

**Bromegrass in Alaska. II.
Autumn Food-Reserve Storage,
Freeze Tolerance, and Dry-Matter
Concentration in Overwintering Tissues
as Related to Winter Survival
of Latitudinal Ecotypes**

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SUMMARY

The objective of this study was to acquire improved understanding of factors that influence winter survival of bromegrass (*Bromus* spp.) at northern latitudes. Four bromegrass strains of diverse latitudinal origins were used: (a) native Alaskan pumpelly brome (*B. pumpellianus* Scribn.) adapted at 61° to 65°N, (b) the Alaska hybrid cultivar Polar (predominantly *B. inermis* Leyss. x *B. pumpellianus*) selected at 61.6°N, and two smooth brome (*B. inermis*) cultivars, (c) Manchar selected in the U.S. Pacific Northwest (43° to 47°N), and (d) Achenbach originating from Kansas (34° to 42°N).

- The temperate-adapted, southern-type Achenbach was lower in dry-matter concentration in crown-rhizome tissues near onset of winter, sustained greater injury from artificial freeze stress, and survived winters much poorer than the three more northern-adapted brome grasses.
- Manchar was less injured by modest freeze stress than Achenbach, had significantly higher dry-matter concentration in overwintering crown-rhizome tissues than Achenbach, and was vastly superior to Achenbach in winter survival. Manchar, however, was more injured by freezing and was significantly poorer in winter survival than the more northern-adapted Polar and pumpelly brome grasses.
- The rapid pre-winter build-up of stored food reserves in pumpelly brome grass, and slow expression of those reserves as etiolated growth when tested near

onset of winter conditions, parallels the early senescence and bleaching of foliage commonly seen in that species late in the growing season.

- The predominantly hybrid, very winter-hardy Polar does not show late-season foliar yellowing but did exhibit dormancy equal to pumpelly brome; moreover, Polar stored highest levels of food reserves, and was more tolerant of moderate freeze stress than Manchar and Achenbach.
- The superior winter hardiness of pumpelly brome and Polar in Alaska is believed due primarily to (a) higher levels of freeze tolerance in overwintering tissues than in more southern-adapted bromes (a characteristic that protects against injury during extreme cold stress), and perhaps also to some extent to (b) superior winter dormancy as indicated by slower expression (as etiolated growth) of high levels of stored food reserves; that dormancy conceivably confers better tolerance to winter temperature fluctuations in the freeze-thaw range common in this area of Alaska.
- These results provide insights into certain facets of the winter-hardening process in brome grass strains that prepare overwintering tissues to tolerate winter stresses.
- These findings also reveal differences in pre-winter changes between northern-adapted, winter-hardy brome grasses and more southern-adapted strains that exhibit marginal to poor winter survival in this subarctic area.

INTRODUCTION

Smooth brome grass (*Bromus inermis* Leyss.), a tall-growing, cool-season, rhizomatous perennial, is the dominant introduced forage grass grown on tilled croplands in Alaska. The closely related pumpelly brome grass (*B. pumpellianus* Scribn.) is native in Alaska (Elliott 1949) and possesses several similar agronomic attributes, including good forage and seed yields (Klebesadel 1970, 1971a, 1984; Klebesadel and Helm 1992). The Alaska cultivar Polar incorporates some characteristics from both species; 11 of the 16 parental clones are *B. inermis* × *B. pumpellianus* hybrids (Wilton et al. 1966).

Native Alaskan pumpelly brome grass is extremely winter hardy in this environment and Polar is only slightly less hardy (Klebesadel 1970, 1971a, 1984, Klebesadel and Helm 1992). A considerable range of winter hardiness in Alaska has been noted, however, among introduced strains of smooth brome grass, especially following highly stressful winters (Klebesadel and Helm 1992; Wilton et al. 1966). Southern-type strains and cultivars usually sustain some degree of winter injury or winter-kill totally during most winters at this location. Northern-type cultivars and regional strains generally are more winter hardy in Alaska than those of the southern type (Klebesadel and Helm 1992). However, although northern-type cultivars survive mild winters without visible injury, they sustain moderate to severe injury during more stressful winters and occasionally winter-kill totally (Klebesadel and Helm 1992; Wilton et al. 1966).

