ADAPTION TO CLIMATE CHANGE AND SMALL GRAIN PRODUCTION SYSTEMS BY *BROMUS TECTORUM*

BY

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DEDICATION

I dedicate my dissertation to my wife Louise. I began dating Louise only a few months before moving to Pullman, proposed to her during the second year of my PhD, married her during my third year, and together we welcomed a son into the world during my fourth year. Louise has been my unfair advantage. Not many graduate students have the benefit of a spouse possessing an advanced degree in the same field. If I needed a document proofread, a sounding board for a presentation, or a second opinion on experimental design, at any hour of the day, she was happy to help. Louise is an outstanding scientist, mother, wife, and best friend.

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ABSTRACT

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Bromus tectorum (downy brome) is an invasive winter annual grass species naturalized throughout western North America. Within the small grain production region of the Pacific Northwest (PNW) downy brome is a ubiquitous and competitive weed. Previous research has documented regional specificity of downy brome phenotypic development, while considerable variation in phenotypic development has been noted between regions. In 2011 and 2012 130 downy brome accessions were collected from across the PNW small grain production region. A genotype-by-sequencing approach was employed to call molecular markers, generate population genetic statistics, and classify 88 of the 130 downy brome accessions into genetically similar clusters. Individuals were assigned to one of six genetic clusters using 384 single-nucleotide polymorphisms and discriminant analysis of principal components clustering approach. Accessions were transplanted to three common garden field sites to document and model the timing of development. The timing of development stages was modeled against cumulative growing-degree-days (GDD) to develop herbicide application thresholds to aid in control of downy brome within small grain fields. The estimate for mature seed production varied from May 18th to June 20th depending upon the

location of the common garden. Earlier production of mature seed was observed following more severe winters compared to mild winters, implying a role of vernalization regulating the timing of development. Greenhouse experiments were initiated to characterize the response of early, intermediate, and late-to-flower downy brome accessions to various vernalization treatments and quantify expression of *Brachypodium distachyon* gene vernalization 1 (*BdVRN1*). Downy brome flowering in response to vernalization treatments was linked to the expression of *BdVRN1*, implying the molecular controls of flowering in downy brome are similar to the controls of other temperate grass species. Downscaled climate modeling was paired with Global Climate Change models to project downy brome development thresholds under future climate scenarios. Downy brome development is anticipated to advance 16 to 34 days across the small grain production region of the PNW. The earlier development of downy brome will require earlier control inputs, which may conflict with earlier rainfall projected under future climate scenarios.

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1.1 Climate Change in the Pacific Northwest Small Grain Production Region

The Intergovernmental Panel on Climate Change (IPCC) characterized the evidence forglobal climate warming as unequivocal. Solomon et al. (2007) attributed global climate change to an increase in radiative forcing, which has increased 1.72 W m^{-2} since 1750, with 1.6 W m^{-2} of that increase attributed to anthropogenic rather than natural sources. Radiative forcing of the climate system is predominantly due to long-lived greenhouse gases (GHGs): carbon dioxide, methane, and nitrous oxide (Solomon et al. 2007). Emissions from GHGs have increased dramatically due to human activities since 1750, with current atmospheric GHG levels exceeding the historic natural range for the past several thousand years. In particular, atmospheric levels of carbon dioxide and methane are higher now than at any point in the last 650,000 years. Solomon et al. (2007) attribute the cause of observed global warming during the last century to anthropogenic sourced GHGs with a >90% likelihood.

Between 1906 and 2005, global average surface temperature increased 0.74°C, with the rate of increase since 1956 nearly twice the rate from 1906 to 1950. Currently, observed changes to global climate since 1850 include decreases in the frequency of cold periods and frosts, and increase in hot periods and heat waves, an increase in heavy precipitation events, and an increase in sea level rise at a global scale (Solomon et al. 2007).

While climate change is often related to observed and projected increases in global mean annual temperature, the impacts of climate change to human and natural systems are expected to vary widely on a regional scale (Field et al. 2014). Experiments have demonstrated increases in photosynthesis rate and improved grain yield among many agronomic crops when carbon dioxide and temperature are increased to levels projected under climate change scenarios (Field et al. 2014). Gains in productivity are most likely to occur in mid to high latitudes where projections include

increased moisture along with increased carbon dioxide and temperatures. Meanwhile, lower latitudes are projected to endure decreasing crop yields in response to decreasing total annual precipitation (Solomon et al. 2007).

When the interaction between increased temperature and water requirements is factored in, models forecast moderate yield increases with moderate temperature changes (0.8 to 1.5°C global average increase) followed by a drop in productivity with average global temperature increases greater than 1.5°C (Kang et al. 2009). Furthermore, factors such as extreme weather events and pest pressure will also be altered by changing climate and have the potential to decrease crop productivity (Tubiello et al. 2007).

The small-grain production area of the Pacific Northwest (PNW) includes central and eastern Washington, parts of northern Oregon, and northern Idaho and totals 2.2 million ha of non-irrigated cropland (Huggins et al. 2012). In 2007, 1.3 million ha of this region were planted into small grains, with around one million ha planted to winter wheat. Annual precipitation in the region ranges from less than 300 mm to greater than 600 mm, with a west to east increasing precipitation gradient. Mean annual temperature varies from 5ºC to 11ºC based on a 30-y average (1971-2000) (Huggins et al. 2012).

Anthropogenic climate forcing is a significant predictor of observed changes in PNW climate during the 20th century. Abatzoglou et al. (2014) reported mean annual temperature increasing 0.6 to 0.8ºC from 1901 to 2012. In addition to the increase in mean annual temperature, trends were observed in increasing temperatures of the coldest night of the year and lengthening annual freeze-free period. Decreased summer and fall precipitation was observed during the last four decades. Together, the increased summer temperature and decreased summer precipitation have led to increased evapotranspiration during the growing season during the 20th century (Abatzoglou et al. 2014). On the other hand, spring precipitation has also increased over the same interval.

Observed climatic trends in the PNW are projected to continue into the future under most climate models. Compared to average annual temperature from 1970-1999, climate models project an increase in annual temperature of 1.1ºC by the 2020s, 1.8ºC by the 2040s, and 3.0ºC by the 2080s (REF). The trends towards drier summers and wetter winters are also projected to continue, driving a continuing increase in the summer evapotranspiration rate (Mote and Salathé 2010).

Climate projections at a regional scale are commonly simulated utilizing 21 global climate models (GCMs) that were coordinated through the IPCC (Solomon et al. 2007). All 21 models integrate atmospheric and oceanographic interactions and are resolved on a horizontal grid (Mote and Salathé 2010). Projections are made utilizing a number of different radiative forcing scenarios, giving a range of possible estimations for future climate. Mote and Salathé (2010) utilized the IPCC integrated models to determine warming and moisture projections for the PNW in the coming century. For temperature projections, Mote and Salathé (2010) averaged all 21 GCMs and radiate forcing scenarios together and compared projections to the average annual temperature from 1970-1999. Rates of warming per decade ranged from 0.1°C to 0.6°C across models. Mote and Salathé's (2010) projected changes in mean annual precipitation were not as extreme as temperature projections, only increasing 1% to 2% to 2080.

To evaluate winter and spring wheat production in eastern Washington under future climate scenarios in the coming century, Stöckle et al. (2010) used four global climate change models (GCCMs) and a cropping system and simulation model (CropSyst) to project crop development and yield. Across eastern Washington, future climate scenarios are expected to be favorable for winter wheat production with higher yields as a result of earlier crop maturity. The earlier crop maturity is anticipated to reduce the coincidence of flowering and grain fill and the projected higher summer temperatures and greater evapotranspiration that could reduce yields. Spring wheat production, in

contrast, is projected to reach critical flowering and grain fill physiological stages when higher temperatures and limited moisture can have detrimental impacts on grain yield (Stöckle et al. 2010).

1.2 Weed Response to Climate Change

There is high confidence in IPCC assessments of how crop yields will respond at a regional scale to rising temperatures, CO_2, and tropospheric O_3 (Smith et al. 2014). However, there is less confidence in the IPCC assessment in the response of agronomically important weeds and of their interaction with crops (Porter et al. 2014). The majority of published studies investigating weed response to climate change have focused on two main areas: plant competition between plants of different photosynthetic functional groups under conditions of increased temperature and CO_2 concentrations (Poorter and Navas 2003), and range shifts of weedy plant species on a regional scale (Peters et al. 2014). Juroszek and von Teidemann (2013) Concluded the majority of publications on weed response to climate change were impossible to generalize and therefore difficult for stakeholders to base decisions upon.

In order to project changes in weed response to climate change which will be relevant to land managers, projections need to be region specific. However, few papers have investigated the response of weeds to climate change in relation to a specific crop or region (Peters et al. 2014). Hanzlik and Gerowitt (2013) did an extensive survey of weed species distributed across Germany. When results were compared to surveys conducted in the 1970s, previously rare weeds had become more common. Winter annual weeds in particular had become common which Hanzlik and Gerowitt (2013) attributed to climate change and the increased frequency of wetter and milder winters.

With increased evapotranspiration rates and milder winters projected for the PNW, winter annual weeds are anticipated to be more successful, compared to spring annual weeds, under future climate scenarios (Concilio et al. 2013, Hanzlik and Gerowitt 2012, Peters et al. 2014) similar to what

has been projected for winter grains compared to spring grains (Ball et al. 1995, Stöckle et al. 2010). When predicting how winter annual weeds may respond to climate change, one of the more critical traits is plasticity in phenology and flowering time (Metcalf et al. 2003, Nicotra et al. 2010). One of the most common and competitive weeds in the PNW small grain production region is Downy brome (*Bromus tectorum* L.), the next section will focus on the biologic and ecologic attributes of downy brome and the species relationship to crop production and climate.

1.3 Downy Brome

Downy brome is an erect grass 5 to 60 cm tall, often growing in large tufts, with a few main stems and a finely divided fibrous root system (Upadhyaya et al. 1986). The leaf sheaths are light green and pubescent. Identifying characteristics of the *Bromus* genus present in *Bromus tectorum* include closed leaf sheaths and a drooping inflorescence (Morrow and Stahlman 1984). Downy brome panicles are dense and soft, and may be purple in color. Downy brome will change from a green to a purple color with cold temperatures, water stress, or as the plant matures (Morrow and Stahlman 1984). Purple coloration can be a useful heuristic to assess seed maturity. Hulbert (1955) found seed collected at the time purple coloration had started forming was viable despite ongoing maturation. Downy brome lemmas are 6.3 to 12.7 mm in length and caryopses are 6.3 to 9.5 mm in length. Glumes are covered in with short barbs that lead spikelets to attach to wool, hair, clothing, and other materials (Stewart and Hull 1949).

Downy brome is an invasive annual grass species naturalized across the United States. Downy brome typically behaves as a winter annual, but can germinate as a spring annual if conditions favor delayed germination. Rarely, downy brome has been reported to exhibit a biennial habit (Thill et al. 1984). Klemmedson and Smith (1964) reported that downy brome does not have very exacting habitat requirements. Downy brome can be found in locations ranging from 15 to 56 cm in annual precipitations, and at elevations as high as 2700 m (Morrow and Stahlman 1984).

Stewart and Hull (1949) described downy brome as a prolific immigrant. Native to Eurasia, genetic evidence suggests downy brome was introduced to North America through multiple immigrations (Novak et al. 1993). The first record of downy brome in the United States occurred in Pennsylvania in 1790 (Muhlenberg 1793). Although the initial finding of downy brome described the species as rare, by 1861 it was a commonly reported species in the Eastern United States (Stewart and Hull 1949). Expansion of downy brome into the west is largely thought to have occurred through movement of contaminated grain and hay. The distribution of downy brome was greatly increased by the depression of 1920 when abandoned wheat farms lead to "spectacular occupations" (Stewart and Hull 1949). Downy brome was first reported in Washington in 1893, Utah in 1894, Colorado in 1895, and Wyoming in 1900. While most of the expansion of brome in the West was accidental, it was deliberately sown at an experiment farm in Pullman, WA in 1898, and sold across the west as a "100 day forage grass" in 1915 (Upadhyaya et al. 1986). By comparing alleles using gel electrophoresis. Novak et al. (1993) were able to identify six unique founder events in the PNW between 1889 and1902. Downy brome currently exists in the western United States as one of the region's most abundant vascular plant species (Novak et al. 1993).

Stewart and Hull (1949) characterized downy brome as an opportunist easily acclimating to local climate due to rapid germination and establishment following variable fall or spring precipitation events. Mack and Pyke (1983) made similar observations during a two year study of demography of isolated populations. A single population can respond simultaneously at the same site with different monocarpic life history strategies (Mack and Pyke 1983). While establishment following initial fall moisture events in some years will result in a large die-off of downy brome if a prolonged dry period occurs after germination, most populations experience a second flush of germination in the spring (Mack and Pyke 1983). Plants that emerge in the fall have far greater fecundity than spring emerged downy brome (Mack and Pyke 1983, Stewart and Hull 1949).

In a study to predict the success of downy brome under conditions of simulated climate change Concilio et al. (2013) manipulated the level depth of winter snowpack through the use of snow fences to simulate projected climate change in the region. As less snow accumulated downy brome was increased in biomass and spikelet production. Snow fall was less influential on Downy brome growth and fecundity then the frequency of rainfall (Conciolio et al. 2013). Downy brome is anticipated to become more successful in regions that experience a shift in winter moisture occurring more frequently as rainfall rather than snow.

While generally described as overwintering in a dormant stage, root growth continues throughout the winter when temperatures are above 3°C (Stewart and Hull 1949). While commonly described as a shallow rooted annual, Hulbert (1955) found downy brome rooting depth exceeding 1.5 m with the majority of roots occupying the upper 20 to 30 cm, and lateral root growth spreading 30 cm. Downy brome can reduce soil moisture to the permanent wilting point to a depth of 0.7 m in natural stands, and 1.1 m in fertilized fields (Hulbert 1955). Because downy brome matures earlier than winter wheat, it often depletes soil moisture and nutrients when winter wheat is at a critical reproductive period (Thill et al. 1984).

Vigorous initial growth and early seed productions compared to other annual grasses allows establishment in disturbed natural areas and agronomic fields. Compared to development of winter wheat downy brome is generally in the early boot stage when wheat is tillering, heading four weeks earlier than wheat (Thill et al. 1984). In a three-year field experiment where downy brome was removed at monthly intervals from emergence to harvest grain, wheat yield was found to be most affected by downy brome competition after March (Rydrych 1974). A 40% reduction in yield was reported when downy brome was removed after March, but only a 6% reduction was recorded for removal prior to March.

Rydrych and Muzik (1968) reported 54 to 538 downy brome plants m^{-2} reduced wheat yield 28 to 92% compared to weed free plots. Rydrych (1974) reported densities of downy brome of 108 to 160 plants m^{-2} reduced winter wheat yield 21 to 47%. Challaiah et al. (1986) reported between 9 and 41% grain reduction among 10 winter wheat cultivars in a field trial at two locations in NE over the course of two years.

Prior to the introduction of herbicides for control of downy brome in winter wheat, burning of stubble followed by inversion tillage in the fall was the preferred method for management (Klemmedson and Smith 1964, Schillinger et al. 2010). Early chemical management options in winter wheat included atrazine alone or atrazine plus amitrole applied in fallow to reduce downy brome in the following winter wheat crop (Peeper 1984, Rydrych and Muzik 1968). The first herbicide to provide consistent control of downy brome within the winter wheat crop was trifluralin applied preplant incorporated (PPI) (Peeper 1984, Rydrych 1974), however poor incorporation or placement of trifluralin could lead to crop injury. The development of metribuzin (Peeper 1984) allowed for effective control without preplant incorporation, but limited crop rotations following the harvest of winter wheat. The arrival of diclofop on the market in the 1970s controlled downy brome applied PPI without the risk of crop injury observed with the use of trifluralin (Peeper 1984, Stahlman 1984). With the development and release of multiple sulfonylurea herbicides for downy brome control in winter wheat (Stahlman 1994), consistent suppression or control of downy brome can be achieved through post emergence applications in the spring or fall (Lawrence et al. 2014) with limited crop rotation restrictions.

1.3.1 Downy Brome Population Genetics. Novak et al. (1991) analyzed genetic variation among 2,141 individuals collected from 60 North American populations using 25 loci identified through gel electrophoresis. No heterozygous individuals were discovered and expected heterozygosity ranged from 0.001 to 0.050, with an average expected heterozygosity across all individuals of 0.012. Novak

et al. (1991) further grouped populations into four geographical regions. Geographical region only explained 2.6% of allelic diversity, while among population allelic diversity explained 45.3% of total diversity, and 52.2% of allelic diversity was accounted for within populations. Given that the majority of allelic variation is accounted for within the population level, that no outcrossing was observed, and considering the relatively short time frame downy brome has taken to colonize North America, Novak et al. (1991), concluded that pre-adapted "all-purpose genotypes" capable of taking advantage of disturbance flourished in North America soon after introduction.

Expanding on previous work, Novak and Mack (1993) compared genetic variation of 51 native populations of downy brome to the 60 introduced populations described in Novak et al. (1991). Inbreeding was as severe in the native range as in the introduced range despite the discovery of several heterozygous individuals in the native range (Novak and Mack 1993). Total genetic diversity across the entire native range is higher than the introduced range, but within population genetic diversity is greater in the introduced range. Novak et al. (1991) reported a limited number of generalist genotypes were found distributed widely across the introduced range. Novak and Mack (1993) were able to identify the source of the widely distributed genotypes in the introduced range as originating from Southern Europe and Southwest Asia however in the native range no genotypes were found to be widely distributed. Genetic differences between native and introduced ranges can be best explained by contrasting forces of reduced genetic variability produced through the founder effect and the increase in within-population variation due to independent introductions leading to mixed population of inbred individuals within the introduced range.

In an effort to trace the location of founder events within western North America, Novak et al. (1993) identified historic records of downy brome invasions prior to 1903. By comparing allelic variation of downy brome from historic sites to downy brome populations identified in Novak et al. (1991) and Novak and Mack (1993), Novak et al. (1993) identified unique introduction events

occurring at the turn of the 20th century at six locations: Cache Creek, B.C, Ritzville, WA, Juniper Flat NV, Emigrant Pass, NV, Dubios, ID, and Provo, UT.

Merrill et al. (2012) investigated microsatellite variation of downy brome populations collected from across the Mountain West. The degree of variation between populations was better explained by ecologic distance of habitat rather than geographical distance of populations. Ramakrishnan et al. (2006) concluded that natural selection on downy brome assemblages selected for similar inbred lines from similar habitats. Evidence of local adaptation from pre-adapted genotypes was also reported by Scott et al. (2010) utilizing microsatellites and populations sourced exclusively from a limited geographical region in western Utah. Across North America, downy brome is well established, but genetic variation is limited (Novak et al. 1991). By analyzing microsatellite variation of 1920 individuals from 96 locations spread across the western United States, Merrill et al. (2012) found 14 biotypes accounted for 79% of total individuals.

While many studies (Ashley and Longland 2009, Bartlett et al. 2002, Kao et al. 2008, Leger et al. 2009, Merrill et al. 2012, Novak et al. 1991, Novak and Mack 1993, 2001, Ramakrishnan et al. 2004, 2006, Scott et al. 2010) compared genetic variation between populations both in native and introduced regions, none have collected accessions from agronomic fields. Indeed much of the research focusing on downy brome variation is in the context of invasive biology rather than agronomy. Yet, genetic diversity of related species sourced from agronomic fields has been reported in the literature.

Green et al. (2001) compared diversity of the inbreeding annual or biennial weed *Bromus sterilis* L. (barren brome) between farms located in the United Kingdom. Similar to what has been reported for downy brome in western North America (Merrill et al. 2012, Scott et al. 2010), barren brome exists as an assemblage of unique but inbreed biotypes within agronomic fields. When low

genetic diversity was found within a field, Green et al. (2001) attributed diversity to selection of locally adapted inbred biotypes.

1.3.2 Variation in Downy Brome Development. In a series of common gardens containing geographically diverse collections of downy brome, Hulbert (1955) recorded variation in winter hardiness, plant size, shoot and root morphology, timing of development thresholds, seed production, and seed dormancy. Minor differences in plant height were described between accessions, however differences in phenology were described as "striking" (Hulbert 1955). The first appearance of panicles, the change to purple coloration, and the change to brown coloration (signaling senescence) were used to differentiate biotypes. The date of sowing, October 1st vs November 11th, had little influence on the timing of different phonological stages (Hulbert 1955). Comparing common garden data of the timing of phenological development from a study in Boise, ID to data reported from Lewiston, ID (Hulbert 1955), Klemmedson and Smith (1964) found considerable variation between common garden sites which was attributed to differences in genotype rather than climatic variation.

In a glasshouse study, Rice and Mack (1991a) evaluated variation of life history traits between populations, within populations, and among siblings. Variation in seed number, seed weight, and plant dry weight was found between siblings, within population, and between populations. Rice and Mack (1991a) interpreted high variation among siblings as evidence of plastic response to environmental influences. Flowering time was found to be highly stable among siblings and within populations, but variable between populations. Rice and Mack (1991a) concluded that variation in flowering time is an environmentally stable trait reflecting local adaptation between populations.

Rice and Mack (1991b) reciprocally sowed downy brome seed from four seed sources in a common garden to observe if the variation among siblings with respect to dry plant weight, seed

weight, seed number, and phenology would adapt to local climate. Seed number, seed weight, and plant weight did vary from year to year, which Rice and Mack (1991b) attributed to local adaptation of traits in response to environment. Flowering time, however, was stable year to year among plants from the same seed source (Rice and Mack 1991b). Rice and Mack (1991b) concluded the stability observed within flowering time was evidence of strong genetic controls, compared with other traits.

Ball et al. (2004) described a model for predicting mature seed set of downy brome based on growing degree days (GDD) starting January 1st and with a base of 0°C. The model was developed using a series of experiments in which panicles from downy brome accession were periodically collected following the first emergence of panicles. Following panicle collection, seeds were allowed to after-ripen for 6 mo to overcome dormancy. Seeds collected from panicles were planted in a greenhouse and the total number of germinated seeds from each panicle and each collection date was counted. Using a non-linear breakpoint analysis, seeds germinated per panicle were regressed against the GDD at each collection date to estimate the GDD required to produce a mature seed from each accession.

The first experiment conducted by Ball et al. (2004) collected panicles from two naturally occurring populations, one in Pullman, Washington and one in Pendleton, Oregon. The second experiment was expanded to four accessions collected within Eastern Oregon and Washington which were planted in the fall at a common garden in Pendleton, Oregon and Pullman, Washington. The final experiment consisted of downy brome collected from naturally occurring populations in Kansas, Oklahoma, Nebraska, Colorado, Montana, and Idaho. While considerable variation in mature seed set in the Western United States was found (582 to 1,287 GDD), little variation was observed in the time to produce mature seeds from accessions collected and grown in the PNW (983 to 1,151 GDD). Ball et al. (2004) concluded mature seed set of downy brome in the PNW could be predicted to occur around 1,000 GDD. The 20 y average (1984-2003) for all PNW

accessions fell on June 11th. Ball et al (2004) hypothesized that variation in flowering time may be regulated genetically by variation in vernalization requirements.

After collecting downy brome accessions from contrasting habitats, Meyer et al. (2004) examined vernalization response and requirement under greenhouse conditions. Treatments included vernalization for 0 to 10 weeks, and response variables included percent individuals flowering after 10 weeks and weeks required for vernalization following plant removal from vernalization (Meyer et al. 2004). Little variation was seen from sibling plants collected from the same maternal plant, however considerable variation was seen between seed sources. Plants collected from warm dry desert regions required little vernalization to flower and no difference was observed from increasing vernalization. However, plants collected from cold high altitude locations required longer vernalization periods, and the weeks required to induce flowering and the proportion of individuals flowering following the end of vernalization treatments increased with the length of vernalization. Meyer et al. (2004) concluded that vernalization response and requirements are adaptively significant and differences observed between seed sources reflect habitat-specific selection operating on an array of founder genotypes within a population

1.4 Vernalization Genetics in Model and Crop Species

Ball et al. (2004), Meyer et al. (2004), and Rice and Mack (1991b) have attributed variation in downy brome phenology to genetic variation in vernalization control. Vernalization can be defined as 'the acquisition or acceleration of the ability to flower by a chilling treatment' (Chouard 1960). Although no prior research has been conducted on the genetic controls of vernalization in downy brome, a great deal is known about the genetic control of vernalization in the cereals *Triticum aestivum* L. and *Hordeum vulgare* L, and the model species *Arabidopsis thaliana* (L.) Heynh. and *Brachypodium distachyon* (L.) P. Beauv.

The genetic control of flowering in the model species *A. thaliana* involve five different pathways; the vernalization pathway, the ambient temperature pathway, the photoperiod pathways, the gibberellic acid (GA) pathway, and an autonomous pathway; and dozens of genes (Srikanth and Schmid 2011). Not all *A. thaliana* biotypes require vernalization to flower, and by comparing genetic variation of winter and spring annual biotypes two genes: *Frigida (FRI)* and *Flowering Locus C (FLC)* were identified as conferring winter annual growth types. *FRI* upregulates *FLC*, which is a suppressor of certain flowering time genes (Geraldo et al. 2009, Johanson et al. 2000, Koornneef et al. 1994, Lee et al. 1994). In biotypes of *A. thaliana* that do not require vernalization, *FRI* and *FLC* have undergone a loss of function mutations, indicating that a winter annual life history is the ancestral state of *A. thaliana* (Johanson et al. 2000).

Additional genes influence the expression or repression of *FLC*, including *Vernalization 1* (*VRN1*), *Vernalization 2* (*VRN2*), and *Vernalization Insensitive 3* (*VIN 3*). *VIN 3* suppresses *FLC* when initial cold exposure occurs, however when temperatures later increase *VIN 3* no longer suppresses *FLC* (Sung and Amasino 2004). *VRN1* and *VRN2* do not initially suppress *FLC* following cold treatments, but do provide long term suppression of *FLC* following a vernalization period and a return to normal temperatures (Gendall et al. 2001, Levy et al. 2002).

In *A. thaliana*, the vernalization and ambient temperature flowering pathways are expressed within the leaf tissues, whereas other flowering pathways are expressed in the meristem. The process of flowering is a whole plant response and flowering signals ultimately need to be expressed throughout different plant tissues. The main gene responsible for the integration of multiple flowering pathways and long distance signaling of flowering is *Flowering Locus T* (*FT*). *FT* is the main gene repressed by *FLC*, and *FT* is expressed following long days by the photoperiod pathway. While initial expression of *FT* occurs in the leaves, *FT* is transported to the meristems where it interacts with proteins from the GA signaling pathway and the autonomous pathway to signal *Apetala1* (*AP1*)

expression. *AP1* expression marks the beginning of floral organ expression in *A. thaliana* (Srikanth and Schmid 2011).

The flowering pathways of temperature cereals, including wheat and barley, share similarities with *A. thaliana*, however there are distinct differences (Greenup et al. 2009). A homologue of *FT* has been identified in the temperature cereals (Yan et al. 2006). The *FT* homologue, *FT like 1* (*FT1*) integrates the vernalization and photoperiod pathway in the cereals and is responsible for long distance signaling of flowering (Greenup et al. 2009). However, no homologous genes of *A. thaliana FLC* genes have been found in temperate grass species, indicating that a requirement for vernalization to flower across diverse taxa may be a result of convergent evolution (Srikanth and Schmid 2011).

In wheat and barley, vernalization is regulated by the genes *VRN1* and *VRN2*. *VRN2* suppresses *FT1* in the presence of long days (Hemming et al. 2008, Takahashi and Yasuda 1971). *VRN1* is activated with vernalization and both upregulates *FT1* and down regulates *VRN2* (Hemming et al. 2008, Trevaskis et al. 2006). *VRN1* mediates flowering through two mechanisms (1) acceleration of the transition to reproductive growth at the shoot apex (2) activation of the long day response in the leaves (Greenup et al. 2009). Spring varieties of wheat and barley that do not require vernalization to flower are the result of a mutation in the *VRN1* promoter or because of deletion or insertion within the first intron of *VRN1* (Cockram et al. 2007, Fu et al. 2005, Szucs et al. 2007, Von Zitzewitz et al. 2005, Yan et al. 2003). In wheat most natural variation in vernalization requirements can be attributed to variation in VRN1 gene (Chen and Dubcovsky 2012).

Similar to wheat and barley, *B. distachyon* contains a homologous gene to *A. thaliana FT*, and a homologous gene to wheat and barley *VRN1*. However, no homologous gene of wheat or barley *VRN2* has been discovered (Higgins et al. 2010). A *VRN2 Like* (*VRN2L*) gene has been identified in *B. distachyon* and is thought to integrate vernalization and photoperiod response, however

BdVRN2L behaves in a dissimilar way from wheat or barley *VRN2* (Ream et al. 2014). In wheat and barley *VRN2* expression decreases sharply following a vernalization treatment, while in *B. distachyon VRN2L* mRNA levels do not drop following the vernalization and more work is needed to characterize the role of *VRN2L* in *B. distachyon* (Ream et al. 2012, 2014).

Although little is known regarding the genetic controls of vernalization in downy brome, inferences may be made based on the relatedness of downy brome with wheat, barley, and *B. distachyon*. All four species are within the subfamily Pooideae. Wheat barley and downy brome additionally all belong to the supertribe Triticodae (Soreng et al. 2015). Comparing known vernalization gene sequences between wheat, barley, and *B. distachyon* greater than 80% homology is found. The close relatedness and high homology may allow for downy brome vernalization genes to be identified and sequenced by developing primers from related species.

1.5 A Need for Applied Research on Weed Response to Climate Change

The IPCC identified a need for more research focusing on the response of agronomically important weeds to climate (Porter et al. 2014). Peters et al. (2014) outlined three possible scales at which weeds respond to climate change: shifts of a weed species range on a landscape scale, shifts in the niche occupied by species at a community scale, and shifts in traits at a species scales. Of these three scales, shifts in niches within communities and traits within species are likely to have the largest impact on weed management in agronomic systems under future climate scenarios. The majority of published research does not focus on shifts in niches or traits and instead the focus has been on projecting range shifts of invasive weeds. If Weed Science as a discipline is to help producers adapt to climate change, more applied research that is region and crop specific must be conducted.

Hanzlik and Gerowitt (2012) described an increase in the abundance of winter annuals in response to climate change within German rapeseed fields, likely due to mild winters becoming

more common. As the PNW is expected to also experience more mild winters in the coming decades (Mote and Salathé 2010) and given downy brome has already demonstrated greater fecundity following milder winters (Concilio et al. 2013) the niche occupied by downy brome within PNW small grain fields will likely expand in the coming decades. Peters et al. (2014) identified plasticity in the timing of germination and flowering as two traits that may predict the success of species in adapting to climate change. Rice and Mack (1991a, 1991b) and Meyer et al. (2004) have observed specificity of downy brome flowering time between contrasting habitats as a result of selection. Adaptability of flowering time in response to novel habitats is evidence for plasticity of flowering time traits. Given that the molecular controls for flowering time of species related to downy brome are well characterized (Greenup et al. 2009), there is opportunity to investigate the capacity of downy brome flowering time to shift in response to climate change.

The potential response of downy brome to climate change within the small grain production region of the PNW can serve as a case study for the type of research that has been called for in the literature (Peters et al. 2014; Porter et al. 2014; Juroszek and von Teidemann 2013). Understanding how the niche downy brome currently occupies within small grain fields may shift under future climate scenarios will help inform the management practices of PNW producers in the communing decades. Modeling a shift in downy brome development time traits under future climate scenarios may allow growers to adapt control practices in response as climate changes by changing the timing or type of control inputs. Projecting downy brome response to climate change within the PNW may serve as an example of how prior research on weed biology and knowledge of region specific climate projections can be leveraged to benefit producers in the coming decades.

