure is the result.

Aside from pollen sterility, high temperatures also had a discernable impact on anther, tiller, and spike growth and development in both *Bromus* species, although each species responded differently. Reduction in anther size above 30°C corresponded to reduced pollen production in both species, although to a greater degree in *B. tectorum*. Smaller anther size at high temperature was partly due to a reduction in cell division given that fewer pollen grains were present in anthers at higher temperatures. Tiller growth was also impacted by temperatures above 30°C. Suppression of cell elongation in *B. rubens* and reduction in cell division in *B. tectorum* resulted in diminished tillers and compressed spikes in both species, respectively. Cell division was significantly reduced in tillers at high temperatures in *B. tectorum*; however, in *B. rubens*, cell division in the tillers was increased. The proliferation in cell division in *B. rubens* enabled this species to exhibit a lower reduction in tiller and spike length at high temperatures than *B. tectorum*, despite having a greater inhibition in tiller cell elongation. When comparing the two *Bromus* species grown at 32°/22°C and 36°/24°C, spikelet production was affected less in *B. rubens* at the higher

temperature, possibly reflecting different thermal optima in cell division in the two species. The greater tolerance of cell division to elevated temperatures in *B. rubens* relative to *B. tectorum* may have permitted the former to maintain greater spikelet meristem productivity than the latter at 32°/22°C. This difference in meristem activity could contribute to a competitive advantage in seed production of *B. rubens* over *B. tectorum* below 1200m elevation.

The complete collapse of reproductive potential in all populations of *B. rubens* and *B. tectorum* at 36°C mirrors similar patterns observed at 34°C to 36°C for rice, wheat, maize and numerous other crop species (Herrero & Johnson, 1980; Saini & Aspinall, 1982; Ziska & Manalo, 1996; Sakata *et al.*, 2000; Vara Prasad *et al.*, 2006; Jagadish *et al.*, 2010). As shown by the *Bromus* populations, rice and wheat, this thermal threshold holds for both species of warm and cool climates, and in accessions from high and low elevations. These observations suggest that 35°C represents a physiological barrier in higher plants that evolution has not been able to directly overcome. Instead, plants may have evolved mechanisms to avoid heat sterility. Flowering at night or early in the year are two possible ways to avoid temperatures causing pollen sterility (Satake & Yoshida, 1978; Nishiyama & Blanco, 1980). Our results also indicate for the first time that heat sterility could be an important contributor to species range distributions in natural ecosystems.

Global climate change and the distribution of *Bromus tectorum* and *Bromus rubens*

The significance of heat induced failure of reproduction for natural populations has been largely ignored, despite the problems noted by agronomists (Peng *et al.*, 2004). Variation in heat sensitivity of pollen production could be expected between species differing in range distributions in the warmer regions of the globe. Species from warmer environments such as the Mojave zone could be expected to have greater tolerance to heat during pollen development than species from cooler climates. In the case of *B. rubens* and *B. tectorum* both species show an absolute thermal limit of 36°C for heat sterility, similar to the thermal limits of a range of crop species (Saini & Aspinall, 1982). No population variation was observed in either *Bromus* species in the pollen sterility threshold. By contrast, there was some variation in sensitivity of floral growth and development at 32°C between the species. These results indicate that 35°C threshold for pollen sterility may be widespread, if not ubiquitous, in the plant kingdom. If so, then evolution may have compensated for the threat of heat sterility by shifting anthesis to nights and mornings, earlier in the season, or by using energy balance modifications to avoid heat build-up on flowers.

With climate warming, many of these strategies may fail, particularly, if warmer nights and winters shrink the safety window for reproduction. Most warming in North America is expected during the winter (IPCC, 2007). Thus, cool-season species such as *Bromus* may be particularly vulnerable to heat-induced reproductive failure. Unlike perennials and annuals with persistent seed banks, *Bromus* species cannot withstand two to three successive years of poor seed set (Holm *et al.*, 1977). Seeds of these species have low survival beyond the second year (Wu & Jain, 1978; Smith *et al.*, 2008), in part due to attack

from fungal pathogens such as *Pyrenophora semeniperd* and *Ustilago bullata* within the seed bank (Beckstead *et al.*, 2007; Meyer *et al.*, 2007; Smith *et al.*, 2008). Because of these characteristics, we conclude that heat events during flowering in back-to-back years, or in a year alternating with a dry year, could be sufficient to collapse the seed bank, and reduce the population size of the *Bromus* species, possibly below invasive thresholds. Based on these results we infer that climate change research should address the heat sterility issue in natural populations, as heat sterility may be a particularly sensitive aspect of the biodiversity response to global warming.

Chapter Four - Thermal acclimation of photosynthesis in two winter annual grass invaders from semi-arid regions of North America

Abstract

Bromus tectorum and *Bromus rubens* are closely-related grass invaders that segregate by latitude and elevation. While *B. tectorum* forms dominant stands in the “cold desert” steppes of western North America, *B. rubens* is a severe invader in the “hot desert” regions of American southwest. We examined the photosynthetic temperature acclimation in these species and evaluated whether photosynthetic responses to temperature explain their different geographic distribution and dominance in the semi-arid regions. We grew *B. tectorum* and *B. rubens* at low (12°/5°C; LT), moderate (22°/14°C; MT), warm (32°/22°C; WT) and high (36°/24°C; HT) temperatures. Both species showed similar temperature response of photosynthesis and patterns of acclimation. The thermal optimum of net CO₂ assimilation (A_{net}) in *B. tectorum* and *B. rubens* grown under (36°/24°C) was 8 degrees higher than under (12°/5°C). In LT and MT treatments, the reduction in the oxygen concentration from 21% to 2% resulted in substantial stimulation of A_{net} down to 2°C in both species. By fitting our experimental data to the photosynthetic models we can explain the limitation on photosynthesis of LT plants of both species as being limited by Rubisco carboxylation capacity from 2°C to 42°C. High-temperature plants, by contrast, appear

RuBP regeneration limited above 30°C and co-limited by Rubisco and RuBP regeneration at the thermal optimum photosynthesis. In the high temperature treatment A_{net} in both species was O_2 insensitive below 15°C, indicating it was limited by Pi regeneration capacity. Acclimation to HT caused an increase in Rubisco carboxylation capacity (V_{cmax}) at supra-optimal temperatures; this contributed to an increase in A_{net} above the thermal optimum in WT and HT plants. Overall, our study shows that both *Bromus* species have good acclimation capacity over broad thermal ranges which might contribute to their success in semi-desert biomes. Yet, their photosynthetic response to temperature cannot explain their current range separation.

Introduction

Photosynthetic capacity frequently depends upon growth temperature and thus varies with seasonal temperature change (Mooney *et al.*, 1978; Berry & Björkman, 1980; Sage & Reid, 1994; Hikosaka *et al.*, 2006). In many plants, a change in growth temperature induces an acclimation response characterized by a shift in the thermal optimum of photosynthesis towards the growth temperature (Berry & Björkman, 1980; Yamasaki *et al.*, 2002; Sage & Kubien, 2007). The extent of acclimation and an ability to optimize photosynthetic performance in response to seasonal changes differs significantly between species. While some species, such as *Saxifraga cernua* are able to shift their thermal optimum by the same number of degrees as the growth temperature (Mawson *et al.*, 1986), others, such as *Eucalyptus pauciflora* and *Eucalyptus nitens* change their optimum temperatures by 1/3 of the growth temperature change (Slatyer, 1977; Battaglia *et al.*, 1996).

Two general patterns of photosynthetic thermal acclimation are recognized—‘quantitative’ (type I) and ‘qualitative’ (type II) acclimators (Atkin & Tjoelker, 2003; Way & Sage, 2008). ‘Quantitative acclimators’ vary the content of all photosynthetic enzymes in roughly equal proportion during thermal acclimation, while the ‘qualitative acclimators’ alter the relative amounts of various photosynthetic enzymes or modify their thermal sensitivity (Way & Sage, 2008). The latter happens when the plant is able to reallocate available resources (e.g. nitrogen) away from non-limiting to the limiting photosynthetic components and reduce the control of a single limiting factor (Sage & Kubien, 2007). Qualitative acclimation allows the plant to rapidly respond to changes in temperature and does not require the generation of new tissue (Atkin & Tjoelker, 2006). Certain species such as winter annuals from arid and semi-arid regions experience wide variation in temperature during their growth period and usually exhibit high phenotypic plasticity (Monson & Szarek, 1979; Toft & Pearsey, 1982). The photosynthetic apparatus of winter annuals first adjust to chilling temperatures during early growth, and then acclimates to higher temperatures as the growing season progresses into late winter and spring (Mooney, 1976; Batanouny, Stichler & Ziegler, 1988, Seeman *et al.*, 1980 a, b).

In this study we evaluated the photosynthetic temperature acclimation in two opportunistic winter annual grasses, *Bromus tectorum* and *Bromus rubens*. These species are among the most aggressive invaders in western North America and are now important species in the semi-arid biomes of the western USA. *Bromus tectorum* and *Bromus rubens* usually germinate in autumn and remain active during the winter (Rogehr & Bazzaz, 1976; Aguirre & Johnson, 1991; Chambers *et al.*, 2007). As soon as temperature rises above zero, they invest all their resources to produce a large leaf canopy, and enter the reproductive

stage by mid-spring (Bradley & Mustard, 2006). Both species experience substantial daytime temperature variation, ranging from below 0°C during mid-winter warm spells to 30°-35°C in the late spring; hence their photosynthetic apparatus should have high potential to acclimate to thermal variation. While both species are winter active, they generally exhibit different geographical ranges. *Bromus rubens* predominantly occurs in the Mojave Desert region of the south-western USA, forming dense stands at 500-1500m elevation (Beatley, 1966;Chapter 3). It sporadically occurs above 1800m elevation and in the cold desert regions between the Rockies and Sierras (Salo, 2005). *Bromus tectorum* is predominant in the cold desert, and above 1500m in the Mojave Desert region. It can occur at lower elevations in the Mojave, but usually in disturbed fields and roadside margins (Chapter 3). The clear segregation along a thermal cline indicates these species may have different thermal optima of photosynthesis. Since both species are closely related, comparing their photosynthetic thermal response should provide a unique opportunity to compare patterns of both thermal acclimation and adaptation in response to different temperature regimes.

To study photosynthetic adaptation and acclimation to varying temperature, it is necessary to evaluate photosynthetic limitations. In general, light-saturated photosynthesis of C₃ species is limited by one of three processes: 1) Rubisco-catalyzed carboxylation of RuBP (Rubisco limitation); 2) the capacity to regenerate RuBP (RuBP regeneration limitation); and 3) the capacity of starch and sucrose synthesis to regenerate Pi for ATP synthesis or triose-phosphate utilization (Pi regeneration limitation) (von Caemmerer & Farquhar, 1981; Sage & Reid, 1994; Leegood & Edwards, 1996; Yamasaki *et al.*, 2002; Sage & Kubien, 2007). Pi regeneration limitation is a subcategory of RuBP-regeneration

limitation, yet is usually describe as a separate limiting process. In C_3 plants, RuBP regeneration and Pi regeneration limitations are often the major limiting processes at sub-optimal temperatures. At elevated temperatures and ambient to elevated CO_2 concentrations, RuBP regeneration or Rubisco activase can limit A (Sage, 2002; Cen & Sage, 2005). Changes in growth temperature can trigger substantial changes in the photosynthetic apparatus of C_3 species; these changes could include the expression of different Rubisco isoforms, increased expression of enzymes of starch and sucrose synthesis, changes in Rubisco content and improved electron transport capacity (Vu *et al.*, 1995; Goulas *et al.*, 2006; Hikosaka *et al.*, 1999; Sage & Kubien, 2007). Recently, Yamori *et al.* (2008) demonstrated that acclimation of spinach grown at 30°/25°C to cool temperatures of 15°/10°C ameliorated a limitation of RuBP-regeneration and allowed Rubisco capacity to limit A above 10°C.