When aerial growth is routinely killed by low temperatures in autumn, the subterranean rhizomes and tillers in the region of the plant crown must remain viable as the principal overwintering tissues. New aerial growth is produced from the terminal ends of rhizomes and tiller branches during the following growing season. Those overwintering plant parts serve as storage tissues for carbohydrate food reserves that accumulate during autumn. Moreover, such overwintering tissues must develop very high levels of cold tolerance to withstand freezing stresses during winter.

Objectives of this study were to learn more about specific characteristics of brome grass overwintering tissues that influence winter survival in Alaska. This was accomplished by comparing brome grasses from diverse latitudinal origins for (a) food reserve levels stored during late summer and early autumn, (b) changes in dry-matter concentration during the cold-hardening period, and (c) levels of freeze tolerance in overwintering tissues just prior to onset of winter. The study was conducted at the University of Alaska's Matanuska Research Farm (61.6°N) near Palmer in southcentral Alaska.

EXPERIMENTAL PROCEDURES

All field plantings were made in areas with good surface drainage in Knik silt loam (coarse-silty over sandy or sandy-skeletal, mixed, nonacid Typic Cryochrept). Commercial fertilizer disked into plowed seedbeds supplied nitrogen (N), phosphorus (as P_2O_5), and potassium (as K_2O) at 32, 128, and 64 lb/acre, respectively.

Four brome grass strains representing three provinces of diverse latitudinal adaptation were compared. These were native Alaskan pumpelly brome grass (adapted at 61° to 65°N), the Alaska cultivar Polar of similar latitudinal adaptation, Manchar from the U.S. Pacific Northwest (43° to 47°N), and Achenbach originating from Kansas (34° to 42°N). All were seeded in rows 50 feet long and 12 inches apart in consecutive years on 22 June (Experiment 1) and 20 June (Experiment 2). Randomized complete block experimental designs with four replications were used in both experiments. When seedlings were two to three inches tall, rows were thinned by hand to leave individual seedlings six to eight inches apart.

Seedlings were withdrawn from the field to determine levels of stored food reserves and changes in dry-matter concentration in overwintering tissues during late summer and autumn of the year planted. In both years, 10 to 15 seedlings were dug from each row on each of three sampling dates (10 August, 30 August, and 10 October in Experiment 1; 22 August, 19 September, and 10 October in Experiment 2). Aerial growth beyond two inches above the soil surface was severed and discarded (to arrest transpirational moisture loss) just before plants were dug. Immediately after digging, seedlings were wrapped in water-saturated paper toweling within water-saturated burlap and kept wrapped, except during washing (to remove soil) and trimming, until each was weighed for potting.

All roots were severed and discarded and the main stem and tillers were severed one inch above the seminal node. Final traces of soil and loose plant debris were removed by washing. Surface moisture was blotted from plants with cloth toweling. Prepared crowns were weighed individually and embedded in water-saturated vermiculite in pots (one plant per pot) so that tissues that had been below the soil surface were also below the vermiculite surface; pots measured seven inches in both diameter and height. Five plants of each entry were potted from each replication on each sampling date.

Five to eight plants from each row, prepared in the same manner as those potted, were weighed fresh, dried to constant weight at 60°C, and reweighed. Percent dry matter in each lot was used to derive extrapolated oven-dry weight of crowns potted.

