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Patterns of Variation in a Collection of Timothy Accessions

M. D. Casler*

ABSTRACT

Cultivated timothy (*Phleum pratense* L.) is an important grass for hay production in temperate North America. It is under utilized in management-intensive rotational grazing systems because of its poor persistence when frequently defoliated. The objective of this study was to evaluate the USDA-NPGS collection of timothy accessions for agronomic traits, including persistence under frequent defoliation. Unlike previous reports for infrequent harvest systems, diploid and tetraploid species of *Phleum* were similar in forage yield to hexaploid *P. pratense*, suggesting their potential value in livestock agriculture. Cultivated accessions tended to have fewer, but larger leaves; longer and narrower panicles with a greater number of smaller seeds; lower survival under frequent defoliation; and higher forage yield than natural accessions. A considerable amount of phenotypic variation among accessions was explained by geographic source of the accessions. Twenty-one unique phenotypic clusters were formed to account for 50% of the phenotypic variability among accessions, providing the basis for development of a core collection for *P. pratense*. Clusters were highly differentiated on the basis of geographic origin of the accessions, underscoring the potential importance of the relationship between phenotype and geography in hexaploid timothy.

T IMOTHY was the first of the cool-season forage grasses to be introduced intentionally to North America by European colonists in the 18th century (Berg et al., 1996). It is the most important forage grass of the Nordic countries (Jönsson et al., 1992) and, among the cool-season forage grasses, has the longest history of formal breeding activity in both Europe and North America (Casler et al., 1996). Over 150 named cultivars have been developed in or imported to North America alone (Alderson and Sharp, 1994; Lawrence et al., 1995; GRIN, Germplasm Resources Information Network; internet address: http://www.ars-grin.gov/npgs/; verified April 18, 2001).

Cultivated forage-type timothy (*P. pratense*) is hexaploid with $2n = 6x = 42$. There are numerous diploid and tetraploid species within *Phleum*, three of which are considered to be progenitors of *P. pratense*. The diploid *P. alpinum* (alpine timothy) is the most likely donor of the A genome of *P. pratense*, perhaps after tetraploidization to *P. commutatum*, while the diploid *P. bertolonii* (turf timothy) is the most likely donor of the B genome of *P. pratense* (Cai and Bullen, 1991; Joachimiak and Kula, 1997; Walton, 1983; Wilton and Klebesadel, 1973). These three species are all members of section *Phleum* Griseb., are geographically widespread, and are virtually indistinguishable morphologically, except for the generally larger plant size of *P. pratense* (Joachimiak and Kula, 1997). *Phleum alpinum* and *P. commutation* appear to be the only members of the genus that are indigenous to North America, with widespread occurrence in subalpine regions (Wilton and Klebesadel, 1973).

Timothy cultivars are well adapted to hay management practices based on relatively infrequent harvests. The frequent harvests (three or four per year) and persistent competition in a timothy-alfalfa (*Medicago sativa* L.) mixture reduces persistence of timothy cultivars (Casler and Walgenbach, 1990; Smith et al., 1973). Cultivars with earlier heading tend to be more persistent under frequent harvests, but earliness is not a warranty against low persistence (Casler and Walgenbach, 1990). Timothy reproduces vegetatively by swollen sections of basal culms, called corms. Individual corms are biennial, so long-term persistence of timothy plants is highly dependent on continual production of new corms (Childers and Hanson, 1985). Frequent harvesting of timothy can increase the number of dead corms per plant by up to 93% and decrease living corm mass by 29% (Peters, 1958).

Relatively few timothy cultivars developed or commercialized in North America were bred to withstand frequent defoliation. Timothy cultivars show considerable variation for persistence in mixture with alfalfa under hay management (Casler and Walgenbach, 1990) or under management-intensive rotational grazing in pure or mixed stands (Casler et al., 1998). Most timothy cultivars ranked low in persistence compared with cultivars of other species in both studies. Jönsson et al. (1992) concluded that hay-type timothy plants should be erect, tall, and early heading, with long, wide leaf blades. Conversely, timothy plants that are most persistent under long-term frequent defoliation are relatively prostrate and late heading (Van Dijk, 1955). As suggested by these conclusions, cultivar $\times$ management interactions

Abbreviations: SEC, standard error of calibration; SEV, standard error of validation.

are common for forage yield of timothy cultivars (Surprenant et al., 1993).

The agricultural importance of timothy in the Nordic countries has led to many studies of genetic variation and adaptation. A considerable amount of genetic variation within timothy is adaptive in nature, resulting from long-term natural selection under localized stressful conditions (Cenci, 1980; Helgadóttir, 1989; Rognli, 1988; Yumoto et al., 1984). The USDA-NPGS collection of timothy accessions contains over 650 accessions from 30 countries, including both cultivated and natural accessions originating from a considerable range of habitats and climates. While this collection likely contains genetic variability for traits that would confer tolerance to frequent defoliation, it has never been systematically evaluated for agronomic traits. A subset of 75 Japanese accessions was evaluated under hay management at five locations in the northeastern USA, illustrating considerable variation for a range of agronomic traits (Bryan et al., 1988). The objectives of this study were to quantify genotypic variation for agronomic traits within the USDA-NPGS collection of timothy accessions, including an assessment of persistence under frequent defoliation, and to relate that variation to geographic source of the accessions.

