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RESEARCH PAPER

Vernalization responses and subsequent fertility of two climatically distinct populations of rattail fescue (*Vulpia myuros* [L.] C.C. Gmel.)

CATHERINE S. TARASOFF[1]★, CAROL MALLORY-SMITH[2] and LYNN INGEGNERI[3]

[1]*School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, Michigan,* [2]*Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon and* [3]*Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado, USA*

Rattail fescue has become increasingly problematic in North America as a result of the greater adoption of no-till practices. While rattail fescue is described generally as a winter annual, there exists a wide variation in the life history documentation of the species. In some instances, rattail fescue has been observed behaving as a spring annual. In order to assess the vernalization plasticity of rattail fescue, laboratory-germinated seedlings from two climatically different rattail fescue populations, eastern versus western Oregon, were exposed to 4, 7 or 10°C temperatures for 0, 2, 4, 5, 6, 6.5, 7 or 8 weeks of vernalization. Following vernalization, the seedlings were transferred to a greenhouse and the developmental stages were recorded. After 13 weeks, the emergent inflorescences were clipped and the seeds were tested for germinability. The initiation of sexual development in the eastern population was affected significantly by the vernalization temperature and length, while the western population only responded to the vernalization length. In general, a faster progression through the reproductive stages of development was associated with longer vernalization lengths. Lastly, there was an increase in the germination rate of the seeds that were produced on the parent plants that were subjected to longer vernalization lengths, regardless of the population or vernalization temperature. The level of plasticity in the vernalization response between the two tested populations indicates that land managers should monitor local population life history traits in order to ensure that effective weed control is implemented at the correct growth stage.

Keywords: chilling, cold treatment, fitness, phenology, *Vulpia myuros*.

Rattail fescue (*Vulpia myuros*) is a weedy grass species that is prevalent within no-till/direct-seeded cropping systems. Native to Eurasia and Northern Africa, it has become widespread around the globe, including in Europe, Asia, Africa, Australia and the Americas. Rattail fescue (or rattail sixweeks) probably was first introduced to California before the 1800s (Hitchcock *et al.* 1969) and was well established across the west of the USA by the 1890s. Until recently, rattail fescue was abundant in localized populations, occurring from Alaska to southern Mexico and east to Nevada and Arizona (Hitchcock *et al.* 1969), dominating on thin, dry and/or sandy soils (DiTomaso & Healy 2007). However, in Australia and the Pacific northwest USA, rattail fescue populations have expanded dramatically in wheat production systems under no-till practices, occurring in regions ranging from high to very low precipitation (Ball *et al.* 2008).

Rattail fescue is commonly described as an obligate winter annual as a vernalization period must be met to achieve successful sexual reproduction and seed set (Wallace 1997). On the San Joaquin Experimental Range, central California, rattail fescue in the early leaf stage was documented from mid-December to early

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★*Correspondence to: Catherine S. Tarasoff, Michigan Technological University, School of Forest Resources and Environmental Science, Houghton, MI 49931, USA.*
Email: ctarasof@mtu.edu

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February, flowering in late March and dispersing seed from June to August (Gordon & Sampson 1939). However, at the Hopland Field Station, northwest California, rattail fescue was in the early leaf stage in mid-March, flowering from mid-April to late May and setting seed by mid-May (Heady *et al.* 1992). The results from these two studies indicate that there is some plasticity, probably linked to differences in the environmental conditions around the vernalization requirements for this species.

The timing of the transition from vegetative to reproductive development is influenced by both developmental and environmental cues, including the photoperiod and temperature (Vince-Prue 1983), the combination of which ensures that not only has the plant accumulated sufficient resources but also that the environmental conditions are suitable (Simpson *et al.* 1999). Extensive work on arabidopsis (a model flowering plant) shows many responses in the timing of flowering to the environmental conditions, including cold treatment, or vernalization. Arabidopsis flowering can be accelerated following a cold treatment, signaling the onset of spring (Simpson *et al.* 1999). Vernalization can be considered to be a quantitative response as the plant system literally accumulates cold treatment 'units' (Simpson *et al.* 1999). The temperature range of vernalization is typically accepted as 0–10°C, with a decreasing influence of the temperature up to 15–18°C (Fowler *et al.* 1996). Vernalization does not initiate the development of flowers directly, as in the development of the primordia; rather, it inhibits the repressors of floral transition (Simpson *et al.* 1999; Michaels & Amasino 2000).