In this study we used leaf gas exchange measurements to examine the photosynthetic performance and thermal acclimation of *Bromus tectorum* and *Bromus rubens* grown under four temperature regimes: low (LT; 12°/5°C, day/night temperature) moderate (MT; 22°/14°C), warm (WT; 32°/22°C) and high (HT; 36°/24°C). The temperature regimes represent much of the temperature range that both species experience in the natural environment. To determine the limiting process for photosynthesis, we first analysed the response of net CO_2 assimilation rate (A_{net}) to temperature and intercellular CO_2 concentration using a modified model of Farquhar *et al.* (1980) (Medlyn *et al.*, 2002). We then compared the measured A_{net} to a 90% reduction in oxygen partial pressure with theoretical predictions assuming Rubisco, RuBP regeneration capacity or Pi regeneration capacity is limiting (Sage & Sharkey, 1987). When C_3 photosynthesis is Rubisco or RuBP-

limited the rate of CO₂ assimilation increases significantly with the reduction in O₂ concentration. By contrast, A changes little and may decline following O₂ reduction if Pi regeneration capacity is limiting (Sharkey, 1985; Sage & Sharkey, 1987).

Materials and methods

Growth conditions

Bromus tectorum seeds were collected along Hidden Valley Drive in Reno, Nevada, USA (39°50' N, 119°78' W). *Bromus rubens* seeds were collected from National Desert Wildlife Refuge, Clark County, NV, USA (36°20' N, 115°08' W). Seeds were germinated in 5 litre pots in a green house at approximately 26°C day, 20°C night temperature. Plants were grown in the green house for 8 weeks before they were transported to the growth chambers (model GC-20; Enconair Ecological Chambers, Winnipeg, Manitoba, Canada) at four temperature regimes: low - 12°/5°C day/night (LT), moderate - 22°/15°C day/night (MT), warm 32°/22°C and high (HT) 36°/24°C. Two growth chambers were used for each treatment; each chamber contained 6 plants in total (3 plants per species). Light was provided for 14 hours daily, with light intensity of 100-200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ at canopy height during the first and the last hour of the light period, and 500-600 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ during the remaining 12 hours. Plants were fertilized weekly with half-strength Hoagland's solution (Hoagland & Arnon, 1950). Leaf temperatures were recorded in the growth chambers with 36 gauge copper-constantan thermocouples attached to a data logger (model SP-1700, Veriteq Instruments, Richmond, BC, Canada). Plants were allowed to

acclimate for four weeks before measurements commenced. During the course of experiment the plants were periodically rotated between the chambers to minimize chamber effects.

Gas exchange analysis

Temperature response curves

The gas exchange measurements were conducted using an open-type gas exchange machine previously described (Kubien & Sage, 2007). Net CO₂ assimilation rate (A_{net}) was measured on 20 to 25-day-old leaves that emerged in the growth chambers on three-month old plants. A_{net} was measured on two to three leaves from one plant at the same time. The temperature response was measured at a saturating light intensity of 1200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, with an exception of measurements taken at 2°C and 5°C, when the light intensity was 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Intercellular CO₂ partial pressure (C_i) during the A vs T curve measurements was maintained at $290 \pm 5 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, which corresponded to an atmospheric CO₂ concentration of 390 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. The VPD during the experiment was 1.4 to 1.7 kPa above 10°C and around 1.0 kPa below 10°C. All temperature response measurements were initiated at 20°C and 21% O₂ for the cool and moderate temperature treatments and at 23°C and 21% O₂ for plants from the warm and high temperature treatment. After the measurements were recorded at 20°C and 23°C, the leaf temperature was decreased in 5°C steps to lower temperatures (with an exception of measurement taken at 2°C when it was decreased by 3°C from 5°C) and then increased in 5°C steps to higher

temperatures (with an exception of measurement taken at 42°C and 48°C when it was increased by 2°C from 40°C and 3°C from 45°C). At each measured temperature, leaves were allowed to equilibrate for at least 20 minutes. A_{net} was first measured at 21% O₂ then the concentration of O₂ was reduced to 2% and A_{net} was re-measured. Once the measurements were taken at 42°C and 48°C, A_{net} was re-measured at 20°C and 23°C. After gas exchange was completed, leaves of both species from both treatments were sampled for leaf nitrogen content, which was assessed with a CN analyser (Costech Elemental Combustion System CHNS-O; Costech, Valencia, CA, USA). Gross CO₂ assimilation rates (A_{gross}) at all measured temperatures were estimated by adding day respiration rates to the respective A_{net} values.

The oxygen sensitivity of A_{net} was calculated as $1 - A_{net21\%}/A_{net2\%} \times 100\%$ (Sage & Sharkey 1987), where $A_{net21\%}$ and $A_{net2\%}$ are net CO₂ assimilation was measured at ambient (21%) and low (2%) O₂ concentrations.

Respiration

Day respiration (R_d) rates were estimated using the Kok method and the light response curves (Wang *et al.*, 2001). We extrapolated the linear part of the light response curve to the intercept with the y-axis and determined the R_d . The light response curves were measured at 5°, 15°, 25°, 38° and 42°C in cool and moderately grown plants and at 10°, 20°, 30° and 40°C and at 21% O₂ in warm and high temperature grown plants. The ambient CO₂ was maintained at 390 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. Net CO₂ assimilation was first measured at saturated light intensity at 21% O₂, then at 100, 80, 60, 40, 30 and 20 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. At each

measured light intensity, leaves were allowed to equilibrate for 20 minutes. To estimate rates every 1°C, measured day respiration rates were fitted to exponential curves.

CO₂ response curves

CO₂ response curves in cool and moderately grown plants were measured at 5°, 15°, 25° and 40°C at 21% O₂. CO₂ response curves in warm- and high-temperature-grown plants were measured at 10°, 20°, 30° and 40°C and at 21% O₂. The measurements were taken at saturated light intensity 1200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, with an exception of measurements taken at 5°C, when the light intensity was lowered to 800 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. All CO₂ response curves were initiated at atmospheric CO₂ of 390 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$, then the CO₂ concentration was decreased to near the CO₂ compensation point and returned in steps to the current atmospheric CO₂ content. After ensuring that the second A_{net} value measured at the atmospheric CO₂ value equalled the initial value, the ambient CO₂ was increased in steps to >1000 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$. Measurements were made at each measured temperature after a 30-min equilibration period. The initial slope of the CO₂ response was calculated from the 4-5 data points below a C_i of 200 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ that fell on its initial linear response of A_{net} vs C_i . The apparent V_{cmax} was calculated from the initial slope following von Caemmerer & Quick (2000) and using the Rubisco kinetics parameters for winter wheat (Cousins *et al.*, 2010). The temperature response of V_{cmax} was modelled using V_{cmax} value measured at 25°C and applying the Arrhenius function for the “model” plant to obtain V_{cmax} at all temperatures (von Caemmerer & Quick, 2000). The apparent J_{max} was calculated from

the gas exchange data using the assimilation rate when C_i was equal to $1000 \mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$ (Medlyn *et al.*, 2002).

Photosynthetic modeling

To determine the major processes limiting light-saturated photosynthesis over the entire temperature range we compared theoretical and measured responses of A_{net} to temperature and intercellular CO_2 concentrations using the Farquhar *et al.* (1980) model as modified by von Caemmerer (2000). A_{net} vs C_i response of Rubisco-limited and RuBP regeneration-limited photosynthesis was modelled using the temperature responses of Γ^* , K_c and K_o of Rubisco (Cen & Sage 2005) and estimated V_{cmax} and J_{max} values from A vs C_i responses (von Caemmerer & Farquhar, 1981). Since K_c and K_o of Rubisco are not known for *Bromus* species, they were assumed to equal that of winter wheat, a cool-climate species in the same grass sub-family as *Bromus* (Pooideae) (Pillay, 1995; Cousins *et al.*, 2010) and calculated using the Arrhenius equation for a “model” plant (von Caemmerer & Quick, 2000). The temperature dependence of Γ^* was taken from Brooks & Farquhar (1985) obtained for spinach, another cold-adapted species. The temperature response of A_{net} was modelled using the temperature responses of Γ^* , K_c and K_o of Rubisco from Cousins *et al.* (2010) and Brooks & Farquhar (1985) as described above. The apparent J_{max} at all temperatures was obtained by fitting the second-order polynomial equation to the estimated values. The apparent V_{cmax} at all temperatures for HT *B. tectorum* and *B. rubens* was obtained by fitting the third-order polynomial regression to the estimated values. To disregard the Rubisco activation adjustments at elevated temperatures in LT plants, the

temperature responses of V_{cmax} in LT plants was corrected using the normalized HT V_{cmax} responses.

The O_2 sensitivities of Rubisco-limited, RuBP regeneration-limited and Pi regeneration-limited photosynthesis were modelled using the temperature responses of I^* , K_c and K_o of Rubisco from Cousins *et al.* (2010) and Brooks & Farquhar (1985) using calculations described before (Sage & Sharkey, 1987).

Statistical analysis

Data were analyzed using SIGMASTAT (v.3.0.1, SPSS). Due to the absence of a growth chamber effect, each plant was treated as a unit of replication. The thermal optimum of the A/T curves was calculated from the second-order polynomial regression fitted to each temperature response curve. The differences in the thermal optimums (T_{opt}) of A_{net} and A_{gross} over the measured temperature range were tested using two-way ANOVA with growth temperature and replicate as the factors. Differences in A_{net} and A_{gross} at each measured temperature between plants from four temperature regimes were assessed using a t-test. The effect of growth conditions and measured temperature on the responses of A_{net} , A_{gross} , R_d , O_2 sensitivity, the apparent J_{max} , V_{cmax} , J_{max}/V_{cmax} were assessed with two-way ANOVAs followed by Holm-Sidak multiple comparison test with leaf temperature, growth temperature and replicate as variables (the chamber and species effect was assessed with two-way ANOVA). To compare V_{cmax} and J_{max}/V_{cmax} at common temperatures we estimated an exponential regression response to the existing data. To compare A_{net} , A_{gross} , A_{gross}/N and

J_{max} , at common temperatures we used a second-order polynomial regression response (Way & Sage, 2008).

Results

Thermal response of photosynthesis

The optimum temperature of A_{net} in both *Bromus* species was around 21°C in LT and MT plants, 23°-24°C in WT plants and 28°-29°C in HT plants ($P<0.05$; Table 4.1). Below 20°C, A_{net} and A_{gross} were up to 100% higher in LT than in HT grown *B. tectorum* and *B. rubens*, 60% higher than in MT *B. tectorum* and *B. rubens*, and up to 15% higher than in MT plants ($P<0.001$; Fig. 4.1a-d). Above 25°C, A_{net} values were up to 30% lower in LT *B. tectorum* than in HT *B. tectorum*. As the growth temperature had no effect on rates of day respiration (R_d) (Fig. 2), the addition of respiration rates to A_{net} had no effect on the temperature dependence of A_{gross} in *B. tectorum* and *B. rubens* (Fig 4.1e,f).