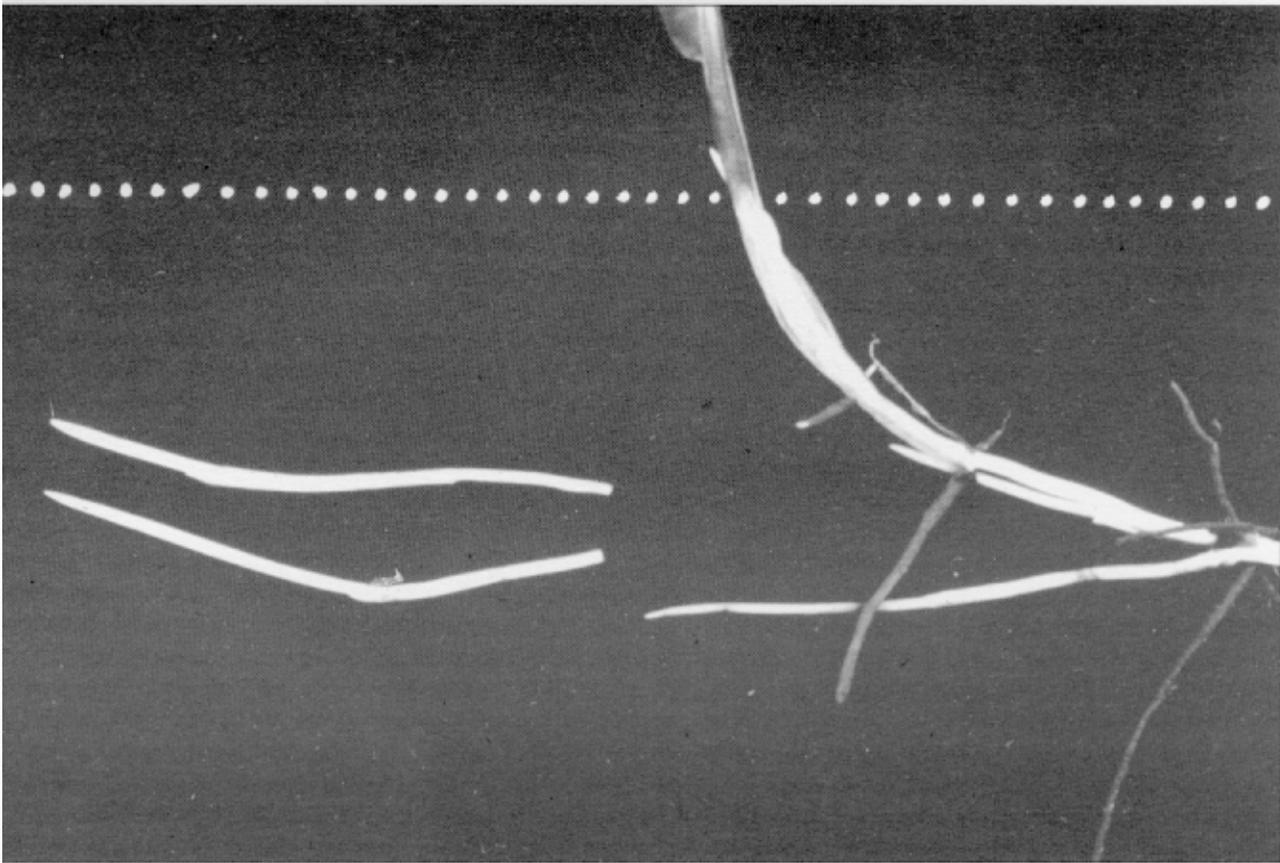


Figure 1. At left: Two excised distal portions of bromegrass rhizomes (underground stems), the overwintering plant tissue subjected to artificial freeze stress. At right: Two other branch rhizomes are shown attached to a portion of a larger rhizome that has emerged through the soil surface (dotted line) to form an aerial culm. Darker outgrowths from nodes on the larger rhizome are roots.

All pots were placed into a dark chamber maintained at $18 \pm 1.5^\circ\text{C}$ with the base of each pot immersed in one-quarter to one-half-inch of water. Fungicide (PCNB) in water spray was applied as needed, about three times per week, to prevent mold development. All etiolated growth from potted plants was harvested (severed at the point where it emerged from the tiller stubble or rhizomes) at successive two-week intervals after potting and until exhaustion of food reserves and death of plants. Harvested etiolated growth was dried at 60°C . Stored food reserves were calculated as milligrams (mg) oven-dry regrowth per oven-dry gram (g) of plant tissue potted.

On 25 October (Experiment 1) and 20 October (Experiment 2), after killing frosts, aerial growth of seedling plants remaining in the field was clipped and removed leaving a two-inch stubble to prevent differential snow retention during winter. In mid-May of each spring following the year of planting and after spring growth had started, living and dead plants were counted in all rows planted the previous year and percentages of winter survival were calculated.

Data on percent winter survival were transformed

to angles prior to statistical analysis to obtain a more normal distribution of values. Duncan's multiple range test comparisons were calculated on angle values. Actual field survival percentages are reported; all indications of statistical significance are based on 95% confidence limits.

To compare bromegrasses of diverse latitudinal adaptation for tolerance of overwintering tissues to different levels of freeze stress, pumpelly brome, Polar hybrid brome, and smooth bromegrass cultivars Manchac and Achenbach were seeded in rows 18 feet long and 24 inches apart with four replications on 10 June (Experiment 3). On 30 October of the same year, aerial growth of all seedlings was severed to preclude any further transpirational loss of water from plants and, immediately thereafter, plants were dug from each row. Cold water was used to wash soil from roots, then the distal segments of rhizomes that had not emerged from the soil were severed for use in freeze-tolerance tests. Individual rhizome segments were from 1 1/2 to 3 inches long (Figure 1). Rhizome segments were rinsed in a cold-water spray to remove all traces of soil and organic debris, rinsed three times in

Table 1. Two-year means of dry-matter concentration near onset of winter in overwintering tissues (crowns + rhizomes) of four *Bromus* strains of diverse latitudinal adaptation, and percent winter survival of individual plants of each strain left in the field over winter.