Plants can be categorized as having a facultative vernalization response, meaning that they do not require vernalization for flowering, but they will transition more quickly through their reproductive life cycle if a cold treatment occurs (Michaels & Amasino 2000). Or, as obligate vernalization responders, they will not flower without a vernalization treatment (Michaels & Amasino 2000). Having a facultative response to vernalization can be more desirable as the variation in vernalization response would allow for the spread of plants from northern to southern climates and vice versa.

Ball *et al.* (2008) published a reciprocal seeding study (eastern *vs* western Oregon [OR] biotypes) and found that, while rattail fescue benefits from cool winters, year-to-year variation and seedbed conditions seem to obscure the location (east *vs* west), biotype (east *vs* west) or date of planting (October to March). The results were difficult to decipher as seasonal variability and cultural practices overpowered the experiment. For this reason, the authors of this article chose to study vernalization under controlled conditions, using growth chambers and a greenhouse.

This study explored the issue of vernalization plasticity by exposing two populations that were collected from eastern and western OR to two sets of environmental conditions: the length of vernalization and the vernalization temperature. Populations from eastern and western OR were chosen as these areas represent climatically different conditions. Eastern OR has a colder, longer and drier winter than western OR (Fig. 1). Thus, it is hypothesized that the eastern climate could promote a stronger vernalization response in rattail fescue.

This study addressed the following question: Are there differences in the vernalization length and temperature required to promote sexual development, as witnessed by 'jointing' for two distinct populations of rattail fescue? It

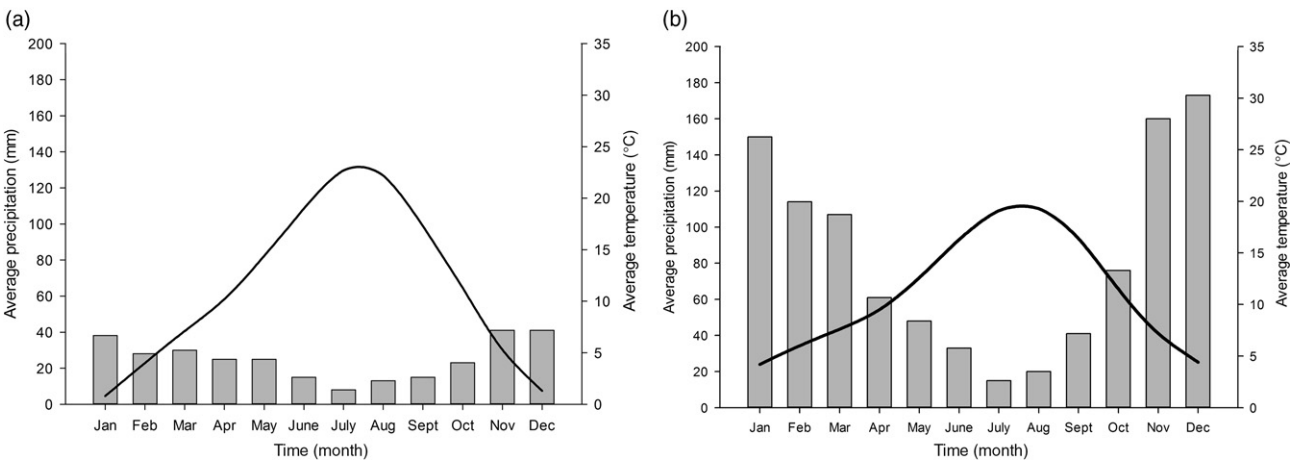

Fig. 1. Average climate data (the bars represent precipitation, the line indicates the temperature) for (a) Pendleton, eastern Oregon (OR), and (b) Salem, western OR. The data were obtained from http://www.climate-zone.com.

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was hypothesized that both populations would respond positively to increases in vernalization length and decreases in vernalization temperature; however, it was expected that the populations would vary in their responses. The results of this study allow us to better understand how rattail fescue could be able to colonize croplands across diverse climates as we move towards the greater adoption of no-till practices.

MATERIALS AND METHODS

Mature inflorescences from two rattail fescue populations in OR (eastern and western) were collected in the summer of 2001. The western population was derived from a seed-cleaning facility in the Willamette Valley, OR. The seed-cleaning facility cleans local winter and spring wheat. Unfortunately, seed from a cleaning facility in eastern OR could not be accessed. Thus, the eastern population was collected from a single large site near Hermiston, OR. In Hermiston, the inflorescences were collected from hundreds of individuals and then bulked. Salem, OR (see Fig. 1), lies within the Willamette Valley and Hermiston is ~46 km north-east of Pendleton, OR. The climate is comparable between the weather data presented in Figure 1 and that of the climates in Corvallis and Pendleton.