In cold-grown *B. tectorum* and *B. rubens* (LT), the leaf nitrogen content was 15% higher than in plants grown under higher temperatures ($P<0.05$; Table 4.1). When A_{gross} was expressed on the nitrogen basis, differences in A_{gross} between the LT and HT treatments were not ameliorated and the growth treatment effect in both species was still apparent ($P<0.001$). In LT plants of both species A_{gross}/N was 20-100% higher than in HT plants below 20°C and 20-35 % lower above 20°C.

CO₂ response of net photosynthesis

Between 5°C and 20°C, the LT and MT initial slopes of both species were 5% to 30% higher than WT and HT initial slopes ($P < 0.05$; Fig 4.3-4.6). Above 20°C, WT and HT initial slopes of *B. tectorum* and *B. rubens* were 20% to 35% higher than LT and MT initial slopes of both species ($P < 0.001$; Fig 4.3-4.6). Low-temperature-grown and MT *B. tectorum* and *B. rubens* had higher apparent V_{cmax} values than WT and HT *B. rubens* and *B. tectorum* below 20°C, but lower above 20°C ($P < 0.001$). From 5° to 30°C the measured response of V_{cmax} in LT, MT plants were consistent with measured response of WT, HT plants of *B. tectorum* and *B. rubens*. Nonetheless, LT and MT plants had 35% lower apparent V_{cmax} than WT and HT plants at 38°C and 40°C (Fig. 4.7a, b). An expression of V_{cmax} on the nitrogen basis revealed that temperature had effect on the allocation of nitrogen to carboxylation processes (V_{cmax}/N) in *B. tectorum* and *B. rubens* (Table 4.1). Low-temperature-grown plants of *B. rubens* and *B. tectorum* had 15% to 35% higher V_{cmax}/N ratio than MT and HT plants ($P < 0.05$; Table 4.1). The apparent J_{max} was affected by the growth conditions ($P < 0.001$, Fig 4.7c, d). Low-temperature-grown and MT plants had higher apparent J_{max} than WT and HT plants of both *Bromus* species between 5° and 40°C. The ratio of J_{max}/V_{cmax} increased with decreasing growth and measured temperatures ($P < 0.001$) in both species. Low-temperature-grown and MT plants of both species had higher J_{max}/V_{cmax} ratios than WT and HT plants between 15°C and 40°C (Fig 4.7e, f).

Oxygen sensitivity

Due to photorespiration, C_3 photosynthesis is inhibited by 21% O_2 and the reduction in O_2 to 2% should increase A ; however, the presence of a Pi regeneration limitation reduces this stimulation (Sharkey, 1985). Low O_2 sensitivity is an indicator of limiting rate of Pi regeneration limitation and the appearance of O_2 sensitivity after the long-term acclimation to sub-optimal temperatures demonstrates a removal of a Pi regeneration limitation (Sharkey, 1985; Sage & Kubien, 2007). Comparison of measured O_2 sensitivity to the modelled response can also identify whether Rubisco or RuBP regeneration capacity limit A_{net} (Sage & Sharkey, 1987).

In *Bromus tectorum* and *Bromus rubens* the oxygen sensitivity of A_{net} increased with increasing temperature ($P < 0.001$), was affected by the growth temperature below 20°C ($P < 0.05$) and was not different between species ($P = 0.271$; Figure 4.8). In LT and MT *B. tectorum* and *B. rubens*, the measured oxygen sensitivity rose from 15-20% to 40% between 2 and 42°C and never went below 0% in both species indicating the absence of Pi regeneration limitation. At all measured temperatures, the modeled Rubisco-limited O_2 sensitivity were similar to the measured O_2 sensitivity of LT and MT plants (Fig 4.8a, b). The O_2 sensitivity of WT and HT plants was below LT and MT plants at all temperatures; below 15°C, the HT and WT response declined to near 0, similar to modeled values of O_2 sensitivity assuming a Pi regeneration limitation at low temperatures. Above 20°C, the measured O_2 sensitivity of A_{net} was similar to modeled Rubisco-limited response and was also close to RuBP-regeneration limited O_2 sensitivity.

Modeling A vs T curves

In LT *B. tectorum* and *B. rubens*, the measured temperature response of A_{net} coincided with Rubisco limited response below the thermal optimum and was slightly deviated from it at supra-optimum temperatures (Fig 4.9a, c). At the thermal optimum, HT plants of both species appeared to be co-limited by Rubisco and RuBP regeneration capacities. Below and above the thermal optimum the measured A_{net} of HT plants was similar to the modelled RuBP regeneration limited photosynthetic rate (Fig 4.9b, d)

Table 4.1 - Leaf characteristics and gas exchange parameters of *B. tectorum* and *B. rubens* plants grown at 12°/5°C (LT), 22°/14°C (MT), 32°/22°C (WT) and 36°/24°C (HT)

Treatment	Species	Leaf N content (mmol N m ⁻²) for all measurements	Thermal optimum of A_{net} , °C	(V_{cmax}/N) at 25°C
LT	<i>B. tectorum</i>	154±12a	21±1a	33.9±4.6a
MT	<i>B. tectorum</i>	129±13b	21±1a	42.3±3.0b
WT	<i>B. tectorum</i>	127±16b	23±2b	48.3±9.7c
HT	<i>B. tectorum</i>	133±19b	28±1c	45.0±6.7b
LT	<i>B. rubens</i>	140±10a	21±2a	39.2±5.5a
MT	<i>B. rubens</i>	120±11b	21±1a	41.4±6.3a
WT	<i>B. rubens</i>	118±18b	24±3b	46.2±7.4b
HT	<i>B. rubens</i>	119±23b	29±3c	46.6±10.6b

Means ± SD, different letters indicate significant differences at $P < 0.05$ level, for each trait independently (two-way Anova, Holm-Sidak); $n=12$ plants for nitrogen content for all treatments and $n=6$ plants for the rest of the measurements. V_{cmax} values at 25°C were obtained by fitting the third-order polynomial regression to the measured data

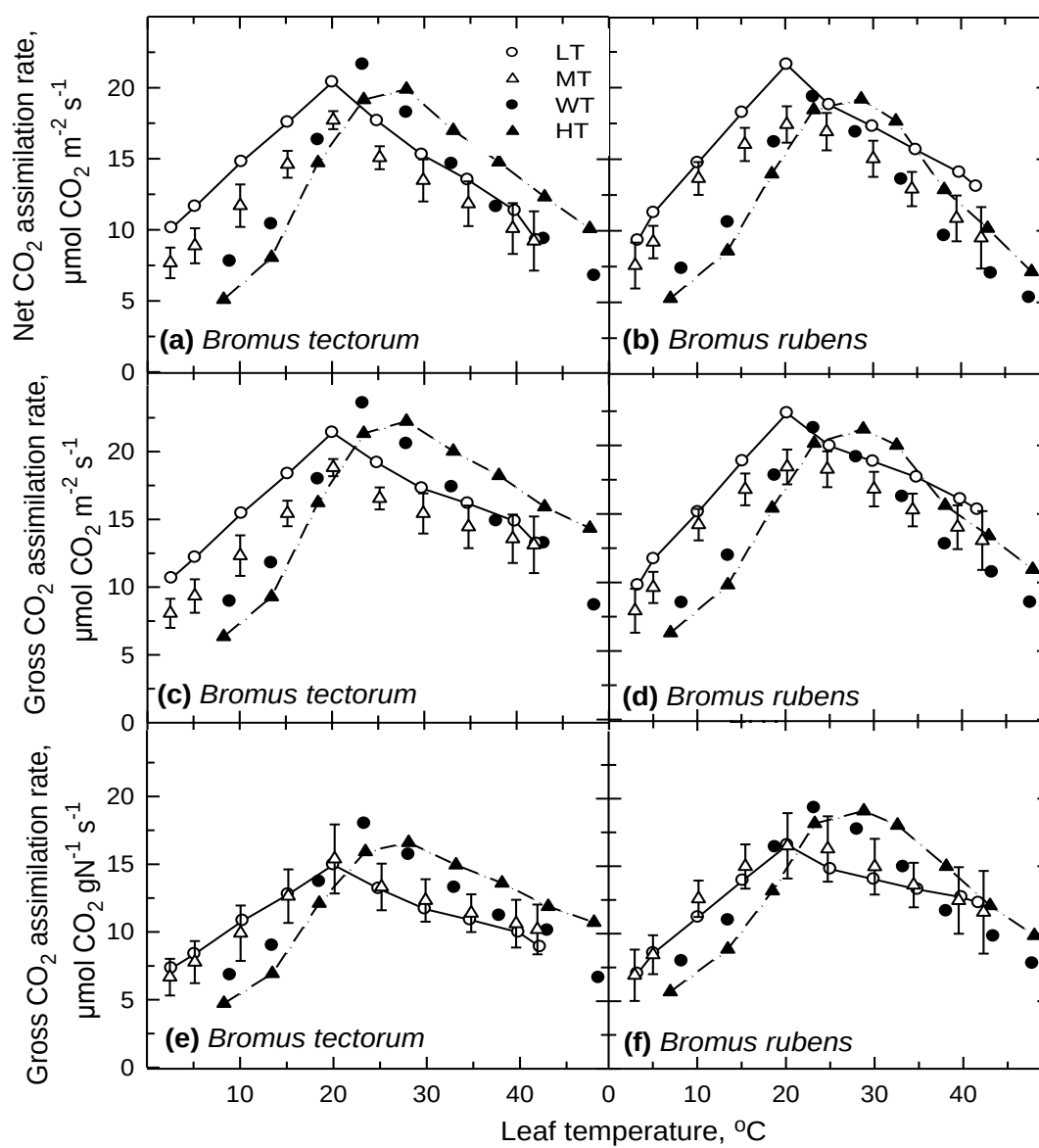


Figure 4.1

Figure 4.1 - The temperature response of photosynthesis in *Bromus tectorum* and *Bromus rubens* grown under 12°/5°C (LT), 22°/14°C (MT), 32°/22°C (WT) and 36°/24°C (HT). **(a)** net CO₂ assimilation rate in *B. tectorum* and **(b)** in *B. rubens*; **(c)** gross assimilation rate in *B. tectorum* and **(d)** in *B. rubens*; **(e)** gross assimilation rate on a nitrogen basis in *Bromus tectorum* and **(f)** in *B. rubens*. Opened circles and solid represent LT plants, opened triangles – MT plants, closed circles -WT plants, closed triangles and dashed line – HT plants. Means $\pm$ SE; $n = 6$. The measured data points are connected with solid lines.