1.6 Literature Cited

Abatzoglou JT, Rupp DE, Mote PW (2014) Seasonal climate variability and change in the pacific northwest of the united states. J Clim 27:2125–2142

Ashley MC, Longland WS (2009) Assessing cheatgrass (*Bromus tectorum*) genetic diversity and population structure using RAPD and microsatellite molecular markers. West North Am Nat 69:63–74

Ball DA, Frost SM, Gitelman AI (2004) Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. Weed Sci 52:518–524

Ball DA, Klepper B, Rydrych DJ (1995) Comparative above-ground development rates for several annual grass weeds and cereal grains. Weed Sci 43:410–416

Bartlett E, Novak SJ, Mack RN (2002) Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. Am J Bot 89:602–12

Challaiah, Burnside OC, Wicks GA, Johnson VA (1986) Competition between Winter Wheat (*Triticum aestivum*) Cultivars and Downy Brome (*Bromus tectorum*). Weed Sci 32:689–693

Chen A, Dubcovsky J (2012) Wheat TILLING Mutants Show That the Vernalization Gene VRN1 Down-Regulates the Flowering Repressor VRN2 in Leaves but Is Not Essential for Flowering. PLoS Genet 8:1–13

Chouard P (1960) Vernalization and its Relations to Dormancy. Annu Rev Plant Physiol 11:191–238

Cockram J, Chiapparino E, Taylor SA, Stamati K, Donini P, Laurie DA O'Sullivan DM (2007) Haplotype analysis of vernalization loci in European barley germplasm reveals novel VRN-H1 alleles and a predominant winter VRN-H1/VRN-H2 multi-locus haplotype. Theor Appl Genet 115:993–1001

Concilio AL, Loik ME, Belnap J (2013) Global change effects on *Bromus tectorum* L. (Poaceae) at its high-elevation range margin. Glob Chang Biol 19:161–172

Field CB, Barros VR, Mach KJ, Mastrandrea MD, van Aalst M, Adger WN, Arent DJ, Barnett J, Betts R, Bilir TE, Birkmann J, Carmin J, Chadee DD, Challinor AJ, Chatterjee M, Cramer W, Davidson DJ, Estrada YO, Gattuso J-P, Hijioka Y, Hoegh-Guldberg O, Huang H-Q, Insarov GE, Jones RN, Kovats RS, Romero Lankao P, Larsen JN, Losada IJ, Marengo JA, McLean RF, Mearns LO, Mechler R, Morton JF, Niang I, Oki T, Olwoch JM, Opondo M, Poloczanska ES, Pörtner H-O, Redsteer MH, Reisinger A, Revi A, Schmidt DN, Shaw MR, Solecki W, Stone DA, Stone JMR, Strzepek KM, Suarez AG, Tschakert P, Valentini R, Vicuña S, Villamizar A, Vincent KE, Warren R, White LL, Wilbanks TJ, Wong PP, Yohe GW (2014) Technical Summary. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. 35-94 p

Fu D, Szucs P, Yan L, Helguera M, Skinner JS, Von Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletions within the first intron in VRN-1 are associated with spring growth habit in barley and wheat. Mol Genet Genomics 273:54–65

Gendall AR, Levy YY, Wilson A, Dean C (2001) The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell 107:525–535

Geraldo N, Bäurle I, Kidou S-I, Hu X, Dean C (2009) FRIGIDA delays flowering in Arabidopsis via a cotranscriptional mechanism involving direct interaction with the nuclear cap-binding complex. Plant Physiol 150:1611–1618

Green JM, Barker JH, Marshall EJ, Froud-Williams RJ, Peters NC, Arnold GM, Dawson K, Karp A (2001) Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within- and between-farm scales. Mol Ecol 10:1035–45

Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Ann Bot 103:1165–1172

Hanzlik K, Gerowitt B (2012) Occurrence and distribution of important weed species in German winter oilseed rape fields. J Plant Dis Prot 119:107–120

Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol 147:355–366

Higgins JA, Bailey PC, Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate Grasses. PLoS One 5

Huggins D, Rupp R, Gessler P, Pan W, Brown D, Machado S, Abatzoglou J, Walden V, Eigenbrode S (2012) Dynamic Agroecological Zones for the Inland Pacific Northwest,. *in* American Society of Agronomy Annual Meeting, Oct. 21-24, Cincinnati, OH

Hulbert L (1955) Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecol Monogr 25:181–213

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C (2000) Molecular analysis of FRIGIDA, a major determinant of natural variation in Arabidopsis flowering time. Science 290:344–347

Juroszek P, von Tiedmann A. (2013) Plant pathogens, insect pests and weeds in a changing global climate: a review of approaches, challenges, research gaps, key studies and concepts. J Agr Sci. 151:163-188

Kang Y, Khan S, Ma X (2009) Climate change impacts on crop yield, crop water productivity and food security - A review. Prog Nat Sci 19:1665–1674

Kao RH, Brown CS, Hufbauer RA (2008) High phenotypic and molecular variation in downy brome (*Bromus tectorum*). Invasive Plant Sci Manag 1:216–225

Klemmedson JO, Smith JG (1964) Cheatgrass (*Bromus Tectorum* L.). Bot Rev 30:226–262

Koornneef M, Vries H, Hanhart C (1994) The phenotype of some late flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg erecta wild-type. Plant J 6(6):911–919

Lawrence NC, Burke IC, Yenish JP (2014) 15 Years of Downy Brome Control in Eastern Washington. Pages 12–13 in Proceedings of the 67th Annual Meeting of the WSWS, Colorado Spring, CO: Western Society of Weed Science

Lee I, Aukerman MJ, Gore SL, Lohman KN, Michaels SD, Weaver LM, John MC, Feldmann KA, Amasino RM (1994) Isolation of LUMINIDEPENDENS : A gene involved in the control of flowering time in Arabidopsis 6:75–83

Leger EA, Espeland EK, Merrill KR, Meyer SE (2009) Genetic variation and local adaptation at a cheatgrass (*Bromus tectorum*) invasion edge in western Nevada. Mol Ecol 18:4366–4379

Levy YY, Mesnage S, Mylne JS, Gendall AR, Dean C (2002) Multiple roles of Arabidopsis VRN1 in vernalization and flowering time control. Science 297:243–246

Mack R, Pyke D (1983) The demography of *Bromus tectorum*: variation in time and space. J Ecol 71:69–93

Merrill KR, Meyer SE, Coleman CE (2012) Population genetic analysis of *Bromus tectorum* (Poaceae) indicates recent range expansion may be facilitated by specialist genotypes. Am J Bot 99:529–37

Metcalf JC, Rose KE, Rees M (2003) Evolutionary demography of monocarpic perennials. Trends Ecol Evol 18:471–480

Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). Ann Bot 93:653–63

Morrow LA, Stahlman PW (1984) The History and Distribution of Downy Brome (*Bromus tectorum*) in North America. Weed Sci Soc Am 32:2–6

Mote PW, Salathé EP (2010) Future climate in the Pacific Northwest. Clim Change 102:29–50

Muhlenberg H (1793) Index florae Lancastriensis. Trans Am Philos Soc Philadelphia 3:157–184

Nicotra a. B, Atkin OK, Bonser SP, Davidson a. M, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692

Novak S, Mack R, Soltis D (1991) Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. Am J Bot 78:1150–1161

Novak S, Mack R, Soltis P (1993) Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. Can J Bot 71:1441–1448

Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* introduced populations. Heredity (Edinb) 71:167–176

Novak SJ, Mack RN (2001) Tracing Plant Introduction and Spread: Genetic Evidence from Bromus tectorum (Cheatgrass). Bioscience 51:114

Peeper TF (1984) Chemical and Biological Control of Downy Brome (*Bromus tectorum*) in Wheat and Alfalfa in North America. Weed Sci 32:18–25

Peters K, Breitsameter L, Gerowitt B (2014) Impact of climate change on weeds in agriculture: a review. Agron Sustain Dev 34:707–721

Poorter H and Navas M (2003) Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytol 157:175-198

Porter JR, Xie L, Challinor AJ, Cochrane K, Howden SM, Iqbal MM, Lobell DB, Travasso MI, (2014) Food security and food production systems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the *Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, and White

LL (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 485-533.

Ramakrishnan A, Meyer S, Waters J, R SM, Coleman CE, Fairbanks DJ (2004) Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae), an inbreeding annual grass. Am J Bot 91:797–803

Ramakrishnan AP, Meyer SE, Fairbanks DJ, Coleman CE (2006) Ecological significance of microsatellite variation in western North American populations of Bromus tectorum. Plant Species Biol 21:61–73

Ream TS, Woods DP, Amasino RM, Eam TSR, Oods DPW, Masino RM a (2012) The molecular basis of vernalization in different plant groups the molecular basis of vernalization in different plant groups. Cold Spring Hrb Symp Quant Biol 77:105–115

Ream TS, Woods DP, Schwartz CJ, Sanabria CP, Mahoy JA, Walters EM, Kaeppler HF, Amasino RM (2014) Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon*. Plant Physiol 164:694–709

Rice K, Mack R (1991a) Ecological genetics of *Bromus tectorum*. I. A hierarchical analysis of phenotypic variation. Oecologia 88:77–83

Rice KJ, Mack RN (1991b) Ecological Genetics of *Bromus tectorum*. III. The Demography of Reciprocally Sown Populations. Oecologia 88:91–101

Rydrych D, Muzik T (1968) Downy brome competition and control in dryland wheat. Agron J 60:279–280

Rydrych DJ (1974) Competition between winter wheat and downy brome. Weed Sci 22:211–214

Schillinger WF, Young DL, Kennedy AC, Paulitz TC (2010) Diverse no-till irrigated crop rotations instead of burning and plowing continuous wheat. Fld Crop Res 115:39–49

Scott JW, Meyer SE, Merrill KR, Anderson VJ (2010) Local population differentiation in *Bromus tectorum* L. in relation to habitat-specific selection regimes. Evol Ecol 24:1061–1080

Smith P, Bustamante M, Ahammad H, Clark H, Dong H, Elsiddig EA, Haberl H, Harper R, House J, Jafari M, Masera O, Mbow C, Ravindranath NH, Rice CW, Robledo Abad C, Romanovskaya A, Sperling F, Tubiello F, (2014) Agriculture, Forestry and Other Land Use (AFOLU). In: Climate Change 2014: *Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, Adler A, Baum I, Brunner S, Eickemeier P, Kriemann B, Savolainen J, Schlömer S, von Stechow C, Zwickel T, Minx JC (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA

Solomon S, Qin D, Manning M, Alley RB, Berntsen T, Bindoff NL, Chen Z, Chidthaisong A, Gregory JM, Hegerl GC, Heimann M, Hewitson B, Hoskins BJ, Joos F, Jouzel J, Kattsov V, Lohmann U, Matsuno T, Molina M, Nicholls N, Overpeck J, Raga G, Ramaswamy V, Ren J, Rusticucci M, Somerville R, Stocker TF, Whetton P, Wood D, Wratt RA(2007) Technical Summary. Climate Change 2007 The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 23-78 p

Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O (2015) A worldwide phylogenetic classification of the Poaceae (Gramineae). J Syst Evol 53:117–137

Srikanth A, Schmid M (2011) Regulation of flowering time: All roads lead to Rome. Cell Mol Life Sci 68:2013–2037

Stahlman P (1984) Downy brome (*Bromus tectorum*) control with diclofop in winter wheat (*Triticum aestivum*). Weed Sci 32:59–62

Stahlman PW (1994) Sulfonylurea Herbicides Suppress Downy Brome (Bromus tectorum) in Winter Wheat (Triticum aestivum). Weed Technol 8:812–818

Stewart G, Hull A (1949) Cheatgrass (*Bromus tectorum* L .) --An ecologic intruder in southern Idaho .Ecology 30:58–74

Stöckle CO, Nelson RL, Higgins S, Brunner J, Grove G, Boydston R, Whiting M, Kruger C (2010) Assessment of climate change impact on eastern Washington agriculture. Clim Change 102:77–102

Sung S, Amasino RM (2004) Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. Nature 427:159–164

Szucs P, Skinner JS, Karsai I, Cuesta-Marcos A, Haggard KG, Corey AE, Chen THH, Hayes PM (2007) Validation of the VRN-H2/VRN-H1 epistatic model in barley reveals that intron length variation in VRN-H1 may account for a continuum of vernalization sensitivity. Mol Genet Genomics 277:249–261

Takahashi R, Yasuda S (1971) Genetics of earliness and growth habit in barley. Pages 388–408 *in* RA Nilan, ed. Proceedings of the Second International Barley Genetics Symposium. Pullman, WA, Washington State University Press.

Thill DC, Beck KG, Callihan RH (1984) The biology of downy brome (*Bromus tectorum*)Weed Sci 32:7–12

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) HvVRN2 responds to daylength, whereas HvVRN1 is regulated by vernalization and developmental status. Plant Physiol 140:1397–1405

Tubiello FN, Soussana JF, Howden SM (2007) Crop and pasture response to climate change. Proc Natl Acad Sci USA 104:19686–19690

Upadhyaya M, Turkington R, McIlvride D (1986) The biology of Canadian weeds: 75. *Bromus tectorum* L. Can J Bot 66:689–709

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci U S A 103:19581–19586

Yan L, Loukoianov a, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci U S A 100:6263–6268

von Zitzewitz J, Szucs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, Casas A, Chen THH, Hayes PM, Skinner JS (2005) Molecular and structural characterization of barley vernalization genes. Plant Mol Biol 59:449–467

Chapter 2: Population Genetics and Structure of *Bromus tectorum* from within the small grain production region of the Pacific Northwest.

2.1 Previous work on downy brome population genetics

Downy brome (*Bromus tectorum* L.) is an invasive winter annual grass and cleistogamous species naturalized across the United States. The first record of downy brome in the United States occurred in Pennsylvania in 1790 (Muhlenberg 1793). By the end of the 19th century downy brome had expanded throughout the western United States (Upadhyaya et al. 1986). Capable of thriving in a wide range of habitats, downy brome readily establishes in novel locations following disturbance (Morrow and Stahlman 1984). Downy brome currently exists in the western United States as one of the region's most abundant vascular plant species (Novak et al. 1993).

A number of previous studies have investigated downy brome population genetics in the context of downy brome as an ecological invader. Consequently, previous research has focused on the predominance of generalist versus specialist genotypes across the landscape (Novak et al. 1991b), genetic differences between native and invasive populations (Novak et al. 1993, Novak and Mack 1993) and evidence of local adaption to ecosystems (Leger et al. 2009, Merrill et al. 2012, Scott et al. 2010).

Novak et al. (1991) reported that a limited number of generalist genotypes were found distributed widely across North America when using 21 loci and starch gel electrophoresis. Within the introduced range, geographical region only explained 2.6% of allelic diversity, while among population allelic diversity explained 45.3% of total diversity, and 52.2% of allelic diversity was accounted for within populations (Novak et al. 1991). Novak et al (1991) concluded that downy brome often persists as inbred populations with limited evidence for spatial adaptation or outcrossing.

Novak and Mack (1993) compared genetic variation of 51 native populations of downy brome to the 60 introduced populations described by Novak et al. (1991). Total genetic diversity across the entirety of the native range was higher than the introduced range, however, within population genetic diversity was greater in the introduced range. Genetic differences between native and introduced ranges can be best explained by contrasting forces of reduced genetic variability produced through the founder effect and the increase in within-population variation due to independent introductions leading to mixed populations of inbred individuals within the introduced range (Novak and Mack 1993).

While widely spread genotypes across the introduced range can be attributed to generalist biotypes, evidence for local adaptation by specialist biotypes has been reported within the literature. Ball et al. (2004), Meyer et al. (2004), Rice and Mack (1991a, 1991b) described adaptively significant variation in downy brome life history traits including flowering time, vernalization requirements, and timing of mature seed set. Ramakrishnan et al. (2006) found that ecological distance better predicted genetic distance of populations than physical distance, indicating that similar habitats select self-pollinating lines from widely distributed downy brome assemblages. Scott et al. (2010) described evidence of downy brome invading new habitats as both broadly adapted generalist genotypes and preadapted specialist genotypes. Merrill et al. (2012) came to a similar conclusion, finding historically invaded land was largely occupied by generalist genotypes, while recently invaded land was dominated by distinct specialist genotypes.

Previous studies have identified low heterozygosity, with mean expected heterozygosity ranging from 0.002 (Bartlett et al. 2002) to 0.336 (Meyer et al. 2013) per populations. While heterozygous individuals have reported in the literature (Leger et al. 2009, Meyer et al. 2013, Novak and Mack 1993, Valliant et al. 2007) out crossing is exceedingly rare. Meyer et al. (2013) established a

common garden experiment to encourage and quantify outcrossing and detected outcrossing rates of 0.75%.

Despite the wealth of information regarding downy brome population genetics, previous studies have not focused on, or made comparisons of downy brome genetics as it persist in individual agroecosystems. The lack of downy brome population genetic studies within agroecosystems is significant given downy brome is a widely distributed and serious pest in small grain and other crops across western North America. Additionally, the yearly disturbance of tillage, planting, and herbicide applications may drive selection on downy brome genotypes differently from the forces acting in non-agronomic ecosystems. While downy brome population genetics has not been investigated within agroecosytems, Green et al. (2001) compared diversity of the inbreeding annual or biennial weed *Bromus sterilis* L. (barren brome) between farms located in the United Kingdom. Similar to what has been reported with downy brome in western North America (Merrill et al. 2012, Scott et al. 2010), barren brome exists as an assemblage of unique but inbreed biotypes within agronomic fields. When low genetic diversity was found within a field, Green et al. (2001) attributed diversity to selection of locally adapted inbred biotypes.

Previous investigations of downy brome population genetics has been conducted using protein electrophoresis, Random Amplified Polymorphic DNA (RAPD) markers, Single Sequence Repeats (SSR) markers; and a single manuscript utilizing Single Nucleotide Polymorphisms (SNPs) markers (Meyer et al. 2013). Recent studies on downy brome population genetics predominately utilize SSR markers, however Merrill et al. (2012) found that SSR markers may overestimate common descent and suggest SNPs as a superior alternative. In comparing the performance of SSR and SNP markers in population structure analysis, Liu et al. (2005) reported that when comparing a large number of markers, SNPs tend to include the more informative genomic regions as they include a greater ratio of non-neutral to neutral sites. Through high-throughput next-generation-

sequencing (NGS) thousands of SNPs can be "called" from species lacking in references genomes (Ekblom and Galindo 2011), such as downy brome, allowing for the generation of population genetic statistics and structure analysis.

2.1.1 Small Grain Production Region of the PNW. The small grain production area of the PNW includes Central and Eastern Washington, parts of Northern Oregon, and Northern Idaho and totals 2.2 million ha of non-irrigated cropland (Huggins et al. 2012) (Figure 1). In 2007 1.3 million ha of the small grain production region were planted with small grains of which 1.0 million ha was planted to winter wheat (*Triticum aestivum* L.). Agronomic fields are continuously distributed throughout the small grain production region of PNW. Additionally, downy brome is endemic within fields in the region. Annual precipitation in the region ranges from less than 300 mm to greater than 600 mm, with precipitation increasing on a west to east gradient. Mean annual temperature also varies on an west to east gradient, with the western portion at 11ºC and decreasing to 5 ºC to the east based on a 30 year average on (1971-2000) (Huggins et al. 2012). In addition to a continuous gradient in temperature and precipitation, the region is not characterized by any natural or manmade barriers to seed or gene flow. It is therefore difficult to assign likely population boundaries prior to conducting a genetic analysis.

The objectives of this study were to assess the genetic variability of downy brome sourced exclusively from within small grain production regions of the Pacific Northwest (PNW). Population genetics metrics were calculated and population structure estimated using a genotyping-by-sequencing (GBS) approach. Of particular interest is if downy brome persists within the small grain production regions of the PNW as specialist or generalist genotypes, and if downy brome genotype distribution is driven by climatic factors or grower practices.

2.2 Materials and Methods

2.2.1 Sampling of Plant Materials. Given the ubiquitous distribution of downy brome within the PNW, continuous environmental gradients, and lack of landscape features to block gene flow a systematic-random sampling design (Storfer et al. 2007) was used where a 10km grid was laid over the small grain production region and a point was randomly assigned for sampling within each grid. One hundred and ninety total sampling points were generated and then viewed using satellite images. If the sampling point had not been assigned to a small grain field, the sampling point was moved to the nearest small grain field. If there was no small grain field within 3 km of the original sampling point, the location was discarded. Following reassignment of the original sampling locations, 130 sampling locations were retained.

In June of 2010 and 2011, trips were made to each of the 130 reassigned sampling locations from which collections from a single downy brome plant was collected as either mature panicles or as live plants. Each collection was made within a small grain field at least 10 m from the field border. Live plants were transplanted into a greenhouse at the Washington State University Plant Growth Facility located in Pullman, WA and allowed to grow until mature panicles could be collected. Panicles that were collected either directly from the field or from maturing plants in the greenhouse were stored at room temperature in a paper bag until 1 March 2014 at which time panicles were planted to produce plants for DNA extraction.

On 21 March 2014, as plants were at the 2-3 leaf stage, a single ~4cm leaf was collected from 96 (Table 1) of the 130 emerged downy brome collections for DNA extraction. OA related species to downy brome, *Bromus diandrus* Roth (ripgut brome), was included as a check to see if population structure analysis could detect the related species as an outlier. Collected leaf tissue was placed into -80°C freezer for 24 hours and then lyophilised in a Multi-Dry™ (FTS® Systems INC., Stone Ridge, NY) freeze dryer for 48 hours. DNA was extracted using a BioSprint 96 Plant Kit and

BioSprint 96 workstation (Qiagen, Valencia, CA). DNA was quantified with the PicoGreen® assay (Invitrogen™, Carlsbad, CA) using a Synergy™ HT (BioTek®, Winooski, VT) microplate reader.

2.2.2 Genotype-by-Sequencing.

A reduced representation genotype-by-sequencing (GBS) approach was employed to identifying SNP molecular markers. The GBS approach used in this study relied upon a combination of a regular and a methylation-sensitive restriction enzyme to avoid repetitive regions of a target genome and instead favor lower copy regions in order to chance of identifying informative genetic markers (Elshire et al. 2011). A two-enzyme approach originally developed for species lacking a reference genome (Poland et al. 2012) was utilized, where a rare (*Pst1,* CTGCA^G) and common (*Msp1,* C^CGG) restriction enzyme were used in combination. A modified GBS protocol developed by Mascher et al. (2013) for use with semi-conductor sequencing platform was followed.

DNA was normalized to 10 ng μl^{-1} in 20 µl of volume in 96-well plates. Restriction digest buffer (NEB4) was added along with both restriction enzymes and samples were incubated at 37°C for 2 hours to digest the DNA. Samples were incubated at 65°C for 2 hours to inactive the enzymes. A unique adapter with an embedded sequence barcode at a volume of 0.1 pmol was added to each sample followed by 15 pmol of a common adapter. A ligation master mix was added to each well and samples were incubated for 2 hours at 22°C, followed by incubation at 65°C for 20 minutes. After these steps samples where pooled and purified with a PCR purification kit (Qiagen, Valencia, CA) prior to PCR. PCR was performed using 5 µl of the PCR product as template, 5 µl of NEB 5x master mix, 2 µl of Ion PCR forward and reverse primers at 10 µM, and 13 ul of H_20. The resulting amplicons were sequenced on an Ion Proton™ sequencer using an Ion P1™ Chip (Life Technologies, Carlsbad, CA).

The master FASTQ file obtained from the sequencer was parsed by barcode to separate each accession into a separate file. FASTQ file size ranged from 5 to 112 MBs with an average size of

45.6 MBs. Three accessions that had less than 10 Mb of data were discarded due to lack of adequate coverage. The first 8 DNA bases of each sequence were removed using the FASTX-Toolkit (0.0.13, Hannon Laboratory, Cold Spring Harbor, NY) to rid sequences of cut site remnants. Data were then checked using the program FastQC (0.11.3, Babraham Bioinformatics, Babraham, Cambridgeshire, UK) to determine the ideal length (Supplemental Figure 1) to trim sequences for SNP calling and assess quality (Supplemental Figure 2-6). Average sequence length was 100 bp and all sequences shorter than 100 bp were subsequently discarded while sequences longer than 100 bp were trimmed using FASTX to provide a uniform sequence length.

2.2.3 SNP Calling. After trimming sequences to a uniform length, SNP calling was conducted using the program Stacks (1.22, Cresko Laboratory, Eugene, OR). The Stacks program aligns identical or, nearly identical, loci into "stacks" across individuals and a catalogue file is written. Each locus from each individual is matched against the catalog to determine the allelic state at each locus in each individual (Catchen et al. 2013). Using the "denvo_map.pl" command a minimum sequencing depth of three was used for creating a stack and with three allowed nucleotide mismatches before merging multiple loci into a single stack. In total, 16,382 SNPs were called across all individuals. Comparisons were then made between each individual using the "population" command in stacks. Each locus had to be found in at least 70 of the 93 individuals (75% of individuals) for further analysis, reducing the total remaining SNPs to 384.

2.2.4 Analysis of Population Genetics, Spatial Structure, and Population Structure. The output from stacks was converted to a "genid" object using the R (R Development Core Team 2014) package adegenet (1.42, Jombart et al.) (Jombart and Ahmed 2011, Jombart 2008) for further analysis. Heterozygosity was investigated across all loci and individuals and a t-test was used to determine if observed heterozygosity (H_{OBS}) differed from expected heterozygosity (Hexp).

Spatial principal component analysis (sPCA) was conducted using the R package adegenet package to identify positive and negative autocorrelation, global and local structure, and to identify genetic clines (Jombart et al. 2008). A Gabriel Graph (Gabriel and Sokal 1969, Legendre and Fortin 1989) connection network was used to develop a spatial weighting matrix based upon the GPS coordinates of each individual. sPCA calculates the variance and spatial structure of each allele using the spatial weighting matrix and Moran's *I* (Moran 1950). Each allele is assigned either a positive or negative eigenvalue, corresponding to global or local structure. Eigenvalues which deviate the greatest from zero are more likely to be significant. Principal components with the largest absolute eigenvalues were retained and tested for significance using a Monte-Carlo simulation.

The positive and negative autocorrelation values of retained PCs that were found to be significant were mapped over the small grain production region of the PNW to aid in visually identifying spatial patterns and genetic clines. To formally identify genetic clines, Monmonier's algorithm, a statistical method for identifying boundaries of maximum differences in continuous polygons, was used to determine a boundary between neighbors reflecting strong genetic differences. The Monmonier's algorithm utilizes the same special weighting matrix used in sPCA (Manni et al. 2004, Monmonier 1973).

Using the adegenet package, Discriminate Analysis of Principal Components (DAPC) (Jombart et al. 2010) was used to describe population structure of collected downy brome accessions. DAPC consists of two general steps. Principal Component Analysis (PCA) is first used to find the optimal number of clusters (*k*) and to initially assign individuals to each cluster. In the second step synthetic variables called linear discriminants, consisting of linear combinations of alleles, are used to discriminate the cluster membership of each individual. If $T(x)$ is total genetic variation, then $B(x)$ is between group variation, and $W(x)$ is within group variation (equation 1). PCA maximizes $T(x)$ to determine the optimal number of clusters and initial group membership.

DAPC optimizes $B(x)$ and minimizes $W(x)$ while ignoring $T(x)$ to assign the probability of group membership for each individual.

$$T(x) = B(x) + W(x) \quad [1]$$

After retaining the principal components which explain the majority of $T(x)$, the optimal number of clusters is chosen based upon which level of k has the lowest Bayesian Information Criteria (BIC) value. Using the adegenet package an a-score, identifying the optimal number of principal components to retain for DAPC, was calculated by running multiple DAPC simulations with differing number of retained principal components. The a-score is useful as retaining too many principal components with DAPC can result in over fitting and poor discrimination. After selecting the number of principal components to retain, linear discriminants are created and assigned an f-statistic corresponding to the relative power of each linear discriminant in discriminating group membership. Finally linear discriminants are used in combination to assign probabilities of group membership of each individual.

To complement cluster assignments based upon DAPC, the fixation index (F_{ST}) between each population was calculated (Nei 1973). Closely related population clusters should be indicated by both a small F_{ST} value, <0.05, and limited dispersion across linear discriminants. Distantly related population clusters should be inferred by large F_{ST} value, >0.25, and be largely dispersed across linear discriminants.

2.3 Results

2.3.1 Heterozygosity. Across all loci expected heterozygosity (H_{EPX}) ranged from 0.012 to 0.5 with a mean value of 0.262. Observed heterozygosity (H_{OBS}) across all loci ranged from 0.0 to 1.0 with a mean value of 0.188 (Figure 2). H_{OBS} across all loci is significantly lower (p-value <0.001) than $H_{EPX,}$ which is not surprising in a highly inbreed species. While completely heterozygous loci were found,

no recent outcrossing was found at the individual level with observed heterozygosity ranging from 0.039 to 0.226, and a mean value of 0.121 (Table 1).

2.3.2 Spatial Structure. Following sPCA the first two PCs, corresponding to global structure, and last PC, corresponding to local structure, were retained as they had the most substantial absolute values (Figure 3). Using the three retained PCs global structure was found to be significant (p-value = 0.04) (Figure 4), however local structure was not significant. Interpolation of the first principal component reveals three pockets of strong spatial autocorrelation at the south, west, and in the north-central portions of the small-grain production region of the PNW (Figure 5). Interpolation of the second principal component indicated broader areas of positive autocorrelation, observed most intensely in south east and south west portions of the small-grain production region (Figure 5). Interpolating both principal components together, pockets of positive autocorrelation are apparent but spatial patterns or genetic clines are not apparent on a regional scale (Figure 5). Monmonier's algorithm was used to identify genetic patches or patches, however, no relevant clines were identified. The lack of a strong or easy-to-interpret genetic cline may be an indication that climate is not driving the distribution of downy brome clusters.

2.3.4 DAPC. For the PCA to identify the number of clusters and provide the initial assignment of group membership, 35 PCs were retained corresponding to roughly 85% of cumulative variance (Figure 6). Seven population clusters were identified as being the optimal number based upon BIC value (Figure 7). Through the use of multiple DAPC simulations, 6 PCs were found to be optimal in assigning group membership without overfitting the model (Figure 8). Three linear discriminants were retained to calculate the probability of group membership (Figure 9). Figure 10 indicates the probability of membership of each individual. 13 of the 93 individuals were assigned to a cluster with less than 90% probability (Figure 11), however only individual 20 was equally likely to be a

member of more than one cluster. Cluster six only contains ripgut brome (Table 2, Figure 10) as would be expected with a separate species.

The distribution of individuals and clusters across the first and second discriminant function (Figure 12A) indicate separation of clusters 3, 6, and 7. Cluster 2 overlapped considerably with cluster 4, as did cluster 1 with cluster 5. When individuals and clusters were distributed on first and third discriminant function (Figure 12B) clusters 2, 5, 6 and 7 were separated, and cluster 1 was overlapping with cluster 4. Figure 12C displays the distribution of clusters and individuals across the second and third discriminant function indicating overlap of the 3 and 4 cluster while clusters 1, 2, 5, 6 and 7 are distributed. Regardless of which discriminate functions are used to describe distribution, clusters 6 and 7 are the most distant clusters. Cluster 4, however, overlaps with cluster 1, 2, and 3 depending on the linear discriminates used to describe the distribution of individuals.

F_{ST} values (Table 2) between each cluster reflect the relationships between clusters described by DAPC in Figures 16-18. Small F_{ST} values were returned for cluster 4 in relation to all other clusters, .003 to .057 (excluding cluster 6). While the loci containing the most informative SNPs were found across all downy brome clusters, cluster 4 did not contain any of the polymorphisms in the limited genomic regions used for the analysis. The lack of identifying SNPs for cluster 4 somewhat explains the limited dispersion of cluster 4 and low pairwise F_{ST} values. Cluster 6, which contained the single ripgut brome accession was more dispersed across the linear discriminants relative to other population clusters, and the dispersion indicated by DAPC was also represented by F_{ST} values (Table 2).

Figure 13 displays the spatial distribution of all individuals color coded by assigned cluster. While no patterns are evident, a close comparison of Figure 15 with Figure 5c helps explain the overlapping of some clusters observed when interpolating the DAPC results. Clusters 1, 5 and 7 in Figure 5c are indicated by white squares, with no positive spatial autocorrelation evident in their

distribution. Clusters 2, 3 4, and 6 are represented by black squares and do indicated varying degrees of spatial autocorrelation. The spatial principal components that most strongly indicate positive autocorrelation contain SNP markers, which are also informative in assigning individuals to cluster 2, 3, and 4. The strong autocorrelation indicated by the single ripgut brome accession is likely due to the spatial isolation of cluster six in the southeast area of the sampling region.

2.4 Discussion

Ramakrishnan et al. (2002) used 7 SSR markers for their analysis and reported that the observed heterozygosity values in downy brome ranged from 0.000 to 0.006 (Ashley and Longland 2009, Valliant et al. 2007). A recent investigation of downy brome population genetics performed using 91 to 93 SNPs and greater than 300 individuals in each population reported that the observed heterozygosity ranged from 0.0011 to 0.0088 across four populations (Meyer et al. 2013). The observed heterozygosity in this study which utilized 384 SNPs across 93 individuals ranged from 0.094 to 0148 and was greater than what was reported by Meyer et al. (2013). It is unclear if the increase is due to the differences in genotype, environment, or simply a reflection of a larger number of genetic markers. No recent evidence of outcrossing was found however manuscripts reporting outcrossing have sampled a larger number of individuals, >185 (Leger et al. 2009, Meyer et al. 2013, Novak and Mack 1993, Valliant et al. 2007), than the 93 individuals investigated in this study.

As the western PNW is considerably dryer than the eastern PNW and the temperature also tends to be warmer in the eastern portion, evidence of specialist genotypes is most likely when comparing the eastern and western portions. While spatial autocorrelation was found, there is no spatial pattern suggesting that certain genotypes are better adapted or more common in these two contrasting portions of the small grain production region.

The DAPC defined clusters of downy brome distribution were successful in identifying the ripgut brome individual as an outlier and helping to inform the spatial autocorrelation results. The

presence of spatial autocorrelation (Figure 9) is explained by the presence of either cluster 2, 3, or 4 (Figure 19) at each sampling point. However, similar to what was observed in terms of spatial autocorrelation, the distribution of genetic clusters does not appear to be driven by climate. While some cluster contain greater numbers of individuals, it appears all clusters are distributed throughout the small grain production region and none of clusters can be described as specialist genotypes in relation to climatic variables or spatial distribution.

