

## ANALYSES OF *PHLEUM* L. (POACEAE) SPECIES IN THE BLACK HILLS OF SOUTH DAKOTA AND WYOMING

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### ABSTRACT

While investigating the flora of the Black Hills we noted numerous specimens of *Phleum* species in herbaria that appeared to be incorrectly identified. Some keys to plant species in the area use plant height or inflorescence length as primary characters to distinguish between the two species in our region. Analyses of 15 characters for 203 specimens of *Phleum* from Colorado, Wyoming and the Black Hills of South Dakota and Wyoming revealed that plant height and inflorescence length of *P. pratense* and *P. alpinum* form continua with heights ranging 18 to 152 cm, and inflorescence lengths of 9 mm to 85 mm. While 97 specimens were clearly *P. pratense* and 88 specimens were *P. alpinum*, 18 Black Hills specimens were intermediate between the two species based on plant height and inflorescence length. Results from the analyses show that culm base width, inflation of the flag leaf sheath, and inflorescence length/width ratios were the most useful characters for discerning species in the Black Hills.

### Keywords

botany, grass, timothy, taxonomy, Great Plains

### INTRODUCTION

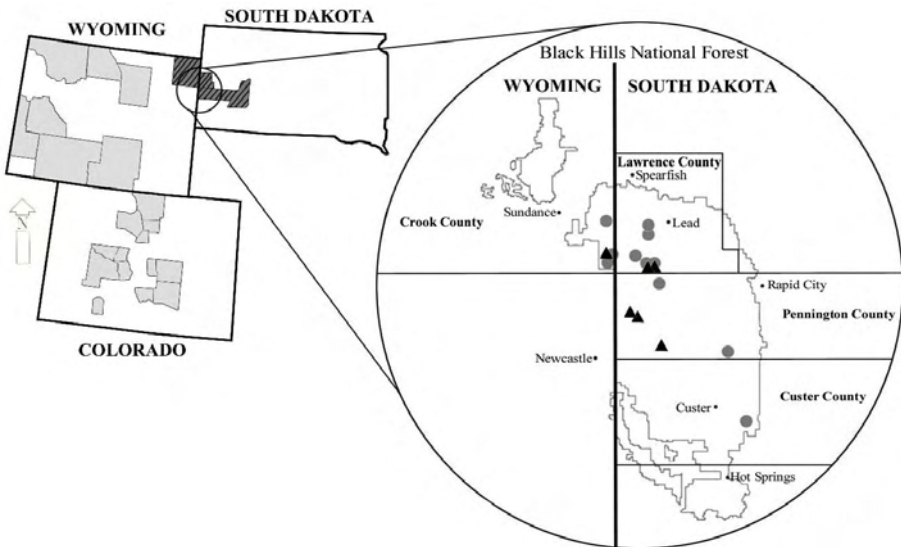
*Phleum* L. is a genus of grasses (Poaceae) found mostly in Eurasia (Conert 1981) that includes about 15 species. Of those, *P. alpinum* L., which originated in Asia (Stewart et al. 2009), is the only species native to North America, while *P. pratense* L. and other species have been introduced (Barkworth et al. 2007). Both *P. alpinum* and *P. pratense* are perennial grasses with dense panicles. The native species *P. alpinum* differs in morphology from *P. pratense* by an inflated sheath of the flag leaf, panicles 1-6 cm long, a 1.5-3 inflorescence length/width ratio, and a lower internode that lacks an enlarged or bulbous base. The flag leaf

sheath of *P. pratense* is often not inflated, and the species has panicles that range from 2-14 cm in length with length/width ratios of 5-20, and a lower internode that is bulbous or partially enlarged.

Historically, Gray (1880) listed no species of *Phleum* in the Black Hills. Rydberg (1896) included only *P. pratense* for the Black Hills. Hayward (1928) did not mention *P. alpinum* in his publication, nor were there specimens of that species in his collections (BHSU database). McIntosh (1931) noted both species of *Phleum* in his checklist. *Phleum alpinum* was "frequent on Harney Range" while *P. pratense* was "common in higher hills, especially in Limestone district."