MATERIALS AND METHODS

This study consisted of an evaluation of 483 timothy accessions from the USDA National Plant Germplasm System (NPGS) collection plus 67 additional cultivars (Table 1). All accessions were classified according to species and country source of origin (obtained from GRIN, Germplasm Resources Information Network, http://www.ars-grin.gov/npgs/) and cultivated status (cultivar or breeding material vs. wild or natural collection and based on published accounts of collecting expeditions, e.g., USDA-ARS, 1990). The 67 additional cultivars were assigned a local accession number and were henceforth treated identically to the NPGS numbered accessions classified as cultivated. Seeds of all accessions were germinated in a greenhouse at Madison, WI, in January 1993 and raised as individual seedlings.

Overseeded Plots

Seedlings of all accessions and cultivars were transplanted to the field near Arlington, WI, in May 1993. The soil type was Plano silt loam (fine-silty, mixed, mesic Typic Argiudoll). The experimental design was a randomized complete block with two replicates. Plots consisted of a row of 10 plants, spaced 45 cm apart. Plots were 45 cm apart and organized into tiers that were 90 cm apart. Ten additional (non-test) plots were added to the end of each replicate; these plots consisted of random plants remaining after transplanting the 550 test entries. Immediately after transplanting, the entire experimental area was broadcast seeded with a prostrate-growth form of white clover (*Trifolium repens* L.) at a rate of 7 kg ha^{-1}.

Plots were clipped four times in 1993 and six times each in 1994 and 1995 to simulate the defoliation frequency of a management-intensive grazing system. Plots were clipped to a 10-cm stubble height when the tallest timothy plants were approximately 25 cm tall. Prior to each clipping, forage yield was rated on a visual scale of 0 = missing plant to 20 = the greatest apparent biomass at that point in time. Following

Table 1. Species of *Phleum* included in the germplasm evaluation, including number and source of 550 accessions or cultivars.

Species	No. of accessions	Country sources of accessions†
P. alpinum L.	4	AZB (1), CAN (1), ITL (1), USA (1)
P. arenarium L.	7	TRK
P. bertolonii DC.	9	TRK (1), SPN (8)
P. boisseri Bornm.	2	TRK (1), IRQ (1)
P. commutatum Gaudin	1	ARG
P. exaratum Hochst. ex Griseb.	1	TRK
P. hirsutum Honck.	1	ITL
P. montanum K. Koch	6	AFG (1), AZB (1), TRK (4)
P. paniculatum Huds.	3	AFG (1), AZB (1), USA (1)
P. phleoides (L.) H. Karst.	27	AFG (1), AZB (1), IRN (1), KZK (1), RUS (17), TRK (5), UZB (1)
P. pratense L.‡	489	23 countries

† AFG = Afghanistan, ARG = Argentina, AZB = Azerbaijan, CAN = Canada, IRN = Iran, IRQ = Iraq, ITL = Italy, KZK = Kazakhstan, RUS = Russia, SPN = Spain, TRK = Turkey, and UZB = Uzbekistan.
‡ Includes 67 cultivars not specifically included in the USDA-NPGS collection.

the forage yield rating, 40 plants (two from each nonzero numerical rating category) were harvested from the non-test plots at a 1-cm stubble height. These plants were dried at 60°C and weighed. Following the last forage yield rating in October 1995, each plant was coded as living or dead. Plant survival percentage was computed on an individual plot basis.

Each set of 40 harvested plants was divided into two sets of 20, each set including one plant of each size class. A linear regression calibration was computed between forage yield ratings and dry matter yield of the 20 harvested plants for each of the 32 sets. Paired sets for each of the 16 cuttings were used to validate the calibration developed in the alternate set of the pair. Regressions were judged adequate for predicting forage yield from visual ratings, with calibration r^2 ranging from 0.64 to 0.92 and validation r^2 ranging from 0.62 to 0.87. On the basis of these results, a single linear regression was computed from the 40 harvested plants of each cutting and used to predict forage yield of that cutting for all plants. Forage yield estimates were summed over six cuttings in 1994 and 1995 prior to any statistical analysis. Previous work has shown that visual ratings of forage yield made by an experienced researcher had high genetic correlations ($r = 0.93$ to 0.97) with forage yield of meadow fescue, *Festuca pratensis* Huds. (Aastveit and Aastveit, 1989). Fertilization with P and K was done according to recommendations derived from soil tests. Nitrogen fertilizer was not applied to this experiment.

Following the visual forage yield rating in August of 1994 and 1995, a bulk forage sample was harvested from each 10-plant row at a 10-cm stubble height. Ten accessions had plants that did not exceed a 10-cm height. For these plants, forage samples were clipped at a stubble height of approximately half their maximum leaf height. Bulk samples consisted of approximately 50 g fresh leaf tissue per plant. Samples were dried and ground through a 1-mm screen of a Wiley-type mill and reground through a 1-mm screen of a cyclone mill. Near-infrared reflectance spectra (NIRS) were obtained on each sample with a Pacific Scientific 6500 scanning monochromator (Pacific Scientific, Rockford, IL). A subset of 120 calibration samples were chosen by the NIRS software using a cluster analysis of reflectance spectra (Shenk and Westerhaus, 1991). These samples were analyzed for neutral detergent fiber (NDF) concentration using the procedure of Van Soest et al. (1991) with the exceptions that sodium sulfite and α-amylase were excluded. Predicted NDF values were generated from a single calibration equation (SEC = 7.9 g kg^{-1}, R^2_{cal} = 0.92, SEV = 10.8 g kg^{-1}, R^2_{val} = 0.86).

Non-Overseeded Plots

The experiment was repeated, with the same design and plot size, adjacent to the overseeded experiment. The second experiment was also established in May 1993, but was not overseeded with white clover. Annual weeds were controlled by pre-emergence herbicide applications in 1993 and 1994, as described by Falkner and Casler (1998). Kentucky bluegrass (*Poa pratensis* L.) and annual bluegrass (*P. annua* L.) provided sufficient ground cover after 1994 that additional weed control was unnecessary. Plants were harvested twice in 1993 and three times in 1994 without data collection. Fertilization with P and K was done according to recommendations derived from soil tests.