The distinction between clusters is evident in Figures 12-14 and the F_{ST} values (Table 2). While ripgut brome is distinct from all other clusters, depending on the linear discriminant used or the pairwise F_{ST} value, the genetic distinction between clusters is often slight. However, population clusters can be separated based upon SNP distribution. Efforts were made to evaluate cluster membership with a different number of retained PCs or with arbitrarily selected k-values, and those efforts failed to identify ripgut brome as an outlier. Similarly, a Bayesian clustering approach based upon Hardy-Weinberg and linkage equilibrium as implemented with the software program STRUCTURE (Falush et al. 2003) also failed to distinguish the downy brome individuals from the ripgut brome sample. The results returned by DAPC may accurately reflect the state of downy brome genotypes within the small grain production region of the PNW. The population consists of, distantly related selfing individuals with little evidence of outcrossing, varying degrees of shared genetic history, and without strong evidence of adaption to various environmental and human indicted selection pressures.

While genetic markers linked to neutral gene regions, and SNPs in particular, are well suited to neutral evolutionary process such as genetic drift and gene flow (Hylar et al. 2011), genetic markers linked to neutral gene regions are poor at detecting active evolutionary processes (Narum et al. 2013). Previous studies have demonstrated neutral markers can fail to detect local adaptation of population to habitats (Storz el al. 2009; Narum et al. 2010). The genetic markers used in this study

may have no genetic linkage to genes which would be influenced by climate. An alternative or complimentary approach would be to utilize molecular markers that are lined to genes which are known to respond to selection by climate. As previous literature has demonstrated flowering time is adaptively significant and influenced by local climate, the genes responsible of regulating downy flowering pathways are a promising target to investigate potential adaptation of downy brome to climate Ball et al. 2004; Meyer et al. 2004; Rice and Mack 1991a, 1991b).

Downy brome population genetics and genetic structure from within an agronomic system indicates that the heterozygous state of downy brome is similar, if not marginally greater, to what has been reported in previous literature. Additionally, downy brome exists within the PNW small grain production region as a series of generalist genotype clusters with limited evidence of spatial adaptation, as was previously reported for all of North America (Novak et al. 1991). Given the apparent random spatial distribution of downy brome clusters at the spatial scale of this analysis, unique genotypes may be well mixed within small grain fields, similar to what was reported for *Bromus sterilis* (Green et al. 2001).

To further expand upon the above findings, future efforts should include more samples of individuals from the same field to increase the spatial resolution of genetic inferences. Additionally, collection of individuals from nearby rangeland and natural areas may allow for the control of climate and the comparison of land use among accessions. Finally, phenotyping of collected individuals in a common garden across several years would provide traits to be compared across individuals and elucidate the results of DAPC clustering by correlating the separation of genotypes with traits.

2.5 Literature Cited

Ashley MC, Longland WS (2009) Assessing cheatgrass (*Bromus tectorum*) genetic diversity and population structure using RAPD and microsatellite molecular markers.West North Am Nat 69:63–74

Ball DA, Frost SM, Gitelman AI (2004) Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. Weed Sci 52:518–524

Bartlett E, Novak SJ, Mack RN (2002) Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. Am J Bot 89:602–12

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool set for population genomics. Mol Ecol 22:3124–40

Ekblom R, Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. Heredity 107:1–15

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–87

Gabriel KR, Sokal RR (1969) A new statistical approach to geographic variation analysis. Sytematic Zool 18:259–278

Green JM, Barker JH, Marshall EJ, Froud-Williams RJ, Peters NC, Arnold GM, Dawson K, Karp a (2001) Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within- and between-farm scales. Mol Ecol 10:1035–45

Helyar, SJ, Hemmer-Hansen J, Bekkevold D, Taylor MI, Ogden R, Limborg MT, Cariani A, Maes GE, Diopere E, Carvalho GR, Nielsen EE (2011) Application of SNPs for population

genetics of nonmodel organisms: New opportunities and challenges. Mol Ecol Resour. 11(1) 123-136

Huggins D, Rupp R, Gessler P, Pan W, Brown D, Machado S, Abatzoglou J, Walden V, Eigenbrode S (2012) Dynamic Agroecological Zones for the Inland Pacific Northwest,. *in* American Society of Agronomy Annual Meeting, Oct. 21-24, Cincinnati, OH

Jombart T (2008) Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405

Jombart T, Ahmed I (2011) Adegenet 1 . 3-1 : new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070–3071

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:1–15

Jombart T, Devillard S, Dufour a-B, Pontier D (2008) Revealing cryptic spatial patterns in genetic variability by a new multivariate method. Heredity 101:92–103

Legendre P, Fortin M (1989) Spatial pattern and ecological analysis. Vegetatio 80:107–138

Leger EA, Espeland EK, Merrill KR, Meyer SE (2009) Genetic variation and local adaptation at a cheatgrass (*Bromus tectorum*) invasion edge in western Nevada. Mol Ecol 18:4366–79

Liu N, Chen L, Wang S, Oh C, Zhao H (2005) Comparison of single-nucleotide polymorphisms and microsatellites in inference of population structure. BMC Genet 6 Suppl 1:S26

Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. Hum Biol an Int Rec Res 76:173–190

Mascher M, Wu S, Amand PS, Stein N, Poland J (2013) Application of genotyping-by-sequencing on semiconductor sequencing platforms: a comparison of genetic and reference-based marker ordering in barley. PLoS One 8:e76925

Merrill KR, Meyer SE, Coleman CE (2012) Population genetic analysis of Bromus tectorum (Poaceae) indicates recent range expansion may be facilitated by specialist genotypes. Am J Bot 99:529–37

Meyer SE, Ghimire S, Decker S, Merrill KR, Coleman CE (2013) The ghost of outcrossing past in downy brome, an inbreeding annual grass. J Hered 104:476–90

Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). Ann Bot 93:653–63

Monmonier MS (1973) Maximum-difference barriers: an alternative numerical regionalization method. Geogr Anal 3:245–261

Moran PAP (1950) Notes on continuous stochastic phenomena. Biometrika, 1/2:17–33

Morrow LA, Stahlman PW (1984) The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci Soc Am 32:2–6

Muhlenberg H (1793) Index florae Lancastriensis. Trans Am Philos Soc Philadelphia 3:157–184

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA (2013) Genotyping-by-sequencing in ecological and conservation genomics. Mol Ecol 22(11): 2841-2847

Narum SR, Campbel NR, Kozfkay CC, Meyer KA (2010) Adaption of redband trout in desert and montane environments. Mol Ecol 19(21): 4622-4637

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci U S A 70:3321–3323

Novak S, Mack R, Soltis D (1991a) Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. Am J Bot 78:1150–1161

Novak S, Mack R, Soltis P (1993) Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. Can J Bot 71:1441–1448

Novak SJ, Mack RN (1993) Genetic variation in *Bromus tectorum* introduced populations. Heredity 71:167–176

Novak SJ, Mack RN, Soltis DE (1991b) Genetic variation in *Bromus tectorum* (Poaceae): Population differentation in its North American range. Am J Bot 78:1150–1161

Poland JA, Brown PJ, Sorrells ME, Jannink J (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253

Ramakrishnan AP, Coleman CE, Meyer SE, Fairbanks DJ (2002) Microsatellite markers for *Bromus tectorum* (cheatgrass). Mol Ecol Notes 2:22–23

Ramakrishnan AP, Meyer SE, Fairbanks DJ, Coleman CE (2006) Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*. Plant Species Biol 21:61–73

Rice K, Mack R (1991a) Ecological genetics of *Bromus tectorum*. I. A hierarchical analysis of phenotypic variation. Oecologia 88:77–83

Rice KJ, Mack RN (1991b) Ecological Genetics of *Bromus tectorum*. III. The demography of reciprocally sown populations. Oecologia 88:91–101

Scott JW, Meyer SE, Merrill KR, Anderson VJ (2010) Local population differentiation in *Bromus tectorum* L. in relation to habitat-specific selection regimes. Evol Ecol 24:1061–1080

Storfer A, Murphy MA, Evans JS, Goldberg CS, Robinson S, Spear SF, Dezzani R, Delmelle E, Vierling L, Waits LP (2007) Putting the "landscape" in landscape genetics. Heredity 98:128–42

Storz JF, Runck AM, Sabatino SJ, Kelly JK, Ferrand N, Moriyama H, Weber RE, Fago A (2009) Evolutionary and function insights into the mechanism underlying high-altitude adaptation of deer mouse hemoglobin. Proc. Natl. Acad. Sci. U.S.A. 106(34): 14450-14455

Upadhyaya M, Turkington R, McIlvride D (1986) The biology of Canadian Weeds: 75. *Bromus tectorum* L. Can J of Bot 66:689–709

Valliant MT, Mack RN, Novak SJ (2007) Introduction history and population genetics of the invasive grass *Bromus tectorum* (Poaceae) in Canada Author. Am J Bot 94:1156–1169

2.6 Tables and Figures

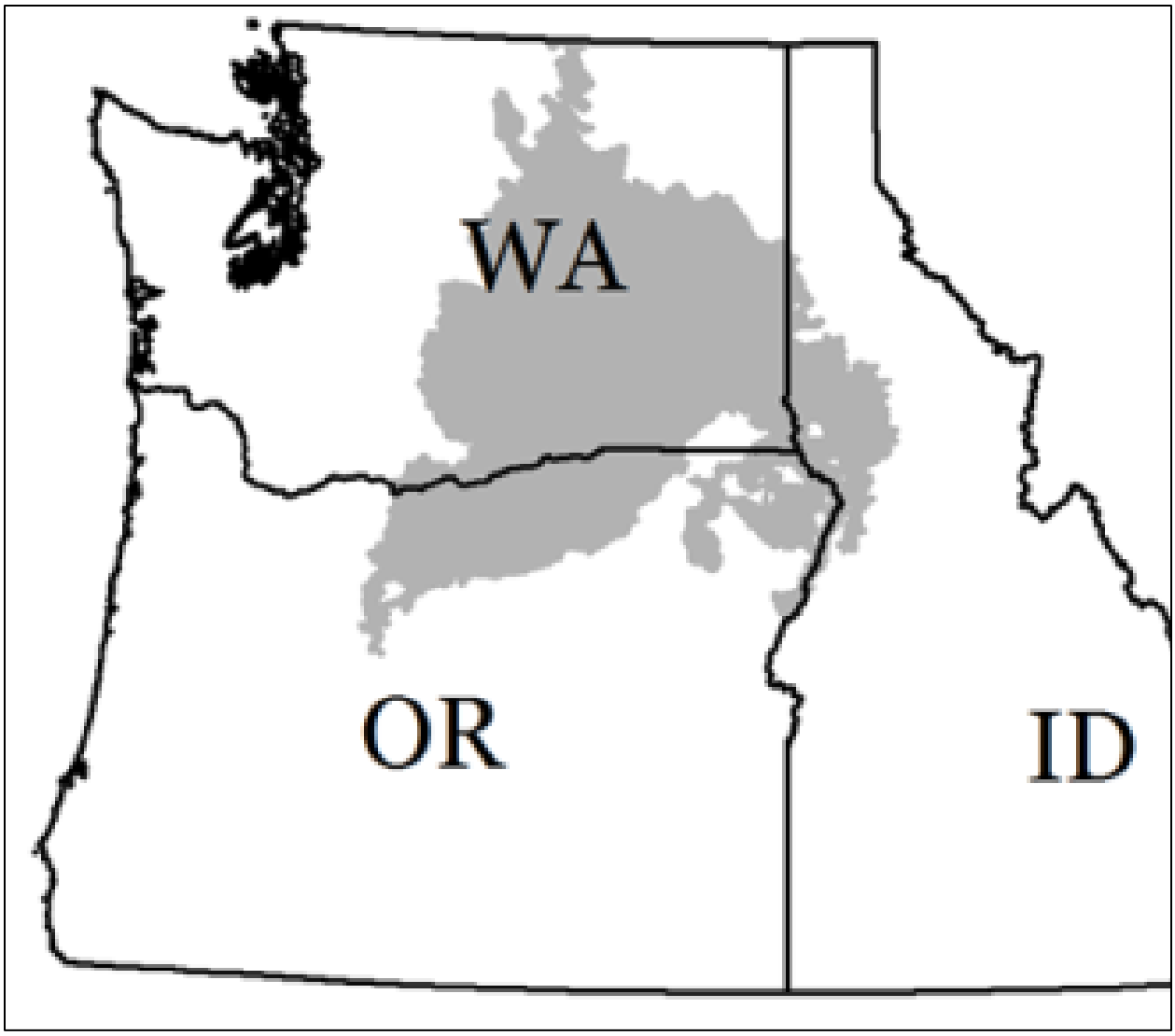


Figure 2.1. The small grain production region of the PNW.

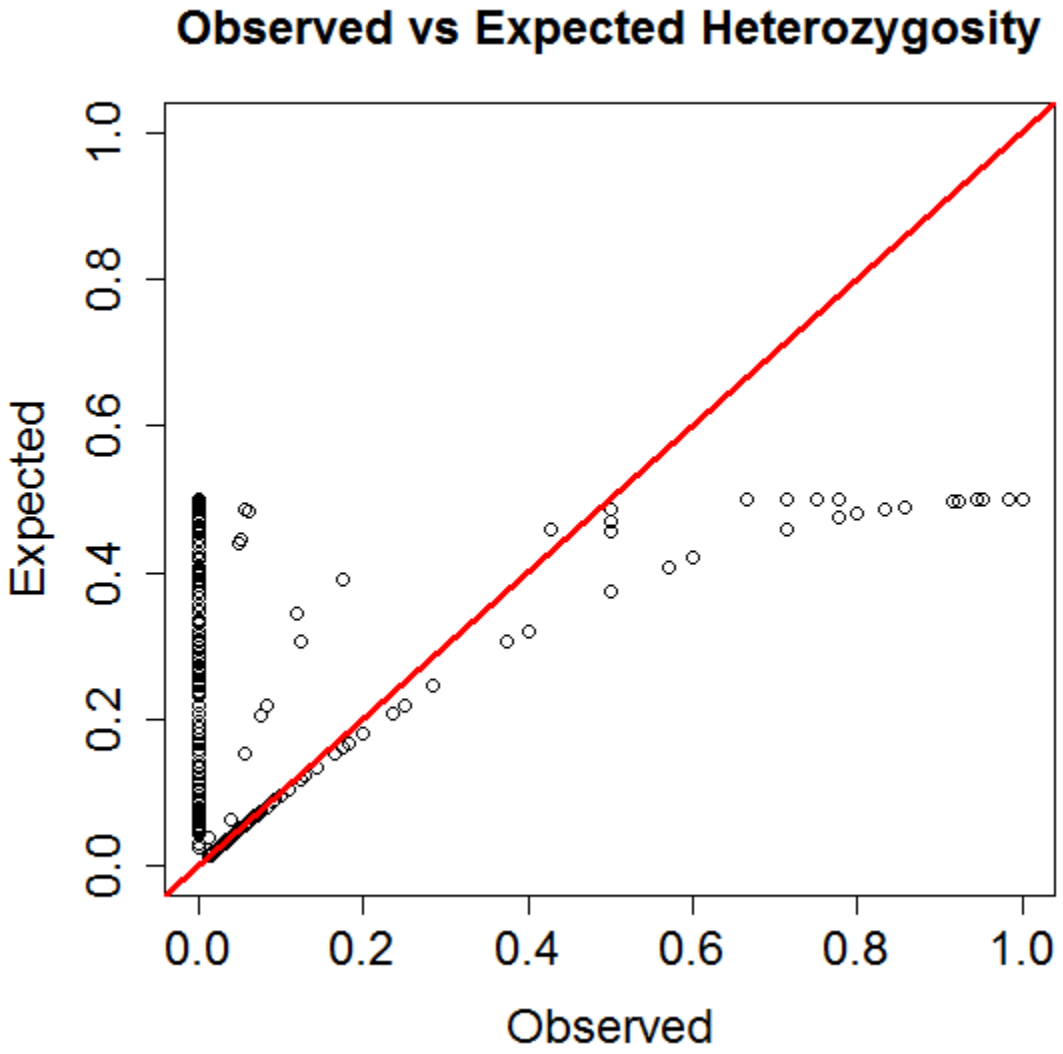


Figure 2.2. Observed versus expected heterozygosity across all loci and individuals.

Table 2.1. Accession ID number, GPS coordinates of collection locations, year of collection, heterozygosity of each accession, and cluster membership as determined by DAPC.

Accession	Longitude	Latitude	Year	[a]H_{OBS}	Cluster	Accession	Longitude	Latitude	Year	H_{OBS}	Cluster
1	-119.215	46.387	2010	0.150	3	49	-118.370	46.677	2011	0.173	4
2	-118.989	46.825	2011	0.165	4	50	-119.853	46.671	2010	0.092	7
3	-116.466	46.252	2010	0.177	3	51	-118.310	47.456	2011	0.092	1
4	-118.916	47.785	2011	0.109	7	52	-119.605	46.182	2010	0.134	4
5	-120.938	45.673	2010	0.094	5	53	-117.949	46.429	2010	0.111	1
6	-118.600	45.995	2011	0.104	3	54	-117.748	47.903	2011	0.083	4
7	-120.646	46.400	2010	0.142	3	55	-117.872	47.214	2010	0.120	4
8	-118.794	46.744	2011	0.143	4	56	-119.935	45.388	2010	0.170	4
9	-118.098	46.370	2010	0.195	4	57	-117.674	47.124	2010	0.114	4
10	-118.785	47.475	2011	0.133	1	58	-119.175	47.420	2011	0.119	4
11	-120.489	45.482	2010	0.166	3	59	-117.477	46.953	2010	0.070	4
12	-118.403	45.760	2010	0.090	4	60	-119.218	46.561	2010	0.117	4
13	-120.184	45.620	2010	0.108	2	61*	-117.162	46.375	2011	—	—
14	-118.358	46.335	2011	0.123	4	62	-119.150	47.179	2011	0.128	7
15	-120.336	46.886	2011	0.101	7	63	-117.251	47.390	2010	0.130	1
16	-117.883	47.515	2010	0.128	3	64	-118.992	45.765	2011	0.102	4
17	-116.870	46.396	2011	0.090	7	65	-116.710	46.917	2010	0.129	4
18	-118.127	46.656	2011	0.170	1	66	-119.049	46.999	2011	0.087	2
19	-119.872	47.102	2011	0.105	4	67	-115.963	46.100	2010	0.117	1
20	-118.135	47.686	2011	0.106	4	68	-119.164	47.990	2011	0.117	1
21	-119.851	46.737	2011	0.104	2	69	-120.616	45.469	2010	0.118	5
22	-117.804	46.629	2010	0.122	4	70	-118.610	46.082	2011	0.153	4
23	-119.441	45.638	2010	0.123	4	71	-120.699	46.676	2010	0.130	4
24	-118.464	47.490	2011	0.121	4	72	-118.651	46.769	2011	0.130	5
25	-119.864	46.263	2010	0.079	5	73	-120.655	46.559	2010	0.110	7
26	-117.632	47.715	2011	0.170	3	74	-118.851	47.523	2011	0.102	2
27	-119.281	45.761	2010	0.123	7	75	-120.561	46.462	2010	0.122	4
28*	-117.378	47.263	2010	—	—	76	-118.436	46.006	2011	0.102	4
29	-119.279	46.708	2011	0.226	4	77	-120.164	46.261	2010	0.117	7
30	-117.165	47.102	2010	0.094	4	78	-118.363	47.235	2011	0.098	2
31	-119.320	47.468	2011	0.039	4	79	-120.412	46.996	2011	0.118	4
32	-117.092	47.484	2010	0.101	4	80	-117.661	46.898	2010	0.148	1
33	-119.691	46.742	2010	0.140	4	81	-120.130	46.375	2010	0.066	5
34	-116.836	46.924	2010	0.133	3	82	-118.168	46.803	2011	0.092	5
35	-119.078	47.203	2011	0.067	4	83	-119.799	45.353	2010	0.089	2
36	-120.965	45.483	2010	0.160	3	84	-117.906	46.394	2010	0.133	2
37	-118.859	46.478	2010	0.149	7	85*	-119.711	47.337	2011	—	—
38	-120.746	45.635	2010	0.126	7	86	-118.180	46.913	2011	0.046	1
39	-118.742	46.343	2011	0.089	4	87	-119.373	46.107	2010	0.129	4
40†	-120.184	46.041	2010	0.094	6	88	-120.241	46.003	2010	0.126	7
41	-118.642	47.261	2011	0.136	7	89	-119.411	46.849	2011	0.170	4
42	-120.358	45.419	2010	0.134	1	90	-117.518	46.492	2010	0.122	4
43	-118.679	47.796	2011	0.211	4	91	-119.241	46.029	2010	0.075	1
44	-120.162	45.396	2010	0.158	4	92	-117.551	47.524	2011	0.127	7
45	-118.491	46.138	2011	0.136	1	93	-119.199	47.014	2011	0.101	4
46	-119.908	46.702	2010	0.101	7	94	-117.245	47.307	2010	0.143	2
47	-118.465	47.490	2011	0.162	4	95	-119.370	47.888	2011	0.118	4
48	-120.346	45.895	2010	0.091	4	96	-118.895	46.669	2011	0.118	4

[A]Nomenclature: H_{OBS}; observed heterozygosity.

*Accession were removed from further analysis following GBS.

†Accession is *Bromus diandrus* Roth.

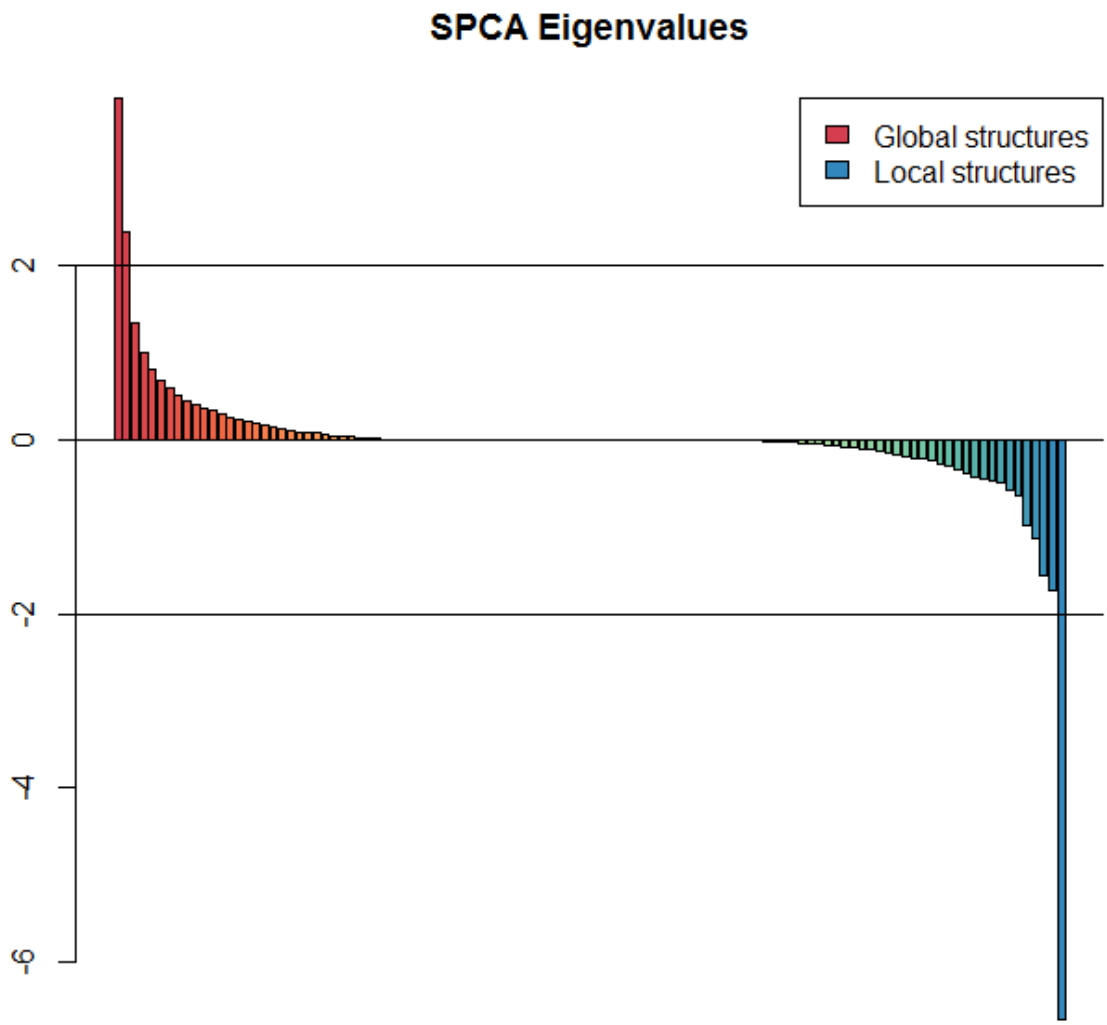


Figure 2.3. Positive and negative eigenvalues, corresponding to global and local spatial structure.

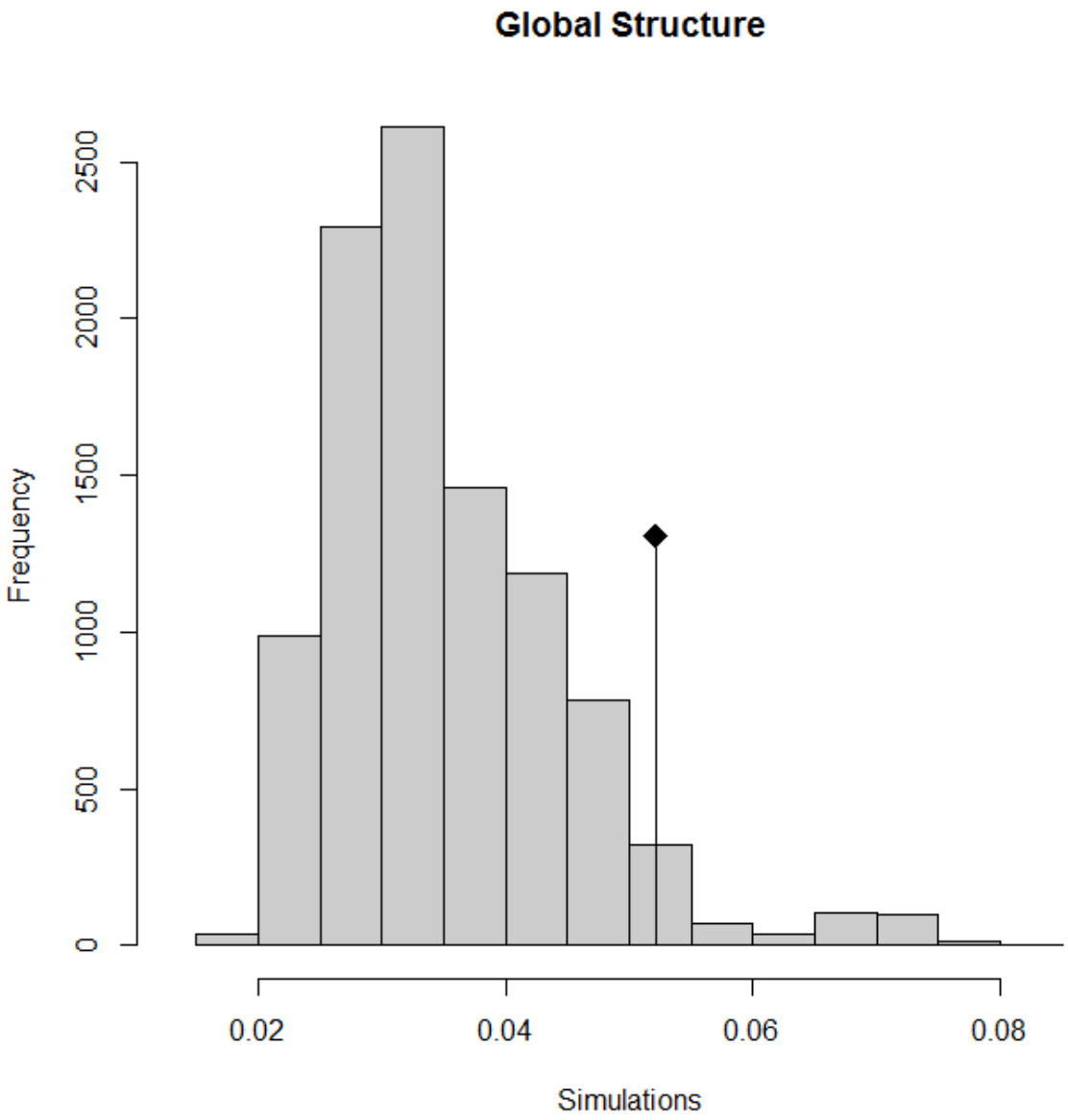


Figure 2.4. Histogram of permuted test statistics from 10,000 Monte Carlo simulations and the observed global structure.

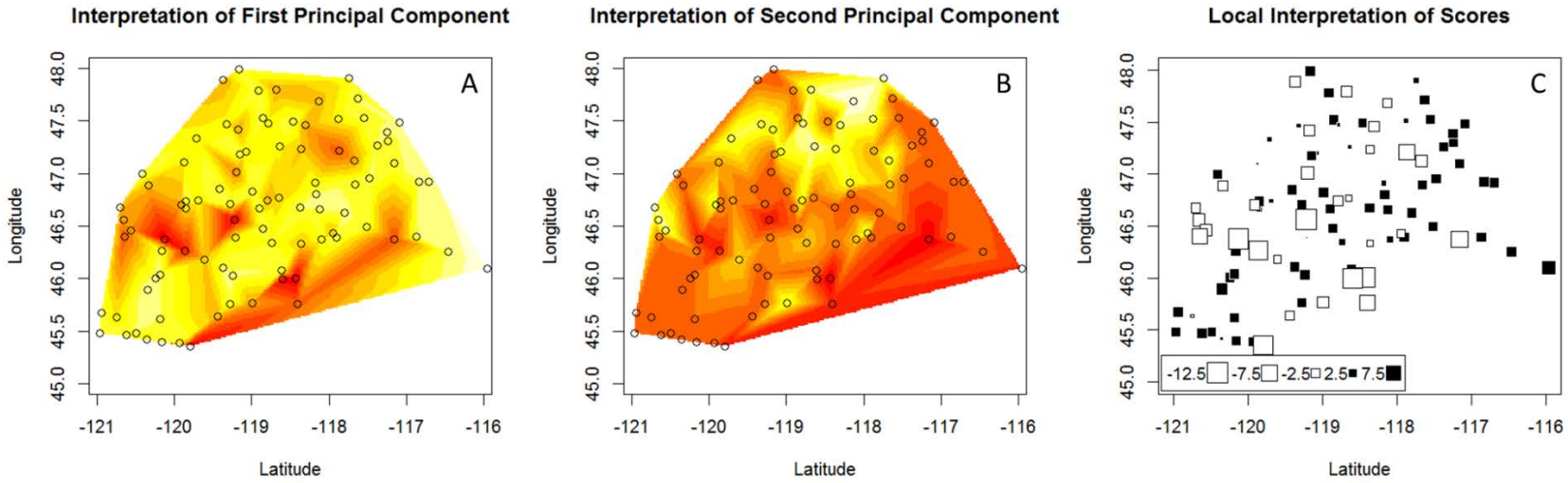


Figure 2.5. Interpolation of the global structure across the first (A) and second (B) principal components. (A and B) Red indicates areas of stronger autocorrelation compared to white and yellow. Circles identify the location of collections. (C) Local scores of all retained principal components with black corresponding to positive autocorrelation and white to negative autocorrelation. The size of squares is proportional to the intensity of autocorrelation.