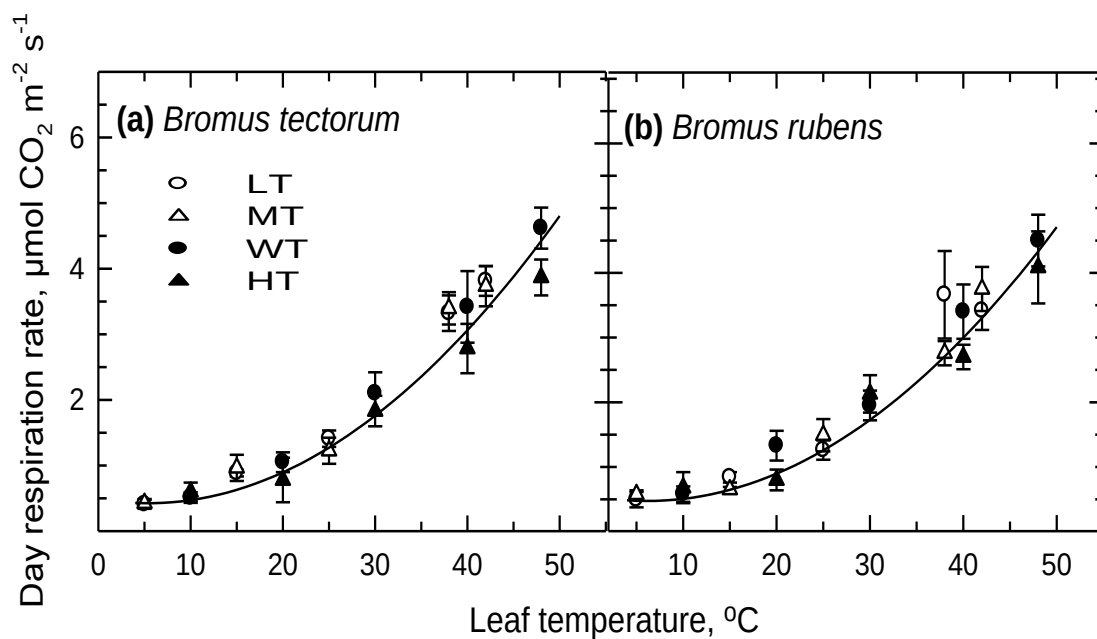


Figure 4.2 - The temperature response of day respiration (R_d) in *Bromus tectorum* (a) and *Bromus rubens* (b) grown under 12°/5°C (LT), 22°/14°C (MT), 32°/22°C (WT) and 36°/24°C (HT). Opened circles represent LT plants, opened triangles – MT plants, closed circles -WT plants, closed triangles – HT plants. Means $\pm$ SE; $n = 6$. Regression: $y=0.0022x^2-0.0105x+0.4379$ (a); $y=0.0025x^2-0.0183x+0.5559$ (b).

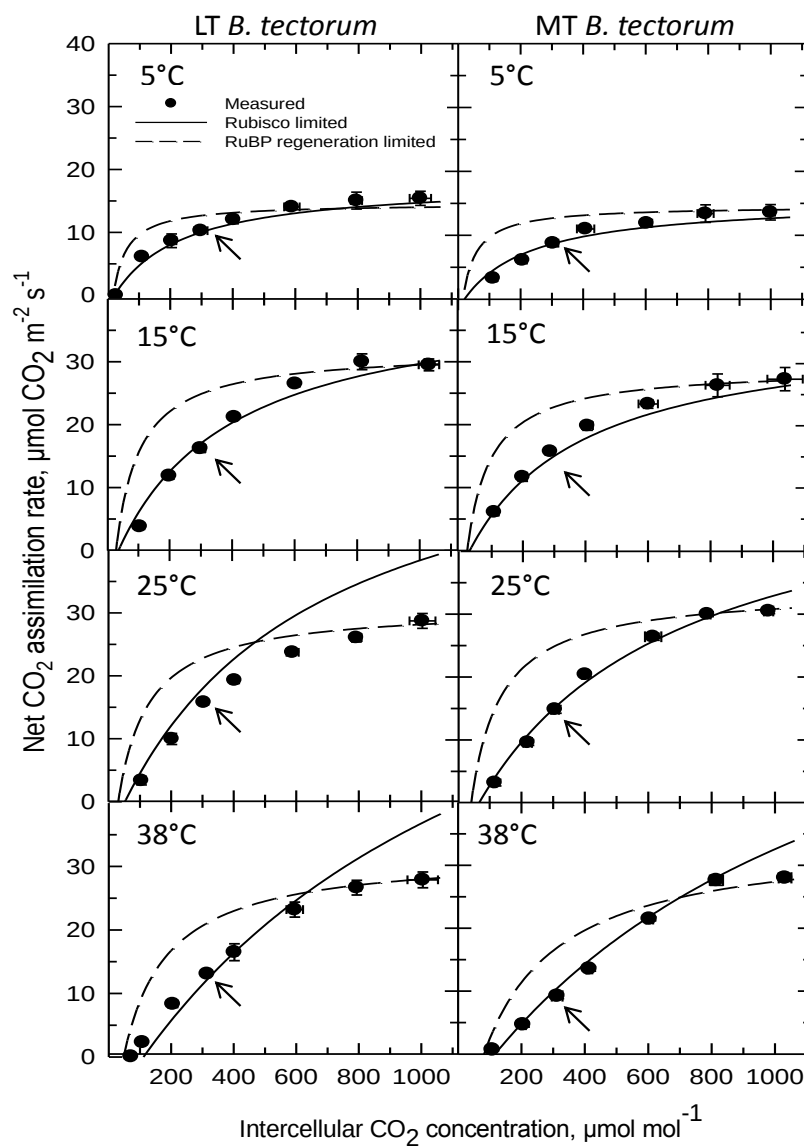


Figure 4.3

Figure 4.3 - The measured and modelled temperature response of net CO₂ assimilation rate (A_{net}) in *Bromus tectorum* grown under 12°/5°C (LT, left panel) and 22°/14°C (MT, right panel). The responses were measured at 5°, 15°, 25° and 38°C. Filled circles are mean measured values, solid line –modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response. Arrows indicate points corresponding to an ambient CO₂ partial pressure of 400 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$.

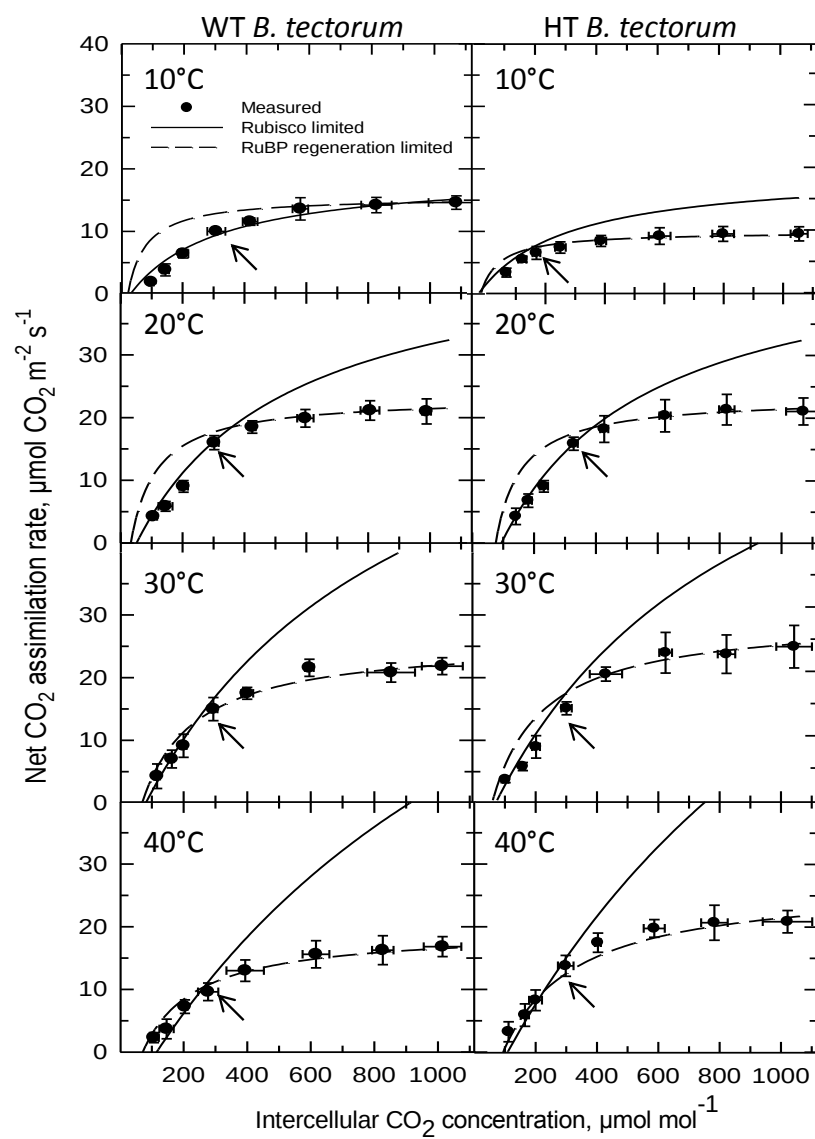


Figure 4.4

Figure 4. 4 - The measured and modeled CO₂ response of net CO₂ assimilation rate (A_{net}) in *Bromus tectorum* grown under 32°/22°C (WT, left panel) and 36°/24°C (HT; right panel). The responses were measured at 10°, 20°, 30° and 40°C. Solid line –modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response, Means $\pm$ SE; $n = 6$. Arrows indicate points corresponding to an ambient CO₂ partial pressure of 400 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

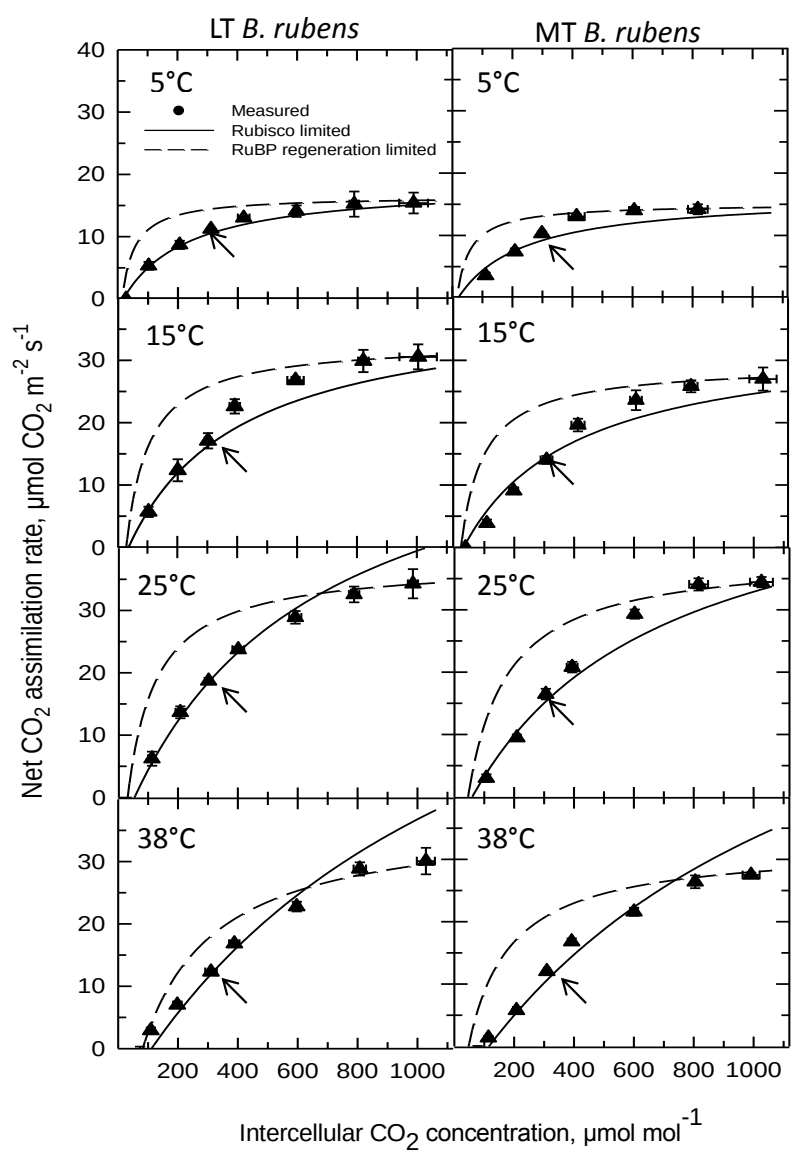


Figure 4.5

Figure 4.5 - The measured and modeled CO₂ response of net CO₂ assimilation rate (A_{net}) in *Bromus rubens* grown under 12°/5°C (LT, left panel) and 22°/14°C (MT, right panel). The responses were measured at 5°, 15°, 25° and 38°C. Filled circles are mean measured values, solid line –modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response. Arrows indicate points corresponding to an ambient CO₂ partial pressure of 400 $\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$