Bromegrass	Latitude of origin	Two-year means	
		Dry-matter concentration 10 October	Winter survival
	°N	%	%
Native pumpelly brome	61-65	20.20 a ¹	100 a
Polar ²	61-62	21.11 a	100 a
Manchar	43-47	20.92 a	94 b
Achenbach	34-42	18.96 b	12 c

¹Within each column, means not followed by a common letter are significantly different (5% level) using Duncan's Multiple Range Test.
²Predominantly of hybrid origin (*B. inermis* x *B. pumpellianus*).

distilled water, and dried of surface moisture.

Five-gram samples of the prepared rhizomes were placed into 1- x 8-inch stoppered test tubes and frozen for 20 hours. Separate samples of each strain were frozen at each of three temperatures, -12°C, -24°C, and -36°C (= +10.4°F, -11.2°F, and -32.8°F). After freezing, test tubes were placed in a refrigerator (+2.5°C) for four hours for temperature equilibration before 50 ml of refrigerated, distilled water was added to each. Test tubes were returned to the refrigerator for 20 hours to allow diffusion of cell electrolytes from freeze-injured and ruptured plant cells. The water was then decanted and brought to 25°C and specific conductivities determined for each sample as described by Dexter et al. (1932). Water samples were returned to their specific test-tubes containing rhizomes and all were boiled for five minutes in a common water-bath to effect complete destruction of plant cells. Samples were again left to diffuse for 20 hours before repeat decanting, equilibration at 25°C, and a second specific conductivity determination on each sample. The ratio of specific conductivity following freezing to specific conductivity following boiling (maximum injury possible) is presented as "percent injury" induced by freezing in rhizome tissues.

RESULTS AND DISCUSSION

Dry-Matter Concentration vs. Winter Survival

On the earliest sampling date during the two years (10 August), mean percent dry matter in overwintering tissues (crowns + rhizomes) for the four strains was 8.3% and differences among strains were not significant. Thereafter, dry-matter concentration in all four brome-grasses increased rapidly between sampling dates, more than doubling from early August to early October. The two-year-mean percent dry matter for all strains on 10 October was 20.3% (Table 1). The southernmost-adapted Achenbach (at 18.96%) was significantly lower than the other three brome-grasses in dry-matter concentration in storage tissues on 10 October and also in percent winter survival (12%) of plants left undisturbed in the field (Table 1).

The results parallel findings with winter wheat and barley crowns where cultivars with highest moisture content (lowest percent dry matter) were most injured by artificial freeze stress (Metcalf et al. 1970). Similarly, studies with other grass species in Alaska have found highest dry-matter concentration in overwintering tissues of the most winter-hardy cultivars

(Klebesadel 1985a; Klebesadel and Helm 1986).

The relationship between dry-matter concentration of crowns + rhizomes and field winter survival of brome grass plants was not totally consistent. Two-year-mean winter survival of Manchlar was significantly poorer than pumpelly brome and Polar, yet the three northernmost brome grasses did not differ significantly in dry-matter concentration when sampled on 10 October near the end of the growing season (Table 1). It is possible, however, that further unmeasured changes in dry-matter concentration in overwintering tissues may have occurred after 10 October to create a more consistent relationship with winter survival.

Stored Food Reserves

Dissimilar sampling dates during the two years precluded calculation of two-year means of stored food reserves, except for the final sampling date of 10 October that was identical in both years. Data from all

sampling dates during the two years are shown in Figure 2. Taken together, these various sampling dates provide a relatively consistent pattern of progressively increasing levels of stored reserves during late summer and autumn. The sampling dates are discussed in chronological sequence, although the actual samplings alternate between the two years.