In April 1995 and 1996, all plants were fertilized with 90 kg N ha^{-1}. Plants were allowed to grow into a reproductive mode, during which time the following measurements were made on all living plants. Heading date was recorded as the day-of-year when the fifth panicle was fully emerged from the boot. For plants that clearly had fewer than five floral primordia, heading date was determined as the earliest day that the majority of panicles were emerged. Plants that never produced panicles were coded as missing values.

Length and maximum width of the first leaf blade below the flag leaf and the total number of leaves were recorded for one tiller per plant in late July, after most plants had reached anthesis. Height to the top of the tallest panicle was recorded in late July. The number of panicles per plant was recorded in early August. Ten random panicles (or fewer, as necessary) were harvested from each plant prior to seed shattering. Total length and maximum width of each panicle were measured during the following winter. Seeds of each 10-panicle sample were threshed and cleaned by one person to maximize uniformity of technique. Seed weight per panicle was determined for each plant. One thousand seeds were counted from each 10-panicle sample and weighed to determine 1000-seed weight. All plants were clipped in late August 1995, following the 1995 seed harvest. Germinating timothy seeds did not interfere with 1996 data collection because of the excellent bluegrass ground cover.

Statistical Analysis

All variables were analyzed by a random effects model analysis of variance by means of generalized least squares to handle missing and unbalanced data (Searle, 1971). The initial ANOVA partitioned accessions into sources of variation for species and accessions within species. A second ANOVA and all additional analyses were based on accessions classified as *P. pratense* (Table 1).

In the second ANOVA, sums of squares for *P. pratense* accessions were divided into three sources of variation related to the source of the accession: region, country(region), and accession(country). Ten geographic regions were defined as JPN = Japan, RUS = Russia (mostly northwestern), NZL = New Zealand, NAM = North America (Canada and USA), SWE = Southwestern Europe (France, Italy, and Spain), NWE = Northwestern Europe (Belgium, Great Britain, and The Netherlands), SCA = Scandinavia (Denmark and Sweden) plus Finland, NEE = Northeastern Europe (The Czech Republic, Germany, Poland, and Slovakia), SEE = Southeastern Europe (Bulgaria, Hungary, Greece, Romania, and the former Yugoslavia), and SWA = Southwestern Asia (Afghanistan, Azerbaijan, Iran, Iraq, Kazakhstan, Turkey, and Uzbekistan). All factors (years, replicates, accessions, countries, and regions) were assumed to have random effects. Separate from the above region/country/accession partition, a single degree of freedom was partitioned from the sum of squares

for accessions to compare the mean of cultivated accessions to the mean of natural accessions.

The 13 variables were standardized and organized into 13 principal components. The principal component scores were subjected to cluster analysis using nearest centroid sorting (Anderberg, 1973; Milligan, 1980). The number of discrete non-hierarchical clusters was arbitrarily determined as that which divided the total sum of squares into 50% among clusters and 50% pooled within clusters. While it would be more desirable to work with a group of clusters that described more of the phenotypic variation, such as 70%, this would have required a minimum of 35 clusters, reducing the effectiveness of presentation and discussion, and placing additional limitations on the structure of a core collection.

RESULTS AND DISCUSSION

Nineteen accessions did not survive the first winter. These accessions were distributed among species as follows: *P. alpinum* (1), *P. arenarium* (1), *P. bertolonii* (1), *P. exaratum* (1), *P. hirsutum* (1), *P. montanum* (2), *P. paniculatum* (2), *P. phleoides* (8), and *P. pratense* (2). An additional 13 accessions did not survive the second or third winter: *P. arenarium* (1), *P. bertolonii* (3), *P. montanum* (1), *P. phleoides* (3), and *P. pratense* (5). These 32 accessions were excluded from all data analyses.

Accession × year interactions were significant ($P <$ 0.05) for 9 of 12 variables (excluding plant height, forage yield, and NDF; also excluding plant survival which was scored in only one year). Variation among accessions was significant at $P < 0.01$ for all 13 variables. Genotypic correlation coefficients between years were generally high ($r \geq 0.8$ for most variables), indicating that accession differences and rankings were generally similar between years. Furthermore, the accession variance component always exceeded the accession × year variance component. Means over years and replicates were used in all tables and discussions to follow.

Minor Species

Analyses of variance demonstrated variation among *Phleum* species means for eight of the 13 variables. There were no differences among species in plant height, leaf number per tiller, panicle length or width, or forage yield. Only those variables that varied among species are presented for the minor species. Because of the immense number of *P. pratense* accessions and the large amount of phenotypic variation among these accessions, the range among *P. pratense* accessions was sufficiently large that it encompassed most of the variability among the minor species' accessions (Table 2). Nevertheless, there were numerous significant deviations of minor species' means from the mean of the *P. pratense* accessions.

P. alpinum

The three accessions of *P. alpinum* were generally quite uniform, despite their diverse origin—Azerbaijan, Canada, and Italy (Table 2). Surprisingly, the Canadian accession had the lowest plant survival in Wisconsin, although this effect was not quite significant at $P <$ 0.05. The Canadian accession also had the lowest NDF concentration, significantly lower than the Azerbaijani

accession, but similar to the average *P. pratense* accession. As a group, *P. alpinum* accessions were 4 d earlier and had 35% more panicles per plant than the average *P. pratense* accession.