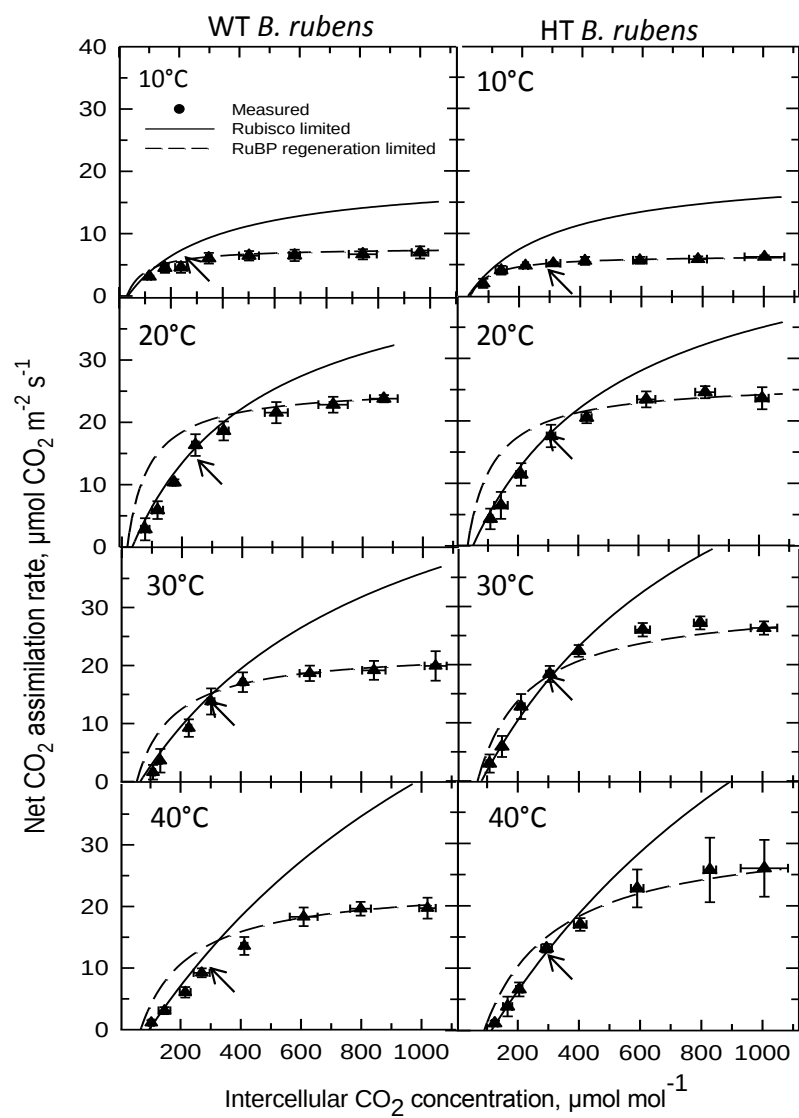


Figure 4.6

Figure 4. 6 - The measured and modeled CO₂ response of net CO₂ assimilation rate (A_{net}) in *Bromus rubens* grown under 32°/22°C (WT, left panel) and 36°/24°C (HT; right panel). The responses were measured at 10°, 20°, 30° and 40°C. Solid line –modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response, Means $\pm$ SE; $n = 6$. Arrows indicate points corresponding to an ambient CO₂ partial pressure of 400 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

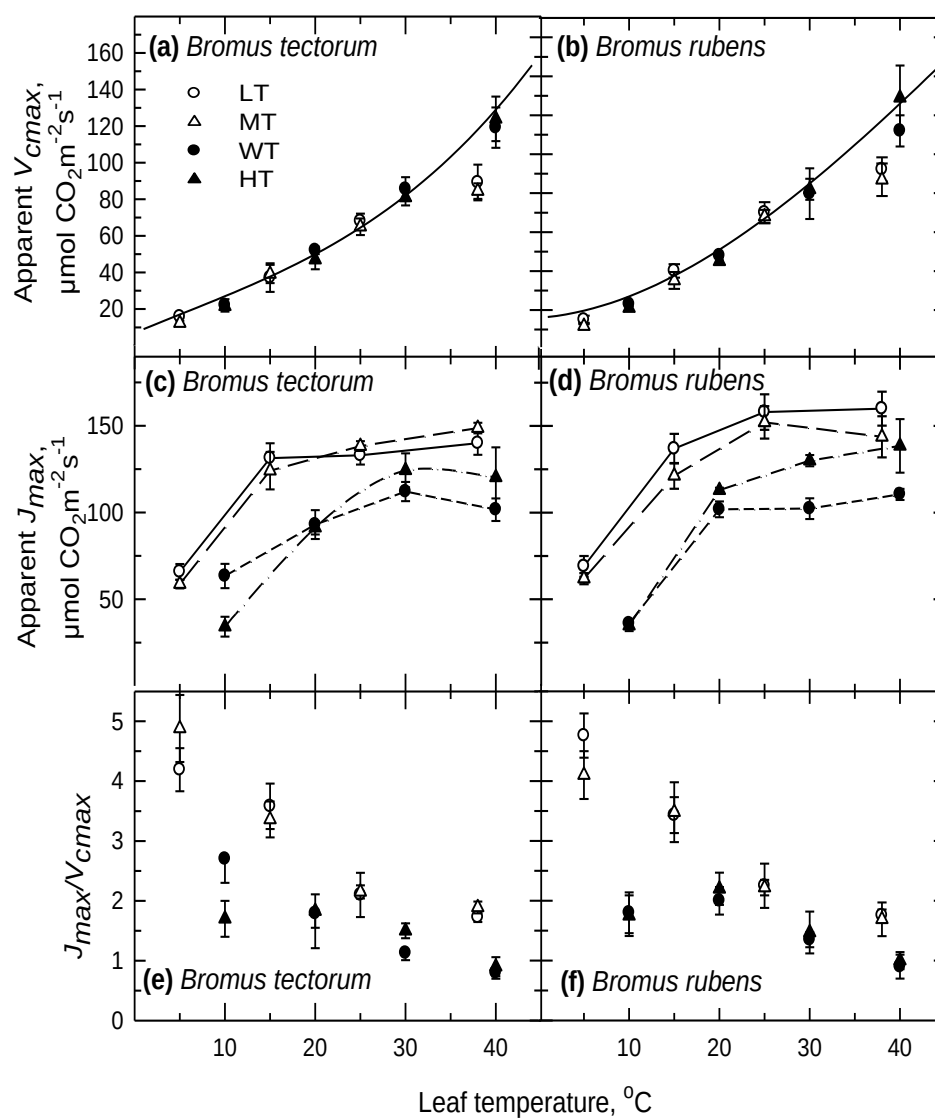


Figure 4.7

Figure 4.7 - The temperature response of V_{cmax} , J_{max} and J_{max}/V_{cmax} in *Bromus tectorum* and *Bromus rubens* grown under 12°/5°C (LT), 22°/14°C (MT), 32°/22°C (WT) and 36°/24°C (HT). **(a)** V_{cmax} in *B. tectorum*, **(b)** V_{cmax} in *B. rubens*, **(c)** J_{max} in *B. tectorum*; **(d)** J_{max} in *B. rubens*; **(e)** J_{max}/V_{cmax} in *B. tectorum*; **(d)** J_{max}/V_{cmax} in *B. rubens*. Opened circles represent LT plants, opened triangles – MT plants, closed circles -WT plants, closed triangles – HT plants. Means $\pm$ SE; $n = 6$. The data of V_{cmax} in HT *B. tectorum* and *B. rubens* were fitted by cubic curves (*B. tectorum*: solid line, $y = 0.001x^3 - 0.015x^2 + 2.05x + 7$; *B. rubens*: solid line, $y = -0.0005x^3 + 0.08x^2 + 0.35x + 16$).

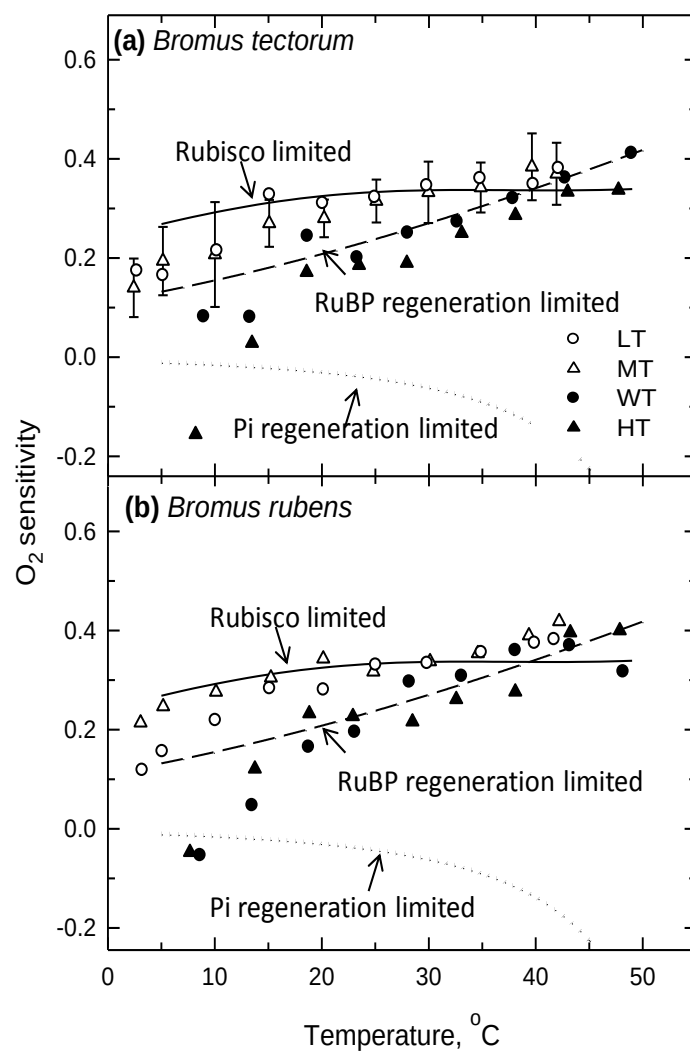


Figure 4.8

Figure 4.8 - The temperature response of O₂ sensitivity of net assimilation rate in *Bromus tectorum* **(a)** and *Bromus rubens* **(b)** grown under 12°/5°C (LT), 22°/14°C (MT), 32°/22°C (WT) and 36°/24°C (HT). Opened circles represent LT plants, opened triangles – MT plants, closed circles -WT plants, closed triangles – HT plants. Solid line –modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response; dotted line - modeled TPU-limited response. Means $\pm$ SE; $n = 6$.