Over (1932) listed both species of *Phleum* in his checklist of the flora of South Dakota. Thelenius (1971) and Dorn (1977) continued to list the two species as present in the Black Hills. While the Flora of the Great Plains (Great Plains Flora Association 1986) does not include a key to separate species of *Phleum*, under the description of *P. pratense* is a note indicating that *P. alpinum* has been found in Colorado, Wyoming and Pennington County of South Dakota. Van Bruggen (1996) included both species in his flora of the state.

The purpose of this study is to determine if *P. alpinum* is present in the Black Hills of South Dakota and Wyoming, and if present, to determine the characteristics that could be used to consistently differentiate this species from *P. pratense* in the Black Hills. We also wanted to determine current range and habitats of *P. alpinum* within the Black Hills.



**Figure 1.** Location of specimen collection sites in this study. Colorado and Wyoming specimens show counties of collection sites lightly shaded; Black Hills counties in which specimens were collected are dark shaded, with cross-hatch showing counties in which specimens were collected. Enlargement of the Black Hills includes gray circles to represent where *Phleum alpinum* has been reported without voucher specimens, and dark triangles where *Phleum alpinum* populations were sampled or verified in this study.

METHODS

Plant specimens of alpine timothy (*P. alpinum*) and timothy (*P. pratense*) from herbaria in South Dakota, Wyoming, and Colorado were examined. Additional specimens were collected from the field in 2010 at locations where alpine timothy had been reported previously on the Black Hills National Forest (Figure 1). Specimens were identified by the authors as *P. alpinum*, *P. pratense*, or intermediate based upon morphological characteristics (Table 1). Included were plant specimens from Colorado (*P. alpinum* only), Wyoming specimens collected outside the Black Hills of Wyoming (*P. alpinum*), and Black Hills plants collected in both South Dakota and Wyoming including *P. alpinum*, *P. pratense* and intermediate specimens. Characteristics of each specimen were categorized or measured (Tables 1 and 2).

Principal component analysis was completed to help identify important variables among the 15 variables collected (SAS 1988; Dunteman 1989; Afifi and Clark 1990). Cluster analyses were then performed on data using ISODATA procedures using a reduced set of variables as input characteristics (Ball and Hall

**Table 1. Characteristics of Phleum L. used for analyses with means and (standard error) displayed. Black Hills specimens contain plants from both South Dakota and Wyoming Black Hills, while Wyoming *P. alpinum* are plant specimens from Wyoming outside the Black Hills.**

Variable	Black Hills <i>P. pratense</i>	Black Hills Intermediate	Black Hills <i>P. alpinum</i>	Colorado <i>P. alpinum</i>	Wyoming <i>P. alpinum</i>
Number of samples	97	18	33	39	16
Plant height (cm)	74.5 (2.2)	60.4 (3.1)	50.0 (2.2)	32.5 (1.9)	28.5 (2.5)
Culm base width (mm)	4.6 (0.1)	3.1 (0.3)	1.4 (0.2)	1.4 (0.2)	2.4 (0.1)
Flag leaf sheath (1 inflated, 0 not inflated, 0.5 interm.)	0.3 <(0.1)	0.5 (0.1)	1.0 <(0.1)	1.0 <(0.1)	1.0 <(0.1)
Length of flag leaf blade (cm)	5.9 (0.3)	5.0 (0.6)	3.4 (0.3)	3.4 (0.3)	5.0 (0.5)
Width of flag leaf blade (mm)	3.7 (0.1)	3.6 (0.3)	3.7 (0.2)	3.2 (0.2)	3.5 (0.3)
Flag leaf l/w ratio ((length (mm)/width (mm)))	16.1 (0.7)	14.3 (1.7)	9.4 (0.7)	11.0 (1.0)	13.6 (0.9)
Inflorescence length (mm)	30.6 (1.8)	20.4 (1.6)	16.8 (0.8)	19.9 (0.8)	23.0 (1.8)
Inflorescence width (mm)	8.0 (0.1)	8.6 (0.3)	8.9 (0.2)	8.4 (0.2)	8.8 (0.2)
Inflorescence length/width ratio	4.0 (0.2)	2.4 (0.2)	1.9 (0.1)	2.3 (0.1)	2.6 (0.2)
Spikelet length (mm)	2.7 <(0.1)	2.7 (0.1)	2.9 <(0.1)	3.0 (0.1)	3.1 (0.1)
Glume awn length (mm)	1.4 <(0.1)	1.6 (0.1)	1.7 <(0.1)	1.6 (0.1)	1.9 (0.1)
Lemma length (mm)	1.8 <(0.1)	2.0 (0.1)	1.9 (0.1)	2.0 <(0.1)	2.1 (0.1)
Extent trichomes are attached on spikelet keel	1.9 <(0.1)	2.0 (0.1)	2.4 (0.1)	2.7 (0.1)	2.6 (0.1)
Maximum length of trichome observed	0.8 <(0.1)	0.9 <(0.1)	0.9 <(0.1)	0.7 <(0.1)	0.9 <(0.1)
Site elevation (m)	1928 (10)	1950 (15)	2002 (7)	3279 (50)	2681 (77)