P. arenarium

The five accessions of *P. arenarium*, all from Turkey, were uniformly 4 d earlier and had 11% shorter leaves than the average *P. pratense* accession (Table 2). These accessions varied for the other six variables presented in Table 2. Only one of the five *P. arenarium* accessions was moderately adapted to southern Wisconsin (40% plant survival; high panicle- and 1000-seed weights), but it had extremely high NDF concentration, limiting its usefulness as a forage crop. The other four *P. arenarium* accessions fell into narrow- or wide-leaf-blade classes, but had very low plant survival. The high panicle- and 1000-seed weights of some accessions indicated that some individual plants were well adapted to this environment, suggesting that selection for improved adaptation might be successful. Nevertheless, the extremely high NDF concentration of all five accessions, relative to the average *P. pratense* accession, would severely limit the value of *P. arenarium* accessions for forage.

P. bertolonii

The five accessions of *P. bertolonii* were uniformly 2 d earlier than the average *P. pratense* accession (Table 2). The relatively long and wide leaf blades of these five accessions, similar to the average *P. pratense* accession, indicated that they are forage phenotypes, rather than turf phenotypes of this species. Only one *P. bertolonii* accession had high plant survival, the Turkish accession. Three of the other four accessions, which were all from Spain, demonstrated some potential for adaptation (high panicle seed weight) or agronomic value (low NDF).

P. boissieri

The two *P. boissieri* accessions averaged 5 d earlier, 28% higher 1000-seed weight, 69% lower plant survival, and 4% higher NDF concentration than the average *P. pratense* accession (Table 2). The Turkish accession had 64% more panicles per plant, 28% higher 1000-seed weight, and 75% lower plant survival than the Azerbaijani accession. Because of their low survival and high NDF concentration, neither *P. boissieri* accession appeared to offer much agronomic potential.

P. montanum

The three *P. montanum* accessions averaged 5 d earlier and 79% lower plant survival than the average *P. pratense* accession (Table 2). Despite their low survival percentages, these three accessions were highly variable for leaf blade length, panicle number per plant, panicle seed weight, 1000-seed weight, and NDF concentration, expressing a range of variation equivalent to that among the 482 *P. pratense* accessions. The Azerbaijani accession had extremely short, but wide, leaf blades and low NDF concentration. The Afghani accession had relatively few panicles per plant, but extremely high panicle seed weight. The Turkish accession contrasted with more panicles per plant than any other accession, but extremely low panicle seed weight. Both the Afghani and Turkish accessions had extremely high NDF concentration, limiting their agronomic value.

P. phleoides

Of the minor species, *P. phleoides* was the most similar to *P. pratense*, but also the most variable, most likely due to the larger number of accessions (Table 2). The greatest differences between *P. phleoides* and *P. pratense* accessions were the lower panicle seed weight, 1000-

Table 2. Means over 2 yr and two replicates for 482 *P. pratense* accessions and 36 *Phleum* accessions representing several minor species.

Phleum species	PI No.‡	Variable†							
		HD	LL	LW	PN	PSW	TSW	PS	NDF
		d-o-y	— mm —		# plant⁻¹	— mg —		%	g kg⁻¹
alpinum	230268	201	229	11.0	88	455	376	95	512
	236891	204	235	10.8	75	368	356	65	468
	251844	204	230	11.1	94	391	359	95	487
	Mean§	*203*	*231*	*11.0*	*85*	*405*	*364*	*85*	*489*
arenarium	203449	205	202	9.6	50	310	432	10	509
	204469	202	189	9.5	98	60	302	10	480
	204472	202	229	10.7	68	453	445	40	513
	204473	202	224	11.2	58	640	570	10	483
	204477	204	204	10.4	51	165	386	15	520
	Mean§	*203*	*210*	*10.3*	*65*	*326*	*427*	*17*	*501*
bertolonii	206718	204	249	10.4	94	410	354	95	493
	287915	204	233	10.7	78	580	524	30	421
	287916	207	238	10.3	56	825	409	20	509
	319076	207	250	11.0	73	105	414	40	453
	319078	206	291	11.8	93	70	396	20	482
	Mean§	*205*	*252*	*10.8*	*79*	*398*	*419*	*41*	*472*
boissieri	206716	201	220	11.5	95	300	566	10	498
	254905	203	254	10.6	58	410	442	40	498
	Mean§	*202*	*237*	*11.1*	*77*	*355*	*504*	*25*	*498*
commutatum	202048	205	219	11.3	66	344	346	*50§*	497
montanum	206719	202	246	11.0	115	150	459	20	537
	223283	201	215	11.0	60	560	334	10	544
	229464	202	186	11.8	80	150	327	20	472
	Mean§	*202*	*216*	*11.3*	*85*	*287*	*373*	*17*	*518*
paniculatum	223676	*201§*	229	10.4	72	423	395	90	499
phleoides	251391	202	229	10.1	63	407	484	65	475
	315488	206	217	10.5	67	178	259	15	509
	325453	201	280	10.5	113	10	213	10	540
	325454	208	211	10.5	76	74	180	80	480
	325456	208	223	11.0	88	20	158	20	498
	383727	205	212	10.5	71	443	356	95	490
	383728	216	256	12.0	58	725	534	20	498
	440586	205	217	10.3	63	75	216	20	484
	440587	206	222	11.1	47	160	225	10	530
	440588	209	243	11.0	76	147	327	55	499
	440589	204	245	11.8	80	177	363	45	485
	440590	205	205	10.1	38	29	247	55	528
	440591	210	219	10.9	68	95	209	45	492
	440592	204	205	12.8	75	108	201	40	494
	440593	207	235	11.0	103	289	298	30	496
	440594	204	236	11.2	57	116	297	25	526
	Mean§	*206*	*228*	*11.0*	*71*	*191*	*285*	*39*	*502*
pratense	Mean	207	236	11.0	63	420	395	82	478
	Maximum	215	301	14.5	137	657	571	100	538
	Minimum	201	174	8.5	23	5	142	10	431
LSD(0.05)¶		5	42	1.5	34	200	85	31	35

† HD = heading date, LL = leaf blade length, LW = leaf blade width, PN = panicle number, PSW = panicle seed weight, TSW = 1000-seed weight, PS = plant survival, and NDF = neutral detergent fiber.
‡ Plant introduction number.
§ Italicized means differ from the mean of *P. pratense* at *P* < 0.05.
¶ LSD for comparing individual plant introduction means.