Rattail fescue requires almost no after-ripening (3 days) to break its dormancy (Ball *et al*. 2008). Thus, the seeds from each rattail fescue population were placed on two 10.2 cm × 10.2 cm germination blotters (BB44; Hoffman Manufacturing, Inc., Albany, OR, USA) that were wetted with 25 mL of deionized water in similarly sized, clear, covered acrylic germination boxes (Cont156C transparent acrylic container; Hoffman Manufacturing, Inc.). The seeds were incubated in growth chambers (SG50SS single growth chamber, two doors, 110 V; Hoffman Manufacturing, Inc.) at 25/15°C day/night temperatures in the dark for 2 days. A total of 144 germinated seeds from each population type with consistent radicle lengths was sown, one seed per pot, 2 cm deep into 3.8 cm diameter by 14 cm depth cones (RLC7 stubby cell depth cone; Stuewe and Sons, Inc., Tangent, OR, USA) that were filled with potting soil (Sunshine mix no. 1/LC 1 potting mix; Sun Gro Horticulture, Agawam, WA, USA). Then, they were placed inside a growth chamber.

The growth chambers were set to constant temperatures of 4, 7 or 10°C (±1°C) with an 8 h photoperiod. Six replicate seedlings were exposed to 0, 2, 4, 5, 6, 6.5, 7 or 8 weeks of vernalization under each temperature treatment. After the vernalization treatment, but before transferring the plants to the greenhouse, all the plants were acclimated for 7 days. The growth chambers were set to 10°C for 2 days, which then was increased to 12°C for 2 days and then 15°C for 3 days. All the plants then were transferred to a greenhouse for the duration of the experiment. The greenhouse conditions were 30/18°C day/night temperatures with a 16 h photoperiod.

In the growth chambers and the greenhouse, the plants were rearranged weekly to minimize any positional effects. The plants were watered when the soil surface appeared to be dry. A commercial water-soluble fertilizer (Miracle-Gro water-soluble all purpose plant food, 15–30–15; Scotts Miracle-Gro Products, Inc., Marysville, OH, USA) was prepared according to the label's directions and was applied weekly throughout the study. Once in the greenhouse, the plants were evaluated weekly for pests and sprayed with nicotine with a Fulex nicotine fumigator (EPA no. 1327–1341; Fuller System, Inc., Woburn, MA, USA) at the labeled rates as needed.

The growth stages of all the plants were recorded twice per week. The number of days from the end of the vernalization period to the first reproductive node (jointing), initial emergence from the boot (awn), full emergence from the boot (full spike) and the first day of anthesis (anthesis) were recorded. The greenhouse minimum and maximum daily temperatures were recorded. The growing degree days (GDDs) were calculated by using a base temperature of 0°C. The accumulated GDDs were calculated as the sum of the daily heat values, beginning with the acclimation period in the growth chamber and ending with the harvest in the greenhouse.

At 13 weeks after being transferred to the greenhouse, the inflorescences were collected from individual plants. The seeds were harvested from each plant and germinated following the procedures outlined above. After 5 days, the germination rates were tallied for each parent plant.

Experimental design and statistical analysis

The experiment was a full-factorial completely randomized design with six replicates per treatment and it was repeated in time. All the statistical analyses were carried out by using SAS statistical software (version 9.1; SAS, Cary, NC, USA). A logistic regression model for the analysis of binary data (joint = 1 and no joint = 0) was used to evaluate the main effects of the vernalization duration and temperature on the probability that a plant would produce a reproductive node. The model consisted of three parts: the response variable, the linear relationship between the vernalization duration and the vernalization temperature and the logit transformation that links the relationship between the main effects to the expected values. The model is described as follows:

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$$logit(p) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_{dur} Duration + \beta_1 Temp(4) + \beta_2 Temp(7) + \varepsilon, \quad (1)$$

where p is the probability that a plant will reach the first reproductive node, β_0 is the estimated x-axis intercept, β_{dur} is the coefficient for the continuous variable, vernalization duration, β_1 and β_2 are the estimated coefficients for the categorical variables, the vernalization temperatures, 4 and 7°C, and ε is the error of the model. The interaction terms were not tested because the use of interaction terms in logit models can lead to erroneous conclusions (Berry *et al.* 2010). Simplifying the model leads to the extrapolation of β_0 as the coefficient for temperature 10°C. The parameter estimates were derived by using the maximum likelihood method of PROC GENMOD and were used to calculate the probability of reaching the first reproductive node ($p - hat$):

$$(p - hat) = \left(\frac{\exp\left[\begin{array}{c}\beta_0 + \beta_{dur} Duration \\ + \beta_1 Temp(4) + \beta_2 Temp(7)\end{array}\right]}{1 + \exp\left[\begin{array}{c}\beta_0 + \beta_{dur} Duration \\ + \beta_1 Temp(4) + \beta_2 Temp(7)\end{array}\right]}\right), \quad (2)$$

The statistical analyses were carried out individually for each population. Comparisons between the populations for their reproductive response in the absence of vernalization were made by using the parameter estimate for the intercept and 83% confidence interval. This interval was chosen because it results in the acceptable Type I error rate of 5% for multiple comparisons between population parameters and approximates a 0.05 hypothesis test (Payton *et al.* 2003).

The phenological and germination data were analyzed with ANOVA by using Tukey's Honestly Significant Difference (HSD) test to separate the means. The data were log-transformed to meet the assumptions of equal variance and normality, where applicable. The results were pooled by treatments when the Type 3 tests for fixed effects failed to show significant differences at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Parent plants: The likelihood of initiating sexual reproduction

The likelihood of initiating sexual reproduction was determined by the initiation of jointing, or the initiation of the first reproductive node. The classification of the eastern rattail fescue population as having a facultative vernalization response was strongly supported by the results of this study. For the eastern population, the vernalization temperature and length of vernalization were both significant ($P < 0.001$) variables in predicting the likelihood of jointing. The reaction of the eastern population is consistent with the authors' expectation of an incremental response by the species to both changes in temperature and in vernalization length. When the likelihood of reaching the jointing stage, based on the vernalization temperature and length of vernalization (Fig. 2), is modeled, a strong relationship

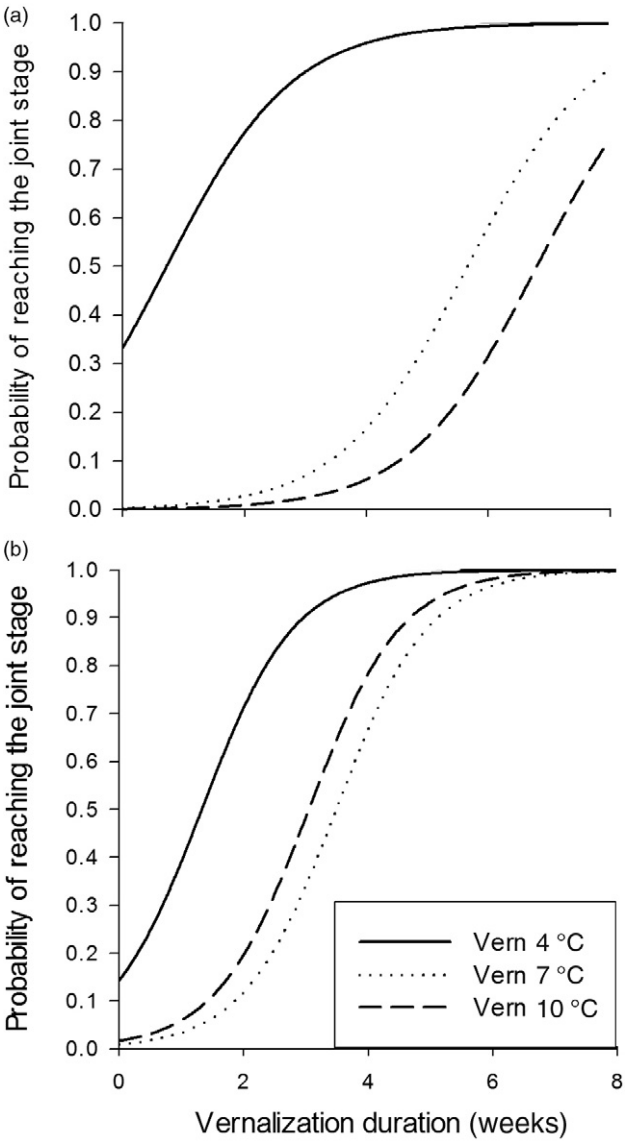

Fig. 2. Modeled response curves for the probability of jointing as a response to vernalization duration and temperature for an (a) eastern Oregon (OR) and (b) western OR population. 'Vern' represents the number of days of vernalization.