**Table 2. Characteristics of Black Hills *Phleum pratense* and *P. alpinum* used for analyses with means and (standard error) followed by range of feature variability. Black Hills specimens contain only plants from both South Dakota and Wyoming Black Hills.**

Variable	<i>P. pratense</i>	Range	<i>P. alpinum</i>	Range
Number of samples	97		33	
Plant height (cm)	74.5 (2.2)	32.7-152.0	50.0 (2.2)	18-88
Culm base width (mm)	4.6 (0.1)	2.2-10	1.4 (0.2)	0-3
Flag leaf sheath (1 inflated, 0 not inflated, 0.5 interm.)	0.3 <(0.1)	0-1	1.0 <(0.1)	1-1
Length of flag leaf blade (cm)	5.9 (0.3)	2-16	3.4 (0.3)	0.9-6.8
Width of flag leaf blade (mm)	3.7 (0.1)	1.9-7	3.7 (0.2)	2.1-6.1
Flag leaf l/w ratio ((length (mm)/width (mm))	16.1 (0.7)	6.8-48.8	9.4 (0.7)	3.2-17.7
Inflorescence length (mm)	30.6 (1.8)	9.1-85.0	16.8 (0.8)	11.3-33.0
Inflorescence width (mm)	8.0 (0.1)	1.7-10.0	8.9 (0.2)	7.2-10.9
Inflorescence length/width ratio	4.0 (0.2)	1.2-12.6	1.9 (0.1)	1.3-3.7
Spikelet length (mm)	2.7 <(0.1)	1.9-3.5	2.9 <(0.1)	2.4-3.5
Glume awn length (mm)	1.4 <(0.1)	0.8-2.1	1.7 <(0.1)	0.9-2.3
Lemma length (mm)	1.8 <(0.1)	0.7-2.4	1.9 (0.1)	0-2.5
Extent trichomes are attached on spikelet keel	1.9 <(0.1)	1.1-3.0	2.4 (0.1)	1.5-3.5
Maximum length of trichome observed	0.8 <(0.1)	0.4-1.1	0.9 <(0.1)	0.5-1.5
Site elevation (m)	1928 (10)	1584-2044	2002 (7)	1892-2044

1967; del Morel 1975). Variables selected to be used in cluster analyses were based on ease to measure and differentiate these two species in the field and to minimize collinearity. Cluster analyses were used to note groups that could quantitatively help identify best characteristics for separating species in the field. Discriminant analysis was also used with cluster groupings to further reduce and identify the best list of variables (Afifi and Clark 1990). The reduced data set of variables (5 characteristics) were analyzed further with cluster analyses among Black Hills specimens only to determine if cluster results would correspond with initial identification of specimens. Discriminant analysis was finally used with our original species identifications (as groups) to determine what variables were most important to identify Black Hills specimens. Data were not standardized for analyses. Habitat descriptions are based on qualitative data collected at five Black Hills sites where alpine timothy was collected in 2010.