When taken from the field on 10 August, none of the four brome grasses produced etiolated growth to indicate presence of stored food reserves (Figure 2). Following sampling on 22 August, Polar, Manchlar, and Achenbach produced some evidence of stored food reserves. Surprisingly, the extreme northern-adapted pumpelly brome produced none. When sampled on 30 August, pumpelly brome produced only 9 mg etiolated growth per g of crown-rhizome tissue, while Polar, Manchlar, and Achenbach averaged 83 mg/g (93, 73, and 80, respectively). However, when sampled approximately three weeks later (20 September), the native brome grass showed a dramatic

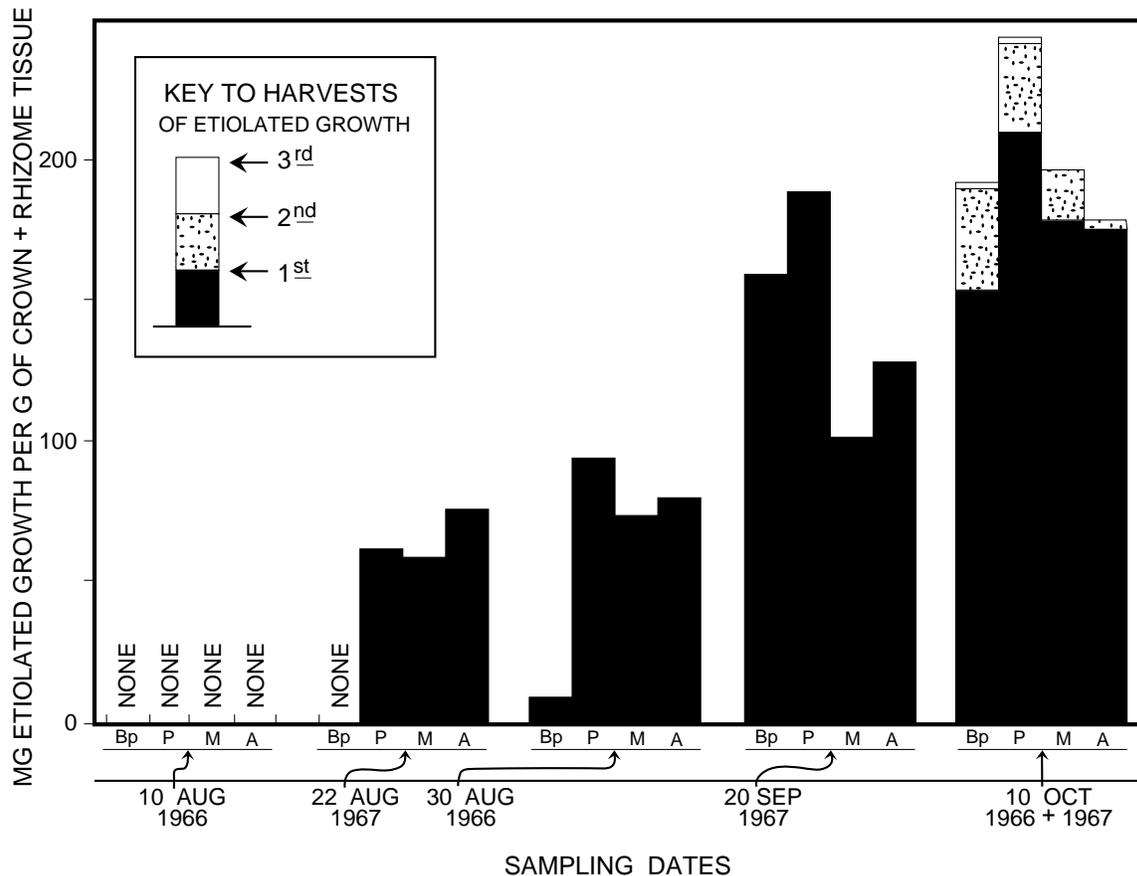


Figure 2. Stored food reserves as measured by etiolated growth harvested at two-week intervals from seeding-year crowns + rhizomes of four brome grass strains of diverse latitudinal adaptation. Data are for three sampling dates in each of two years, with two-year means presented for the final sampling date of 10 October, common to both years. The four-bar groupings are evenly spaced in the graph, but the curved arrow below each is attached to the bottom "calendar line" to indicate the correct chronological relationship of sampling dates. Bp = native Alaskan pumpelly brome grass (*B. pumpellianus*), P = the Alaska cultivar Polar, M = the cultivar Manchlar from the Pacific Northwest, and A = the cultivar Achenbach from Kansas.