Table 3. Means of stated number of *P. pratense* accessions derived from cultivated germplasm (cultivars or breeding lines) or natural germplasm (ecotypes, land races, and wild or semiwild collections).

Group	n	PH	PD	LN	LL	LW	PL	PW	PN	FY	PSW	TSW	SPP	PS	NDF
		cm	d-o-y	#		mm			#	g plant^{-1}	mg		#	%	g kg^{-1}
Cultivated	208	114	206	7.9	239	11.1	128	7.4	64	107	431	385	1121	79	478
Natural	274	115	206	8.1	234	10.9	122	7.8	63	97	413	403	1019	84	477
Significance		ns	ns	**	**	**	**	**	ns	**	*	**	**	**	ns

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
*** Indicates significance at $P < 0.001$.
ns, Not significant.
† PH = plant height, HD = heading date, LN = number of leaves per stem, LL = leaf blade length, LW = leaf blade width, PL = panicle length, PW = panicle width, PN = number of panicles per plant, FY = forage yield, PSW = panicle seed weight, TSW = 1000-seed weight, SPP = seeds per panicle, PS = plant survival, and NDF = neutral detergent fiber.

seed weight, and plant survival and the higher NDF concentration of most *P. phleoides* accessions. Without intensive selection for increased adaptation, the only accession that appears to have agronomic value is PI 251391 (Afghanistan) which had moderately high panicle seed weight, 1000-seed weight, and plant survival, combined with NDF concentration similar to the average *P. pratense* accession.

P. commutatum and *P. paniculatum*

The *P. commutatum* accession (Argentina) had 39% lower plant survival than the average *P. pratense* accession and the *P. paniculatum* accession (Azerbaijan) was 6 d earlier than the average *P. pratense* accession. Otherwise these two accession were phenotypically similar to the average *P. pratense* accession.

Minor Species Summary

These minor species of *Phleum* appear to offer as much variation as present within *P. pratense*, limited primarily by the number of accessions present in the collection. The low NDF concentration, moderate plant survival percentages, and relatively high panicle- or 1000-seed weights indicate some potential for agronomic adaptation to environments similar to southern Wisconsin. The lack of variation among species for forage yield was notable, given previous reports that diploid and tetraploid *Phleum* species are lower in vigor and/or forage yield than hexaploid *P. pratense* (Caradus, 1978; Joachimiak and Kula, 1997). The distribution of forage yield means for *P. pratense* (minimum = 37, maximum = 264, mean = 98, and median = 95 g plant^{-1}) was nearly identical to that for the minor species (minimum = 43, maximum = 254, mean = 107, and median = 100 g plant^{-1}), suggesting that some of these species may have utility for livestock agriculture. Utilization of any of these minor species will likely require additional collection to broaden the genetic base and intensive selection for adaptation both within and among accessions.

Major Species: *Phleum pratense*

Cultivated vs. Natural Populations

Differences between cultivated and natural populations represent the accumulated and combined effects of

over 100 yr of timothy breeding in numerous temperate regions of the world (Casler et al., 1996). Cultivated and natural accessions did not differ in mean plant height, heading date, or number of panicles per plant (Table 3). Cultivated accessions averaged 2% longer leaf blades, 2% wider leaf blades, and 2% fewer leaves per stem than natural accessions. Cultivated accessions averaged 5% longer panicles, 5% narrower panicles, 4% greater panicle seed weight, 4% lower 1000-seed weight, and 10% more seeds per panicle than natural accessions. Cultivated accessions also averaged 10% higher forage yield, but 6% lower plant survival than natural accessions.

These differences suggest that timothy breeders have focused largely on traits related to plant vigor, seed production, and morphology (fewer, but larger leaves), in addition to other traits not measured in this study. The 10% difference in forage yield is intermediate to that observed for perennial ryegrass, *Lolium perenne* L. (Casler, 1995) and orchardgrass, *Dactylis glomerata* L. (Casler, 1991). The significant reduction in plant survival of cultivated accessions suggests that they are not as well-adapted to frequent and severe defoliation as natural accessions. Responses of timothy populations to natural selection pressures under hay, short-term grazing, or long-term grazing managements indicated that long-term (≥21 yr) grazing pressure was necessary to bring about measurable adaptive responses (Van Dijk, 1955). Thus, breeding programs that rely on infrequent mechanical harvesting are unlikely to result in cultivars that are adapted to frequent and/or severe defoliation.

Variation among Region and Country Germplasm Sources

Phleum pratense accessions demonstrated considerable phenotypic variation among region and country sources (Table 4). Variation among country means within regions was significant ($P < 0.05$) for five of 13 variables and variation among region means was significant for the other eight variables (Table 4). The sum of squares attributable to regions always exceeded the percentage of degrees of freedom for regions (1.9%), an indication of phenotypic differentiation among regions. The sum of squares attributable to countries within regions exceeded the percentage of degrees of freedom allocated

Table 4. P-values for mean squares, percentage contribution of sum of squares, and variance component estimates for geographic sources of variation of 482 *P. pratense* accessions collected from 23 countries in 10 geographic regions.