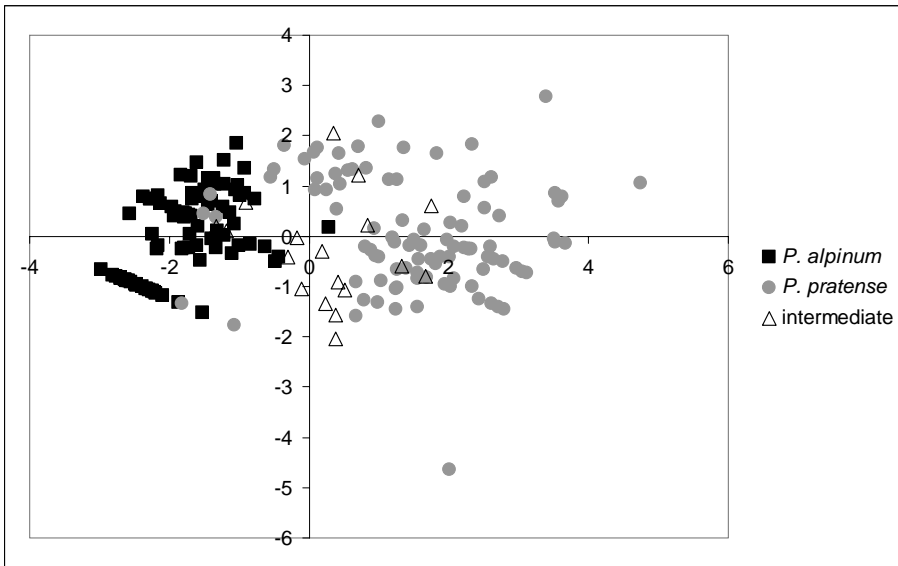
**Table 3. Results of principle component analysis that include 15 characteristics of *Phleum L.* used for initial analyses are displayed with values of the first four principle components (PC1-4).**

Variable	PC1	PC2	PC3	PC4
Plant height (cm)	0.343343	0.096217	-0.133784	0.120498
Culm base width (mm)	0.333109	0.059731	-0.112949	0.057946
Flag leaf sheath (1 inflated, 0 not inflated, 0.5 interm.)	-0.293678	0.086701	0.187063	-0.048330
Length of flag leaf blade (cm)	0.281951	0.321620	0.028517	0.393586
Width of flag leaf blade (mm)	0.100481	0.450128	-0.073911	-0.303559
Flag leaf l/w ratio ((length (mm)/width (mm))	0.279679	0.090421	0.088484	0.659176
Inflorescence length (mm)	0.274118	0.345033	0.269003	-0.189735
Inflorescence width (mm)	-0.159838	0.351119	-0.338663	0.090918
Inflorescence length/width ratio	0.300963	0.205439	0.356717	-0.190733
Spikelet length (mm)	-0.246278	0.428011	-0.020010	0.037549
Glume awn length (mm)	-0.247308	0.110608	-0.242000	0.399559
Lemma length (mm)	-0.182645	0.333157	-0.098624	-0.036561
Extent trichomes are attached on spikelet keel	-0.304264	0.238791	0.254135	-0.020901
Maximum length of trichome observed	0.013933	0.096288	-0.536189	-0.198147
Site elevation (m)	-0.279075	0.078127	0.430314	0.113595
Cumulative percent of variation explained per PC	36%	52%	63%	70%

## RESULTS

Plant characteristic means and standard errors are presented in Table 1. Results of principle component analyses indicated ten variables that include width of culm base, inflation of flag leaf sheath, plant height, flag leaf length/width ratio, inflorescence length, inflorescence length/width ratio, flag leaf length, awn length, maximum trichome length, and elevation explained about 70% of the variation among data (Table 3). Flag leaf width and inflorescence length were excluded from cluster analyses because of collinearity issues with ratios that were included. Maximum trichome length, awn length, and elevation were also excluded because they are more difficult to measure and help little to differentiate species in the field.

Results of cluster analyses using width of culm base, inflation of flag leaf sheath, plant height, flag leaf length/width ratio, and inflorescence length/width ratio grouped alpine timothy specimens together, regardless of collection locality. When two cluster groups were analyzed alpine timothy and timothy groups were formed and when three groups were analyzed the *P. pratense* group was further divided into a timothy group and a robust timothy group. The robust timothy



**Figure 2.** Results of discriminant analysis of entire data set where Black Hills *P. alpinum* are grouped together with Colorado and Wyoming *P. alpinum* specimens. Triangles represent specimens identified as intermediate. The x and y axis represent canonical discriminant function scores.

group included taller plants with larger culm bases. Results of discriminant analysis identified plant height, bulb width, inflorescence length/width ratio, and inflation of flag leaf sheath as the most important variables (in that order) to differentiate specimens among the entire data set. Results of discriminant analysis of the entire data set based on original identification are illustrated in Figure 2.