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is displayed for the eastern OR population (Fig. 2a). If vernalized for 4 weeks at the low temperature of 4°C, the eastern population is predicted to display a 96% likelihood of reaching the jointing stage (78–99%). With the same vernalization length at 7°C, not only does the likelihood drop, to 17%, but the variability around the mean increases (2–73%). At 10°C, the likelihood of jointing decreases even further to 6% (1–50%). The eastern population appears to rely heavily on cold vernalization periods to promote the initiation of the reproductive cycle and this response increases with the vernalization length.

This trait might reflect the extreme growing conditions of Pendleton, OR. By July, the average maximum temperature can reach 32°C. At the same time, the soil water availability probably is decreasing as the region is entering a dry-down period (Fig. 1b). The average amount of precipitation declines throughout the summer and does not improve until October, when the average monthly precipitation increases by ~30 mm. Although the soil moisture deficit cannot be calculated with precipitation and temperature alone, it might be difficult for a spring-germinated plant to access enough water to complete its life cycle by fall.

The western population did not exhibit a clear response to the vernalization temperature ($P = 0.37$), yet the length of vernalization was very significant ($P < 0.001$) (Fig. 2b). The western population responded to the medium and maximum vernalization temperatures of 7 and 10°C, while still benefiting from the low temperature of 4°C. For this reason, the western population appears to be more of a generalist as it responded positively to all the vernalization temperatures. The variability around the western population's response to vernalization can be seen when the same vernalization length of 4 weeks is analyzed. Given a 4 week vernalization period, the likelihood of jointing was not significantly different between the temperature treatments due to high variability. For example, at 4°C, the likelihood is predicted to be 97% (ranging from 71 to 100%); the average likelihood drops to 67% at 7°C (ranging from 11 to 98%) and increases to 78% (ranging from 17 to 99%) at 10°C. The significant response to the vernalization length is a likely response to the quantitative nature of vernalization.

The average temperatures in Salem, OR, are consistently warmer in the winter and cooler in the summer than in Pendleton (Fig. 1a). Additionally, the precipitation in Salem is much more favorable to plant growth. Combining the effects of average temperature and precipitation, one would expect that spring-germinated seedlings in Salem would experience a much lower soil moisture deficit throughout the growing season, relative to the Pendleton seedlings.

Although it appears that seedlings from moderate western climates are evolving away from a vernalization requirement, reciprocal common garden studies by Ball *et al.* (2008) concluded that both populations (eastern and western) grew significantly better under eastern OR conditions, indicating that the western populations have not completely shed their vernalization response.

Parent plant populations: The rate of development

Each stage of reproductive development was recorded. There was no significant difference ($P > 0.05$) between the populations in the GDDs required for each vernalization temperature and length. The plants that were vernalized for <35 days had inconsistent phenological development during the course of this study. Therefore, it was difficult to analyze the complete set of treatments due to missing data. However, analyzing the minimum (35 days) and maximum (56 days) vernalization treatments showed a trend that reflected the population's response to the vernalization length presented in the previous section. The rate of reproductive development was faster for both populations under longer vernalization treatments and the western population responded more strongly than the eastern population to lengthening vernalization treatments (Fig. 3).

For the jointing stage of development, there was no difference between the populations (Tukey's HSD

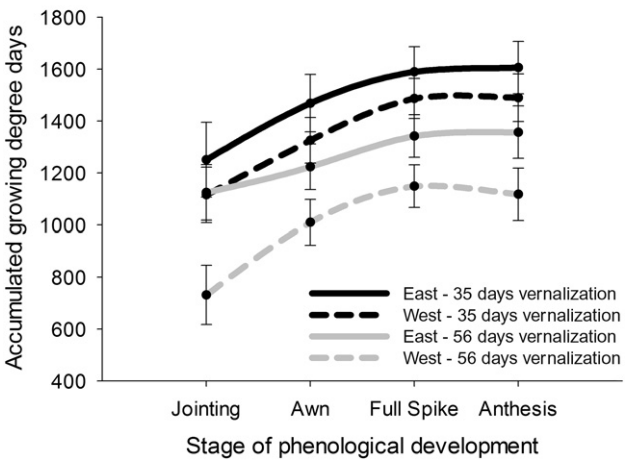

Fig. 3. Phenological development by accumulated growing degree days for two populations (eastern and western Oregon) of rattail fescue that was vernalized for 35 days versus 56 days. The error bars represent a 95% confidence interval.