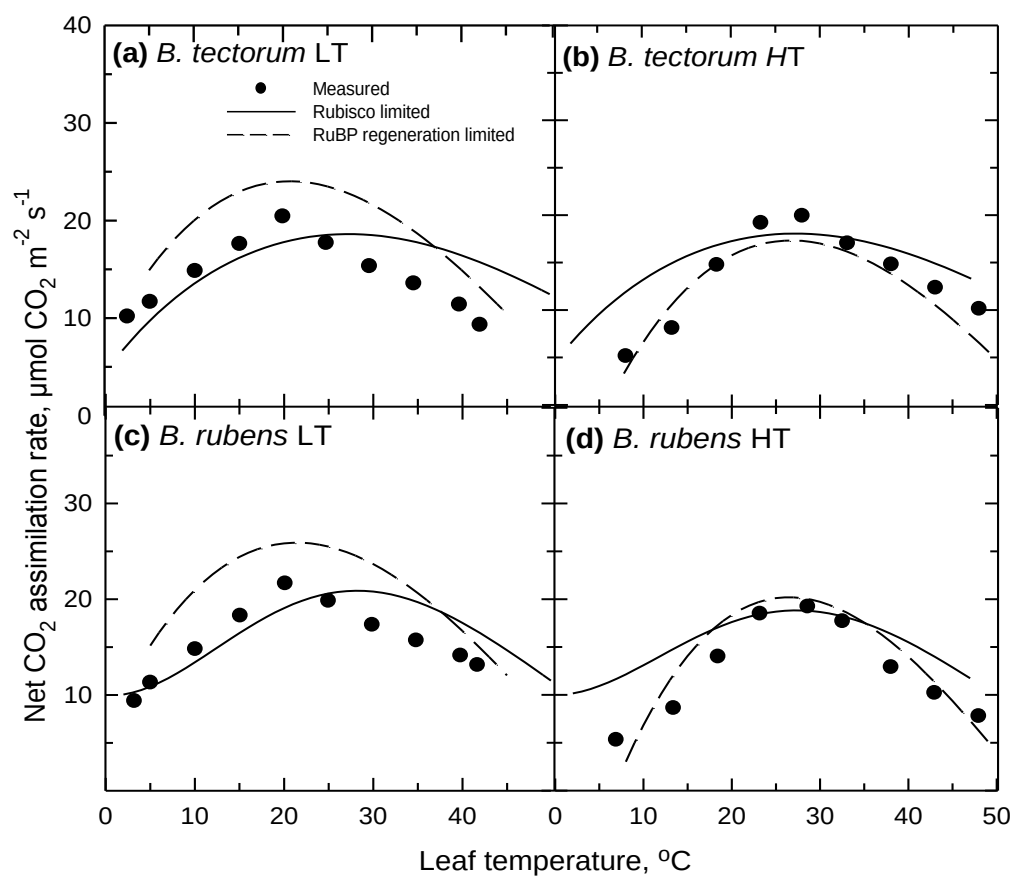


Figure 4.9

Figure 4.9 - The measured and modelled temperature response of net CO₂ assimilation rate (A_{net}) in *Bromus tectorum* (**a, c**) and *Bromus rubens* (**b, d**) grown under 12°/5°C (LT, left panel) and 36°/24°C (HT, right panel). Filled circles are mean measured values, solid line – modeled Rubisco limited response; dashed line – modeled RuBP regeneration-limited response.

Discussion

Based on the current difference in the distribution of *B. tectorum* and *B. rubens* along an altitude and latitude gradient, we hypothesized that these species have distinct temperature responses of photosynthesis. This hypothesis was not supported, as the *Bromus* species differed little in their photosynthetic temperature responses. Both species had similar thermal optima of photosynthesis, values at any given temperature, acclimation response to variation in growth temperature and the same pattern of apparent limitations of A_{net} . When expressed on a nitrogen basis, A_{gross} in LT plants of *B. tectorum* and *B. rubens* was significantly higher than in HT plants below 20°C and lower than in HT plants above 20°C. Moreover, acclimation over broad thermal ranges caused a shift in *B. tectorum* and *B. rubens* photosynthetic limitations. The comparison of measured and modelled A vs T curves indicates that LT plants of *B. tectorum* and *B. rubens* were Rubisco limited at all measured temperatures. Yet, above the thermal optimum this limitation may have been mediated by Rubisco deactivation, since measured A deviated from predicted A (Fig 4.9). High-temperature plants, by contrast, were apparent RuBP regeneration-limited at sub-optimal and supra-optimal temperatures and co-limited by Rubisco and RuBP regeneration at the thermal optimum. Apparent RuBP-regeneration limitation below the thermal optimum in high temperature treatment was likely a result of limited Pi regeneration capacity as was indicated by the absence of oxygen sensitivity below 15°C. Pi-regeneration limitation probably led to inadequate ATP synthesis, the build up of pH gradient and reduced RuBP-regeneration rates in HT *B. tectorum* and *B. rubens* at sub-optimal temperatures (Sharkey, 1985).

Low temperature acclimation

Plants are often limited by Pi regeneration capacity at low temperatures, which imposes a ceiling on A_{net} (Sharkey, 1985; Sage & Sharkey, 1987; Sage, 2002; Cen & Sage, 2005; Sage & Kubien, 2007). Growth under low temperatures can remove this limitation through increased expression of enzymes of starch and sucrose synthesis or elevated cytoplasmic (Pi) content (Sage *et al.* 1989; Sage & Kubien 2007). Yamori *et al.* (2010), for instance, showed that while the photosynthesis of *N. tabacum* and *S. lycopersicum* grown under 30°C was limited by a Pi regeneration capacity, growth at 15°C ameliorated this limitation. In our study, low-temperature acclimation of A_{net} in *B. tectorum* and *B. rubens* was also driven by the alleviation of Pi regeneration capacity at sub-optimal temperatures which was accompanied by up to 100% increase in A_{net} . Pi regeneration limitation was ameliorated down to 2°C in LT and MT plants of both species as indicated by results from oxygen sensitivity analysis of A_{net} and the comparison of measured and modelled A vs T responses. When *Bromus* species were grown under WT and HT, the sensitivity of A to O_2 reduction below 15°C was close to zero and approached the theoretical Pi regeneration limited response. Nonetheless, growth under LT and MT improved oxygen sensitivity in both species up to 20% and resulted in the shift towards Rubisco limitation below the thermal optimum. These changes also lead to improved RuBP regeneration capacity since these limitations (Pi and RuBP regeneration) are interconnected (Sharkey, 1985).

Together with improved Pi regeneration capacity we saw a moderate (15%) increase in leaf nitrogen (N) content in *B. tectorum* and *B. rubens* grown under 12°/5°C. Low-temperature acclimation probably resulted in higher N in the photosynthetic enzymes as indicated by this N increase. The investment of leaf N into the photosynthesis is

proportional to the total leaf N content and any increase in the latter is usually accompanied by an increased level of photosynthetic enzymes (Evans, 1989; Way & Sage, 2008). Nonetheless, this investment was disproportional since LT plants of both species showed reductions in N allocated to carboxylation processes. The LT plants had lower V_{cmax}/N and higher J_{max}/V_{cmax} which might have contributed to Rubisco limitation observed at all measured temperatures. Previously, an increase in the leaf nitrogen content following cool temperature acclimation was demonstrated in forbs, evergreen trees, evergreen shrubs and grasses, including *Bromus* species (*Bromus ramosus*) and was suggested to be a ‘compensatory response’ to a reduced enzymatic capacity (Holaday *et al.*, 1992; Huner *et al.*, 1993; Ryan, 1995; Hikosaka, 2005; Hikosaka *et al.*, 2006; Campbell *et al.*, 2007; Way & Sage, 2008).

High temperature acclimation

High temperature acclimation in *Bromus* species may have been driven by improved Rubisco activation capacity as indicated by reduced deviation between measured and modelled responses of Rubisco-limited A above the thermal optimum. At measurement temperatures of 38° to 40°C, the apparent V_{cmax} in the LT and MT grown plants was ~30% less than in warm- and high-temperature-grown plants, indicating a reduction in carboxylation capacity *in vivo*. The reduction in apparent V_{cmax} in LT and MT *B. tectorum* and *B. rubens* plants at 38°C and 40°C also supports a reduction in carboxylation capacity below the expected capacity. Since Rubisco is heat stable to >50°C, this implicates a loss of activation state as an explanation (Crafts-Bradner & Salvucci, 2000; Salvucci *et al.*, 2001).

Besides, A vs T response in LT plants of both species measured below 35°C was lower than either modelled responses assuming Rubisco or RuBP regeneration limitation, which also supports this hypothesis. Similar decline in V_{cmax} at 40°C accompanied by the reduction in A_{net} was reported in black spruce grown under 22°/14°C (Way & Sage, 2008). Sage *et al.* (2008) proposed that the observed changes were a result of heat induced reduction in Rubisco activase activity (Sage *et al.*, 2008). Rubisco activase is known to be heat labile with dissociation of activase subunits above 35° to 40°C (Salvucci & Crafts-Brandner 2004). If so, acclimation to warm temperature appears to have stabilized the activase enzyme in both *Bromus* species as indicated by an increase in V_{cmax} at 40°C in HT plants.

Thermal optimum of photosynthesis

When plants were grown under LT and HT regimes, their T_{opt} differed by 7° to 8°C. A decrease in V_{cmax} at supra-optimal temperatures and greater light-saturated electron transport rate (J_{max}) at sub-optimum temperatures, resulted in higher J_{max}/V_{cmax} ratios in LT and MT *B. tectorum* and *B. rubens* and a subsequent shift in T_{opt} . Similar adjustments in the thermal optimum accompanied by increased J_{max}/V_{cmax} ratios in LT plants occur in spinach, *Polygonum cuspidatum* and *Plantago asiatica* (Hikosaka, 2005; Yamori *et al.*, 2005, Onoda *et al.*, 2005a, b). In our study, growth under low temperatures appear to have promoted a disproportional allocation of leaf nitrogen towards RuBP regeneration processes and triggered a subsequent shift to Rubisco limitation over the entire temperature range in both species. Higher J_{max}/V_{cmax} together with Rubisco limited photosynthesis is a distinct

characteristic of cold tolerant species that exhibit high photosynthetic rates at chilling temperatures (Yamori *et al.*, 2010).

Photosynthesis and ecological success of *B. tectorum* and *B. rubens* in semi-arid regions

Within the last 100 years *B. tectorum* and *B. rubens* reached their dominance (invasive zones) in semi-arid regions of western North America characterized by significant seasonal temperature fluctuations. *Bromus tectorum* is the dominant invasive grass in the “cold desert” steppes of the Intermountain region of western North America, while *B. rubens* is one of the severe grass invaders in the “hot deserts” of south-western North America. A clear trend in dominance of *Bromus* species in “cold” and “hot deserts” is partially a result of their capacity to acclimate over broad thermal ranges. Their photosynthetic apparatus first adapts to chilling temperatures during the early growth, and then adjusts to elevated temperatures as the growing season progresses. Once temperature rises above zero, *B. tectorum* and *B. rubens* produce large canopy and outcompete their competitors due to the ability to ameliorate Pi regeneration limitation and a subsequent increase in A_{net} at sub-optimal temperatures. As temperature goes up and exceeds 30°-35°C, *Bromus* species improve their photosynthetic rate at elevated temperatures through the stabilization of Rubisco activity at elevated temperatures and an upward thermal optimum shift.

Our results demonstrate that photosynthetic response to temperature does not explain current range separation of *B. tectorum* and *B. rubens*, since both species differ little in their

photosynthetic temperature responses, thermal optimums, and acclimation response to variation in growth temperature.