Cluster analyses results generally placed Black Hills *Phleum* specimens into two or three groups, regardless of input variables used. There was considerable overlap of many measured characteristics among all Black Hills specimens making cluster analyses less useful for Black Hills specimens only. Results of cluster analyses failed to separate Black Hills specimens based on initial plant identification and a reduced set of variables (width of culm base, inflation of flag leaf sheath, flag leaf length/width ratio, inflorescence length/width ratio, and plant height) determined most important for field identification. Data are displayed in Table 1 based on initial identification of plant specimens as Black Hills (SD and WY) plants and *Phleum alpinum* from areas of Wyoming outside the Black Hills and *P. alpinum* from Colorado. Characteristics of plants identified as intermediate had values in between values recorded for Black Hills *P. alpinum* and *P. pratense* for 75% of the measured variables.

Variables identified with principle component analysis as important were width of stem base (bulb), inflation of flag leaf sheath, plant height, flag leaf length/width ratio, inflorescence length, inflorescence length/width ratio, flag leaf width, awn length, maximum trichome length, and elevation. The width of culm base, inflation of flag leaf sheath, plant height, flag leaf length/width ratio, inflorescence length, inflorescence length/width ratio, top leaf length were

determined to be better variables for field identification of *Phleum* in the Black Hills. Other variables identified as important from principle component analysis included awn length, maximum trichome length, and elevation. Awn length and maximum trichome length differ little between timothy and alpine timothy within the Black Hills and would require magnification to accurately measure (Table 2). Since *P. pratense* was observed at every location where *P. alpinum* was observed in the Black Hills, elevation was determined to be a poor variable to differentiate these two species. Inflorescence length and flag leaf length were excluded from further analyses because of potential problems with collinearity and inflorescence and flag leaf length/width ratios were included as variables.

Plant height of timothy was greater than for alpine timothy, but there was considerable overlap in plant size, especially for Black Hills specimens (Table 2); this variable was used in initial cluster analyses and selected as most important for the entire data set, but failed to benefit further analyses of Black Hills only specimens. Therefore, of the ten variables identified as most important, the width of culm base, extent flag sheath was inflated, flag leaf length/width ratio, and inflorescence length/width ratio were the reduced variables set that was used for cluster analyses among Black Hills specimens. Results of these cluster analyses failed to create reliable group separation that matched initial specimen identification.

The final discriminant analysis using our initial plant identification of Black Hills specimens as groups yielded a discriminant model that selected width of culm base (bulb) as most important. This was followed by extent flag sheath was inflated and inflorescence length/width ratio as the three variables important for plant identification. The discriminant model correctly classified 75% of our initial identifications correctly.

Ten sites in the Black Hills were revisited where *P. alpinum* had been previously reported (Table 4). Three of ten sites had timothy, intermediate, and alpine timothy recorded. We failed to find alpine timothy at 50% of the sites where it

**Table 4. Black Hills reported *P. alpinum* locations re-visited in 2010 and number of specimens sampled and identified at each site.**

Site	Black Hills <i>P. pratense</i>	Black Hills intermediate	Black Hills <i>P. alpinum</i>
04H051C	19	0	2
04H053A	12	5	2
04H062A	7	0	0
04M034A	7	3	2
04M035A	5	6	0
04M043A	7	0	0
05W045A	5	0	0
05W046A	7	2	0
08W008C	12	0	11
10CISSIE	14	1	16



**Table 5. Range of variability observed plant height (cm) for Black Hills populations of *Phleum alpinum* and *P. pratense*.**

Site	<i>P. alpinum</i>	<i>P. pratense</i>
04C051C	62-81	57-127
04H053A	36-48	33-69
04M034A	72-88	75-110
08W008C	40-75	59-116
10CISSIE	18-66	41-74

had been reported previously. At the sites where alpine timothy was relocated in 2010, 30% of them also had intermediate specimens.