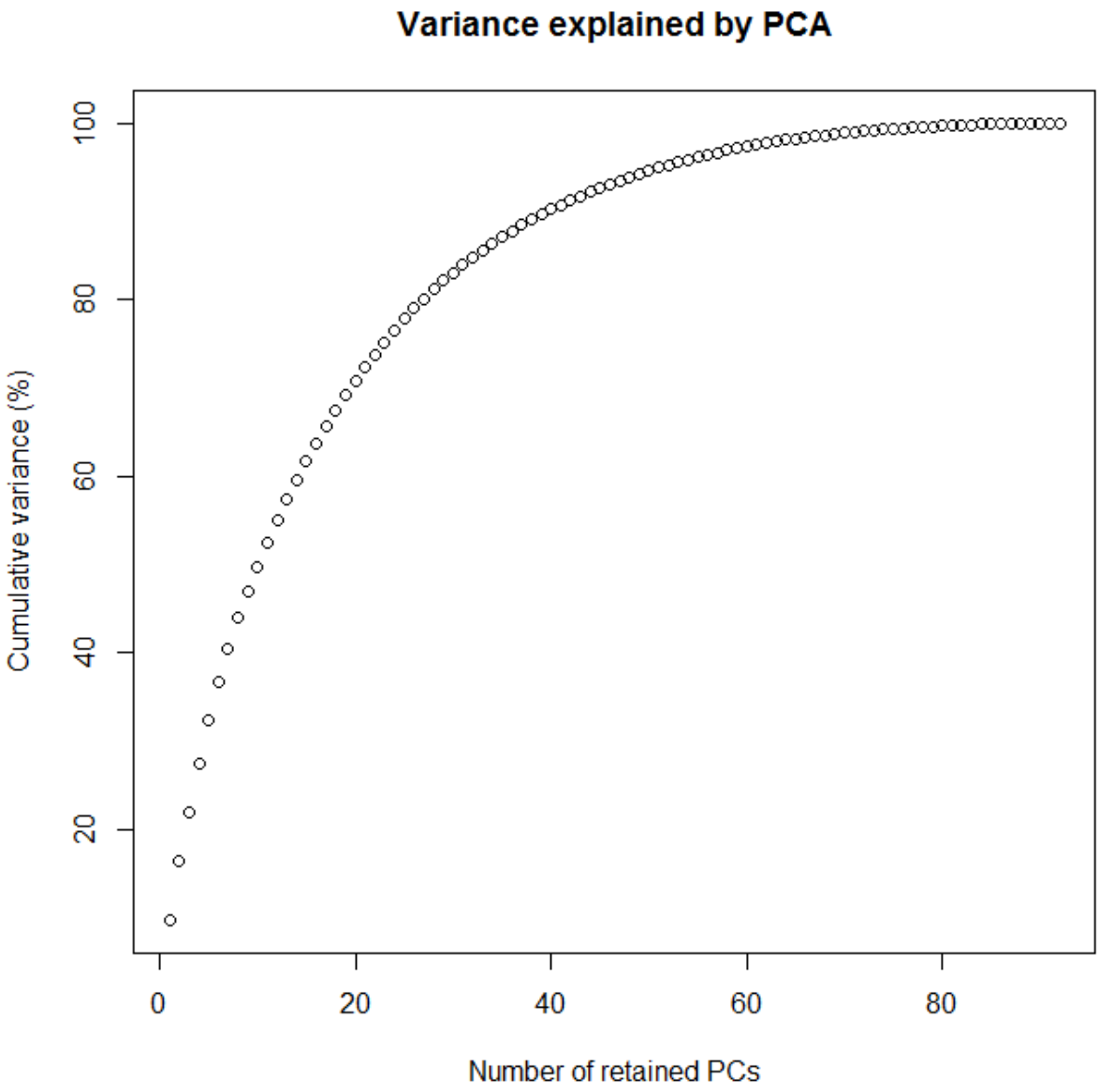


Figure 2.6. Cumulative variance explained by retaining each additional Principal Component.

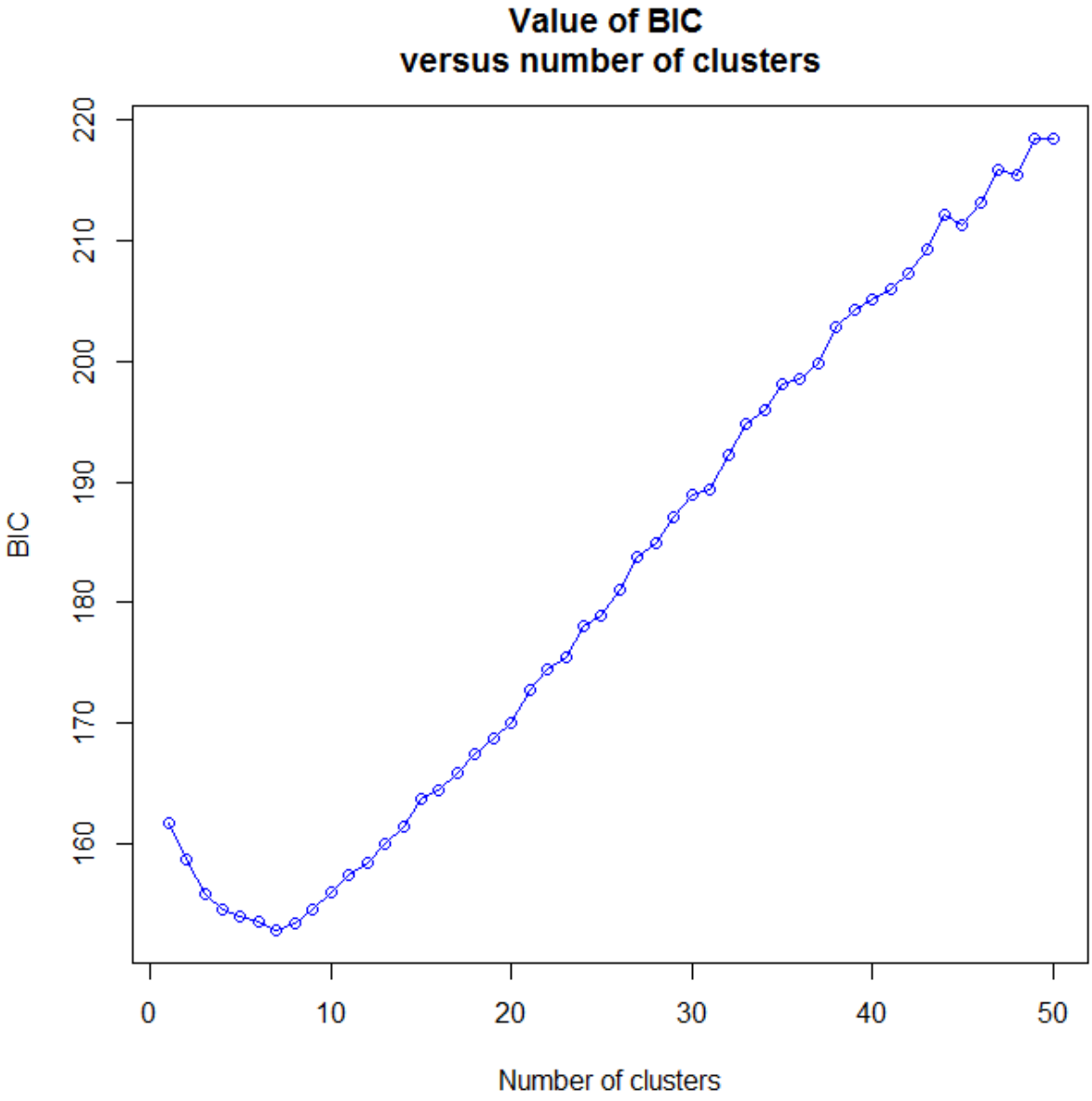


Figure 2.7. Bayesian information criterion value for increasing values of k.

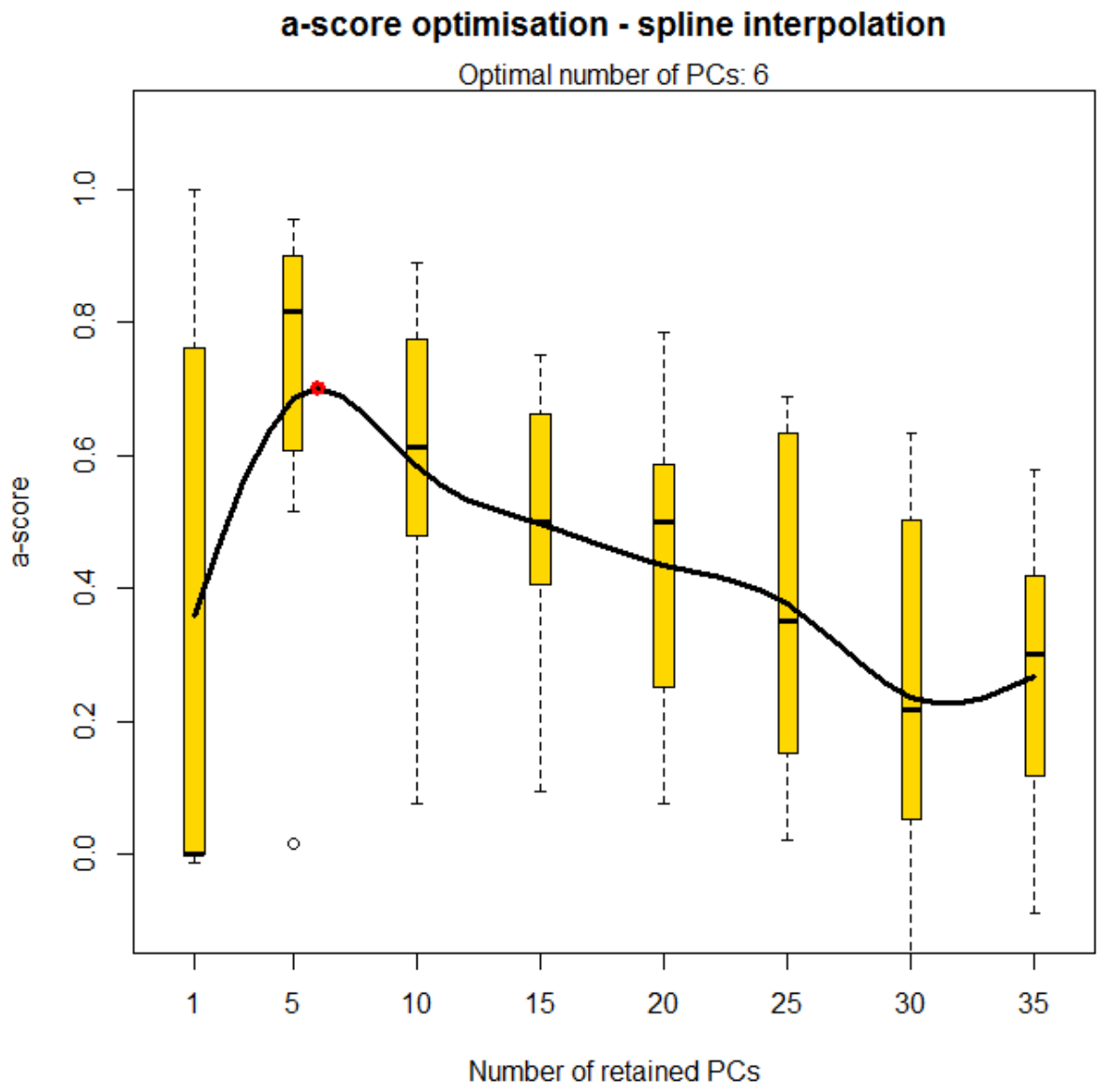


Figure 2.8. Optimal number of retained principal components for assigning cluster membership probabilities using discriminate analysis of principal components.

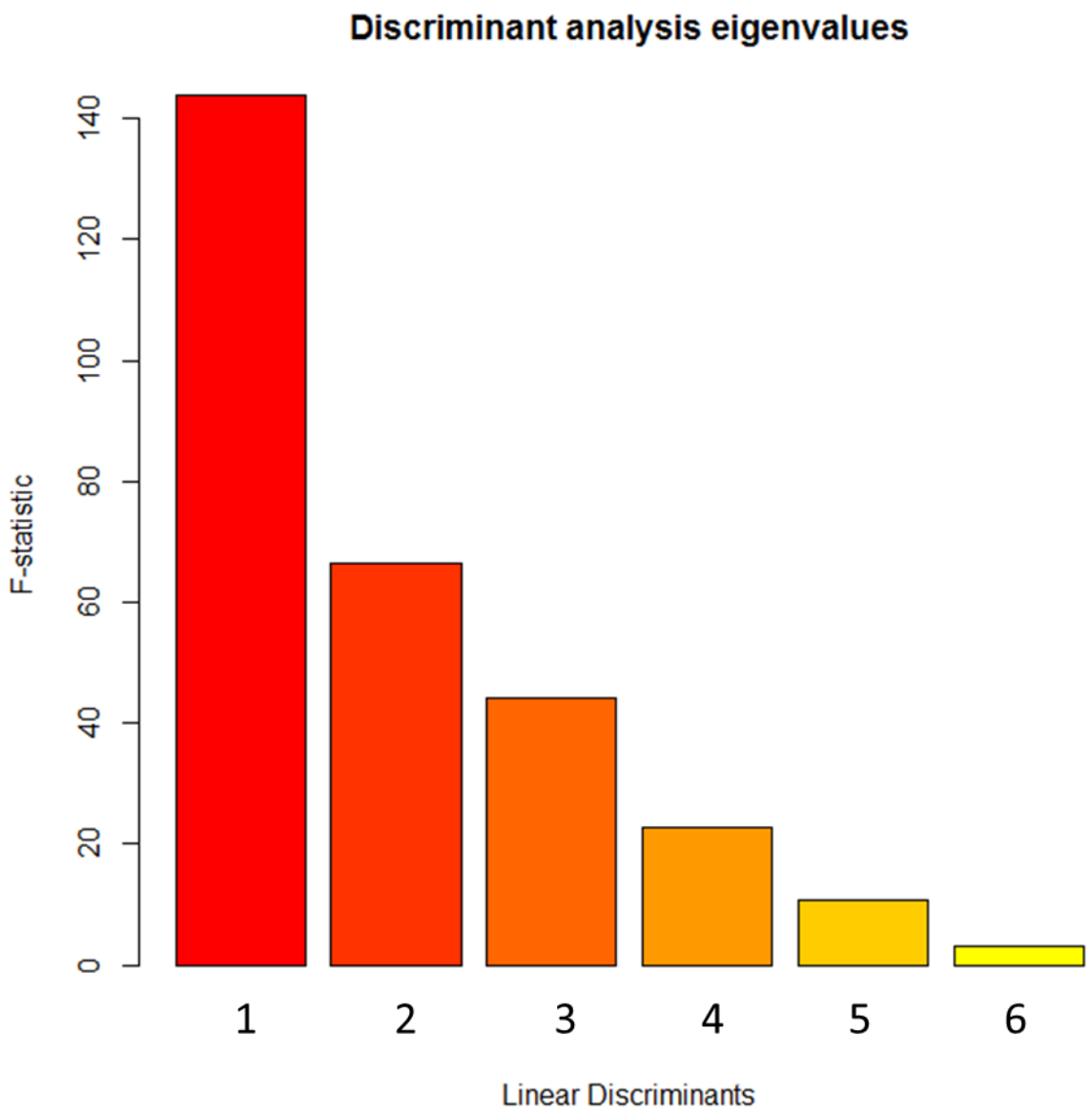


Figure 2.9. F-statistic associated with each discriminant function. The first three linear discriminants were retained.

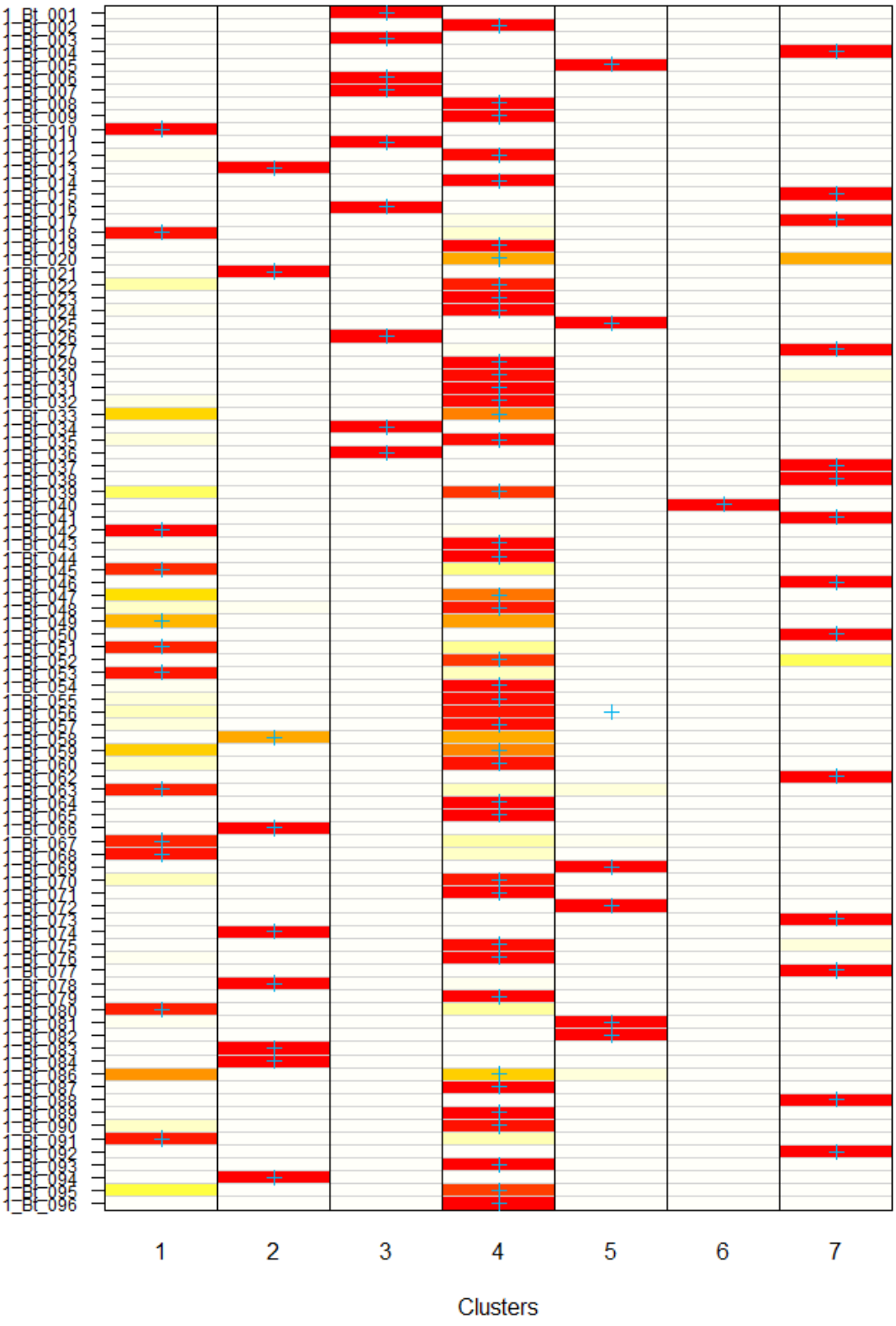


Figure 2.10. Probability of cluster membership for each individual. Red indicates 100% probability while white indicates 0% probability of cluster membership. Blue crosses indicate original cluster assignment obtained from principal component analysis.

Figure 2.11. Individuals that were assigned to a cluster with less than 90% probability. Each color represents the probability of assignment to a particular cluster.

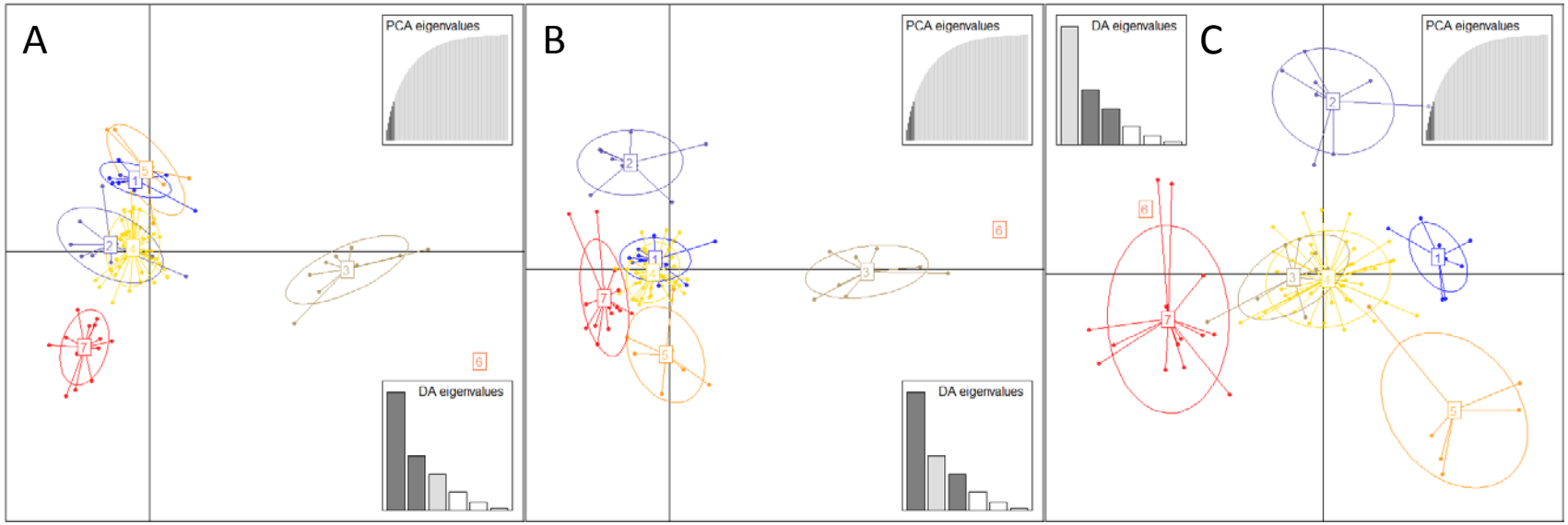


Figure 2.12. Distribution of individuals and clusters across the first, second, and third linear discriminates. PCA eigenvalues is the cumulative variance explained by the six retained principal components. DA eigenvalues represents which linear discriminants are being compared in each scatter plot, with the height of each bar representing the relative contribution in explaining total variance. Scatter plot A represents linear discriminant 1, x-axis, and linear discriminate 2, y-axis. Scatter plot B represents linear discriminant 1, x-axis, and linear discriminate 3, y-axis. Scatter plot A represents linear discriminant 2, x-axis, and linear discriminate 3, y-axis. Each point on each scatter plot represents an individual. Each color is used to distinguish a separate cluster, which is identified by number. The ellipses around each number represent were 67% of the variance of each cluster assuming a bivariate distribution

Table 2.2. Pairwise Fst values of the 7 described population clusters.

	Fixation Index (F_{ST})					
	1	2	3	4	5	6
2	0.226	—	—	—	—	—
3	0.148	0.187	—	—	—	—
4	0.006	0.003	0.006	—	—	—
5	0.146	0.364	0.238	0.057	—	—
6	0.751	0.713	0.805	0.134	0.680	—
7	0.151	0.162	0.121	0.011	0.258	0.705

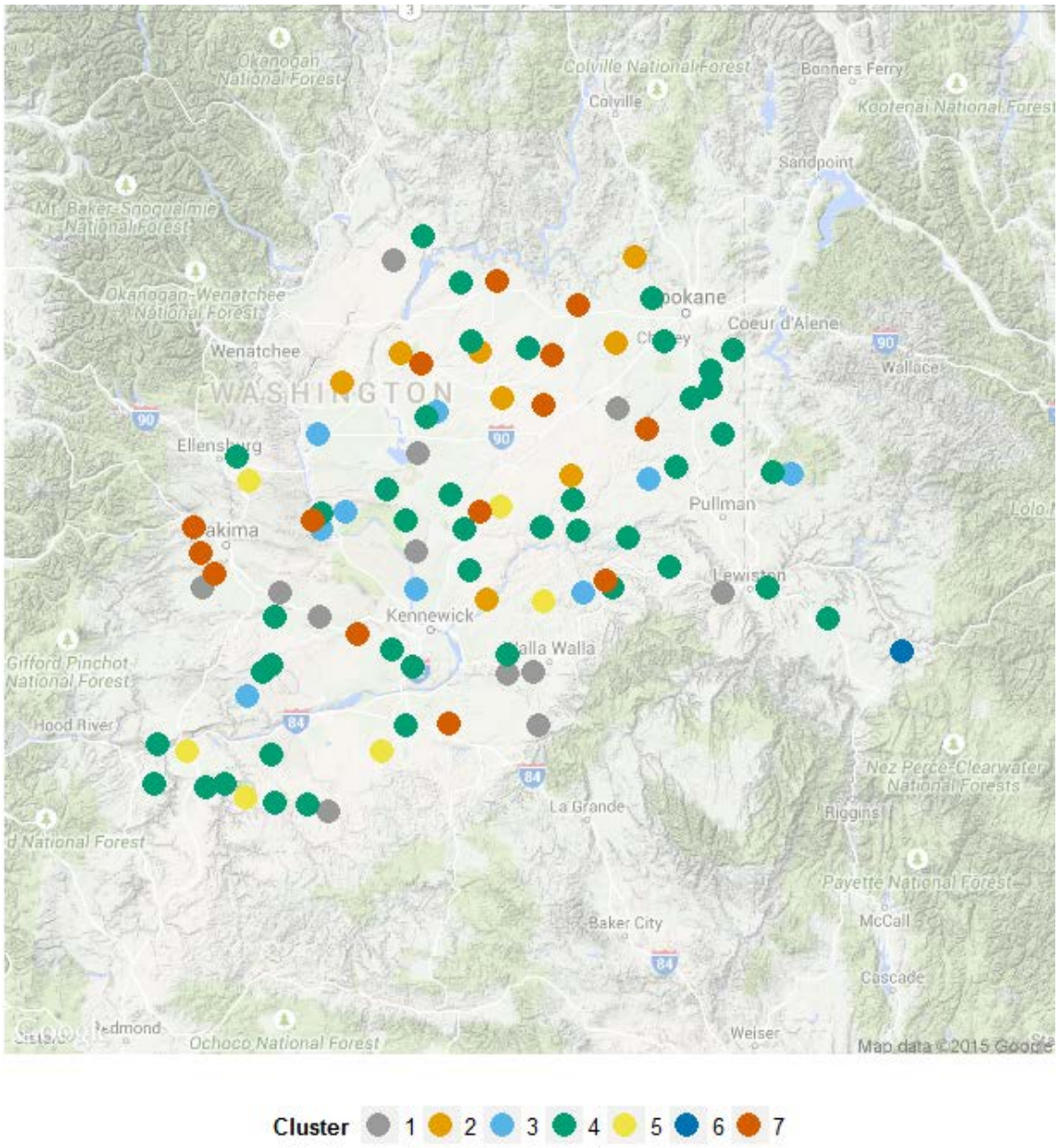


Figure 2.13. Spatial distribution of individuals and cluster membership as determined by discriminate analysis of principal components.

Chapter 3: Modeling *Bromus tectorum* Phenology from the PNW Small Grain Production Region

3.1 Downy Brome Phenology

Downy brome (*Bromus tectorum* L.) is an invasive winter annual grass species naturalized throughout the Western United States (Upadhyaya et al. 1986). Stewart and Hull (1949) characterized downy brome as an opportunist, easily acclimating to local climate due to rapid germination and establishment following variable fall or spring precipitation events. Capable of thriving in a wide range of habitats, downy brome readily establishes in novel locations following disturbance (Morrow and Stahlman 1984).

Compared to the development of winter wheat (*Triticum aestivum* L.) downy brome is generally in the early boot stage when wheat is tillering, heading four weeks earlier than wheat (Thill et al. 1984). The early development of downy brome results in depleted soil moisture and nutrients at a critical reproductive period for winter wheat (Thill et al. 1984). Studies comparing winter wheat competition with downy brome have reported yield reductions of up to 92% (Rydrych and Muzik 1968), with 21% reduction occurring from densities of 108 plants m-2 (Rydrych 1974). As the competitiveness of downy brome with winter wheat is a result of relative early development, improved knowledge of downy brome phenology is of critical importance for winter wheat producers.

Variation in downy brome phenology has been previously investigated through common garden experiments and through observations of naturally occurring populations (Ball et al. 2004, Hulbert 1955, Klemmedson and Smith 1964, Meyer et al. 2004, Rice and Mack 1991a, 1991b). Hulbert (1955) conducted a series of common garden experiments using downy brome accessions collected from within and outside North America. Winter hardiness, plant size, shoot and root morphology, timing of development thresholds, seed production, and seed dormancy were all

measured by Hulbert (1955). A great deal of variation was observed between populations and within populations with respect to most traits. The exception was timing of development, which was consistent within populations while differences between populations were described as "striking" (Hulbert 1955). Klemmedson and Smith (1964) also found considerable variation in phenology between populations from a common garden study which was attributed to differences in genotype within populations.

In a greenhouse study, Rice and Mack (1991a) evaluated variation of life history traits between populations, within populations, and among siblings. Flowering time was found to be highly stable among siblings and within populations, but variable between populations. Rice and Mack (1991a) concluded that variation in flowering time is an environmentally stable trait reflecting local adaptation between populations. Rice and Mack (1991b) reciprocally sowed downy brome seed from four seed sources in a common garden to observe if the variation among siblings with respect to dry plant weight, seed weight, seed number, and flowering time would adapt to local climate. Most traits varied considerably based on climate. Flowering time however, was stable year to year among plants from the same seed source (Rice and Mack 1991b). Rice and Mack (1991b) concluded flowering time was under strong genetic controls compared with other traits

Ball et al. (2004) described a model for predicting mature seed set of downy brome based on cumulative growing degree days (GDD) (Equation 1). The model begins 1 January with a base of 0°C, and was developed from a previous model for projecting cereal crop development (Klepper et al. 1988). The model was developed using a series of experiments in which panicles from downy brome accessions were periodically collected following the first emergence of panicles. Following panicle collection, seeds were allowed to after ripen for 6 mo to overcome dormancy. Seeds collected from panicles were planted in a greenhouse and the total number of germinated seeds from each panicle and each collection date was counted. Using a non-linear breakpoint analysis,

seeds germinated per panicle were regressed against the GDD at each collection date to estimate the GDD required for initial mature seed production.

The first experiment conducted by Ball et al. (2004) collected panicles from two naturally occurring populations, one in Pullman, WA and one in Pendleton, OR. The second experiment was expanded to four accessions collected within Eastern Oregon and Washington which were planted in the fall at a common garden in Pendleton, OR and Pullman, WA. The final experiment consisted of downy brome collected from naturally occurring populations in Kansas, Oklahoma, Nebraska, Colorado, Montana, and Idaho. While considerable variation in mature seed set in the Western United States was found (582 to 1,287 GDD), little variation was observed in the time to produce mature seeds from accessions collected and grown in the PNW (983 to 1,151 GDD). Ball et al. (2004) concluded mature seed set of downy brome in the PNW could be predicted to occur around 1,000 GDD. The 20-year average (1984-2003) for the 1,000 GDD threshold fell on 11 June in the PNW.

Meyer et al. (2004) examined vernalization response and requirements under greenhouse conditions among downy brome populations collected from contrasting habitats. Plants collected from warm, dry desert regions required little vernalization to flower and no difference in flowering response was observed from increased vernalization. However, plants collected from cold, high elevation locations required longer vernalization periods, and the weeks required to induce flowering and the proportion of individuals flowering following the end of vernalization treatments increased with the length of vernalization. Meyer et al. (2004) concluded that vernalization response and requirements are adaptively significant and differences observed between seed sources reflect habitat-specific selection operating on an array of founder genotypes within a population.

The small grain production area of the Pacific Northwest (PNW) includes Central and Eastern Washington, parts of Northern Oregon, and Northern Idaho and totals 2.2 million ha of

non-irrigated cropland (Huggins et al. 2012) (Figure 1). In 2007, 1.3 million ha of the small grain production region were planted with small grains of which 1.0 million ha was planted to winter wheat. Within the small grain production region, downy brome is among most widely distributed weeds.

When predicting how winter annual weeds may respond to climate change, one of the more critical traits is plasticity in phenology and flowering time (Metcalf et al. 2003, Nicotra et al. 2010). Few papers have investigated the response of weeds to climate change in relation to a specific crop or region (Peters et al. 2014). With increased evapotranspiration rates and milder winters projected for the PNW, winter annual weeds are anticipated to be more successful, compared to spring annual weeds under future climate scenarios (Concilio et al. 2013, Hanzlik and Gerowitt 2012, Peters et al. 2014) similar to what has been projected for winter grains compared to spring grains (Ball et al. 1995, Stöckle et al. 2010). A thorough understanding of downy brome phenology may aid in grower adaptation to climate change.

Ball et al. (2004) described little variation in time to mature seed set from downy brome accessions collected from the PNW. However Ball et al. (2004) only sourced a limited number of downy brome accessions from the PNW. A series of common garden and greenhouse studies were therefore conducted in 2012, 2013 and 2014 to characterize the variation in downy brome phenology within the PNW. A similar approach to that outlined by Ball et al. (2004) was followed, but with a greatly expanded number of collections and with common garden experiments capturing a diverse range in winter vernalization temperatures. Study objectives were to observe the full range in variation of downy brome phenology in the PNW small grain production region, utilize molecular tools to identify the influence of genotype on phenology, and capture what impact climate has on phenology.

3.2 Methods and Materials

3.2.1 Plant Materials. A systematic-random sampling design (Storfer et al. 2007) was utilized where a 10 km grid was laid over the small grain production region and a point was randomly assigned for sampling within each grid. A total of 190 sampling points were generated and then viewed using satellite images. If the sampling point had not been assigned to a small grain field, the sampling point was moved to the nearest small grain field. If there was no small grain field within 3 km of the original sampling point, the location was discarded. Following reassigning of the original sampling locations, 130 sampling locations were retained.