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test). But, within the western population, the GDD requirement to reach jointing was significantly greater ($\alpha = 0.05$) for those plants that were vernalized for 35 days than for those that were vernalized for 56 days (1399 *vs* 1029, respectively). Differences in the vernalization treatments between the populations were no longer significant by the awn stage of development. These results support those of Fandrich *et al.* (2008), who found that the vernalization treatment effects were most pronounced in the early stages of reproductive development (jointing).

Offspring: Seed viability

The results were pooled by run and population when the Type 3 tests for fixed effects failed to show significant differences at $\alpha = 0.05$. The plants that were vernalized for 0 and 2 weeks did not produce seed heads within the time frame of the study. Therefore, the germination values are presented for 4–8 weeks of vernalization only as all the inflorescences had produced viable seed.

The germination values were strongly affected by the vernalization length of the parent plant (Fig. 4) ($P < 0.001$), while the vernalization temperature showed a weak, but not significant, effect on germination ($P = 0.08$). There was no interaction between the vernalization length and the vernalization temperature ($P = 0.11$). The seeds from the parent plants that were subjected to longer vernalization periods had greater germination rates than those that came from parent plants with shorter vernalization periods, regardless of the vernalization temperature or the original population source (eastern *vs* western biotype). It is possible that, although the inforescences had produced viable seed, the seeds from the plants that were subjected to longer vernalization might have been physiologically more mature, or less dormant. It is not uncommon for vernalization of the parent plant to affect the germination of the offspring (Fandrich *et al.* 2008). The greater germination rates associated with longer parental vernalization could reflect an adaptive feature to ensure that a long vernalization requirement remains in the population. This could be particularly useful in regions with long winters (i.e. Pendleton). However, the mechanism(s) driving the differences in germination as a result of parental vernalization is unknown.

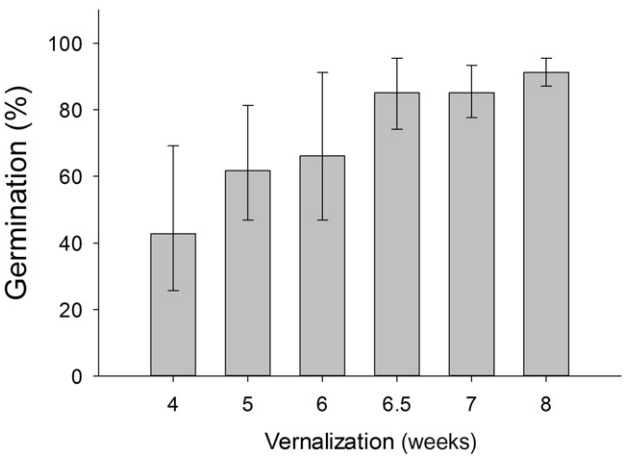

Fig. 4. Average germination of the offspring of the vernalized parent plants when both populations are combined. The error bars indicate a 95% confidence interval.

CONCLUSIONS

This study verifies that significant differences in vernalization requirements exist between ecologically distinct (eastern *vs* western OR) populations of rattail fescue. These results support those found by Chauvel *et al.* (2002) and Meyer *et al.* (2004). In a study of downy brome (*Bromus tectorum*), Meyer *et al.* found that most of the variation in the species' response to vernalization was differences between populations. Similarly, Chauvel *et al.* found that there was dissimilar variation within populations of black-grass (*Alopecurus mysuroides*) than between populations. Within agronomic cultivars, the vernalization requirements might be genetically fixed to ensure predictability (Fowler *et al.* 1996). But, for wild populations, maintaining vernalization plasticity between populations is an advantageous trait as regional climatic conditions can vary and the ability to survive in new environments hinges on genetic variability. The two populations might have come from different founding populations or they could be the result of evolution within one population. Regardless, there exists enough variability in the vernalization requirements of the studied populations that land managers would be prudent to observe the life history of local populations in order to ensure effective weed management. Further research of the vernalization responses along a climatic gradient, or cline, would be useful to fully understand the genetic variation within the species.

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