Chapter Five – Concluding Discussion

The role of physiological processes and their tolerance limits in species range formation was first formulated by Ronald Good in his “Theory of Tolerance” (Good, 1931). Good suggested that species distribution is closely linked to their physiological responses to climate and implied that even closely related sister species can have distinct tolerance ranges. Species’ tolerance, according to Good (1931) is comprised of many tolerance parameters, but only one of them limits its distribution. In reality, however, a single species might be limited by more than one factor and process since the nature of geographical ranges and niches is multi-dimensional (Hutchinson, 1957). In order to predict changes in vegetative cover, one has to look at a large number of proximal variables and understand their effect on major physiological and reproductive processes. My thesis contributes to this area by looking at the effects of temperature on range formation of two closely-related grass invaders, *B. tectorum* and *B. rubens*, through the assessment of their freezing tolerance, male reproductive development and photosynthetic performance under varying temperatures.

Bromus tectorum and *Bromus rubens* are of the particular interest to me because of their critical role in North American semi-arid biomes and the allopatric character of their geographical ranges. Based upon the spatial separation of *B. tectorum* and *B. rubens* along a thermal cline it is reasonable to assume that physiological mechanisms might contribute to geographical isolation of these sister species. As bioinvaders, *B. tectorum* and *B. rubens*, should have escaped biological controls and therefore are more likely to be controlled by the climatic factors, such as temperature (Blaney & Kotanen, 2001).

Upper and northern range limits

In the second chapter of my thesis I investigate freezing tolerance of *B. tectorum* and *B. rubens*, compare their physiological tolerance limits and identify the factor that controls their different distributions. I demonstrate that the mechanism explaining the distinct northern range limits is different acquisition time of freezing tolerance. While *B. rubens* has a slower rate of freezing acclimation that leads to intolerance of sudden, late-autumn drops in temperature $\leq -12^{\circ}\text{C}$, its colder-adapted sister species, *B. tectorum*, rapidly hardens and so is not impacted by the sudden onset of severe late-autumn cold. *Bromus tectorum* rapidly acclimates to sub-zero temperatures and reaches its maximum cold tolerance level of -30°C when minimum air temperature falls below -10°C . These results establish a mechanistic means for modelling the impact of future climate on the worst invasive species in the world. Instead of modelling distribution based on winter minimums, severe cold events in autumn appear to be more critical predictor. With anthropogenic warming, isoclines for minimum winter temperature and the range limits of many species will progressively shift to higher latitudes and altitudes. If so, *Bromus rubens* should follow the modulation of the -12°C minimum autumn isocline and potentially infest areas currently occupied by *B. tectorum* (Chapter 2). Once the likelihood of early frost events $\leq -12^{\circ}\text{C}$ in the regions currently occupied by *B. tectorum* decreases, *B. rubens* should heavily invade new habitats and potentially replace *B. tectorum* (Chapter 2; Bradley *et al.* 2009). Besides, *Bromus rubens* could take advantage of an earlier onset of non-lethal autumn cold events ($\leq -5^{\circ}\text{C}$), frost harden and survive freezing temperatures of $\leq -12^{\circ}\text{C}$.

Along with the shift of *B. rubens* range limits, my data suggests that climate changes should affect the northern ranges of *B. tectorum* distribution. *Bromus tectorum* will probably track the northward shift of the -30°C winter isocline and also infest higher elevations where the average daily minimum temperatures are already increasing (Forister *et al.*, 2010). The abundance of *B. tectorum* at high elevation sites is already increasing (Bromberg *et al.*, 2011). Over the last 15 years its cover and frequency at 2300m to 4300m elevations more than doubled in the Rocky Mountain National Park (Bromberg *et al.*, 2011). Although the reported cover of *B. tectorum* at these elevations is relatively low with an average of 4%, climate warming might amplify its current abundance and increase the overall frequency. If so, within the next 100 years high elevation plant communities in the western United States might undergo significant structural changes and biodiversity loss. Projected increases in nutrient inputs into high-elevation sites will reduce the competitive advantage of native species and promote the establishment of exotic species (Pauchard *et al.*, 2009). Although high elevation sites usually have the lowest non-native species plant richness, recent studies demonstrated that alien species with broad tolerance ranges are gradually colonizing increasingly higher elevations (Pauchard & Alaback, 2004; Becker *et al.*, 2005; Pauchard *et al.*, 2009, Alexander *et al.*, 2011) and *B. tectorum* with its capacity to acclimate to rapid frost events is one of the best candidates for such invasions. In addition to high elevation infestations, *B. tectorum* could invade boreal and tundra ecosystems in western North America. The boreal forest in particular may be prone to *B. tectorum* infestation in a warmer climate. Fire frequency and total burned area in the North American boreal region has more than doubled between the 1960/70s and the 1980/90s (Kasischke & Turetsky, 2006), and General Circulation Models project that under high atmospheric CO₂ concentrations, the areal extent of boreal fires might increase relative to the 1990s (Stocks *et*

al., 1998). Predicted increases in fire disturbance in the boreal zone should disrupt the life cycles of native species and promote aggressive establishment of fire adapted grasses such as *B. tectorum*. Once introduced into boreal ecosystems, *B. tectorum* could readily colonize burned areas and reduce fire return intervals (Hobbs, 1989). Reductions in summer precipitation will significantly increase their success and promote range expansion (Bradley, 2009). Elevated CO₂ will also promote grass fire cycles, since *B. tectorum* is highly responsive to elevated CO₂ and will more rapidly establish the fuel layers that drive frequent destructive fires (Smith *et al.*, 1987; Sage, 1996; Ziska *et al.*, 2005). As a consequence, if climate warming with drying facilitates the establishment of a *B. tectorum* infestation, then the boreal zone could be altered to a much greater degree than it would without the occurrence of *Bromus* invaders. Dominance of these species in the boreal forest will lead to poor forest re-establishment and as a consequence, replacement of boreal forests by grasslands and changes in the surface albedo (Rost & Mayer, 2006).

Bromus species appear to be controlled by winter temperatures only in western North America where summer drought and fire promote the success of these species. In eastern North America, by contrast, other environmental variables might contribute to the success of *B. tectorum* and *B. rubens*. The failure of *B. tectorum* and *B. rubens* to extend their distribution to the -30°C winter and -10°C autumn isoclines in eastern North America has no explanation, but may relate to antagonistic factors such as greater summer moisture, increased competition and greater snowpack, which discourage the success of *B. tectorum* (Nasri & Doescher, 1995; Bradley, 2008; Griffith *et al.*, 2010).

Lower and southern range limits

Along with range expansion, global warming might facilitate severe range contractions of *Bromus* species at their lower and southern range limits. In my third chapter I look at the effect of high temperatures on the reproductive development of *B. tectorum* and *B. rubens* and show that heat-induced sterility could affect their geographical ranges if the warming of the climate progresses. Plant reproductive processes are extremely sensitive to elevated temperatures (Sharkey & Shrader, 2006). This sensitivity affects reproductive outputs of numerous economically important crops (e.g. Saini & Aspinall, 1982, Sakata et al., 2000). Nonetheless, heat induced sterility of natural populations has been little studied by the research community. It was demonstrated that most cultivated plants reach their reproductive capacity near 30° with complete failure at 36°C. If the wild species have the same threshold, heat sterility could be critical factor controlling the response of warm-climate ecosystems and species southern range limits to global warming. To my knowledge, no previous studies have looked at the effect of elevated temperatures on the reproductive output of wild species, particularly invasive ones. In chapter three, I found that *Bromus tectorum* and *Bromus rubens* share the same threshold of heat sterility of 36°C. Exposure to 36°/24°C during the flowering triggered complete pollen abortion and a subsequent seed set failure in all populations of *B. tectorum* and *B. rubens*. Grain yield reductions were also observed under 32°/22°C but were associated with the reductions in spikelet numbers. At 32°C the reductions in seed yield were more pronounced in *B. tectorum* than in *B. rubens* which probably reflects different sensitivity of growth in the two species.

A field survey performed by R.F. Sage and T. L. Sage demonstrates that during the flowering period, both *Bromus* species already experience temperatures close to their reproductive threshold (Chapter 3). This indicates that in a warmer world *B. tectorum* and *B. rubens* might undergo severe contraction of their range limits. Yet, lethal heat events are episodic by nature and therefore their effect on species fecundity will depend on a number of factors, such as the timing, extent and frequency of heat events. If they are to impact a *B. tectorum* and *B. rubens* distribution, they must either occur frequently enough to have a long-term impact on a population, or should be severe enough to reduce the population to low levels from which recovery is slow. Because both *Bromus* species are annuals and their seed bank is short lived (Holm *et al.*, 1977; Wu & Jain, 1979; Smith *et al.*, 2008), they appear to be particularly vulnerable to heat effects on reproduction. Temperatures $\geq 36^{\circ}\text{C}$ during flowering in back-to-back years may reduce seed banks of *Bromus* species and diminish their cover in the semi-desert biomes. As a result, the shift of *Bromus* species towards higher latitudes and elevations described above will be offset by a contraction of the distribution at the southern and lower end of their ranges (IPCC, 2001; Bradley *et al.*, 2009; Chapter 3). Moreover, high latitude populations of *B. tectorum* and *B. rubens* might also be prone to heat-induced sterility if climatic changes promote delayed flowering of these species. With global warming heat sterility at lower elevations could be common, unless plants flower very early in the year, in which case they may have insufficient reserves for high fecundity. The interactions between vegetative growth during winter at low elevations, resource levels and flowering may be a large part of the mechanism affecting the low elevation distributions of these species.

Photosynthetic performance during the growth season

Along with excellent frost tolerance capacity *Bromus tectorum* exhibits high photosynthetic rates at chilling temperatures. In the fourth chapter I demonstrate that both *Bromus* species can alter relative investments into their photosynthetic components and rapidly acclimate to growth under the sub-optimal temperatures. Low-temperature acclimation ameliorates Pi regeneration limitation in *B. tectorum* and *B. rubens* and increases net CO₂ assimilation rate at sub-optimal temperatures. By fitting the experimental data to the modelled response I can conclude that photosynthesis of LT and MT *B. tectorum* and *B. rubens* was limited by Rubisco at all measured temperatures. If *Bromus* species expand their distribution ranges towards higher latitudes and experience increasing frequency of chilling temperatures during their growth period, an ability to overcome Pi regeneration limitation and exhibit Rubisco limitation at sub-optimal temperatures could enhance their competitive advantage.

Photosynthesis of *Bromus* species, unlike their reproductive development, will not be affected by future climatic changes. In my fourth chapter I have shown that *B. tectorum* and *B. rubens* acclimate to broad thermal ranges and are able to grow under extremely high temperature conditions (36°C). Growth under higher temperatures improved their carboxylation capacity above the thermal optimum which probably resulted from increased thermostability of Rubisco activase. Surprisingly, but I found no difference in their photosynthetic responses as well as acclimation capacity which indicates that photosynthesis-related traits do not explain current range separation of *Bromus* species.

Understanding physiological and reproductive responses in order to predict species range shifts

Kearney & Porter (2009) recommend using a mechanistic approach to study a fundamental niche of species and to predict their future range shift. They suggested linking physiological responses of the organism with spatial data in order to create biophysically based species distribution models (SDMs). My thesis demonstrates that physiological and reproductive processes could be important determinants of species distribution and therefore should be considered in these models. The similarity between the freezing tolerance of *B. tectorum* and *B. rubens* and minimum winter/autumn temperature isoclines corresponding to their northern-most distributions supports the hypothesis that aggressive weedy invaders expand their realized niche to correspond to their fundamental niche. Yet, the presence of distinct tolerance thresholds in *Bromus* species indicates that distribution models should take into account a larger suite of temperature variables when predicting ecosystem responses to climatic changes.