In June of 2010 and 2011 each of the 130 reassigned sampling locations was visited and a single downy brome plant and was taken as either a mature panicle or a live plant. Each collection was taken within a small grain field at least 10 m from the field border. Live plants were transplanted into a greenhouse at the Washington State University (WSU) Plant Growth Facility located in Pullman, WA and allowed to grow until mature panicles could be collected.

To limit the influence of maternal effects, all accessions collected during June of 2010 and 2011 were planted in a greenhouse located in Pullman, WA and brought through one complete life cycle. Accessions were planted into 10 cm^3 pots containing a peat based growth media (LC1 Mix. Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA) and placed in a greenhouse kept at between 26° C and 22° C on December 1st 2011. By 1 January 2012 all emerged individuals had reached 2-3 true leaves. Between 1 January and the 31 March 2012 the heating system in the greenhouse was turned off and cooling vents were opened to bring in outside air to allow vernalization. Seed was collected from each individual when shattering was first observed, between May and June depending on the accession. No supplemental lighting was provided during from the time of planting to harvesting. Collected seeds were stored at room temperature in paper envelopes until the beginning of common garden experiments.

3.2.2 Common Garden Experiments and Greenhouse Seed Maturity Experiments. Loose smut [*Ustilago bromivora* (Tul. & C. Tul.) A.A. Fisch. Waldh] was observed on around 30% of the 130 individuals during the course of the December 2011 to June 2012 seed increase. Based on the incidence of smut and availability of seed, 85 (Table 1) out of the 130 accessions were used in future common garden experiments. On October 15th of 2012, seed from 85 accessions was treated with a premade mixture of mefenoxam and difenoconazole (CruiserMaxx Cereals®, Syngenta Crop Protection, LLC, Greensboro, NC, USA) to prevent smut and planted into 48-cell inserts with around 20 seeds planted per cell. Each cell was 6 cm^3 in volume and filled with a peat moss-based planting mix. No supplemental lighting was provided and the greenhouse was maintained at 26° C to 22° C. Plants were thinned to one plant per cell and on 14 November 2012 and 10 cells from each accession were transplanted to a common garden location located in Central Ferry, WA (46.73, -117.54, elevation of 533 m). Another 10 cells from each accession were transplanted to a separate common garden located at the Cook Agronomy Farm (46.78, -117.09, elevation of 795 m) located north of Pullman, WA on 16 November 2012. At the time of transplanting, individual plants ranged in growth stage from 1 to 3 true leaves.

A third common garden experiment was initiated in the fall of 2013 and terminated in the spring of 2014. The same 85 accessions and seed sources (Table 1) were used in all three common garden experiments. Seeds were treated with mefenoxam and difenoconazole and planted on 15 October 2013 at the WSU Plant Growth Facility. Seeds were planted in 48 cell inserts with around 20 seeds planted per cell. Due to the limited availability of seed the number of replicates was reduced from 10 to 6 per accession, with each cell representing one replication. Each cell was thinned to one plant per cell after emergence and transplanted to a field site located in Central Ferry, WA on 12 November 2013. At the time of transplanting, each individual had between 1 to 3 true leaves.

The experimental design at all three locations was a RCBD with ten replications. No supplemental irrigation was provided at either location. Prior to planting, all three locations were roto-tilled to control weeds and to facilitate transplanting. Each site was kept weed free by hand weeding for the duration of the study. In January, February, March, and April, survival was assessed for each individual along with growth stage. Four growth stages were utilized to classify individuals: pre-tillering, tillering, boot stage, as indicate by visible reproductive tillers that are swollen and purple, and emerged panicles. Beginning in May, each study location was visited weekly. Both common garden study sites were located near a weather station that recorded air temperature on an hourly basis for the duration of each study.

The most mature panicle from each plant was collected at the first observation of panicle emergence. Sampling of the most mature panicle for each individual occurred weekly until the completion of each study. All studies were concluded when all individuals had started shattering seed. Collected panicles were stored at room temperature in Pullman, WA for at least 3 mo to allow after ripening. After 3 mo of storage seed from the last sampling date were planted to assess if seed had after ripened. The three earliest and three latest accessions were included in the after-ripening germination test. If emergence was observed from all six accessions seed was considered to be after-ripened. If emergence was not observed from all six accessions, the after-ripening germination test was repeated again two weeks later. Once germination of all tested accessions was observed, a series of seed maturity experiments was initiated to determine the GDD required for each individual to produce mature seed. Greenhouse seed maturity experiments began between October and December depending upon the common garden experiment that seed had been sourced from.

Individual panicles were broken apart by hand to separate caryopses which were subsequently planted into 48-cell inserts and flood irrigated as needed. The greenhouse was

maintained at between 22° C and 26° C with no supplemental lighting. After two weeks, emergence was scored on a binary scale to indicate seed was or was not mature at the time of collection.

3.2.3 Genetic Clustering. Depending upon the study year, winter survival ranged between 0 and 90% of transplanted individuals. To compensate for the high number of winter-killed replicates, response variables for each replication were pooled across genetic clusters. Within each replication all individuals of a particular cluster were treated as subsamples yielding 6 to 10 replications per cluster depending on study year. Genetic clusters were determined by grouping like individuals based upon distribution of 384 single nucleotide polymorphisms (SNPs).

A two-enzyme (*Pst1* and *Msp1*) genotyping-by-sequencing (GBS) approach originally developed for species lacking a reference genome (Poland et al. 2012) was utilized. A modified GBS protocol developed by Mascher et al. (2013) for use with semi-conductor sequencing platform was followed according to protocol. Sequencing was conducted on an Ion Proton™ using an Ion P1™ Chip (Life Technologies, Carlsbad, CA, USA).

SNP calling was conducted with the program Stacks (1.22, Cresko Laboratory, Eugene, OR, USA) using the "denovo_map.pl" command in stacks. In total, 16,382 SNPs were called across all individuals. The "population" command in stacks was used to reduce the total number of SNPs by only selecting genetic markers that were called from the majority of individuals. Each locus had to be found in 75% of individuals for further analysis, reducing the total remaining SNPs to 384. With the R (R Development Core Team, version 3.0.2, R Foundation for Statistical computing, Vienna, Austria) package adegenet (Jombart et al., Version 2.0.0). Discriminate Analysis of Principal Components (DAPC) (Jombart et al. 2010) was used to describe population structure of collected downy brome accessions. DAPC is multivariate approach that first relies on principal component analysis to determine the optimal number of population clusters. DAPC then creates linear

combinations of the most informative genetic markers to maximize the between-cluster variation and assign each individual to a particular cluster.

3.2.4 Statistical Modeling. Modeling the timing of different development thresholds was conducted using the same GDD model developed by Ball et al. (2004), starting 1 January and with a base temperature of 0ºC [1].

$$GDD = \sum(\frac{Tmax+Tmin}{2} - Tbase) \quad [1]$$

Using the R package DRC, a two-parameter log-logistic model [2] (Ritz and Streibig 2005; DRC R package version 2.3-96) suitable for non-linear regression of binary response variables was employed to estimate the GDD required for mature seed set. Where "*x"* refers to cumulative GDD, "*e"* refers to the GDD required to produce a 50% response, and "*b"* refers to the slope of the model at "*b"*. Accessions from each block were treated as sub-samples for each genetic cluster.

$$f(x) = \frac{1}{1+\exp(b(\log(x))-e))} \quad [2]$$

3.3 Results

3.3.1 Genetic Clustering. Results of DAPC returned seven genetic clusters (Table 1). Cluster six contains a single individual that is a congener to downy brome, *Bromus diandrus* Roth (ripgut brome), included as check to see if clustering analysis could detect the related species as an outlier. The number of individuals detected in each cluster varied by cluster. None of the clusters was more commonly found in any particular part of the small grain production region and distribution appears random.

3.3.2 Winter Survival and Timing of Development. Survival of individuals varied considerably between experiments, and was evenly distributed between clusters. The Central Ferry experiment that was completed in 2013 had a very low rate of winter kill with over 90% of individuals surviving until the end of the experiment. The Cook Agronomy Farm experiment that was completed in 2013

had considerably more winter kill with 60% of individuals surviving until the end of the experiment. Cluster six, containing only the outlier accession, was completely killed during the winter and no data were collected for that cluster at the 2013 Cook site. The Central Ferry experiment concluded in 2014 had complete winter kill of all individuals.

Comparing the GDD accumulation at all three sites (Table 2), GDD accumulation was similar in 2013 and 2014 at the Central Ferry location, but slower accumulation of GDD was observed at the 2013 Cook Agronomy Farm location. The slower GDD accumulation at the Cook Agronomy Farm is indicative of a colder winter and likely the cause of the greater degree of winter kill observed at the Cook Agronomy Farm location. The complete winter kill at the 2013 Central Ferry location is thought to be due to a December cold snap not described by a cumulative GDD model beginning on 1 January.

Survival was assessed at the 2014 Central Ferry Location in January and February and no living individuals were found. In March, downy brome plants were observed emerging from transplanted potting soil. When emerging plants were further examined, the caryopses were clearly stained with the commercial seed treatment used to control loose smut. While the 2013 Central Ferry and Cook Agronomy Farm experiments documented development from November transplanted seedlings, the 2014 Central Ferry location documented spring emergence of individuals that did not germinate in the fall. While nearly every replicate had been transplanted in the fall, only 40% of the replications emerged in the spring.

At all visits to experiment sites, growth stage was recorded. At the 2013 Central Ferry and Cook Agronomy Farm locations all individuals had tillered by the first visit in January. Reproductive tillers were not observed at the 2013 Central Ferry Location until 16 April (709 GDD) at which time most individuals were still tillering. By 8 May (1014 GDD) all individuals had progressed to

producing reproductive tillers or displaying panicles. On 15t May (1147 GDD) greater than 90% of individuals were displaying panicles.

Compared to the 2013 Central Ferry location, little variation of development stage was observed at the 2013 Cook Agronomy Farm Site. All individuals at that site had progressed from tillering to displaying reproductive tillers by 17 May (679 GDD). When the site was revisited on 22 May (734 GDD) all individuals had progressed to displaying panicles, and no differences in development stage between genetic clusters was observed.

Emergence at the 2014 Central Ferry location was observed between 1 March and 2 May (211 to 908 GDD). Timing of plant emergence from the soil did not differ among genetic clustering. The majority of individuals had produced reproductive tillers by 18 April (717 GDD) although some individuals had yet to emerge from the soil. By 2 May (908 GDD), individuals ranged in development stage from pre-tillering to emerged panicles. All individuals had displayed panicles by 3 June (1467 GDD).

3.3.3 Modeling Panicle Emergence. It was not possible to develop a GDD model for panicle emergence at the 2013 Cook Agronomy Farm location as no variation in panicle emergence was observed. Panicle emergence by cumulative GDD was modeled at both Central Ferry locations (Figure 2). With the exception of cluster six, the GDD required for panicle emergence at the 2013 Central Ferry location did not vary by cluster. The GDD required for panicle emergence for clusters one, two, three, four, five, and seven ranged from 850 to 870 (Table 3).

Greater variation was observed at the 2014 Central Ferry location with an estimated 960 to 1330 GDD required for panicle emergence (Table 3). Standard errors of the GDD estimates for the 2014 Central Ferry location were smaller (with the exception of cluster six) than the 2013 site. From the 2014 Central Ferry location cluster, one and three, can distinguished from clusters two and four as early to develop panicles.The greater GDD requirements for panicle development at the 2014

Central Ferry location compared with the 2013 Central Ferry location is likely a result of spring emergence versus winter emergence.

3.3.4 Modeling mature seed set. GDD estimates for mature seed set varied by genetic cluster and study location (Figure 3). The GDD required for mature seed set could not be estimated for cluster six at the 2013 Cook Agronomy Farm as cluster six was completely winter killed. Only two individuals emerged in the spring at the 2014 Central Ferry location and the standard error is quite large in comparison to the other clusters (Table 4). The GDD required for mature seed set at the 2013 Central Ferry location ranged from 1150 to 1250. Clusters one and three matured earlier compared to clusters four and five when comparing estimates (Table 4). Estimated GDD required for mature seed set at the 2013 Cook Agronomy Farm ranged from 990 to 1040 (Table 4). GDD estimates varied little compared to the 2013 Central Ferry location. Mature seed set estimates from the 2014 Central Ferry Location ranged from 1340 to 1450 GDD. Comparable to the 2013 Central Ferry Location, cluster 1 and 3 set seed early relative to cluster four.

3.4 Discussion

Study objectives were to observe the full range in variation of downy brome phenology in the PNW small grain production region, utilize molecular tools to identify the influence of genotype on phenology, and capture what impact climate has on phenology. Downy brome phenology was variable among genetic clusters, but the range in variation depended upon the climate of the common garden location. At the 2013 Cook Agronomy Farm, which had the coldest winter, average GDD required for mature seed set was 1010. The At the 2013 Central Ferry location, with a more mild winter compared to the Cook Site, was requirement for seed set was 1200 GDD. The At the 2014 Central Ferry location with the least exposure to winter temperatures the average estimated GDD for mature seed set was 1400.

The patterns in panicle emergence and mature seed set observed at all three common garden locations suggests that differential vernalization requirements interact with exposure to vernalizing temperatures to drive the rate of downy brome development. Clusters 1 and 3 were the earliest to set mature seed at each common garden, while clusters 2 and 4 were consistently delayed in setting mature seed relative to other clusters regardless of growing conditions, indicating that genetics and environment act additively to influence seed maturation. On the other hand, variation in development timing among accessions was greater in spring emerging downy brome and least in downy brome exposed to the most severe (coldest) winter conditions. Thus, genetically based differential vernalization requirements are obscured if winters are severe enough winter to saturate the vernalization response of all individuals. Conversely, a mild winter or spring emergence would result in differential saturation of the vernalization pathways, and greater variation in the rate of development. Differential vernalization requirements have been previously suggested (Ball et al. 2004; Meyer et al. 2004; and Rice and Mack 1991b) as an explanation for the observed variation in downy brome development. Schwartz et al. (2010) studied variation in time to flowering with differing levels of vernalization exposure from a diverse collection of *Brachypodium distachyon* ((L.) P. Beauv) were. As the length of vernalization exposure was increased, variation in time to flowering was decreased (Schwartz et al. 2010). The expression of two vernalization genes, *Vernalization 1* (*BdVRN1*) and *Vernalization 2* (*BdVRN2*) were also correlated with increased cold exposure. Given that downy brome and *Brachypodium distachyon* are both in the Triticodae group within the Pooideae subfamily (Soreng et al. 2015), genetic controls of vernalization are likely similar.

In the north of the PNW small grain production region, the GDD required for mature seed set are likely lower than the 1010 GDD average estimate from the Cook Agronomy Farm. The GDD accumulation observed at the 2013 Cook Agronomy Farm location is similar to the 30 year average for both Pullman and Ritzville. The 30 year average GDD accumulation at Wilbur, located

in the North of the Small grain production region, was slower than the GDD accumulation at the any of the common garden location. In the south of the small grain production region, the 30 year average for GDD accumulation at Walla Walla was in between the observations at the Cook Agronomy Farm and Central Ferry locations. The 1010 GDD average likely captures the requirements for all but the norther part of the PNW small grain production the bulk of the small grain production region in normal years. The 30 year average for GDD accumulation at Walla Walla fell in between what was recorded at the Central Ferry and Cook Agronomy farm locations, and the GDD requirements for the southern range of the small grain production region likely are between 1010 and 1200 GDD.

The estimates from the Central Ferry location, which is at a lower elevation and hence warmer than most of the small grain growing areas in the PNW, may represent downy brome development during atypically mild winters across the entire region. An increased frequency of mild winters are anticipated for the PNW under climate change projections (Mote and Salathé 2010), suggesting that the GDD estimates for mature seed set in the 2013 Central Ferry common garden are representative of more typical downy brome development rates under future climate change. Although increased GDD were required for downy brome mature seed set at the 2013 Central Ferry location (Table 4), mature seed set occurred in mid-May, rather than mid-June as occurred for most accessions at the Cook Agronomy Farm. This suggests that despite the increased GDD requirement for downy brome mature seed set that may occur as the climate warms, mature seed set will still likely occur earlier in the year than it does currently, since GDD will accumulate more rapidly under warmer conditions.

With an expanded collection of downy brome accessions from across the region and with a different modeling approach, the 1000 GDD mature seed set threshold proposed by Ball et al. (2004) appears to describe the development of downy brome for most of the region following an

average winter. Ball et al. (2004) used a nonlinear regression model that estimated the GDD required for the first mature seed set by describing where the model deviates from the lower asymptote. The log-logistic regression model estimates the GDD required for 50% of replications from one genetic cluster to set mature seed. The model used by Ball et al. (2004) likely is more accurate at describing the GDD required for the absolute first mature seed set from a population. While the log-logistic model is likely more accurate at describing the average GDD threshold for an entire population. The average estimate for mature seed set from the Cook Agronomy Farm, 1010 GDD, is very close to the 1000 GDD estimate proposed by Ball et al. (2004).

Downy brome phenology has implications for the efficacy of its control in agronomic fields. Ball et al. (2004) proposed the 1,000 GDD threshold as a heuristic for the last opportunity to control downy brome in fallow fields to prevent additional recruitment (or deposition) of downy brome seed into the seed bank. While the 1,000 GDD estimate appears to be a sound threshold for most of the region, at the north end of the small grain region, control likely would need to take place at an earlier GDD threshold as increased exposure to vernalizing temperatures likely would lead to earlier mature seed set relative to cumulative GDD.

Control of downy brome within small grain fields is largely reliant on applications of spring applied ALS-inhibiting herbicides (Stahlman 1994). Blackshaw (1991) reported decreased efficacy of spring applied herbicides as downy brome matured. Control of downy brome after the boot stage, when reproductive tillers are visible, was less efficacious compared to herbicide applications at earlier development stages. Reproductive tillers were first observed at the 2013 Central Ferry, 2013 Cook Agronomy Farm, and 2014 Central Ferry locations were 709, 679, 717 GDD, respectively. At the 2013 Cook Agronomy Farm reproductive tillers were likely visible several d to w earlier than the first observation which occurred at 679 GDD. Reproductive tillers may be first visible between 500

and 650 GDD although further study is necessary to confirm the required GDD under similar conditions of vernalization.

Lawrence et al. (2014) summarized 15 years of herbicide efficacy trials for the control of downy brome in the PNW. Little variation in winter applied ALS-inhibiting herbicides was reported over 15 years. The efficacy of spring applied herbicides was considerably more variable. A possible explanation for the observed variability in efficacy of spring applied herbicides may be differential development resulting in variability in growth stage on date of herbicide application. It is likely that the biotypes of downy brome present in a particular field, variation in emergence, and yearly differences in vernalization intensity may all influence the results of spring applied herbicide applications.

Variation in downy brome development is dependent on a combination of genetic factors, local climate, and their interaction. Despite yearly variation in development time, the control of downy brome can be aided by developing management thresholds for timing tillage operation and herbicide applications. The 1,000 GDD threshold proposed by Ball et al. (2004) appears to be a sound heuristic for timing tillage operations in fallow fields. A similar GDD threshold accounting indicating the development of reproductive tillers could provide an upper limit for the timing application of spring applied herbicides within small grain fields. Further work is needed to identify the spring application threshold in greater detail and to identify with certainty the genetic mechanism responsible for the observed variation in downy brome vernalization requirements.

3.5 Literature Cited

Ball DA, Frost SM, Gitelman AI (2004) Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. Weed Sci 52:518–524

Ball DA, Klepper B, Rydrych DJ (1995) Comparative above-ground development rates for several annual grass weeds and cereal grains. Weed Sci 43:410–416

Blackshaw RE (1991) Control of downy brome (*Bromus tectorum*) in conservation fallow systems. Weed Technol 5:557–562

Concilio AL, Loik ME, Belnap J (2013) Global change effects on *Bromus tectorum* L. (Poaceae) at its high-elevation range margin. Glob Chang Biol 19:161–172

Hanzlik K, Gerowitt B (2012) Occurrence and distribution of important weed species in German winter oilseed rape fields. J Plant Dis Prot 119:107–120

Huggins D, Rupp R, Gessler P, Pan W, Brown D, Machado S, Abatzoglou J, Walden V, Eigenbrode S (2012) Dynamic Agroecological Zones for the Inland Pacific Northwest,. *in* American Society of Agronomy Annual Meeting, Oct. 21-24, Cincinnati, OH

Hulbert L (1955) Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecol Monogr 25:181–213

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:1–15

Klemmedson JO, Smith JG (1964) Cheatgrass (*Bromus Tectorum* L.). Bot Rev 30:226–262

Klepper B, Rickman R, Zuzel J, Waldman S (1988) Use of growing degree days to project sample dates for cereal crops. Agron J 80:850–852

Lawrence NC, Burke IC, Yenish JP (2014) 15 Years of Downy Brome Control in Eastern Washington. Pages 12–13 *in* Proceedings of the 67th Annual Meeting of the WSWS, Colorado Spring, CO: Western Society of Weed Science

Mascher M, Wu S, Amand PS, Stein N, Poland J (2013) Application of genotyping-by-sequencing on semiconductor sequencing platforms: a comparison of genetic and reference-based marker ordering in barley. PLoS One 8:e76925

Metcalf JC, Rose KE, Rees M (2003) Evolutionary demography of monocarpic perennials. Trends Ecol Evol 18:471–480

Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). Ann Bot 93:653–63

Morrow LA, Stahlman PW (1984) The History and Distribution of Downy Brome (*Bromus tectorum*) in North America. Weed Sci 32:2–6

Mote PW, Salathé EP (2010) Future climate in the Pacific Northwest. Clim Change 102:29–50

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692

Peters K, Breitsameter L, Gerowitt B (2014) Impact of climate change on weeds in agriculture: a review. Agron Sustain Dev 34:707–721

Poland JA, Brown PJ, Sorrells ME, Jannink J (2012) Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS One 7:e32253

Rice K, Mack R (1991a) Ecological genetics of Bromus tectorum. I. A hierarchical analysis of phenotypic variation. Oecologia 88:77–83

Rice KJ, Mack RN (1991b) Ecological genetics of bromus tectorum. III. The Demography of Reciprocally Sown Populations. Oecologia 88:91–101

Ritz C, Streibig JC (2005) Bioassay analysis using R. J Stat Softw 12:1–22

Rydrych D, Muzik T (1968) Downy brome competition and control in dryland wheat.Agron J 60:279–280

Rydrych DJ (1974) Competition between Winter Wheat and Downy Brome. Weed Sci 22:211–214

Schwartz CJ, Doyle MR, Manzaneda AJ, Rey PJ, Mitchell-Olds T, Amasino RM (2010) Natural variation of flowering time and vernalization responsiveness in *Brachypodium distachyon*. BioEnergy Res 3:38–46

Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O (2015) A worldwide phylogenetic classification of the Poaceae (Gramineae). J Syst Evol 53:117–137

Stahlman PW (1994) Sulfonylurea herbicides suppress downy brome (*Bromus tectorum*) in winter wheat (*Triticum aestivum*). Weed Technol 8:812–818

Stewart G, Hull A (1949) Cheatgrass (*Bromus Tectorum* L.) --An ecologic intruder in Southern Idaho. Ecology 30:58–74

Stöckle CO, Nelson RL, Higgins S, Brunner J, Grove G, Boydston R, Whiting M, Kruger C (2010) Assessment of climate change impact on Eastern Washington agriculture. Clim Change 102:77–102

Thill DC, Beck KG, Callihan RH (1984) The biology of downy brome (*Bromus tectorum*). Weed Sci 32:7–12

Upadhyaya M, Turkington R, McIlvride D (1986) The biology of Canadian weeds.: 75. *Bromus tectorum* L. Can J Bot 66:689–709

3.6 Tables and Figures

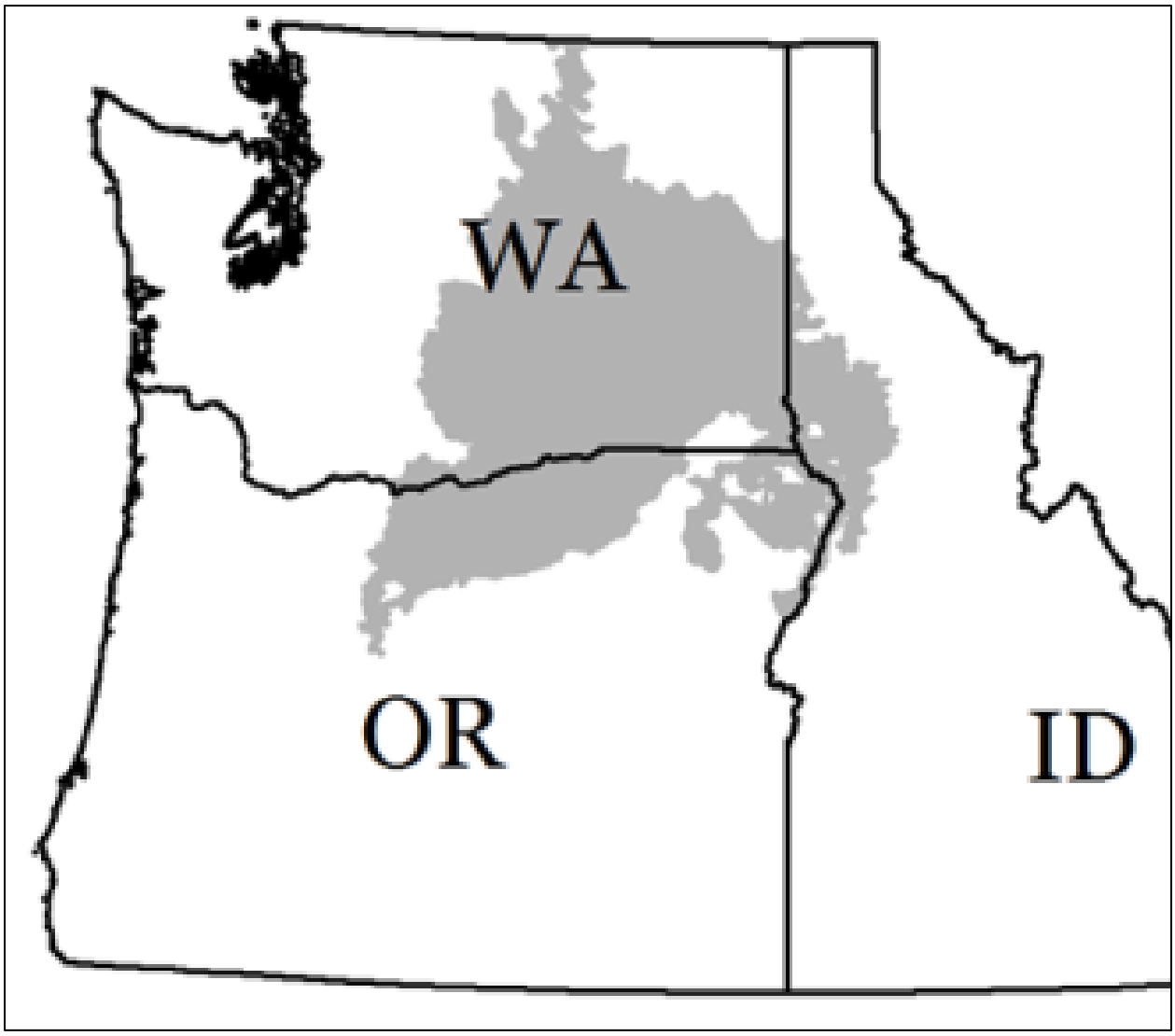


Figure 3.1. The small grain production region of the PNW. All accessions were sourced from within the small grain production region of the PNW.

Table 3.1. Accession ID, year of collection, GPS coordinates of collection, and assigned cluster of each accession evaluated in common garden and greenhouse experiments.

Accession	Year	Longitude	Latitude	Cluster	Accession	Year	Longitude	Latitude	Cluster
5	2010	-120.35	45.90	3	104	2011	-118.61	46.08	4
8	2010	-120.24	46.00	4	106	2011	-118.74	46.34	2
10	2010	-120.66	46.56	7	107	2011	-118.79	46.74	7
12	2010	-120.36	45.42	5	108	2011	-118.65	46.77	5
13	2010	-120.49	45.48	4	110	2011	-118.64	47.26	2
18	2010	-120.56	46.46	7	111	2011	-118.79	47.48	2
21	2010	-120.16	45.40	4	112	2011	-118.85	47.52	4
22	2010	-120.18	45.62	4	113	2011	-118.68	47.80	7
26	2010	-120.16	46.26	4	115	2010	-118.40	45.76	1
28	2010	-119.91	46.70	7	116	2011	-118.44	46.01	1
29	2011	-120.34	46.89	5	118	2011	-118.36	46.33	5
30	2011	-120.41	47.00	4	123	2011	-118.36	47.23	7
32	2010	-119.94	45.39	4	125	2011	-118.46	47.49	4
37	2010	-119.86	46.26	1	127	2010	-118.10	46.37	3
38	2010	-120.13	46.38	1	128	2011	-118.37	46.68	4
39	2010	-119.85	46.67	3	129	2011	-118.13	46.66	4
42	2011	-119.87	47.10	3	130	2011	-118.17	46.80	4
45	2010	-119.80	45.35	1	133	2011	-118.31	47.46	7
53	2011	-119.85	46.74	4	135	2011	-118.14	47.69	7
56	2011	-119.71	47.34	2	136	2010	-117.91	46.39	4
59	2011	-117.75	47.90	2	137	2010	-117.95	46.43	7
60	2010	-119.44	45.64	5	138	2010	-117.80	46.63	4
63	2010	-119.37	46.11	4	139	2011	-118.18	46.91	2
64	2010	-119.22	46.39	3	141	2010	-117.87	47.21	1
65	2010	-119.69	46.74	3	143	2010	-117.88	47.52	2
67	2011	-119.41	46.85	4	147	2010	-117.66	46.90	3
70	2011	-119.18	47.42	7	148	2010	-117.67	47.12	7
74	2010	-119.28	45.76	4	152	2011	-117.63	47.72	4
75	2010	-119.24	46.03	4	154	2010	-117.52	46.49	4
78	2010	-119.22	46.56	1	156	2010	-117.48	46.95	4
79	2011	-119.28	46.71	4	157	2010	-117.38	47.26	4
81	2011	-119.20	47.01	1	159	2011	-117.55	47.52	4
82	2011	-119.15	47.18	4	161	2011	-117.16	46.37	1
83	2011	-119.32	47.47	2	165	2010	-117.17	47.10	4
86	2011	-119.37	47.89	1	166	2010	-117.25	47.31	4
88	2011	-118.99	45.77	7	167	2010	-117.25	47.39	4
93	2011	-118.90	46.67	4	168	2010	-117.09	47.48	4
94	2011	-118.99	46.82	4	169	2011	-116.87	46.40	4
96	2011	-119.08	47.20	3	172	2010	-116.84	46.92	4
99	2011	-118.92	47.79	4	179	2010	-116.71	46.92	3
100	2011	-119.16	47.99	4	182	2010	-116.47	46.25	4
101	2010	-118.86	46.48	4	183*	2010	-115.96	46.10	6
103	2011	-118.60	45.99	1	—	—	—	—	—

*Accession is *Bromus diandrus* Roth.

Table 3.2. Growing degree accumulation by month for the three common garden studies. Accumulation of growing degree days began on January 1st and with a base temperature of 0°C.