Source of variation	df	PH	HD	LN	LL	LW	PL	PW	PN	FY	PSW	TSW	PS	NDF
							P-values							
Region	9	0.006	0.005	0.322	0.042	0.198	<0.001	0.001	0.013	0.097	0.223	0.017	0.103	0.001
Country/R	13	0.393	0.305	0.016	0.922	0.012	0.941	0.999	0.149	0.048	0.008	0.357	0.025	0.211
Accession/C/R	459	<0.001	<0.001	<0.001	0.003	0.063	0.171	0.035	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
						Sum of squares (%)								
Region	1.9‡	8.6	9.8	4.6	2.7	6.0	10.5	2.5	9.3	6.6	6.0	7.1	7.1	13.9
Country/R	2.7‡	2.6	2.8	5.1	1.4	5.3	1.2	0.5	3.4	4.4	5.5	2.8	4.8	3.0
Accession/C/R	95.4‡	88.8	87.3	90.2	96.0	88.7	88.3	97.0	87.2	89.0	88.6	90.2	88.2	83.1
						Variance Component Estimate								
Region		18.6	3.4	0.02	30.8	0.05	110.4	0.12	93.9	154	1303	915	59.1	164.9
Country/R		0.9	0.4	0.11	0.0	0.12	0.0	0.00	29.7	175	3950	97	78.8	21.0
Accession/C/R		18.3	3.3	0.08	68.1	0.04	15.0	0.15	55.9	148	4595	2129	120.2	59.5

† PH = plant height, HD = heading date, LN = number of leaves per stem, LL = leaf blade length, LW = leaf blade width, PL = panicle length, PW = panicle width, PN = number of panicles per plant, FY = forage yield, PSW = panicle seed weight, TSW = 1000-seed weight, PS = plant survival, and NDF = neutral detergent fiber.
‡ Percentage of degrees of freedom for each source of variation.

to countries (2.7%) for nine of 13 variables. Variance components indicated that regions and accessions within countries were the most important sources of variation. The variance component for these two sources of variation were largest, among the three sources, for 5 of 13 variables each. For countries within regions, this variance component was largest for only 3 of 13 variables and there were three zero estimates.

Despite their large number, Japanese accessions had, on average, longer leaf blades, shorter and wider panicles, fewer panicles per plant, and lower NDF concentration than all other regional sources (Table 5). Japanese accessions averaged later in heading than accessions from all but one other region. Russian and Southwestern European accessions tended to be shorter than the other accessions. European accessions (excluding Scandinavia and Southeastern Europe) generally had the longest average panicles, but average panicle width. The two New Zealand accessions had extremely long and wide panicles. Southeastern European, North American, Northeastern European, and Japanese accessions tended to

have the heaviest seeds. Southwestern Asian accessions had the highest average NDF concentration.

Accessions with the greatest number of leaves per tiller tended to come from the Northeastern Mediterranean region: Greece, Italy, Romania, Turkey, and the former Yugoslavia (Table 6). Bulgarian, French, British, and New Zealand accessions had the fewest number of leaves per tiller, on average. The French accessions had the widest leaf blades, while the Bulgarian and Italian accessions had the narrowest leaf blades, on average. The Bulgarian, Finnish, Italian, and Dutch accessions had the highest average forage yield, while the British

Table 5. Means over stated number of *P. pratense* accessions (n) deriving from 10 geographic regions. Means were computed over two replicates, 2 yr, and 10 plants per plot.

Source	n	PH	HD	LL	PL	PW	PN	TSW	NDF
		cm	d-o-y		mm		#	mg	g kg⁻¹
Japan	300	115	207	238	122	7.7	60	396	473
North America	55	116	206	237	127	7.4	69	412	489
Scandinavia	16	115	206	232	125	7.4	75	381	483
NE Europe	19	112	205	228	134	7.4	67	400	481
NW Europe	25	115	206	237	135	7.5	70	366	479
SE Europe	22	114	204	235	127	7.5	68	427	485
SW Europe	6	108	208	236	138	7.5	72	380	483
Russia	31	108	206	232	129	7.4	62	371	481
SW Asia	6	116	204	227	127	7.6	74	348	499
New Zealand	2	116	206	227	142	7.7	69	355	484
*t*0.05*SE_d‡		14	5	42	35	2.4	34	85	35

† PH = plant height, HD = heading date, LL = leaf blade length, PL = panicle length, PW = panicle width, PN = panicles per stem, TSW = 1000-seed weight, and NDF = neutral detergent fiber.
‡ Compute LSD0.05 as $t0.05(SE_d)[(n_1+n_2)/n_1n_2]^{1/2}$, where n_1 and n_2 are the number of accessions included in the means to be compared.

Table 6. Means over stated number of *P. pratense* accessions deriving from 23 countries. Means were computed over two replicates, 10 plants per plot, and (with the exception of plant survival) 2 yr.