Patterns of heat-induced sterility in *Bromus* species demonstrate that their reproductive threshold is similar to the ones reported for crops and might be an important factor controlling the distribution of warm-climate grasses. If the 34°-36°C threshold for heat sterility is widespread in the plant kingdom, then climate warming could potentially have a massive impact on the reproductive ability of the world's flora, particularly at low latitude, and in short-lived species that do not form long-term seed banks. Even species active in cool conditions, such as the winter-active *Bromus* species studied here, can experience heat sterility and thus may have evolved developmental mechanisms to avoid it.

The apparent lack of variation in heat sterility between species and populations of different climate zones indicates evolutionary adaptation is not a likely probability, particularly given the rapid rate of anthropogenic climate change. For species living in climates that approach the thermal threshold for heat sterility, large losses in fecundity are probable with increasing frequency of heat events, as demonstrated by the *Bromus* example.

Gaps in our understanding of species distribution and abundance prevent us from creating a robust framework for assessing potential climate change impact on natural plant communities. Defining every factor limiting distribution is a challenge, yet only a precise analysis of all tolerance parameters and processes gives a clear picture of plants fundamental niche and its responses to climatic changes. To better understand the distribution of *B. tectorum* and *B. rubens* it is important to collect more experimental data on their physiological and reproductive development, and incorporate them into the mechanistic distribution models together with the data presented here. Predicted changes in nocturnal temperatures (IPCC, 2001), for instance, might have a significant effect on the distribution of *Bromus* species. Exposure to elevated night-time temperatures during the reproductive development might trigger spikelet infertility, reductions in the duration of grain filling and low seed yield in *B. tectorum* and *B. rubens* (Vara Prasad *et al.*, 2008). In addition, in order to define mechanisms controlling southern and lower range limits of *Bromus* species it is also important to examine the temperature sensitivity of their germination process. Closely related species with distinct distribution ranges can possess distinct germination requirements (Ascough *et al.*, 2007). If so, the temperature sensitivity germination of *B. tectorum* and *B. rubens* has to be determined and incorporated into the species distribution models.

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Appendix I

To compare the effect of the cooling rate on the INP we replicated the ice nucleation experiment on both species using two cooling rates (4.5°C and 2°C) and found no effect of the cooling rate on the INP (Table A. 1)

Table A. 1. The ice nucleation temperatures of *Bromus tectorum* (R1) grown at 12°/10°C.

	INP (cooling rate of 2°C/h)	INP (cooling rate of 4.5°C/h)
<i>B. tectorum</i> grown in the cool greenhouse at 12°/10°C	-13.8 ± 1.9	-13.3 ± 3.1

To compare the effect of water and ice crystals on the tissue freezing, the electrolyte leakage test was replicated three times with an addition of 5 µl of distilled water into the vials at the beginning of freezing cycle; with small ice crystals placed into the vials at -1.5°C during the freezing cycle (Clement & Van Hasselt, 1996); and without an addition of water/ice crystals (Fig. A. 1). During this trial we found no effect of water/ice on the TEL₅₀ of both species, yet, an addition of water into the vials resulted in a more uniform freezing pattern.

We also replicated the electrolyte leakage test on both species three times using three different cooling rates (2°C, 4.5°C and 6°C per hour) and found that cooling rates of 2° to 6° C per hour had no effect on the results of the electrolyte leakage test (Fig A. 2). This experiment was conducted in order to compare different cooling rates that were used in the literature (e.g. Roden *et al.*, 2009) and to identify whether the difference in the cooling rate affects the results of the test.

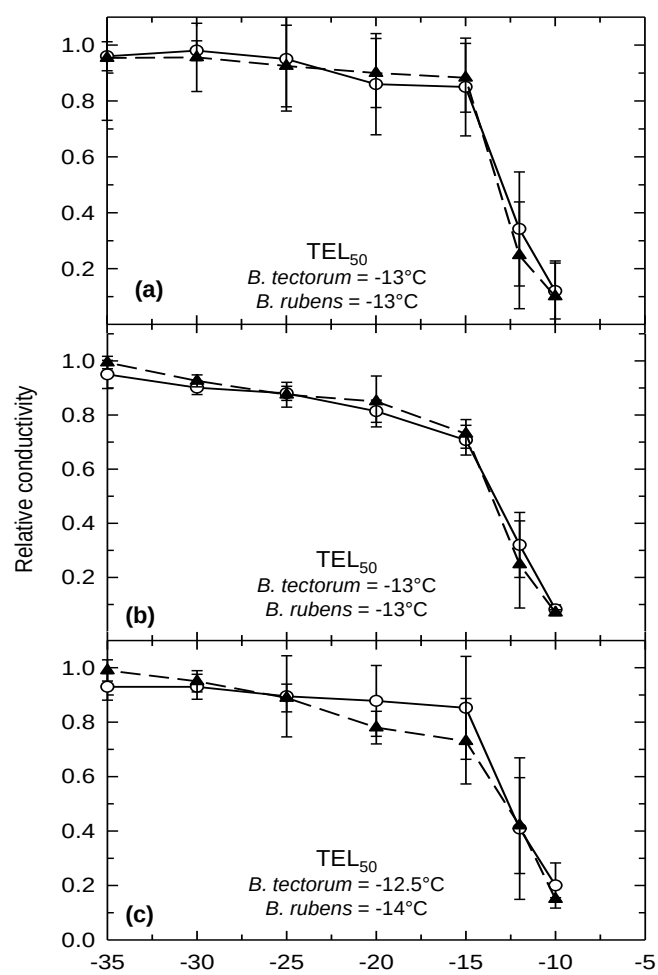


Figure A.1

Figure A.1 - The response of electrolyte leakage to low temperature in leaf samples of *Bromus tectorum* (solid line with opened circles) and *Bromus rubens* (dashed line with closed triangles). Plants were grown outdoors during September 2007 until December 2008. Samples were frozen at 4.5°C/hour without the presence of water/ice (a), with an addition of water (b) and ice crystals (c). Means $\pm$ SE; $n = 6$.

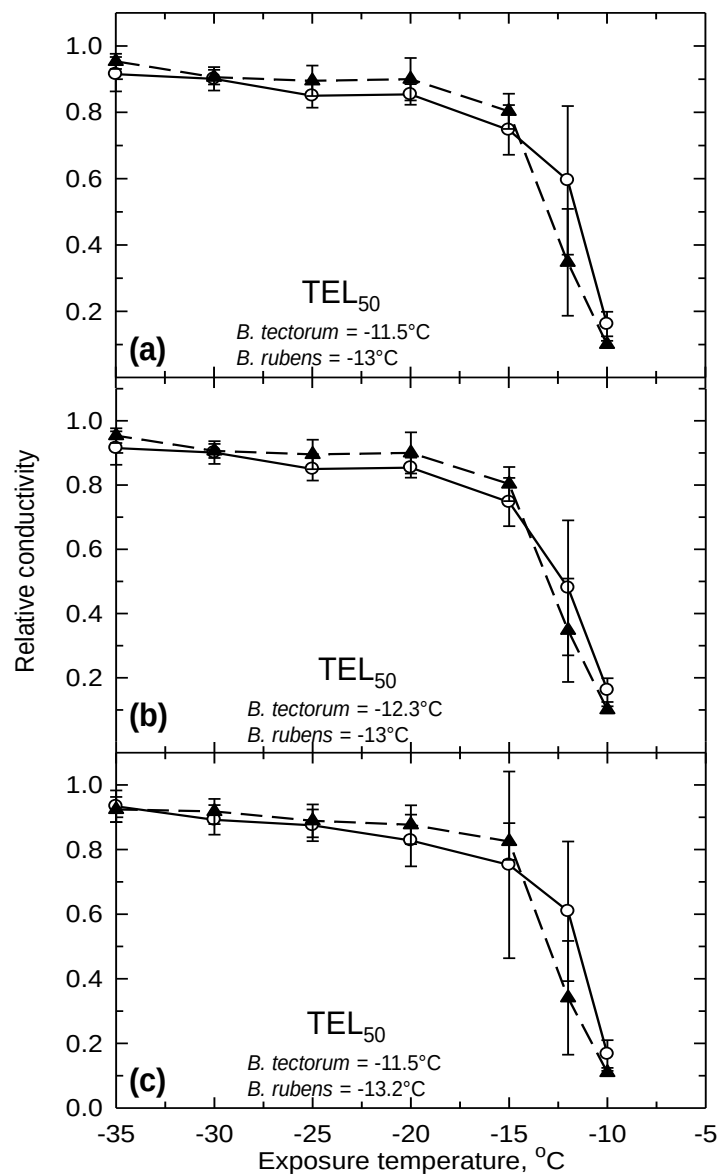


Figure A.2

Figure A.2 - The response of electrolyte leakage to low temperature in leaf samples of *Bromus tectorum* (solid line with opened circles) and *Bromus rubens* (dashed line with closed triangles). Samples were frozen at 2°C/hour **(a)**, 4.5°C/hour **(b)** and 6°C/hour **(c)**. Plants were grown outdoors during September 2007 until December 2008. Means $\pm$ SE; $n = 6$.

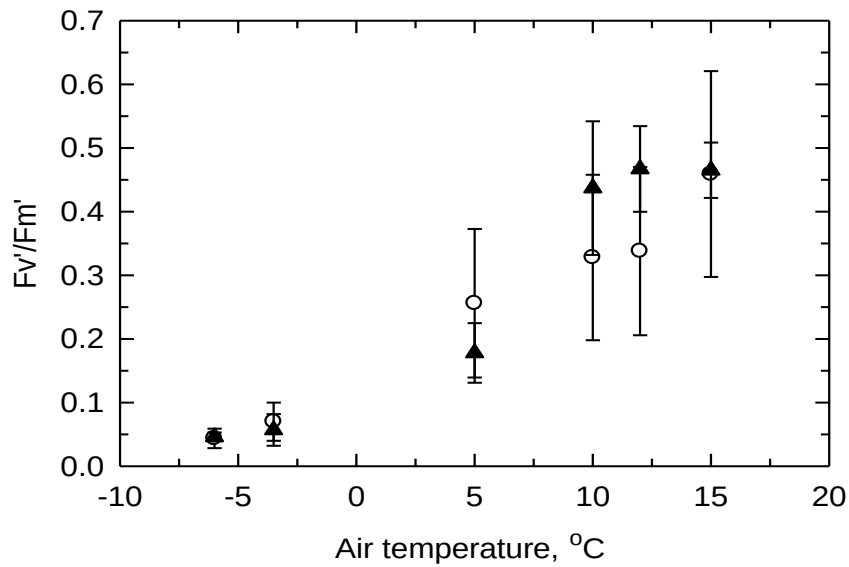


Figure A.3 - Relationship between leaf temperature and the efficiency of excitation energy transfer to open PSII reaction centers (F_v'/F_m') in *Bromus tectorum*, Reno population, T1 (opened circles) and *Bromus rubens*, R1 (closed triangles). The F_v'/F_m' were measured during the fall-winter period 2007-2008, at noon on clear days with high light intensities ($1350 \pm 50 \mu\text{mol photons m}^{-2}\text{s}^{-1}$). Means $\pm$ SD, $n = 8$ plants per species.