	Location and Year		
Date	Central Ferry 2013	Cook 2013	Central Ferry 2014
1 January	1	0	2
1 February	86	23	109
1 March	251	82	211
1 April	544	244	506
1 May	881	438	890
1 June	1403	833	1422
1 July	1976	1266	1979

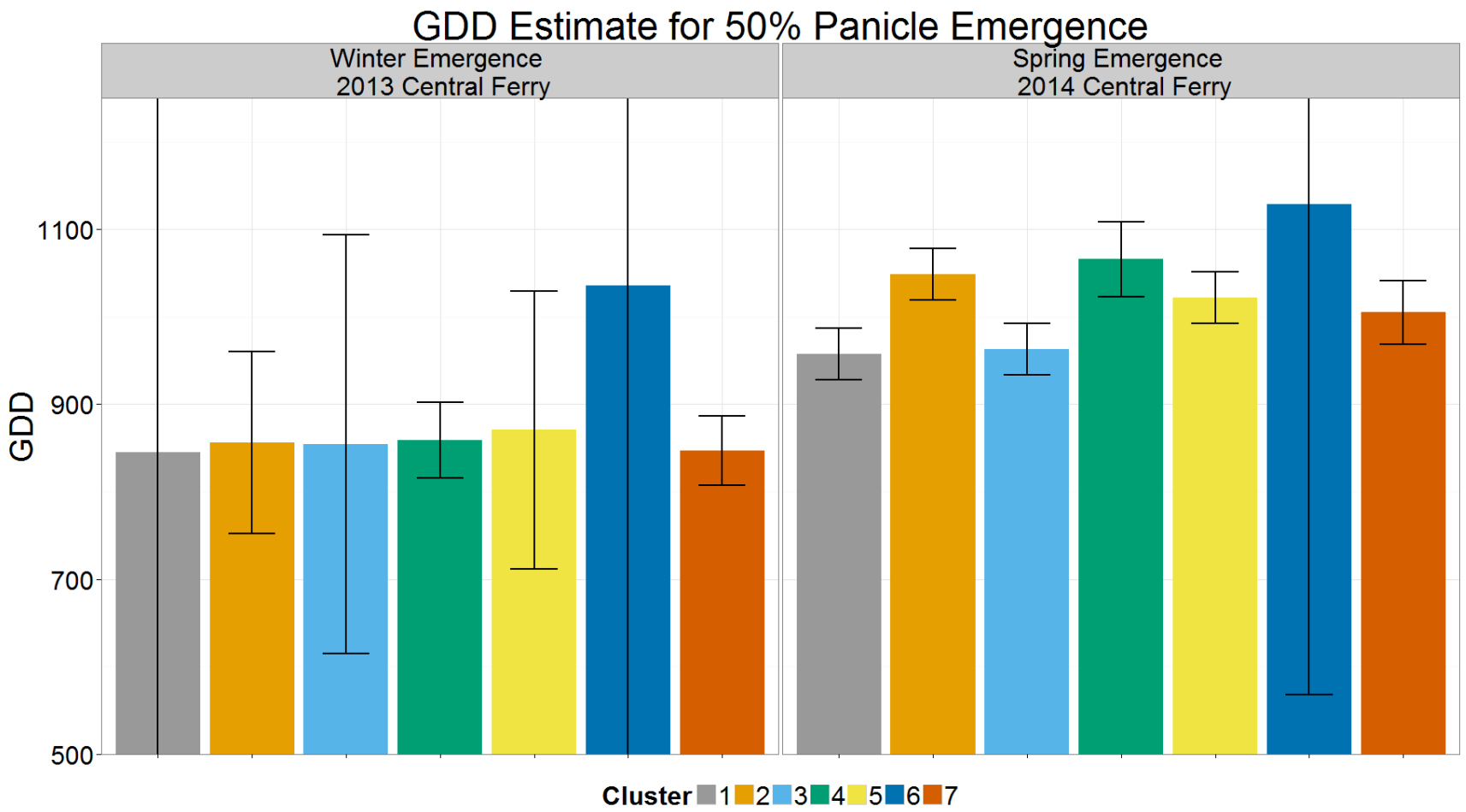


Figure 3.2. Growing degree days required and S.E. for 50% panicle emergence for each genetic cluster. Response of each cluster is separated by common garden location and year the study was terminated. Cluster six is *Bromus diandrus* Roth

Table 3.3. Growing degree days required and S.E. for 50% panicle emergence for each genetic cluster. Response of each cluster is separated by common garden location and year the study was terminated.

	——GDD Estimate (S.E.)——	
Cluster	Central Ferry 2013	Central Ferry 2014
1	850 (460)	960 (30)
2	860 (100)	1050 (30)
3	850 (240)	960 (30)
4	860 (40)	1070 (40)
5	870 (160)	1020 (30)
6*	1040 (2700)	1130 (560)
7	850 (40)	1010 (40)
Average†	860	1010

*Cluster is *Bromus diandrus* Roth.
†Average ignores cluster six.

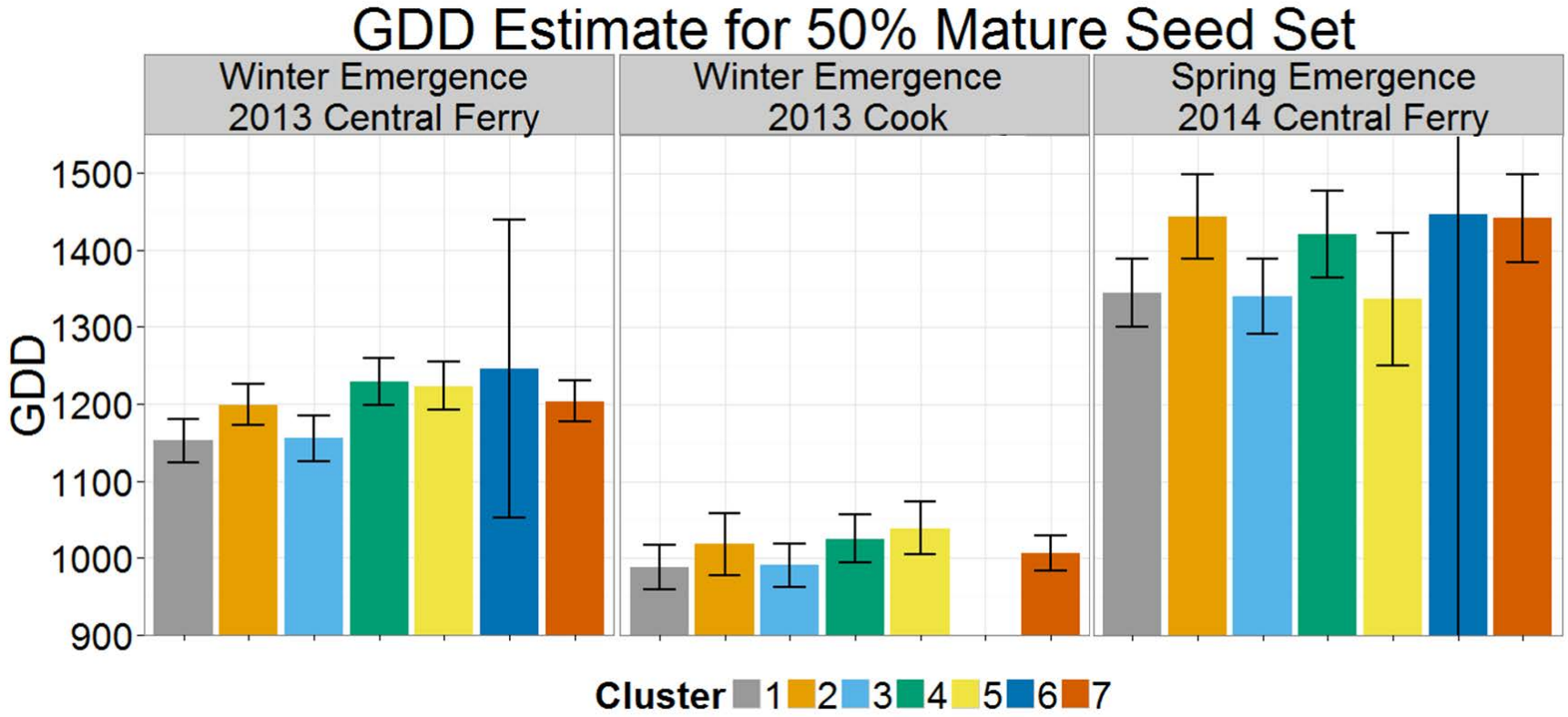


Figure 3.3. Growing degree days required and S.E. for 50% mature seed set for each genetic cluster. Response of each cluster is separated by common garden location and year the study was terminated. Cluster six is *Bromus diandrus* Roth. Absence of a bar indicates all replicates were winter killed.

Table 3.4. Growing degree days required and S.E. for 50% mature seed set for each genetic cluster. Response of each cluster is separated by common garden location and year the study was terminated.

	GDD Estimate (S.E.)		
Cluster	Central Ferry 2013	Cook 2013	Central Ferry 2014
1	1150 (30)	990 (30)	1340 (40)
2	1200 (30)	1020 (40)	1440 (60)
3	1160 (30)	990 (30)	1340 (50)
4	1230 (30)	1030 (30)	1420 (60)
5	1220 (30)	1040 (30)	1340 (90)
6*	1250 (190)	—	1450 (5500)
7	1200 (30)	1010 (20)	1440 (60)
Average†	1200	1010	1400

*Cluster is *Bromus diandrus* Roth. At the 2013 Cook location all individuals from cluster six were winter killed.
†Average ignores cluster six

Table 3.5. Locations of four towns in the PNW small grain production region, thirty year average (1981-2010) GDD accumulation for each town, and the 30 year average calendar date that 850, 1000 and 1500 GDD occurred. Downy brome panicle emergence, averaged across all genetic clusters, occurred near 850 GDD at the 2013 Central Ferry location. The 1000 GDD is the estimated threshold for flowering across the majority of the PNW small grain production region. Averaged across genetic clusters, spring emerging downy brome produced mature seed around 1500 GDD at the 2014 Central Ferry location.

	Walla-Walla	Pullman	Ritzville	Wilbur
Longitude	-118.29	-117.19	-118.37	-118.72
Latitude	46.09	46.76	47.12	47.768
Elevation (m)	355	767	568	689
Date		GDD		
1 January	1	0	0	0
1 February	63	2	0	0
1 March	176	39	33	5
1 April	427	187	192	124
1 May	767	425	445	349
1 June	1243	797	838	721
1 July	1822	1252	1336	1192
GDD		Calendar Date		
850	May 8th	June 2nd	June 5th	June 10th
1000	May 18th	June 16th	June 12th	June 20th
1500	June 16th	July 15th	July 10th	July 18th

Chapter 4. Characterization of Vernalization Requirements and Expression of *BdVRN1* in *Bromus tectorum*

4.1 Introduction

Downy brome (*Bromus tectorum* L.) is a widely distributed winter annual weed across Western North America (Morrow and Stahlman 1984). Native to Eurasia, genetic evidence suggests downy brome was introduced to North America through multiple immigrations (Novak et al. 1993). Vigorous initial growth and early seed production allows establishment in disturbed natural areas and agronomic fields. When wheat is tillering, downy brome is generally in the boot stage, flowering four weeks earlier than winter wheat (Thill et al. 1984). Since downy brome matures earlier than winter wheat, soil moisture and nutrients can be depleted when winter wheat is at a critical reproductive period, resulting in considerable yield losses (Thill et al. 1984).

Multiple studies have characterized variation in downy brome phenotype by observing natural populations, conducting common garden experiments, and through greenhouse studies (Ball et al. 2004, Hulbert 1955, Klemmedson and Smith 1964, Meyer et al. 2004, Rice and Mack 1991a, 1991b). Hulbert (1955) described differences in phenology among downy brome collections from Europe and North America as "striking" in a common garden experiment. The first appearance of panicles, the change to purple coloration, and the change to brown coloration (signaling senescence) were used to differentiate biotypes as winter hardiness, plant size, shoot and root morphology, seed production, and seed dormancy were too variable (Hulbert 1955). Rice and Mack (1991a) also observed phenotypic variation among siblings in respect to several life history traits, which was attributed to plastic response to environmental influences. Traits including plant size, shoot and root morphology, seed production, and seed dormancy have been reported as variable between individuals collected from the same source population and even among siblings (Hulbert 1955, Rice and Mack 1991a, 1991b). Variation in downy brome development time, however, has been reported

as stable within populations, across different environments, and adaptively significant (Hulbert 1955, Meyer et al. 2004, Rice and Mack 1991a, 1991b).

Flowering time was found to be highly stable among siblings and within populations, but variable between populations by Rice and Mack (1991a) who concluded that variation in flowering time is an environmentally stable trait reflecting local adaptation between populations. As part of the same series of studies, Rice and Mack (1991b) reciprocally sowed downy brome seed from four seed sources in a common garden to observe whether variation among siblings with respect to dry plant weight, seed weight, seed number, and phenology would differ with local climate. Seed number, seed weight, and plant weight did vary from year to year, which Rice and Mack (1991b) attributed to local acclimation of traits in response to environment. Flowering time, however, was stable year to year among plants from the same seed source (Rice and Mack 1991b). Rice and Mack (1991b) concluded the stability observed within flowering time was evidence of strong genetic controls, compared with plant size, survival, and seed production.

In a series of experiments to model the cumulative growing degree days (GDD) required to produce mature seed, Ball et al. (2004) observed little variation in the timing of mature seed set from collections in Oregon and Washington. However, when collections were made across Western North America, considerable variation in the GDD was reported. Variation in seed maturity time across western North America might be attributed to: 1) how differences in climate affect the rate of vernalization, 2) varied vernalization requirements in different biotypes regardless of climate, or both (Ball et al. 2004). Ball et al.'s (2004) observed consistent timing of mature seed set within the PNW but variable timing of mature seed set across the western United States (Ball et al. 2004) agrees with the conclusions of Rice and Mack (1991a, 1991b) that downy brome flowering time can reflect local adaption to climate.

Ball et al. (2004), Meyer et al. (2004), and Rice and Mack (1991b) have attributed variation in downy brome phenology to variation in the vernalization requirements. Vernalization can be defined as the acquisition or acceleration of the ability to flower by a chilling treatment (Chouard 1960). Meyer et al. (2004) observed differences in vernalization requirements by downy brome under greenhouse conditions. Downy brome was collected from two dissimilar seed sources, one originating from a cold desert and the other a warm desert environment. Flowering time was measured for individuals from each collection and vernalized for 0 to 14 w. Little variation in flowering response to vernalization treatments was observed among sibling plants collected from the same seed source. However, considerable variation occurred among seed sources. Plants collected from warm dry desert regions required no vernalization whereas plants collected from cold high altitude locations required vernalization to flower (Meyer et al. 2004). Moreover, the proportion of individuals flowering increased with the length of vernalization time. Meyer et al. (2004) concluded that vernalization response and requirements are adaptive and that differences observed between seed sources reflect selection on founder populations.

Although no prior research has been conducted on the molecular controls of vernalization in downy brome, a great deal is known about vernalization genetics in the cereals *Triticum aestivum* L. and *Hordeum vulgare* L, and the model species *Brachypodium distachyon* (L.) P. Beauv. *FT like 1* (*FT1*) integrates the vernalization and photoperiod pathway and is responsible for long distance signaling of flowering in the cereals (Greenup et al. 2009). In wheat and barley, vernalization is regulated by *Vernalization 1* (*VRN1*) and *Vernalization 2* (*VRN2*) genes. *VRN2* suppresses *FT1* in the presence of long days (Hemming et al. 2008, Takahashi and Yasuda 1971). *VRN1* is expressed with vernalization and upregulates *FT1* and down regulates *VRN2* (Hemming et al. 2008, Trevaskis et al. 2006). *VRN1* mediates flowering through acceleration of the transition to reproductive growth at the shoot apex and activation of the long day response in the leaves (Greenup et al. 2009). Spring varieties of

wheat and barley that do not require vernalization to flower are the result of a mutation in the *VRN1* promoter, or because of deletion or insertion within the first intron of *VRN1* (Cockram et al. 2007, Fu et al. 2005, Szucs et al. 2007, Yan et al. 2003, Von Zitzewitz et al. 2005). In wheat, most natural variation in vernalization requirements can be attributed to variation of *VRN1* gene (Chen and Dubcovsky 2012).

Similar to wheat and barley, *B. distachyon* contains a homologous gene to *A. thaliana FT*, and a homologous gene to wheat and barley *VRN1*. However, no homologs of wheat or barley *VRN2* have been discovered (Higgins et al. 2010). A *VRN2 like* (*VRN2L*) gene has been identified in *B. distachyon* and is thought to integrate vernalization and photoperiod response, however *BdVRN2L* behaves in a dissimilar way from wheat or barley *VRN2* (Ream et al. 2014). In wheat and barley *VRN2* expression decreases sharply following a vernalization treatment, while *BdVRN2L* expression does not, suggesting it may play a different role in *B. distachyon* vernalization response (Ream et al. 2012, 2014). By leveraging the current knowledge and genetic resources of cereal and model species molecular controls of vernalization in downy brome can be correlated to variation in development time.

Although little is known regarding the genetic controls of vernalization in downy brome, transitional approaches based on known sequences for vernalization genes and vernalization behavior of wheat, barely, and *B. distachyon* can be used to design primers and experiments for downy brome. All four species are within the subfamily Pooideae. Wheat, barley and downy brome are all members of to the supertribe Triticodae (Soreng et al. 2015). Comparisons of known vernalization gene sequences between wheat, barley, and *B. distachyon* have detected greater than 80% homology (Schwartz et al. 2010). The close relatedness and high homology may allow leveraging VNR genes to use a translational approach to develop primers in brome.

Variation in phenology of downy brome is a key factor in the success of the species as an ecological invader of natural areas and competitor within agronomic fields (Thill et al. 1984). Prior research documented differing vernalization requirements of downy brome collected from different environments (Ball et al. 2004, Meyer et al. 2004, and Rice and Mack 1991b), but no previous work has characterized the connection between phenotypic responses and genotypic control of downy brome vernalization.

As most variation in vernalization requirements of wheat and barley have been attributed to variation of *VRN1* (Chen and Dubcovsky 2012), quantifying the expression of a *VRN1* orthologue in downy brome may help explain the genetic controls regulating downy brome phenology. Therefore a series of greenhouse experiments was conducted to: (1) characterize the vernalization requirements of downy brome accessions which had previously demonstrated differential developmental rates and (2) determine if expression of *VRN1* orthologues can be linked to contrasting rates of development.

4.2 Methods

4.2.1 Greenhouse Vernalization Experiments. A series of previous common garden experiments was conducted involving 85 accessions of downy brome collected from within small grain production fields of Washington, Oregon, and Idaho. Results of previous common garden experiments identified differences in time to flowering of up to 19 d and time required for mature seed production of up to 21 d among accessions with little variation among siblings. Eight accessions from the larger collection of 85, three characterized as early to develop (Early 1, Early 2, and Early 3), two characterized as intermediate (Intermediate 1 and Intermediate 2), and three characterized as late to develop (Late 1, Late 2, and Late 3), were used to quantify vernalization requirements and *VRN1* expression (Table 1). Each accession was exposed to 0, 2, 4, 6, or 8 w of vernalization at 3°C in a growth chamber. The duration of the study lasted 16 w from the start of

vernalization through the observation of flowering. Plants exposed to the 2 w, 4 w, 6w, and 8 w of vernalization were monitored for flowering response an additional 14, 12, 8, and 6 w, respectively. Non-vernalized controls were observed for flowering response for 16 w. The experimental design was two-way factorial, with factor one as accession and factor two as vernalization treatment, set in a completely randomized design with each treatment replicated five times. The entire experiment was replicated twice with separate growth chambers and greenhouses used for each study replicate.

Prior to and after vernalization exposure, plants were kept in a greenhouse maintained at 22 to 26°C. Supplemental lighting prior to, during, and after vernalization was provided for 12 hours per day (d) at 400 μmols m^{-2}. Lighting was kept consistent at all stages to control for the influence of photoperiod on flowering. Biological replicates from each accession were full siblings sourced from the same maternal plant. Individual replicates were planted into 49 cm^3 containers (RLC3, Stuewe & Sons, inc, Tangent, OR, USA) and filled with a peat based growth media (LC1 Mix. Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA). Each container held a separate biological replicate. Biological replicates were planted at a density of 10 to 15 seeds per container at a depth of 2 cm below the surface of the growth media. All containers were thinned to one plant prior to start of vernalization treatments. At the start of the vernalization treatments all individuals were between 2 to 3 true leaves.

4.2.3 VRN1 Primer Design. The *Brachypodium* gene BRADI1g08340 (*BdVRN1*) (Schwartz et al. 2010), 88% identical to barley (*HvVRN1*) and wheat (*TaVTR-1*) VRN1 genes, was used to develop primers to identify expression of a downy brome VRN1 orthologue. BRADI1g08340 was compared to *HvVRN1* and wheat *TaVTR-1* using the BLAST (Altschul et al. 1990) tools available through Phytozome 10.3 (http://phytozome.jgi.doe.gov/) to search the NCBI database (http://blast.ncbi.nlm.nih.gov/). Primers were developed using the first exon from BRADI1g08340 (primers 5'-CATGAGTCGGTGGCGAACT-3' and 5'-CGGGAAGGTGCAGCTGAA-3').

Elongation factor 1-alpha (EF1α) (primers 5'-GTCTGGCCATCCTTGGAGAT-3' and 5'-CCATCGATATTGCCTTGTGG -3') was chosen as a reference gene to compare *VRN1* expression (Hong et al. 2008). No prior publications have studied valid reference genes for use with downy brome. Hong et al. (2008) compared nine genes for stability across different plant tissues, development stages, following hormone treatments, and abiotic stresses. *EF1a* was the third most stable gene across all treatments, and the second most stable gene in response to heat and cold stress (Hong et al. 2008).

VRN1 and *EF1* primers were validated using polymerase chain reaction (PCR) and 100 ng of genomic DNA per reaction. PCR conditions were: 5 min of denaturation 94°C, followed by 35 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 60°C, and 50 s of extension at 72°C, and ending with a final extension at 72°C for 10 min. *VRN1* and *EF1a* amplicons were analyzed using 1% agarose gels and visualized with SYBR® Safe (Thermo Fisher Scientific Inc, Wilmington, DE, USA) according to the manufacturer's recommendations.

4.2.2 RNA Extraction and RT-PCR. Plant tissue was collected from 3 of the 5 biological replicates of each treatment two weeks following the end of each vernalization treatment. The youngest fully expanded leaf was harvested at each interval to maintain consistent size as plants matured. Plant tissue was flash frozen in liquid nitrogen, and later stored at -80°C until RNA extraction. RNA was extracted from frozen leaf tissue using a TRIZOL® Reagent (Thermo Fisher Scientific Inc, Wilmington, DE, USA) according to protocol provided by Thermo Fisher Scientific. RNA concentrations were quantified using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, DE, USA), and normalized to a concentration of 250 ng μl^{-2}. Semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis was used to measure *VRN1* expression between accessions from different vernalization treatments. Complementary DNA (cDNA) was synthesized using 3.8 μg of total RNA using the iScript™ Reverse Transcription

Supermix (BIO-RAD, Hercules, California) according to the manufacturer's protocol. PCR conditions were: 5 min of denaturation 94°C, followed by 35 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 60°C, and 50 s of extension at 72°C, and ending with a final extension at 72°C for 10 min. *VRN1* and *EF1a* amplicons were analyzed using 1% agarose gels and visualized with SYBR® Safe (Thermo Fisher Scientific Inc, Wilmington, DE, USA) according to the manufacturer's recommendations.

4.2.4 Statistical Analysis. The "glm" command in R (R Development Core Team, version 3.0.2, R Foundation for Statistical computing, Vienna, Austria) was used to test for the significance of vernalization and downy brome accession on flowering response after 16 w. Both vernalization treatment and downy brome accession were treated as fixed effects. As downy brome accession is a categorical variable, the glm procedure treats one of the accessions a reference variable and contrasts all other accessions are tested against the reference accession. To provide contrast of each accession against all others, the glm procedure was repeated with each accession serving as a reference variable. Significant interactions between vernalization treatment and downy brome accession were described by regressing the flowering response of each accession aginst weeks of vernalization using the R package "drc" (Ritz and Streibig 2005; DRC R package version 2.3-96) and two-parameter logistic regression model suitable for binomial response variables.

$$f(x) = \frac{1}{1+\exp(b(\log(x))-e))} \quad [2]$$

Where "*x"* refers to weeks of vernalization, "*e"* refers to the weeks of vernalization required to produce a 50% response, and "*b"* refers to the slope of the model at "*e"*.

4.3 Results

4.3.1 Greenhouse Vernalization Experiments. The objective of the greenhouse experiment were to: (1) characterize the vernalization requirements of downy brome accessions which had previously

demonstrated differential developmental rates and (2) identify if expression of *VRN1* orthologues can be linked to contrasting rates of development. There was no difference in variance between the two study replicates and results for both studies were combined. Non-vernalized controls did not flower by the end of the experiment (Figure 1). Only a single replicate from the Early 3 accession flowered within 14 w following vernalization for 2 w. Flowering was observed in 30 to 55% of individuals from the early flowering accessions and from the Late 1 accession following 4 w of vernalization. Greater than 70% of plants from all accessions had flowered following the 6 w vernalization treatment. The Late 2 accession did not flower in response to any of the vernalization treatments and so was removed from further statistical analyses testing the significance of flowering response.

Each categorical variable was compared pairwise using a glm procedure to test which accessions were statistically different from each other. The glm procedure segregated the flowering response of each accession into two groups (Table 2). Accessions Early 1, 2, 3, and Late 1 began flowering after 4 w of vernalization. Accessions Intermediate 1, 2 and Late 3 did not flowering until after 6 w of vernalization. Accessions Early 1, 2, 3, and Late 1 were all different from accessions Intermediate 1, 2, and Late 3; and vice versa. Regression analysis returned an estimate of the weeks of flowering required to induce a 50% flowering response in each accession (Table 3). Results of regression analysis can be grouped by accession into two responses similar to results generated from the glm procedure. Estimates for the number of weeks required to induce a 50% flowering response were identical for accessions Early 1, 2, 3, and Late 1. The estimates for 50% flowering from accessions Intermediate 1 and Late 3 were identical, and similar to the estimate for Intermediate 2.

4.3.2 VRN1 Expression. Expression of *VRN1* from accessions Early 3, Intermediate 2 and Late 2 were evaluated from cDNA which was extracted from each replicate two weeks following the conclusion of vernalization treatments. The control gene, *EF1a*, was expressed from all accessions,

replicates, and vernalization treatments. The gene *VRN1* was expressed following 8 w of vernalization only in accessions Intermediate 2 and Late 2 for all replicates (Figure 2). RNA extracted from other vernalization treatments did not contain expressed *VRN1*.

4.4 Discussion

Study objectives were to characterize the vernalization requirements of downy brome accessions which had previously demonstrated differential developmental rates and determine if expression of *VRN1* orthologues can be linked to contrasting rates of development. The timing of flowering response among accessions induced from growth chamber and greenhouse experiments differed from those observed from a common garden experiment conducted under field conditions Accession Late 1 flowering behavior was more similar to the early to develop accessions than the late to develop accessions. The earliest and latest to develop accessions from common garden experiments, Early 3 and Late 2, respectively remained the earliest and latest accessions to flower was observed under growth chamber and greenhouse conditions Expression of a *VRN1* orthologue was only observed in treatments were flowering did occur, suggesting that the molecular controls of flowering in downy brome are likely similar to related species.

Meyer et al. (2004) conducted a similarly designed experiment to study the variation of flowering response to different lengths of vernalization among accessions from four populations representing two contrasting environments, one a cold desert and the other a warm desert. One hundred percent of individuals from the warm desert environment flowered without any vernalization. From the cold dessert environment between 20 and 30% of individuals flowered within 16 w from a 2 w vernalization treatment, comparable to the response of accession Early 3. Greater than 80% of individuals from cold desert environments flowered from the 4 w vernalization treatments within 10 weeks (Meyer et al. 2004), developing earlier from a comparable vernalization treatment than any of the accessions in the present study collected from the small-grain production

region of the Oregon, Idaho, and Washington. Differences between results reported by Meyer et al. (2004) and results reported above may be an indication adaptation of downy brome to local climate.

Ball et al. (2004) reported little variation in time to develop among accessions collected within Oregon and Washington, but considerable variation among accessions collected from Colorado, Montana, Nebraska, Oklahoma, and Kansas. Meyer et al. (2004) were all collected from desert regions of Nevada and Utah. The different vernalization requirements of plants collected from the Pacific Northwest and the Southwest likely are a reflection selection of founder genotypes to different environments (Ramakrishnan et al. 2006, Scott et al. 2010). Both Ramakrishnan et al. (2006) and Scott et al. (2010) documented genotypes that were commonly found in similar habitats, indicating selection can favor a particular genotype better adapted to a particular environment. Scott et al. (2010) argued the generation of novel genotypes as a substrate for habitat specific selection would be accompanied by evidence of recombination. As recombination was exceedingly rare Scott et al. (2010) argued selection favoring preadapted genotypes was the most parsimonious explanation.

Despite observed flowering in accession Early 2 following 4 w of vernalization, RT-PCR did not detect any expression of *VRN1* from the 4 w treatment. In a study of vernalization and photoperiod requirements for *Brachypodium distachyon,* Ream et al. (2014) reported differences in the expression *VRN1* from saturating (long enough to induce a flowering response) and subsaturating (not long enough to induce a flowering response) vernalization treatments. Subsaturating treatments did not differ significantly from nonvernalized plants with respect to *VRN1* expression but result in delayed flowering compared to fully vernalized individuals. The 4 w vernalization treatment in the present study was likely subsaturating compared to 6 and 8 w vernalization treatments for most accessions. In barley, *HvVRN1* was detectable after 7 or 9 w of vernalization by gel-blot analysis, but difficult to observe after 1, 3, or 5 w of vernalization (Trevaskis et al. 2006). Alternatively,

VRN1 expression may have been detectable in the present study using quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) which is more sensitive than RT-PCR.

Previous research has suggested the relative differences in development time observed among downy brome populations is due to variation in vernalization requirements (Ball et al. 2004, Meyer et al. 2004, and Rice and Mack 1991b). The expression of *VRN1* was only found in vernalized plants. The expression of a *VRN1* orthologue in downy brome is similar to what has been reported for *Brachypodium* (Ream et al. 2014) and barley (Trevaskis et al. 2006). To fully elucidate the flowering requirements of downy brome, the role of other flowering genes, and the role of day length in regulating downy brome flowering still need to be addressed. Variation in flowering time among cereal species can be largely attributed to variation in *VRN1* expression (Chen and Dubcovsky 2012). Within species, other factors may play a role, but not likely molecular or structural differences in *VRN1* proteins, which are highly conserved in cereals (Von Zitzewitz et al. 2005). In barley, variation in the intron length may account for differences in vernalization requirements of different genotypes (Szucs et al. 2007). To determine whether a similar mechanism occurs in downy brome, its *VRN1* homologue should be sequenced in accessions that differ in vernalization requirements.

4.5 Literature Cited

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ball DA, Frost SM, Gitelman AI (2004) Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. Weed Sci 52:518–524

Chen A, Dubcovsky J (2012) Wheat TILLING mutants show that the vernalization gene VRN1 down-regulates the flowering repressor VRN2 in leaves but is not essential for flowering. PLoS Genet 8:1–13

Chouard P (1960) Vernalization and its relations to dormancy. Annu Rev Plant Physiol 11:191–238

Cockram J, Chiapparino E, Taylor SA, Stamati K, Donini P, Laurie DA, O'Sullivan DM (2007) Haplotype analysis of vernalization loci in European barley germplasm reveals novel *VRN-H1* alleles and a predominant winter *VRN-H1/VRN-H2* multi-locus haplotype. Theor Appl Genet 115:993–1001

Fu D, Szucs P, Yan L, Helguera M, Skinner JS, Von Zitzewitz J, Hayes PM, Dubcovsky J (2005) Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol Genet Genomics 273:54–65

Greenup A, Peacock WJ, Dennis ES, Trevaskis B (2009) The molecular biology of seasonal flowering-responses in Arabidopsis and the cereals. Ann Bot 103:1165–1172

Hemming MN, Peacock WJ, Dennis ES, Trevaskis B (2008) Low-temperature and daylength cues are integrated to regulate FLOWERING LOCUS T in barley. Plant Physiol 147:355–366

Higgins JA, Bailey PC, Laurie DA (2010) Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. PLoS One 19;5(4):e10065

Hong SY, Seo PJ, Yang MS, Xiang F, Park CM (2008) Exploring valid reference genes for gene expression studies in *Brachypodium distachyon* by real-time PCR. BMC Plant Biol 8:112

Hulbert L (1955) Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecol Monogr 25:181–213

Klemmedson JO, Smith JG (1964) Cheatgrass (*Bromus Tectorum* L.). Bot Rev 30:226–262

Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). Ann Bot 93:653–63

Morrow LA, Stahlman PW (1984) The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci Soc Am 32:2–6

Novak S, Mack R, Soltis P (1993) Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. Can J Bot 71:1441–1448

Ramakrishnan AP, Meyer SE, Fairbanks DJ, Coleman CE (2006) Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*. Plant Species Biol 21:61–73

Ream TS, Woods DP, Amasino RM (2012) The molecular basis of vernalization in different plant groups the molecular basis of vernalization in different plant groups. Cold Spring Harb Symp Quant Biol 77:105–115

Ream TS, Woods DP, Schwartz CJ, Sanabria CP, Mahoy JA, Walters EM, Kaeppler HF, Amasino RM (2014) Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon*. Plant Physiol 164:694–709

Rice K, Mack R (1991a) Ecological genetics of *Bromus tectorum*. I. A hierarchical analysis of phenotypic variation. Oecologia 88:77–83

Rice KJ, Mack RN (1991b) Ecological Genetics of *Bromus tectorum*. III. The Demography of reciprocally sown populations. Oecologia 88:91–101

Ritz C, Streibig JC (2005) Bioassay analysis using R. J Stat Softw 12:1–22

Schwartz CJ, Doyle MR, Manzaneda AJ, Rey PJ, Mitchell-Olds T, Amasino RM (2010) Natural variation of flowering time and vernalization responsiveness in *Brachypodium distachyon*. BioEnergy Res 3:38–46

Scott JW, Meyer SE, Merrill KR, Anderson VJ (2010) Local population differentiation in *Bromus tectorum* L. in relation to habitat-specific selection regimes. Evol Ecol 24:1061–1080

Soreng RJ, Peterson PM, Romaschenko K, Davidse G, Zuloaga FO, Judziewicz EJ, Filgueiras TS, Davis JI, Morrone O (2015) A worldwide phylogenetic classification of the Poaceae (Gramineae). J Syst Evol 53:117–137

Szucs P, Skinner JS, Karsai I, Cuesta-Marcos A, Haggard KG, Corey AE, Chen THH, Hayes PM (2007) Validation of the *VRN-H2/VRN-H1* epistatic model in barley reveals that intron length variation in *VRN-H1* may account for a continuum of vernalization sensitivity. Mol Genet Genomics 277:249–261

Takahashi R, Yasuda S (1971) Genetics of earliness and growth habit in barley. Pages 388–408 *in* RA Nilan, ed. Proc of the Second International Barley Genetics Symposium. Pullman, WA

Thill DC, Beck KG, Callihan RH (1984) The biology of downy brome (*Bromus tectorum*). Weed Sci 32:7–12

Trevaskis B, Hemming MN, Peacock WJ, Dennis ES (2006) *HvVRN2* responds to daylength, whereas *HvVRN*1 is regulated by vernalization and developmental status. Plant Physiol 140:1397–1405

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci 100:6263–8

Von Zitzewitz J, Szucs P, Dubcovsky J, Yan L, Francia E, Pecchioni N, Casas A, Chen THH, Hayes PM, Skinner JS (2005) Molecular and structural characterization of barley vernalization genes. Plant Mol Biol 59:449–467

4.6 Tables and Figures

Table 4.1. Class, accession ID, GDD estimates and calendar dates of mature seed production and flowering from a 2013 common garden experiment. Mature seed production was determined by sequentially sampling emerging panicles over a period of several weeks and planting seeds sampled from panicles in the greenhouse to test at which date seeds were first mature.