Country source of accessions	Number of accessions	Leaf number	Leaf blade width	Forage yield	Panicle seed wt.	Plant survival
		# stem⁻¹	mm	g plant⁻¹	mg	%
Belgium	6	8.1	11.4	113	319	78
Bulgaria	1	7.0	9.7	129	488	100
Canada	18	8.1	11.2	111	393	73
Czech Republic	1	8.1	10.3	88	449	70
Denmark	4	8.0	10.4	102	535	90
Finland	1	7.9	11.3	136	431	80
France	2	7.1	12.7	98	460	80
Great Britain	5	7.1	10.8	81	261	63
Germany	4	7.7	10.8	91	458	74
Greece	2	8.4	10.5	105	598	85
Hungary	3	7.5	10.3	81	444	90
Italy	3	8.5	9.9	131	235	50
Japan	300	8.0	11.0	98	426	84
The Netherlands	14	8.1	11.0	134	435	84
New Zealand	2	7.1	11.1	104	285	83
Poland	14	8.0	10.6	92	474	86
Romania	4	8.2	10.6	106	431	68
Russia	31	7.7	10.9	95	388	83
Spain	1	8.0	11.0	84	509	90
Sweden	11	8.1	10.9	118	365	81
Turkey	6	8.2	10.6	116	369	70
USA	37	8.1	11.0	116	425	78
the former Yugoslavia	12	8.3	10.6	95	458	83
*t*0.05*SE_d†		1.4	1.5	66	200	31

† Compute LSD0.05 as $t0.05(SE_d)[(n_1+n_2)/n_1n_2]^{1/2}$, where n_1 and n_2 are the number of accessions included in the means to be compared.

Table 7. Number of *P. pratense* accessions from each of 10 geographic regions that comprise each of 21 phenotypic clusters, including chi-square tests for deviations of cluster composition (χ^2_9, right margin) or accession distribution among clusters (χ^2_{20}, bottom margin) from the mean geographic distribution of the entire collection.

Cluster	Japan	North America	Scandinavia	NE Europe	NW Europe	SE Europe	SW Europe	Russia	SW Asia	New Zealand	Sum	χ^2_9	P-value
A	0	0	0	0	1	0	0	0	0	0	1	9.91	0.358
B	2	0	0	0	0	0	0	2	0	0	4	17.64	0.040
C	2	0	0	0	1	0	0	0	0	0	3	15.06	0.089
D	1	0	1	0	1	0	1	0	1	0	5	5.55	0.784
E	0	1	0	0	1	0	0	0	0	0	2	8.82	0.454
F	5	3	0	0	1	0	0	7	0	0	16	40.30	<0.001
G	36	3	1	0	0	1	0	0	1	0	42	296.75	<0.001
H	11	3	0	5	2	2	1	7	0	2	33	36.33	<0.001
I	5	6	4	9	2	10	0	3	1	0	40	31.16	<0.001
J	44	0	0	0	0	0	0	0	0	0	44	436.00	<0.001
K	19	9	3	4	0	4	2	4	2	0	47	67.39	<0.001
L	39	1	1	0	3	0	0	4	0	0	48	302.39	<0.001
M	66	3	1	0	2	0	0	1	0	0	73	579.01	<0.001
N	3	1	0	0	1	0	0	3	0	0	8	18.77	0.027
O	4	4	3	0	3	1	0	0	1	0	16	18.30	0.032
P	26	7	0	0	3	1	0	0	0	0	37	178.15	<0.001
Q	1	0	0	0	0	0	1	0	0	0	2	8.82	0.454
R	3	0	0	0	2	0	0	0	0	0	5	23.15	0.006
S	6	10	0	0	0	2	1	0	0	0	19	60.90	<0.001
T	26	1	2	1	2	1	0	0	0	0	33	193.00	<0.001
U	1	3	0	0	0	0	0	0	0	0	4	23.14	0.006
Sum	300	55	16	19	25	22	6	31	6	2	482		
χ^2_{20}	475.18	71.38	39.13	116.95	19.52	100.18	22.00	72.65	22.00	40.00			
P-value	<0.001	<0.001	0.006	<0.001	0.488	<0.001	0.341	<0.001	0.341	0.005			

and Hungarian accessions had the lowest average forage yield. The highest average panicle seed weights were obtained for Bulgarian, Danish, Greek, and Spanish accessions, illustrating the lack of regional variation for this variable. Although mean plant survival ranged from 50 to 100% among the 23 country sources, this variation appeared only partially related to climatic factors. Bulgarian, Danish, Hungarian, and Spanish accessions had the highest mean plant survival. British and Italian accessions had the lowest mean plant survival. The relatively low survival of the Canadian accessions vs. the Japanese and Dutch accessions (73 vs. 84%; $P < 0.05$) was clearly unrelated to climatic factors, as the timothy-growing regions of Japan and the Netherlands represent milder or more moderate climates than found in southern Wisconsin, which is climatically closer to the timothy-growing regions of Canada. Much of the variation in survival may be related to management factors at the site of origin. The cutting schedule may have favored accessions preferentially adapted to frequent and/or severe defoliation, which may have been more important than edaphic stress tolerances, such as cold and/or freezing tolerance.

Cluster Analysis

Twenty-one nonhierarchical clusters accounted for 50% of the total phenotypic variability among *P. pratense* accessions (Table 7). Cluster composition was strongly related to geographic origin of the accessions. Chi-square tests showed that 16 of the 21 clusters had a geographic composition that deviated from the average of the entire collection ($P < 0.05$) and seven of the 10 regions had accessions that were non-randomly allocated to clusters ($P < 0.05$).

Six clusters (J, G, L, M, P, and T) were dominated by Japanese accessions, which accounted for 86% of the accessions in these six clusters and 79% of the 300 Japanese accessions. Clusters B, F, H, and N all had unusually high frequencies of Russian accessions, accounting for 61% of the Russian accessions. Sixty percent of the accessions from Scandinavia and Eastern Europe (34 of 57) were assigned to Clusters I and K, comprising the dominant component of Cluster K. The two accessions from New Zealand clustered together, indicating a strong phenotypic similarity. Accessions from Western Europe and Southwestern Asia did not deviate from a random distribution among clusters.