Plant height values of alpine timothy were completely within the variability of values measured for timothy at two sites and nearly so for a third site (Table 5). Inflorescence length is often used as one of the key attributes to differentiate timothy (2-14 cm) and alpine timothy (1-6 cm) (Barkworth et al. 2007). However, Black Hills specimens of timothy vary considerably and are often found with inflorescences shorter than or equal in length to alpine timothy. Three characteristics, width of culm base, inflated flag leaf sheath, and inflorescence (l/w) ratio differentiate the two Black Hills species, with the exception of intermediate plants.

*Habitat description* – *Phleum alpinum* within the Black Hills was found at elevations from 1892-2044 m on slopes less than 30% with various aspects. Plants were located on various shaped slopes in open to partial sun on soils characterized as dry-mesic to moist. Bedrock of all sites where alpine timothy was collected for this study was limestone. Tree canopy cover was estimated at less than 40% and plant communities were dominated by quaking aspen (*Populus tremuloides* Michx.), spruce (*Picea glauca* (Moench) Voss)/pine (*Pinus ponderosa* C. Lawson), or pine/aspen/common juniper (*Juniperus communis* L.) with canopy between 10-50% of the same tree and shrub species. Forb cover was estimated between 10-80% and represented many different species. Grass canopy was estimated to be 60% or less and the most common species present was timothy, but rough-leaved rice grass (*Oryzopsis asperifolia* Michx.), false melic (*Schizachne purpurascens* (Torr.) Swallen), nodding brome (*Bromus anomalus* Rupr. ex Fourn.), various bluegrasses (*Poa* spp.), Torrey's sedge (*Carex torreyi* Tuck.) and other *Carex* spp. were recorded. Litter cover varied between 10-65%. Areas where plants were observed were usually less than 50 m<sup>2</sup>, but one site was reported to be about 3 ha. Areas in which alpine timothy was found also had disturbances to habitat that included deadfall trees, old skid trails, roads, regeneration of saplings, and livestock and wildlife grazing activities.



## DISCUSSION

The Black Hills *Phleum alpinum* clustered within a group with Colorado and Wyoming *P. alpinum*, indicating close similarities in plant characteristics of this species (Figure 2). Plant height was determined to be an important variable to differentiate among groups and our initial plant identifications when analyzing the entire data set (WY and CO specimens included), but it was not useful when analyzing only Black Hills specimens. Joachimiak and Kula (1997) reported that species of *Phleum* lack great differences in morphologies and morphological criteria alone fail to identify phylogenetic relationships. Barkworth et al. (2007) also reported that some depauperate specimens of timothy are difficult to differentiate from alpine timothy. We found that 12% of the Black Hills specimens used in this study were difficult to differentiate as either timothy or alpine timothy.

Cluster analyses of only Black Hills specimens failed to produce group separation of species initially identified by us (*P. alpinum*, *P. pratense*, and intermediate). Discriminant analysis using our initial groups for only Black Hills specimens selected width of culm base (bulb) as most important followed by extent flag sheath was inflated and inflorescence length/width ratio as the three best variables important for plant identification of Black Hills specimens. Intermediate specimens may represent only an overlap of characteristics of *P. alpinum* and *P. pratense* or possible hybrids. Origin of intermediate specimens, e.g., through hybridization, was not determined in this study and should be investigated using molecular techniques (Stewart et al. 2009). Much overlap of measured variables, selection of reduced variables list, incorrect initial identification, and possible hybrids may be possible reasons cluster analyses failed to separate Black Hills specimens into groups similar to initial identifications.

One characteristic not measured was color. It was observed in the field that Colorado specimens of *P. alpinum* L. generally were purple tinged on leaf, stem, and inflorescence; Black Hills *P. alpinum* were less so. The purple color in plant tissues is probably from anthocyanins, which are water-soluble pigments (Harborn 1988). This may merely be an artifact of elevation differences between Colorado and Black Hills populations since alpine timothy produces greater amounts of anthocyanins at greater elevations in Colorado compared to elevations observed in the Black Hills. Anthocyanins are thought to protect plant tissues from UV and visible radiation, factors that are greater at higher elevations (Burger and Edwards 1996).

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