			Calendar Date	
Class	Accession ID	GDD Estimate (S.E)[A]	Seed Set	Flowering
Early 1	38	1077 (371)	7 May	1 May
Early 2	127	1112 (31)	13 May	1 May
Early 3	179	1077 (364)	7 May	1 May
Intermediate 1	12	1194 (35)	18 May	8 May
Intermediate 2	88	1215 (31)	19 May	8 May
Late 1	46	1340 (12)	29 May	8 May
Late 2	100	1340 (12)	29 May	20 May
Late 3	112	1324 (33)	28 May	8 May

[A]Growing degree model beings January 1st with a base temperature of 0°C. Estimates were made using a 2-paramter log-logistic regression model and six replications.

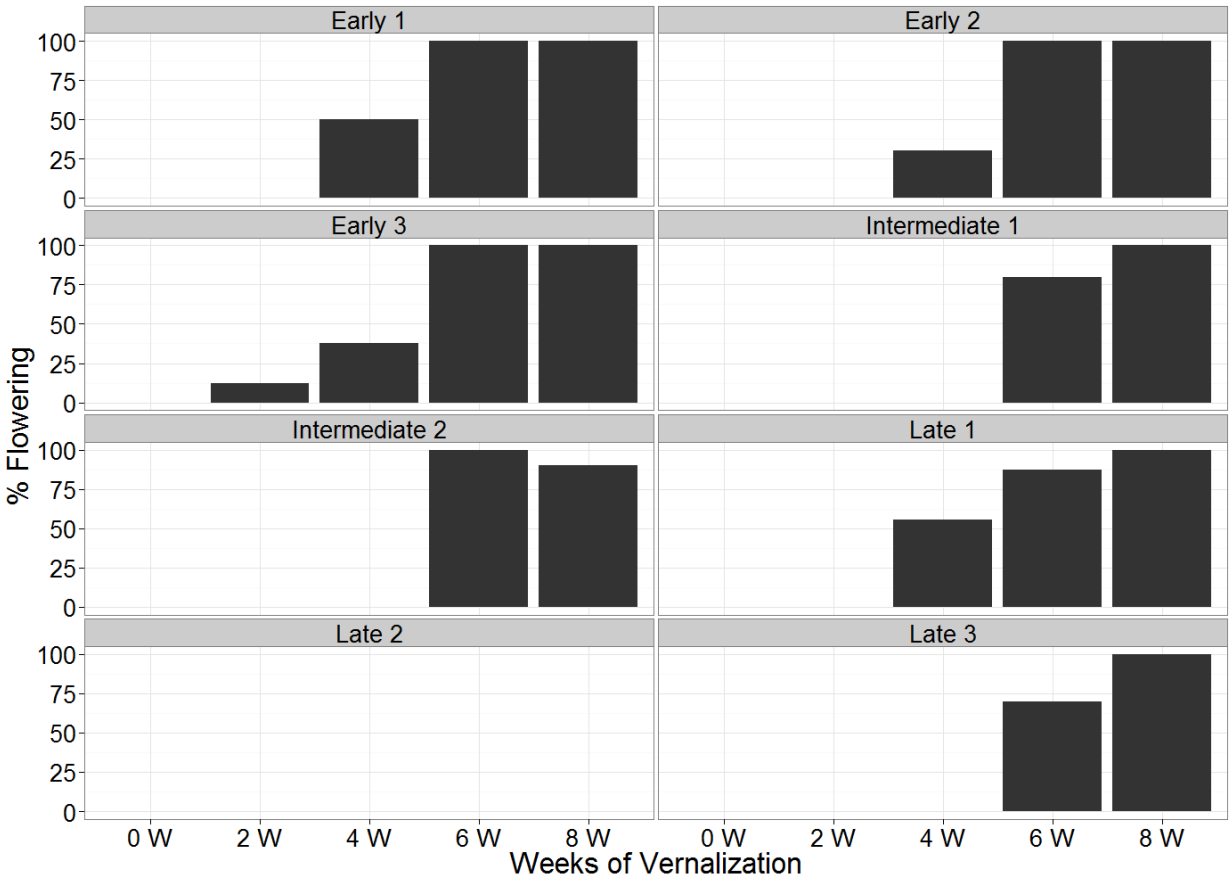


Figure 4.1. Percent individuals flowering from each accession 16 weeks after the beginning of vernalization treatments.

Table 4.2. Matrix of p-values contrasting the flowering response of each accession against all other accessions. A p-value of <0.05 indicates that the flowering response due to vernalization treatments of two accession was different.

	Early 1	Early 2	Early 3	Intermediate 1	Intermediate 2	Late 1	Late 3
Early 1	—	0.46	1.00	0.01	0.02	0.83	0.00
Early 2	0.46	—	0.42	0.03	0.07	0.56	0.01
Early 3	1.00	0.42	—	0.01	0.02	0.82	0.00
Intermediate 1	0.01	0.03	0.01	—	0.64	0.01	0.66
Intermediate 2	0.02	0.07	0.02	0.64	—	0.02	0.36
Late 1	0.83	0.56	0.82	0.01	0.02	—	0.00
Late 3	0.00	0.01	0.00	0.66	0.36	0.00	—

Table 4.3. Regression parameter estimating the weeks of vernalization required to induce flowering in 50% of individuals in each accession.

Accession	Estimate (S.E.)	t-value
	b[a]	
Early 1	-28(521)	-0.05
Early 2	-28(521)	-0.05
Early 3	-28(521)	-0.05
Intermediate 1	-58(848)	-0.07
Intermediate 2	-4(3)	-1.32
Late 1	-58(848)	-0.05
Late 3	-28(521)	-0.07
	e	
Early 1	4 (0.2)	19.88*
Early 2	4 (0.2)	19.88*
Early 3	4 (0.2)	19.88*
Intermediate 1	5(15)	0.33
Intermediate 2	6(1)	4.43*
Late 1	4 (0.2)	19.88*
Late 3	5(15)	0.33

[a]Model Parameters: *b*, slope of the model at *e*; *e*, is the weeks required to induce flowering in 50% of individuals.

*Significant at α of 0.05 compared to the null model.

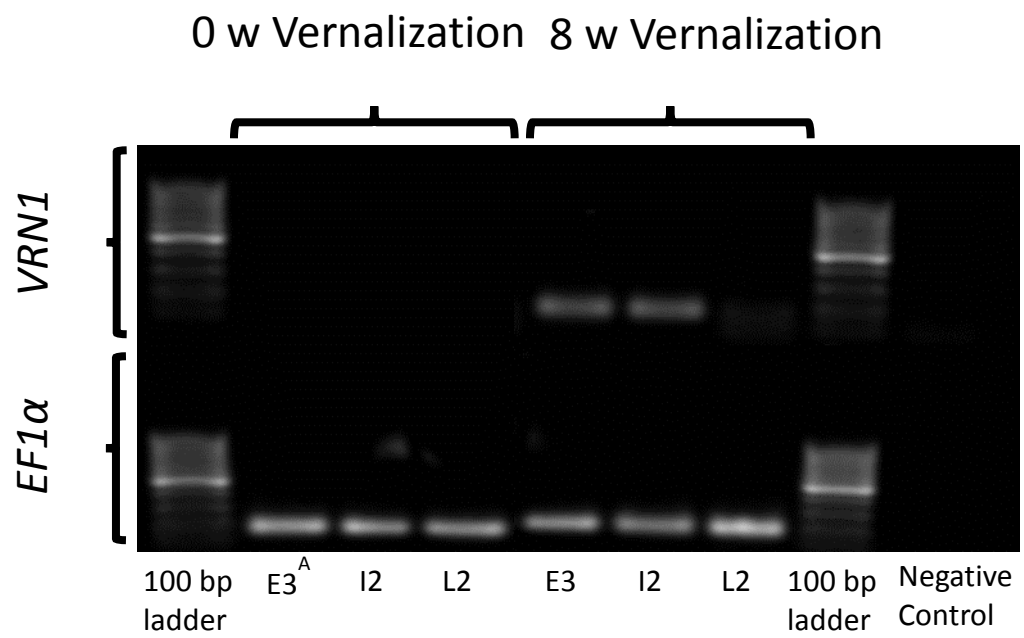


Figure 4.2. Expression of *VRN1* in downy brome following 0 or 8 w of vernalization compared to the expression of *EF1a*. Each lane is representative of three replications.

[A]Abbreviations: E3, Early 3; I2, Intermediate 2; L2, Late 2.

Chapter 5. Projecting *Bromus tectorum* Development in the Pacific Northwest Small Grain Production Region using Downscaled Climate Modeling

5.1 Weed Management For a Changing Climate

The impacts of climate change to human, managed and natural systems are expected to vary widely on a regional scale (Field et al. 2014). Furthermore, assessments of these potential impacts differ among and within sectors. For the agricultural sector, projections by the Intergovernmental Panel on Climate Change (IPCC) of the effects of rising temperatures, CO_2, and tropospheric O_3 on crop yields are much better developed than are responses of agronomically important weeds and their interactions with crops (Porter et al. 2014).

In a review of the literature available on pathogen, weed, and insect pest response to climate change, Juroszek and von Teidemann (2013), projections concerning agronomic weeds are notably lacking compared to pathogens and insect pests. Nonetheless, as climates change land managers will require simple, readily interpretable information on pest, weed and disease response to climate change to guide their management decisions. To be useful, this information must be region-specific. Unfortunately, few studies have investigated the response of weeds to climate change in a specific crop or region (Peters et al. 2014). The majority of published studies investigating weed response to climate change have focused on competition between plants of different photosynthetic functional groups under conditions of increased temperature and CO_2 concentrations (Poorter and Navas 2003), and range shifts of species on a landscape scale (Peters et al. 2014).

The small grain production area of the Pacific Northwest (PNW) includes Central and Eastern Washington, parts of Northern Oregon, and Northern Idaho and totals 2.2 million ha of non-irrigated cropland (Huggins et al. 2012). In 2007 1.3 million ha of the small grain production region were planted into small grains with around 1.0 million ha planted to winter wheat. Annual precipitation in the region ranges from less than 300 mm to greater than 600 mm, with precipitation

increasing on a west to east gradient. Mean annual temperature varies from 5 ºC to 11 ºC based on a 30 year average (1971-2000) (Huggins et al. 2012).

The climate in PNW small grain production region has been changing throughout the past century. Abatzoglou et al. (2014) reported mean annual temperature increasing 0.6 to 0.8ºC from 1901 to 2012. In addition to increases in mean annual temperature, trends of a warmer coldest night of the year and a lengthened freeze-free period were also observed. Decreased precipitation was observed during the summer and fall in the previous four decades; however a long-term increase in spring precipitation was noted. Taken together, increased summer temperature and decreased summer precipitation have led to increased evapotranspiration during the growing season (Abatzoglou et al. 2014). Anthropogenic climate forcing is a significant predictor of observed changes, with the exception of changes in seasonal precipitation in which poor model skill for regional precipitation, in PNW climate during the 20th century and can be used to construct projections into the 21st century. Climatic trends observed within the PNW in the last century are projected to continue under most climate models. Climate models using a balanced emissions scenario which anticipates a future mix of fossil and non-fossil fuel soureces (A1B scenario) project an increase in annual temperature of 1.1ºC by the 2020s, 1.8 ºC by the 2040s, and 3.0 ºC by the 2080s. Recent trends of drier summers and wetter winters are also project to continue, along with an increased summer evapotranspiration rate (Mote and Salathé 2010).

A principal weed in this system is *Bromus tectorum* L. (downy brome). Native to Eurasia, genetic evidence suggests downy brome was entered North America through multiple immigrations introductions (Novak et al. 1993). Vigorous initial growth and early seed productions compared to other annual grasses allows establishment downy brome to establish in disturbed natural areas and agronomic fields. Compared to the development of winter wheat, downy brome infesting winter wheat fields is generally in the early boot stage when the wheat is tillering, and it heading four weeks

earlier than wheat (Thill et al. 1984). Because downy brome matures earlier than winter wheat, it often depletes soil moisture and nutrients when winter wheat is at a critical reproductive period (Thill et al. 1984).

Downy brome is a widely distributed winter annual weed in the small grain production region of the PNW and across Western North America (Morrow and Stahlman 1984). Variation in phenology and flowering time has been extensively documented for *Bromus tectorum* L. (downy brome). Rice and Mack (1991a, 1991b) observed consistent flowering among downy brome populations across multiple years, suggesting flowering time is stable within a population. After collecting downy brome accessions from contrasting habitats, Meyer et al. (2004) examined vernalization response under greenhouse conditions, concluding vernalization response was quite different between contrasting habitats. However, even when a single habitat included plants of different genotypes, similar flowering times were observed, indicating variation in flowering time is constrained by natural selection (Meyer et al. 2004).

In a series of studies, Ball et al. (2004) developed a downy brome development model that could be used to assist farmers in the PNW to time field operations for control of downy brome. In their studies, seed was sequentially sampled over a period of weeks from developing panicles and later germinated in a greenhouse to determine dates of seed maturity. The timing of mature seed development was then regressed against cumulative growing degree days (GDD) starting 1 Jan and with a base temperature of 0°C. While considerable variation in mature seed set from locations in Colorado, Kansas, Montana, Nebraska, and Oklahoma was reported (582 to 1,287 GDD), little variation was observed in the time to produce mature seeds from accessions collected and grown in the PNW (983 to 1,151 GDD).

Ball et al. (2004) concluded that mature seed set of downy brome in the PNW could be predicted to occur around 1,000 GDD. The 20-year average (1984-2003) for the 1,000 GDD

threshold fell on 11 June for the entirety of the PNW. In parts of the PNW where annual precipitation cannot support a crop every year, winter wheat is typically grown alternating with fallow. The 1,000 GDD threshold for mature seed set provides guideline for the last date when downy brome must be controlled in fallow years to prevent an increase of seed into the soil seed bank. During the wheat years in the wheat/fallow rotation, control of downy brome is generally achieved through the use of spring-applied herbicides. Spring-applied herbicide applications can occur between March and May in the PNW (Lawrence et al. 2014), which corresponds to 50 to 1,200 GDD depending on the year and location. Developmental stage at the time of herbicide application can impact herbicide efficacy, with herbicide efficacy decreasing after downy brome enters the boot stage, when reproductive tillers are swollen and purple (Blackshaw 1991).

Ball et al. (2004) found little variation in time to mature seed set in PNW downy brome accessions, but six accessions were evaluated in that study. A series of common garden and greenhouse studies were therefore conducted between 2012 and 2014 to characterize more thoroughly the variation in downy brome phenology across the small grain production region of the PNW. Three study objectives were pursued: (1) to validate the 1000 GDD threshold for mature seed production using a larger number of accession representing the entire region, (2) include other developmental thresholds including visible reproductive tillers and the emergence of panicles to aid timing of herbicide applications in winter wheat for the control of downy brome and, (3) pair downy brome development thresholds with downscaled climate modeling to project downy brome development under future climate scenarios. Downscaled climate models have previously been used to anticipate plant pathogen, insect pest, and beneficial insect distribution and life history under future climate scenarios in various crops on a regional level and with a high degree of spatial resolution (Eigenbrode and Abatzoglou 2013, Hirschi et al. 2012, Srinivasa Rao et al. 2015).

Downscaled climate modeling has also been used to project potential habitat under future climate scenarios of invasive weeds, including downy brome (West et al. 2015).

5.2 Methods and Materials

5.2.1 Expanded Modeling of Development. In November of 2012, 85 downy brome accessions (Table 1) which were collected from across the small grain production region of the PNW were transplanted to two common gardens, one located in Central Ferry, WA (46.73, -117.54, elevation of 533 m) and the second at the Cook Agronomy Farm (46.78, -117.09, elevation of 795 m) north of Pullman, WA. In November of 2013, a repeat of the common garden at Central Ferry was planted. Accessions were originally collected from within small grain production fields in the summer of 2010 and 2011. For each accession ten replicates were planted using a RCBD. All replicates were planted in a greenhouse in mid-October, and later transplanted to the common garden site in mid-November. No irrigation or fertilizer was provided. Both common garden sites were located near weather stations to allow hourly recording of air temperature and other climate variables. Each site was kept weed free by hand weeding for the duration of the study. In January, February, March, and April, survival was assessed for each individual along with growth stage. Four growth stages were utilized to classify individuals: pre-tillering, tillering, boot stage (as indicate by visible reproductive tillers that are swollen and purple), and emerged panicles. Beginning in May, each study location was visited weekly.

Emerged panicles were sequentially sampled and later germinated in the greenhouse to determine the date of mature seed development. All observations of developmental stage were related to the cumulative GDD, starting 1 January and with a base temperature of 0°C. Using the R package drc, a two parameter log-logistic model (Equation 2.) (Ritz and Streibig 2005; DRC R package version 2.3-96) suitable for non-linear regression of binary response variables was employed

to estimate the GDD required for mature seed set and other development stages based on field observations.

5.2.2 Downscaled Climate Projection. The calendar date when cumulative GDD of relevant development thresholds were met was calculated from downscaled climate data covering both a contemporary climate from 1950-2005 and a mid-21st century climate from 2031- 2060. Mid-21st century climate projections considered Global Climate Model (GCM) simulations for representative concentration pathways (RCP) 4.5 Wm^{-2} and 8.5 Wm^{-2}. Climate simulations from 14 GCMs participating in the Fifth Coupled Model Intercomparison Project phase 5 (CMIP5, Table 2) were selected based on the criterion that they have adequately captured characteristics of historical climate for the PNW (Rupp et al. 2014). Daily GCM output was statistically downscaled using the training data of Abatzoglou (2013) at 4-km grid using the Multivariate Adaptive Constructed Analogs (MACA) method (Abatzoglou and Brown 2012).

5.3 Results

5.3.1 Development Thresholds. All three common garden studies differed in the estimated GDD required to produce mature seed (Table 3). GDD at the two Central Ferry locations accumulated more rapidly than the Cook Agronomy Farm location, and more GDD were required to produce mature seeds. Mature seed production at the 2013 Central Ferry location was estimated to occur at 1200 GDD averaged over all accessions. The 2013 average estimates for mature seed production at the 2013 Cook Agronomy Farm and 2014 Central Ferry Location site was were 1010 and 1400 GDD, respectively. Differences among accession were limited at each site relative to differences between study locations. At the 2013 Central Ferry Location, the 2013 Cook Agronomy Farm Location, and the 2014 Central Ferry location accessions differed by 70, 40, 100 GDD respectfully. The two studies conducted common gardens at Central Ferry were located at a low elevation relative to the majority of the small grain production region of the PNW. The growing degree accumulation

and the estimates for both Central Ferry studies may not be representative of the region as a whole. Additionally, at the 2014 Central Ferry location all fall transplanted individuals were winter killed in December. However, seed that had not germinated at the time of transplanting later emerged between March and May. Therefore the estimate for the 2014 Central Ferry Location captured spring emergence patterns. As plants emerging in the spring would be less exposed to vernalizing temperatures delayed development would be expected.

The estimate for mature seed set and the rate of accumulation of GDD at the 2013 Cook Agronomy location is representative of the climate of the small grain production region. The average estimate for mature seed set from the Cook Agronomy Farm, 1010 GDD, is very close to the 1000 GDD estimate proposed by Ball et al. (2004). The results of the Cook Agronomy Farm common garden validate the 1000 GDD mature seed set threshold proposed by Ball et al. (2004) using a different modeling approach and an expanded collection of downy brome.

Modeling the GDD required for the production of visible reproductive tillers was not possible at either of the common garden locations as most individuals had progressed to visible reproductive tillers prior to the weekly May visits. Consequently observations of the transition to reproductive tillers lacked the resolution needed for effective modeling. However, the GDD threshold for appearance of reproductive tillers may be used to develop a heuristic for timing spring applied herbicide applications. At the 2013 Central Ferry locations, reproductive tillers were not observed until April 16th (709 GDD), at which time most individuals were still tillering. By 8 May (1014 GDD), all individuals had progressed to producing reproductive tillers or displaying panicles. At 2014 Central Ferry location the majority of individuals had produced reproductive tillers by 18 April (717 GDD). All individuals at the Cook Agronomy Farm site were displaying reproductive tillers by 17 May, 679 GDD. As current application of spring applied herbicide occur anywhere from 50 to 1200 GDD, depending on the year and location (Lawrence et al. 2014), a 500 GDD spring

application heuristic would provide a conservative date for applying herbicides prior to downy brome developing reproductive tillers.

Projection of both contemporary and mid-century climate were based upon a 500 and 1,000 GDD threshold. The 500 threshold serves as a conservative estimate for applying spring applications prior to downy brome becoming less susceptible to herbicide applications. The 1,000 GDD estimate is based up the threshold proposed by Ball et al. (2004) and confirmed by the 2013 Cook Agronomy Farm common garden experiment which observed and average GDD estimate of 1,010 GDD and a range of 990-1,040 between accessions.

5.3.2 Contemporary Climate Projections. The historic GDD accumulation from 1950-2005 was averaged at six locations within the small grain production region of the PNW (Figure 4). The 500 GDD herbicide application threshold historically fell between 99 to 128 calendar days, corresponding to 9 April and 12 May, depending on location. The 1000 GDD mature seed set threshold historically occurred between 136 to 163 calendar days, corresponding to 14 May and 11 June, depending on location. Variation in GDD accumulation is dependent upon latitude and longitude Walla Walla and Connell, the southernmost locations, accumulated GDD earlier in the year compared to all other locations. Albion and Almira, the most eastern and northern locations, were the slowest to accumulate GDD. Endicott and Ritzville, intermediate in their location within the region, were also intermediate in their accumulation in GDD.

5.3.3 Mid-Century Climate Projections. The average calendar date for accumulation of the 500 and 1000 GDD thresholds by mid-21st century were projected at six locations representing the diversity of climate currently found within the PNW small grain production region (Figure 4). For the 500 GDD application threshold, projected advancement in calendar days from current to mid-21st century ranged from 69 d to 120 d depending on the model and location (Table 5). Averaging across models at each location, 500 GDD was reached between 75 to 105 calendar days,

corresponding to 16 March to 15 April. The 500 GDD herbicide application threshold is projected to advance 24 to 30 d compared to the historical average. Projections under the RCP 8.5 scenario did not differ greatly from the RCP 4.5 scenario for the 500 GDD threshold. For the RCP 8.5 scenario the herbicide application threshold is anticipated to advance 26 to 33 days, corresponding to 5 April to 24 March. The Albion and Endicott locations are projected to experience the largest change in the calendar days required to reach the 500 GDD threshold under both RCP scenarios. The Albion and Endicott locations are also further east (Figure 4) than the other locations.

Projections for advancement of the mature seed set threshold are less extreme under both the RCP 4.5 and 8.5 compared to the herbicide application threshold (Table 6). Mature seed set at the six locations within the small grain production region was projected to advance between 16 to 19 d when averaged across models, corresponding to 28 April to 25 May. Albion and Endicott are projected to experience the largest change. Under the RCP 8.5 scenario, projected calendar days until mature seed set were advanced by 18 to 22 days when models were averaged (Table 4), corresponding to 26 April to 23 May. Similar to projections under the RCP 4.5 scenario, the most easterly accessions experienced the greatest change compared to contemporary averages in reaching the mature seed set threshold with the Albion and Endicott locations advancing seed production 22 and 21 days, respectively.

Across all models, mature seed set is expected to occur earlier with changing climate. To help visualize the spatial pattern of climate projections, a 4 km resolution map projecting the 1000 GDD threshold using the RCP 4.5 scenario was created (Figure 1). The map includes projections from the second most extreme and second least extreme model to provide a representation of the spread among models, along with the projected average across all models. The second most extreme model projected mature seed set occurring 25 to 30 days earlier, while the second least extreme model projected mature seed set occurring 5 to 12 days earlier. Additionally, the advance in mature

seed set is projected to vary along an east to west gradient in the PNW small grain production region with greater changes projected in the east.

5.4 Discussion

Herbicide application for control of downy brome within winter wheat likely will need to be made 24 to 33 days earlier to ensure application are made prior to the boot stage under future climate scenarios. Projections of mature seed set under future climate scenarios indicate that control operations in fallow systems likely will need to be conducted 16 to 22 d earlier by the mid-century (2031-2060). Results of the common garden experiments detected differences between locations in GDD requirements for both seed set and the production of reproductive tillers. However, when comparing the 2013 Central Ferry and Cook Agronomy Farm studies where plants survived the winter, the difference in mature seed set was 200 GDD. The Cook Farm is more representative of the PNW small grain production region today, but the Central Ferry location with a less severe winter may better represent the region under future climate scenarios.

Although increased GDD was required for downy brome mature seed set at the 2013 Central Ferry location, (Table 3) mature seed set occurred in mid-May while mature seed set occurred at the Cook location occurred in mid-June. If climate change leads to milder winters and an increased GDD requirement for downy brome mature seed set, mature seed set will still likely occur earlier in the year as GDD will accumulate more rapidly.

What is not addressed by projecting either the 500 or 1000 GDD mature seed set threshold under future climate scenarios is whether downy brome may adapt to climate change and display different GDD requirements for maturation under future climate scenarios. If GDD requirements for downy brome development thresholds relevant to land managers do change in response to climate, that change is likely to be small. Ball et al. (2004) found considerable variation in development time among downy brome accessions collected from across Colorado, Kansas,

Montana, Nebraska, and Oklahoma, while collections from the PNW were consistent with respect to development time. Rice and Mack (1991a) also reported stability in development timing among accessions collected from the same environment. Rice and Mack (1991b) found differences in flowering time between contrasting populations remained consistent even after planting individuals to common locations. Given that little variation was observed in the time required to produce mature seed set from the 2013 Central Ferry and Cook Agronomy Farm common gardens, ecotypes present in the PNW may have already undergone selection for similar phenology phenotypes.

Projected advancement of downy brome seed set, up to 25 d, is less than then the historical differences among locations, up to 30 d, indicating the rate of downy brome maturation may not exceed the current variation present in the region within the next century; and downy brome will likely continue to develop and mature in a predictable manner. The projected advancement of the 500 GDD threshold, up to 30 d, also does not exceed the current variation in the region. Downy brome development and seed set is projected to advance across the PNW small grain production region regardless of the model used or the RCP scenario employed. Land managers will need to adapt to climate change by controlling downy brome earlier in the year, relative to current control measures, or move to using fall applied herbicide application in winter wheat rather than spring applied applications.

5.5 Literature Cited

Abatzoglou, J.T., 2013, Development of gridded surface meteorological data for ecological applications and modeling, International Journal of Climatology. 33(1):121-131

Abatzoglou JT, Brown TJ (2012) A comparison of statistical downscaling methods suited for wildfire applications. Int J Climatol 32:772–780

Abatzoglou JT, Rupp DE, Mote PW (2014) Seasonal climate variability and change in the pacific northwest of the united states. J Clim 27:2125–2142

Ball DA, Frost SM, Gitelman AI (2004) Predicting timing of downy brome (*Bromus tectorum*) seed production using growing degree days. Weed Sci 52:518–524

Ball DA, Klepper B, Rydrych DJ (1995) Comparative Above-Ground Development Rates for Several Annual Grass Weeds and Cereal Grains. Weed Sci 43:410–416

Blackshaw RE (1991) Control of downy brome (*Bromus tectorum*) in conservation fallow systems. Weed Technol 5:557–562

Concilio AL, Loik ME, Belnap J (2013) Global change effects on *Bromus tectorum* L. (Poaceae) at its high-elevation range margin. Glob Chang Biol 19:161–172

Eigenbrode S, Abatzoglou J (2013) Cereal leaf beetle and its parasitoid under future climates of the PNW. *in* 97th Annual Meeting Pacific Branch of the Entomological Society of America

Field CB, Barros VR, Mach KJ, Mastrandrea MD, van Aalst M, Adger WN, Arent DJ, Barnett J, Betts R, Bilir TE, Birkmann J, Carmin J, Chadee DD, Challinor AJ, Chatterjee M, Cramer W, Davidson DJ, Estrada YO, Gattuso J-P, Hijioka Y, Hoegh-Guldberg O, Huang H-Q, Insarov GE, Jones RN, Kovats RS, Romero Lankao P, Larsen JN, Losada IJ, Marengo JA, McLean RF, Mearns LO, Mechler R, Morton JF, Niang I, Oki T, Olwoch JM, Opondo M, Poloczanska ES, Pörtner H-O, Redsteer MH, Reisinger A, Revi A, Schmidt DN, Shaw MR, Solecki W, Stone DA, Stone JMR, Strzepek KM, Suarez AG, Tschakert P, Valentini R, Vicuña S, Villamizar A, Vincent

KE, Warren R, White LL, Wilbanks TJ, Wong PP, Yohe GW (2014) Technical Summary. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. 35-94 p

Hanzlik K, Gerowitt B (2012) Occurrence and distribution of important weed species in German winter oilseed rape fields. J Plant Dis Prot 119:107–120

Hirschi M, Stoeckli S, Dubrovsky M, Spirig C, Calanca P, Rotach MW, Fischer AM, Duffy B, Samietz J (2012) Downscaling climate change scenarios for apple pest and disease modeling in Switzerland. Earth Syst Dyn 3:33–47

Hulbert L (1955) Ecological studies of *Bromus tectorum* and other annual bromegrasses. Ecol Monogr 25:181–213

Juroszek P, von Tiedemann A (2013) Plant pathogens, insect pests and weeds in a changing global climate: a review of approaches, challenges, research gaps, key studies and concepts. J Agric Sci 151:163–188

Lawrence NC, Burke IC, Yenish JP (2014) 15 Years of Downy Brome Control in Eastern Washington. Pages 12–13 *in* Proceedings of the 67th Annual Meeting of the WSWS, Colorado Spring, CO: Western Society of Weed Science

Metcalf JC, Rose KE, Rees M (2003) Evolutionary demography of monocarpic perennials. Trends Ecol Evol 18:471–480

Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). Ann Bot 93:653–63

Morrow LA, Stahlman PW (1984) The history and distribution of downy brome (*Bromus tectorum*) in North America. Weed Sci Soc Am 32:2–6

Mote PW, Salathé EP (2010) Future climate in the Pacific Northwest. Clim Change 102:29–50

Novak S, Mack R, Soltis P (1993) Genetic variation in *Bromus tectorum* (Poaceae): introduction dynamics in North America. Can J Bot 71:1441–1448

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends Plant Sci 15:684–692

Peters K, Breitsameter L, Gerowitt B (2014) Impact of climate change on weeds in agriculture: a review. Agron Sustain Dev 34:707–721

Poorter H, Navas ML (2003) Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytol 157:175–198

Porter JR, Xie L, Challinor AJ, Cochrane K, Howden SM, Iqbal MM, Lobell DB, Travasso MI (2014) Food security and food production systems. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. 485-533 p

Ramakrishnan AP, Meyer SE, Waters J, Stevens RM, Coleman CE, Fairbanks DJ (2004) Correlation between molecular markers and adaptively significant genetic variation in Bromus tectorum (Poaceae), an inbreedingannual grass. Am J Bot 91:797–803

Rice K, Mack R (1991a) Ecological genetics of *Bromus tectorum*. I. A hierarchical analysis of phenotypic variation. Oecologia 88:77–83

Rice K, Mack R (1991b) Ecological genetics of *Bromus tectorum*. II. Intraspecific variation in phenotypic plasticity. Oecologia 88:84–90

Rice KJ, Mack RN (1991c) Ecological Genetics of *Bromus tectorum*. III. The Demography of Reciprocally Sown Populations. Oecologia 88:91–101

Ritz C, Streibig JC (2005) Bioassay Analysis using R. J Stat Softw 12:1–22

Srinivasa Rao M, Swathi P, Rama Rao CA, Rao K V., Raju BMK, Srinivas K, Manimanjari D, Maheswari M (2015) Model and scenario variations in predicted number of generations of *Spodoptera litura* Fab. on peanut during future climate change scenario. PLoS One 10:e0116762

West AM, Kumar S, Wakie T, Brown CS, Stohlgren TJ, Laituri M, Bromberg J (2015) Using high-resolution future climate scenarios to forecast *Bromus tectorum* invasion in Rocky Mountain National Park. PLoS One 10:e0117893

5.6. Tables and Figures.

Table 5.1. Accession ID, year of collection, and GPS coordinates of collection site for the accessions of downy brome used to model development thresholds.