The cluster analysis identified some striking phenotypic differences among groups of accessions (Table 8). Cluster P was perhaps the most notable, with 44 accessions that had extremely short and wide panicles. Cluster K consisted of accessions that were tall and early heading with high panicle- and 1000-seed weights. Clusters H and T consisted of accessions that were short and late heading with wide leaf blades and low 1000-seed weight. Clusters I, M, and O consisted of accessions with low panicle- and 1000-seed weights. Clusters L, M, Q, and T consisted of accessions with low NDF concentration. However, Clusters M, Q, and T had problems that might limit the use of this low-NDF germplasm, such as extremely low seed weights or forage yield. Cluster L may be a valuable source of germplasm for low NDF, because its accessions had no such obvious agronomic limitations.

Clusters A through G include the bulk of accessions with the greatest agronomic value in a short-term breeding program (Table 8). Six of these seven clusters had mean forage yield significantly higher than the mean of all accessions ($P < 0.05$), with cluster means ranging

Table 8. Cluster means over stated number of *P. pratense* accessions (n) for each of 21 phenotypic clusters. Means are over 2 yr, two replicates, and 10 plants per plot, and are expressed as deviations from the overall mean.

Cluster	n	PH	HD	LN	LL	LW	PL	PW	PN	FY	PSW	TSW	PS	NDF
		cm	d-o-y	#			mm		#	g plant⁻¹		mg	%	g kg⁻¹
A	3	1	−2	−0.9**	41**	0.4	−2	−0.5	22*	115**	−5	−9	−14	−1
B	4	0	1	0.9**	25*	0.4	−34**	−0.2	54**	80**	−99*	30	6	7
C	1	26**	−2	1.0	58**	2.0**	25	0.0	22	76*	308**	92*	−22	−11
D	16	7**	0	0.4*	3	0.6**	3	−0.1	20**	48**	−42	−21*	8	16**
E	2	10*	−4*	−0.9	45**	−1.0	54**	−0.1	9	21	91	23	13	48**
F	19	2	−1	0.3*	5	0.3	1	−0.3	−2	17*	−12	68**	−45**	15**
G	47	5**	−3**	0.0	0	−0.1	1	−0.2	21**	15**	31*	8	3	5
H	8	−5*	6**	−0.2	30**	0.9**	26**	−0.8	5	14	−90**	−44**	4	−7
I	5	6	0	−1.0**	−5	0.2	8	−0.3	4	10	−147**	−100**	−48**	−4
J	37	2	1**	0.6**	10**	−0.1	1	−0.4*	6*	2	−11	11	5	12**
K	42	5**	−2**	−0.4*	−3	−0.1	1	−0.2	−8**	0	89**	32**	3	5
L	73	1	0	0.0	5*	0.3*	2	−0.3	−6**	−3	7	3	5*	−12**
M	48	−2	3**	0.2	5	0.0	1	−0.5**	0	−4	−140**	−71**	3	−10**
N	33	−6**	0	−0.9**	−3	−0.2	7*	−0.3	1	−5	17	12	1	−1
O	5	1	0	0.5	−13	−0.7*	2	−0.4	3	−8	−305**	−166**	−54**	18*
P	44	1	1	0.2	−3	0.2	−24**	2.8**	−13**	−10	20	17*	1	−6*
Q	33	−9**	1**	0.2	−16**	−0.3*	−2	−0.4	−12**	−16*	34	20**	6*	−11**
R	40	−1	−3**	0.2	−13**	−0.8**	6*	−0.1	3	−18**	52**	22**	3	8**
S	16	−9**	−1	−0.7**	−26**	−0.5*	−3	−0.2	−13**	−22**	−93**	−50**	1	12**
T	2	−12*	5**	−1.0*	21	3.3**	9	0.8	−29*	−27	95	−29	−12	−24
U	4	−11**	2	−1.1**	28**	1.1**	5	0.0	−2	−40*	−40	−66**	−19*	10
Mean	482	114	207	8.0	236	11.0	125	7.6	63	102	420	395	82	478

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$
† PH = plant height, HD = heading date, LN = number of leaves per stem, LL = leaf blade length, LW = leaf blade width, PL = panicle length, PW = panicle width, PN = number of panicles per plant, FY = forage yield, PSW = panicle seed weight, TSW = 1000-seed weight, PS = plant survival, and NDF = neutral detergent fiber.

from 15 to 113% of the overall mean. In general, the accessions in these high-yielding clusters were characterized by tall, early heading plants with longer- and wider-than-average leaf blades, more leaves per stem, more panicles, and higher NDF concentration than average. Cluster A was a notable exception to this generalization, with a relatively low number of leaves per stem. Clusters B and E differed largely by having the most extreme mean panicle lengths of the 21 clusters, 27% lower or 43% higher than the mean, respectively. Clusters C, D, F, and G differed largely by extreme values of panicle seed weight or 1000-seed weight. Cluster G was among the lowest clusters in mean plant survival (37%).

The results of this study provide a mechanism to select a core subset of accessions from the USDA-NPGS timothy collection. The core subset for *Phleum* should consist of representatives of each species somewhat proportional to the number of accessions of each species. For *P. pratense*, a core subset can be described based on the cluster analysis, with one to five accessions per cluster, proportional to the number of accessions in each cluster. Such a sampling scheme would produce a core subset of approximately 46 accessions. The greatest utility of this core subset will be for locations similar to southern Wisconsin, for which phenotypic expression and variability should be similar. For extremely different locations, this core subset may represent a more-or-less random sample of the accessions. Phenotypic data and cluster numbers for each accession evaluated in this experiment are available on the internet through the GRIN homepage.

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