Accession	Year	Longitude	Latitude	Accession	Year	Longitude	Latitude
5	2010	-120.35	45.90	103	2011	-118.60	45.99
8	2010	-120.24	46.00	104	2011	-118.61	46.08
10	2010	-120.66	46.56	106	2011	-118.74	46.34
12	2010	-120.36	45.42	107	2011	-118.79	46.74
13	2010	-120.49	45.48	108	2011	-118.65	46.77
18	2010	-120.56	46.46	110	2011	-118.64	47.26
21	2010	-120.16	45.40	111	2011	-118.79	47.48
22	2010	-120.18	45.62	112	2011	-118.85	47.52
26	2010	-120.16	46.26	113	2011	-118.68	47.80
28	2010	-119.91	46.70	115	2010	-118.40	45.76
29	2011	-120.34	46.89	116	2011	-118.44	46.01
30	2011	-120.41	47.00	118	2011	-118.36	46.33
32	2010	-119.94	45.39	123	2011	-118.36	47.23
37	2010	-119.86	46.26	125	2011	-118.46	47.49
38	2010	-120.13	46.38	127	2010	-118.10	46.37
39	2010	-119.85	46.67	128	2011	-118.37	46.68
42	2011	-119.87	47.10	129	2011	-118.13	46.66
45	2010	-119.80	45.35	130	2011	-118.17	46.80
53	2011	-119.85	46.74	133	2011	-118.31	47.46
56	2011	-119.71	47.34	135	2011	-118.14	47.69
59	2011	-117.75	47.90	136	2010	-117.91	46.39
60	2010	-119.44	45.64	137	2010	-117.95	46.43
63	2010	-119.37	46.11	138	2010	-117.80	46.63
64	2010	-119.22	46.39	139	2011	-118.18	46.91
65	2010	-119.69	46.74	141	2010	-117.87	47.21
67	2011	-119.41	46.85	143	2010	-117.88	47.52
70	2011	-119.18	47.42	147	2010	-117.66	46.90
74	2010	-119.28	45.76	148	2010	-117.67	47.12
75	2010	-119.24	46.03	152	2011	-117.63	47.72
78	2010	-119.22	46.56	154	2010	-117.52	46.49
79	2011	-119.28	46.71	156	2010	-117.48	46.95
81	2011	-119.20	47.01	157	2010	-117.38	47.26
82	2011	-119.15	47.18	159	2011	-117.55	47.52
83	2011	-119.32	47.47	161	2011	-117.16	46.37
86	2011	-119.37	47.89	165	2010	-117.17	47.10
88	2011	-118.99	45.77	166	2010	-117.25	47.31
93	2011	-118.90	46.67	167	2010	-117.25	47.39
94	2011	-118.99	46.82	168	2010	-117.09	47.48
96	2011	-119.08	47.20	169	2011	-116.87	46.40
99	2011	-118.92	47.79	172	2010	-116.84	46.92
100	2011	-119.16	47.99	179	2010	-116.71	46.92
101	2010	-118.86	46.48	182	2010	-116.47	46.25

Table 5.2. Global climate change models from the coupled model intercomparison project phase 5 used to project downy brome development thresholds.

Country	Institution	Model	Model Abbreviation
Australia	Commonwealth Scientific and Industrial Research Organization	Commonwealth Scientific and Industrial Research Organization Mark 3.6 Global Climate Model	CSIRO-Mk3.6
Canada	Canadian Center for Climate Modelling and Analysis	Second Generation Earth System Model	CanESM2
China	Bejing Climate Center	Climate System Model 1.1	BCC-CSM1.1
	Bejing National University	Earth System Model	BNU-ESM*
France	Centre National de Recherches Météorologiques	General Circulation Model 5	CNRM-CM5
Japan	Meteorological Research Institute	Global Climate Model	MRI-CGCM3
	Japan Agency for Marine-Earth Science and Technology	Model for Interdisciplinary Research on Climate - Earth System Model	MIROC-ESM
	Ocean Research Institute and National Institute for Environmental Studies	Model for Interdisciplinary Research on Climate - Earth System Model -Atmospheric Chemistry Coupled	MIROC-ESM-CHEM
	Atmosphere and Ocean Research Institute	Model for Interdisciplinary Research on Climate 5	MIROC5
Russia	Institute for Numerical Mathematics	Climate Model 4	inmcm4
UK	Met Office Hadley Centre	Global Environmental Model 2 - Carbon Cycle	HadGEM2-CC
	Met Office Hadley Centre	Global Environmental Model 2 - Earth System	HadGEM2-ES
USA	Geophysical Fluid Dynamics Laboratory	Earth System Model 2 Generalized Ocean System Dynamics	GFDL-ESM2G
	Geophysical Fluid Dynamics Laboratory	Earth System Model 2 Modular Ocean Model	GFDL-ESM2M

*Model not used to project the 500 GDD threshold.

Table 5.3. GDD accumulation estimates for mature seed production at three common garden locations.

	Central Ferry 2013	Cook 2013	Central Ferry 2014
	————GDD Accumulation————		
1 January	1	0	2
1 February	86	23	109
1 March	251	82	211
1 April	544	244	506
1 May	881	438	890
1 June	1403	833	1422
1 July	1976	1266	1979

Table 5.4. Table 4. Average calendar days and date when 500 and 1,000 GDD were reached from 1950-2005 at six locations located in the small grain production region of the PWN.

Location	Albion	Almira	Connell	Endicott	Ritzville	Walla Walla
Latitude	46.84	47.79	46.67	46.9	47.18	46.01
Longitude	-117.24	-118.92	-118.89	-117.66	-118.23	-118.44
			500 GDD			
Calendar days	128	129	111	125	132	99
Calendar date	8 May	9 May	21 Apr.	5 May	12 May	9 Apr.
			1000 GDD			
Calendar days	163	162	143	152	158	136
Calendar date	11 Jun.	10 Jun.	21 May	31 May	6 Jun.	14 May

Table 5.5. Projections of calendar days required to reach 500 GDD under the RCP 4.5 and 8.5 scenarios from 2031-2060, compared to 1950-2005.

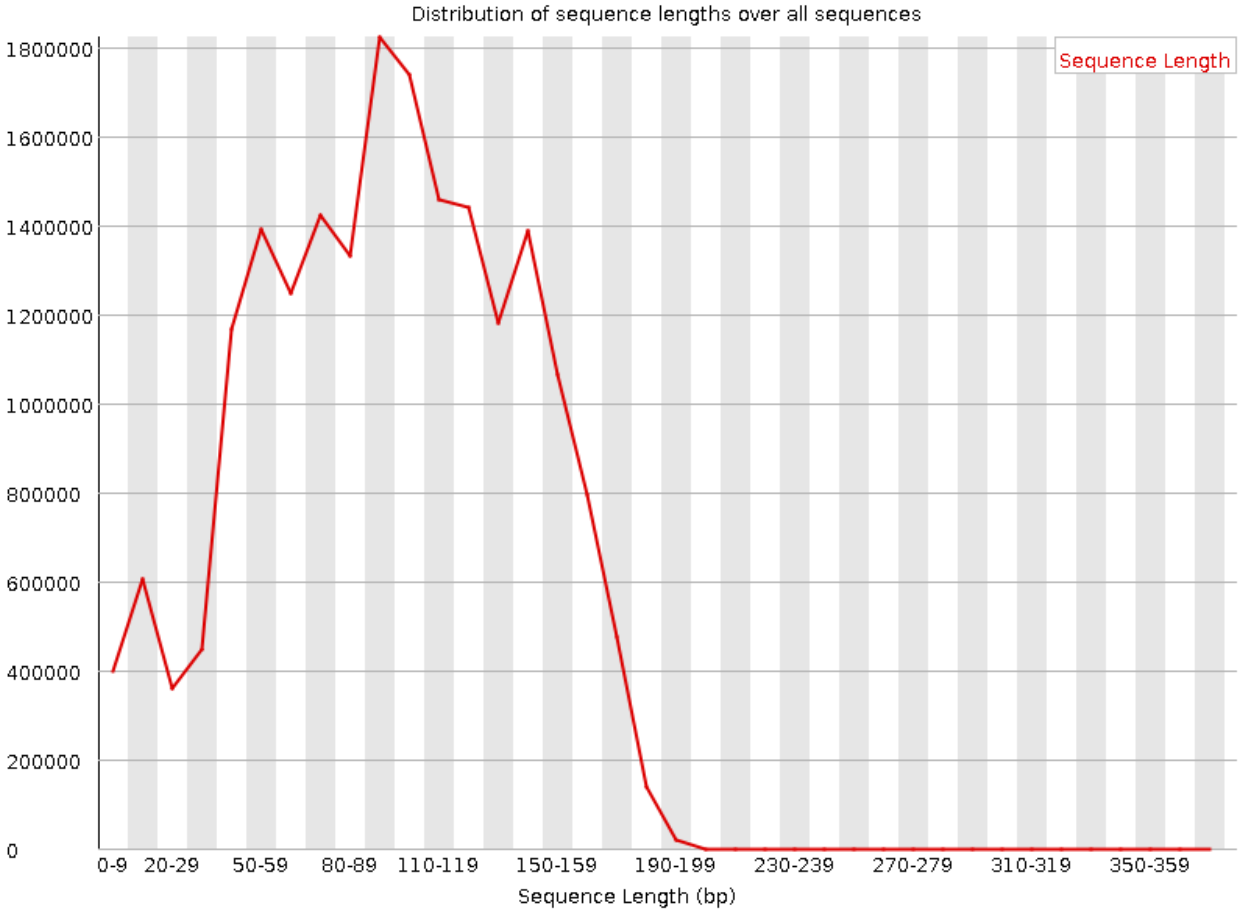
Projection	Albion	Almira	Connell	Endicott	Ritzville	Walla Walla
			RCP 4.5			
BCC-CSM1.1	97	103	85	96	104	74
CanESM2	91	97	80	90	97	70
CNRM-CM5	94	103	81	95	103	71
CSIRO-Mk3.6	99	104	86	97	105	75
GFDL-ESM2G	112	112	89	108	117	77
GFDL-ESM2M	109	111	94	106	113	83
HadGEM2-CC	97	102	84	95	102	75
HadGEM2-ES	96	100	81	94	102	72
inmcm4	113	120	99	112	121	86
MIROC5	94	104	86	93	103	75
MIROC-ESM	83	91	77	83	90	69
MIROC-ESM-CHEM	82	89	76	82	88	67
MRI-CGCM3	112	114	98	109	117	86
Mean calendar days	98	104	86	97	105	75
Mean calendar date	8 Apr.	14 Apr.	27 Mar.	7 Apr.	15 Apr.	16 Mar.
Mean difference from historical data	30	25	25	28	27	24
			RCP 8.5			
BCC-CSM1.1	94	99	81	92	101	71
CanESM2	81	88	73	80	88	63
CNRM-CM5	96	104	83	96	104	73
CSIRO-Mk3.6	103	108	89	100	108	77
GFDL-ESM2G	114	114	92	111	119	79
GFDL-ESM2M	109	111	94	107	113	84
HadGEM2-CC	88	92	76	86	93	68
HadGEM2-ES	86	91	74	84	92	67
inmcm4	108	115	96	108	117	84
MIROC5	92	102	85	92	100	74
MIROC-ESM	77	86	72	77	84	64
MIROC-ESM-CHEM	77	85	72	77	83	64
MRI-CGCM3	106	110	93	104	112	81
Mean calendar days	95	100	83	93	101	73
Mean calendar date	5 Apr.	10 Apr.	24 Mar.	3 Apr.	11 Apr.	14 Mar.
Mean difference from historical data	33	29	28	32	31	26

Table 5.6. Projections of calendar days required to reach 1,000 GDD under the RCP 4.5 and 8.5 scenarios from 2031-2060, compared to 1950-2005.

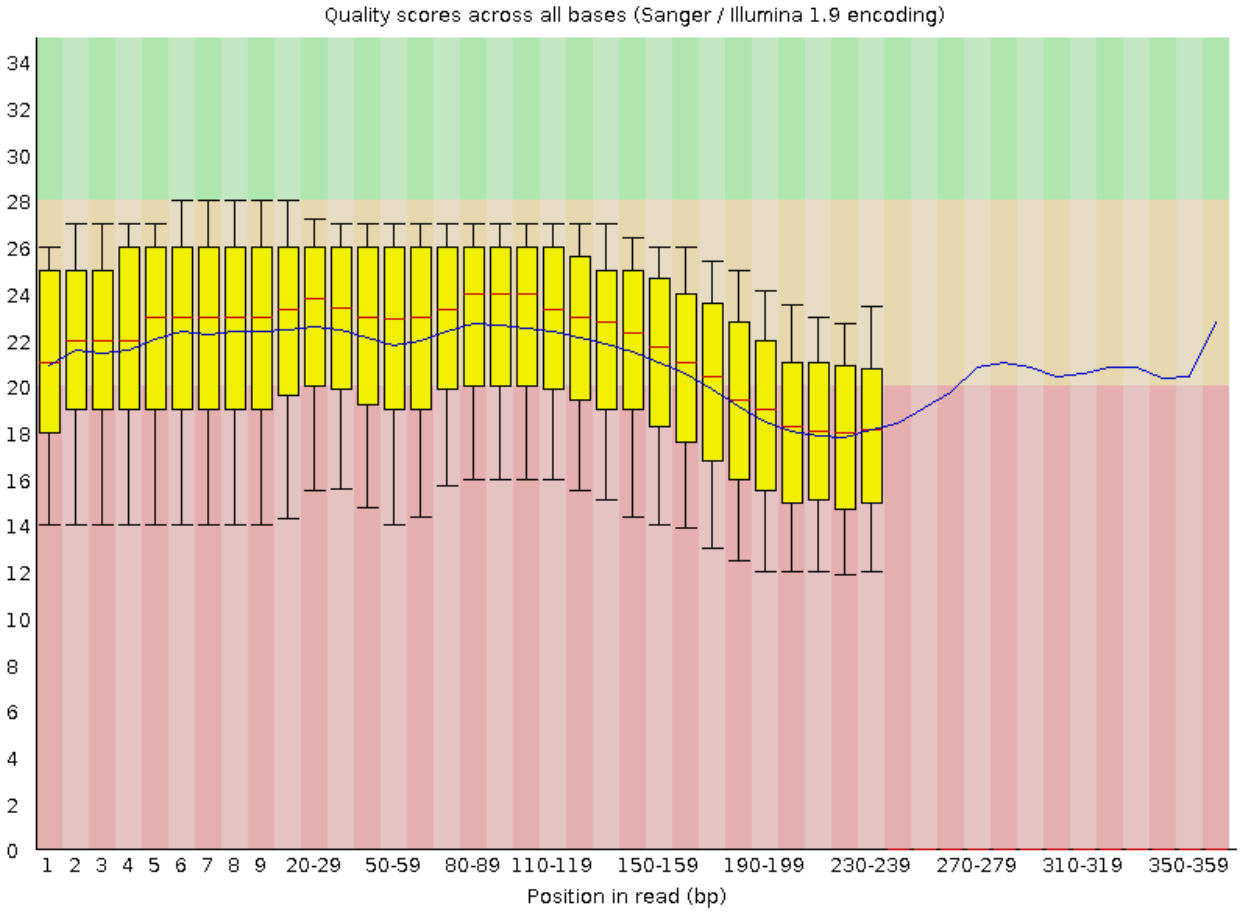
Projection	Albion	Almira	Connell	Endicott	Ritzville	Walla Walla
			RCP 4.5			
BCC-CSM1.1	146	148	128	135	143	120
BNU-ESM	140	143	124	131	138	118
CanESM2	139	143	123	129	138	116
CNRM-CM5	143	146	126	134	142	118
CSIRO-Mk3.6	146	148	129	136	143	122
GFDL-ESM2G	146	148	127	136	143	118
GFDL-ESM2M	149	151	131	138	146	125
HadGEM2-CC	145	145	125	134	142	118
HadGEM2-ES	144	146	126	134	141	119
inmcm4	156	156	137	145	152	129
MIROC5	143	148	129	134	143	120
MIROC-ESM	135	140	121	126	134	115
MIROC-ESM-CHEM	133	138	120	124	132	113
MRI-CGCM3	151	154	135	141	149	127
Mean calendar days	144	147	127	134	142	120
Mean calendar date	23 May	25 May	6 May	13 May	20 May	28 Apr.
Mean difference from historical data	19	16	16	18	17	16
			RCP 8.5			
BCC-CSM1.1	143	146	125	132	141	118
BNU-ESM	133	137	119	124	132	112
CanESM2	132	138	119	123	132	111
CNRM-CM5	143	145	127	134	142	118
CSIRO-Mk3.6	148	150	131	138	145	123
GFDL-ESM2G	146	148	128	137	144	118
GFDL-ESM2M	146	149	130	137	144	123
HadGEM2-CC	139	141	120	128	136	113
HadGEM2-ES	138	140	121	128	135	114
inmcm4	153	154	135	143	150	128
MIROC5	143	147	129	134	142	121
MIROC-ESM	129	136	117	121	130	110
MIROC-ESM-CHEM	130	136	118	121	130	110
MRI-CGCM3	149	152	134	140	148	126
Mean calendar days	141	144	125	131	139	118
Mean calendar date	19 May	23 May	4 May	10 May	18 May	20 Apr.
Mean difference from historical data	22	18	18	21	19	18

Figure 5.1. Mean calendar date when 1,000 GDD were reached from 1950-2005 compared with projected mean calendar date when 1,000 GDD were reached from 2031-2060 using 14 GCMS from the CMIP5 and the under the RCP 4.5 radioactive forcing scenario. Locations not reaching 1,000 GDD by 170 calendar days were masked from projections.

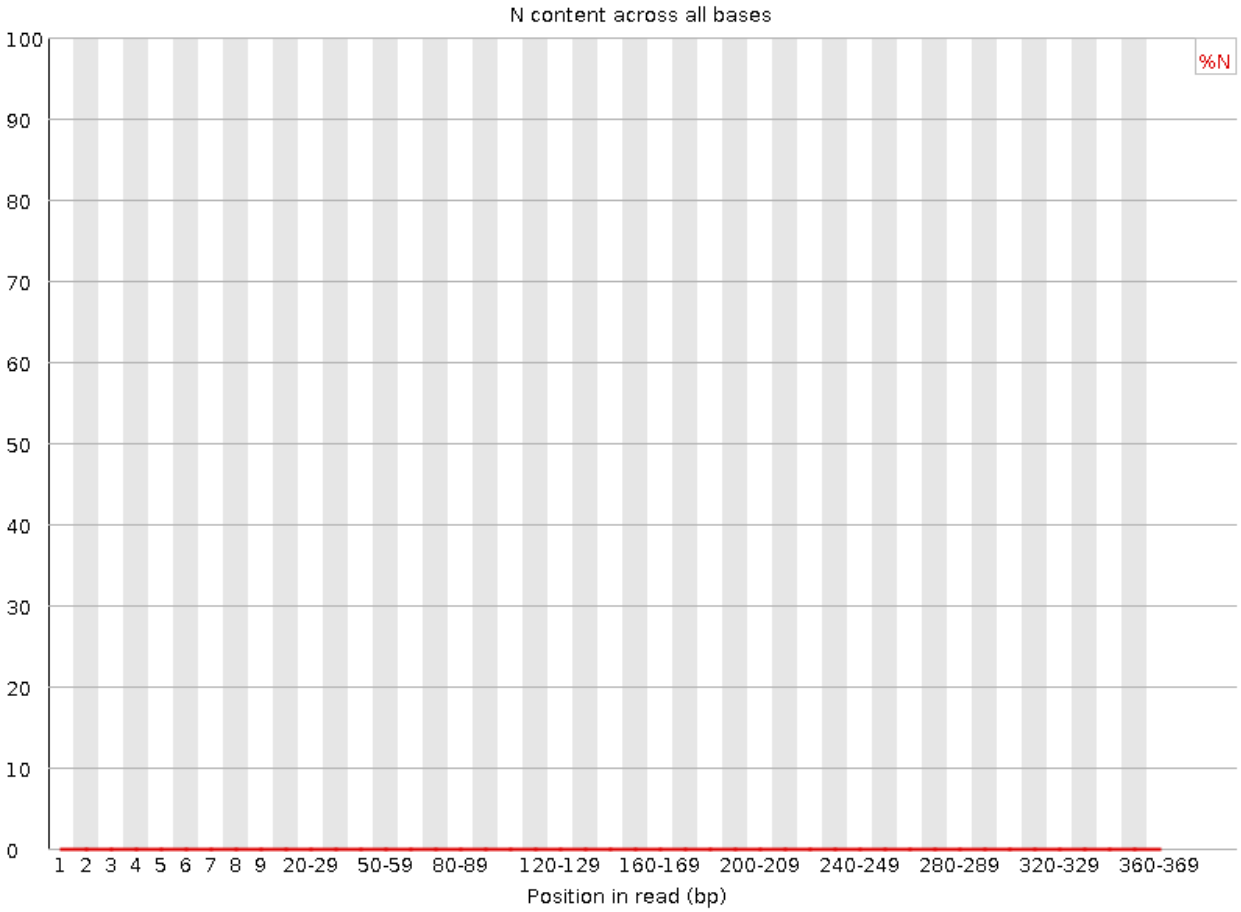
Supplemental Figures



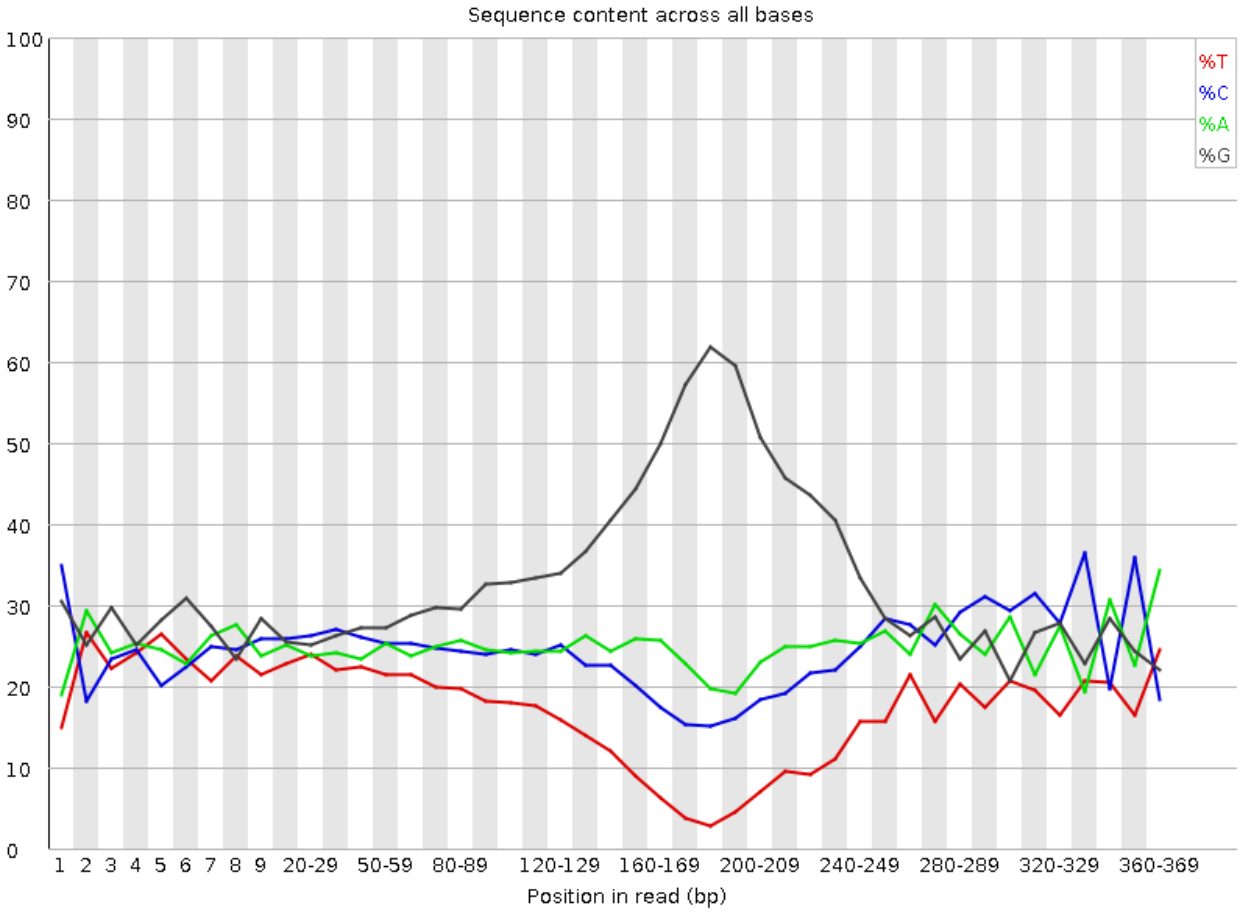
Supplemental Figure 1. The frequency of each sequence length retrieved from GBS.



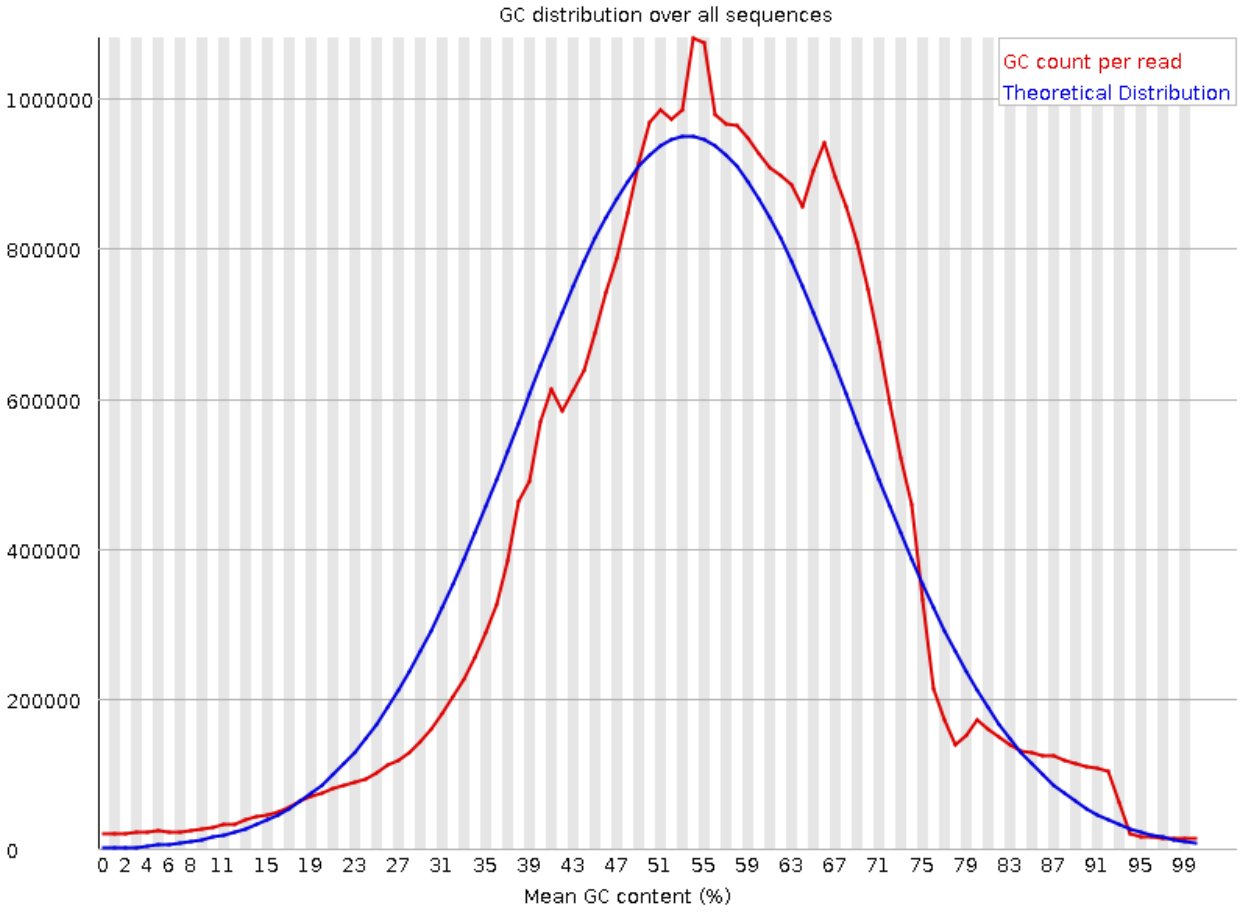
Supplemental Figure 2. Phred quality scores. A score of 10, 20, and 30 correspond to 90, 99, and 99.9% confidence in each base call, respectfully.



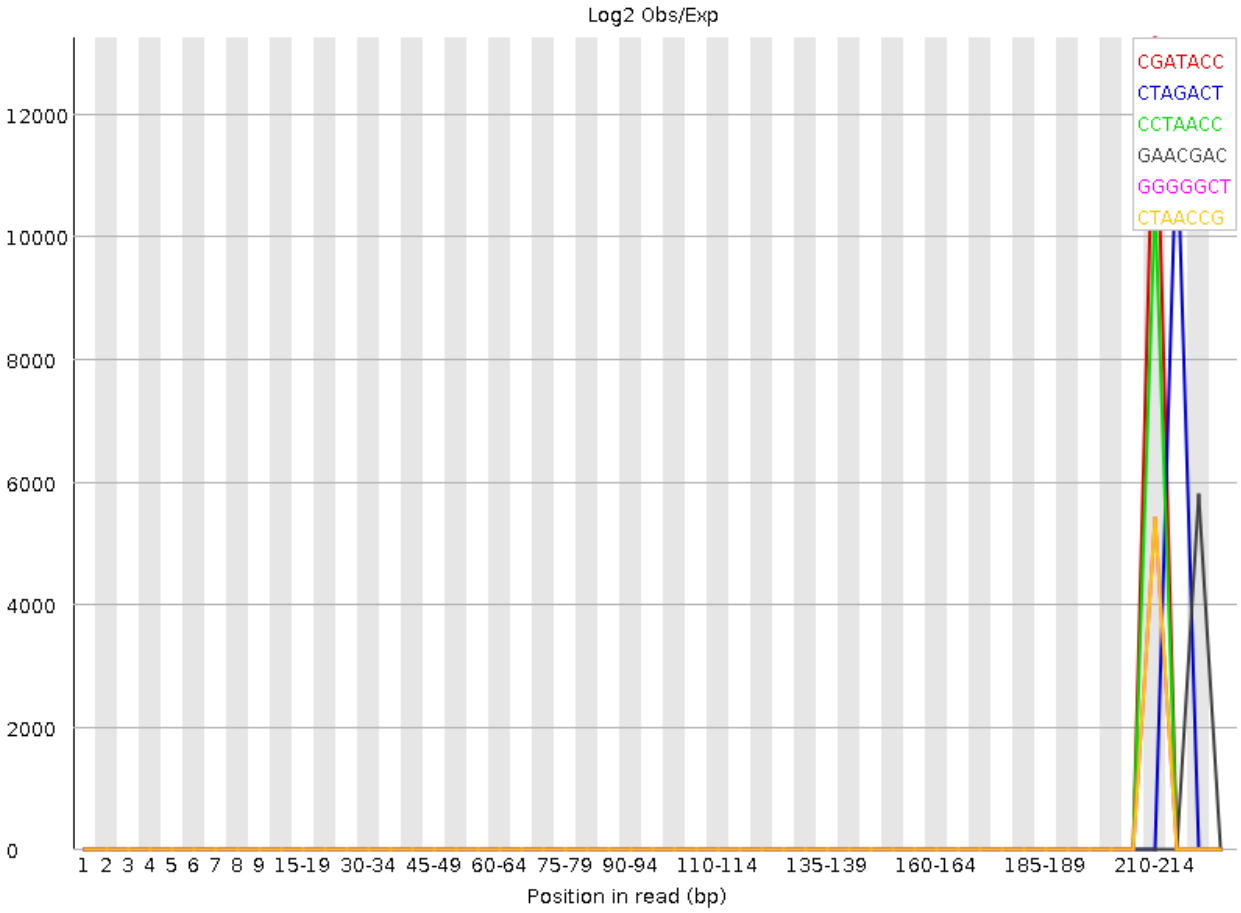
Supplemental Figure 3. Number of ambiguous base calls.



Supplemental Figure 4. Percent distribution of bases across all sequences.



Supplemental Figure 5. Percent GC content across all sequences.



Supplemental Figure 6. Kmer content across all sequences.