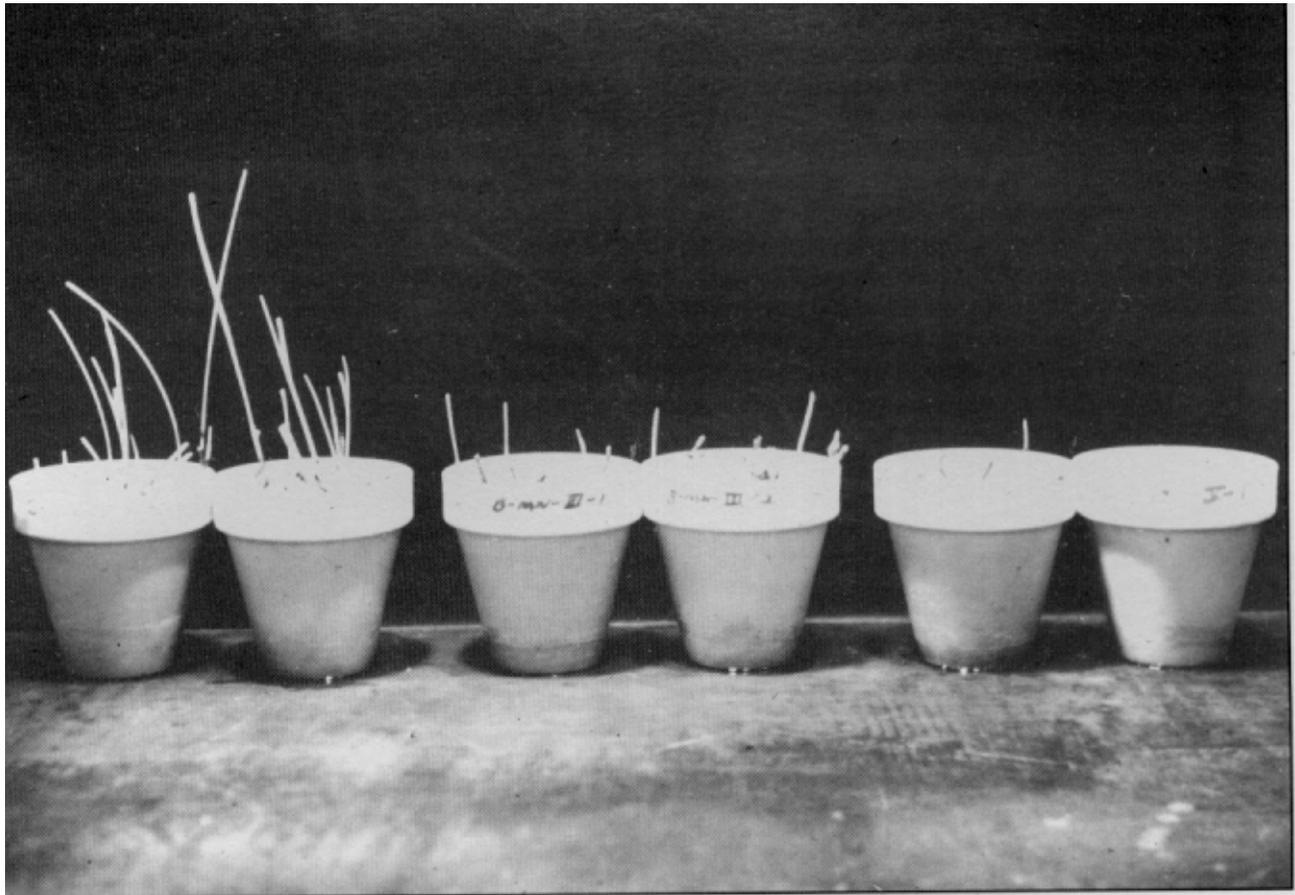


Figure 3. Left to right: Paired pots of three bromegrass cultivars—Polar, Manchar, and Achenbach (pumpelly brome not shown)—just before second harvest of etiolated growth, a measure of stored food reserves. These plants were dug and potted on 10 October; etiolated growth produced during the first two weeks in the warm, dark chamber appears as the solid, basal portion of bars in Figure 2, 10 October sampling. Etiolated growth seen here was produced during the two weeks after the first harvest and appears in Figure 2 as the dotted portion of graph bars.

increase in food reserves (159 mg/g), almost as much as Polar and more than Manchar or Achenbach.

Despite its southernmost origin, Achenbach surpassed all bromes in stored food reserves at the 22 August sampling and showed higher levels than Manchar at the 30 August and 20 September samplings. Only at the final sampling (10 October) was Achenbach surpassed by all three of the more northern-adapted strains.

With samplings earlier than 10 October in both years, no production of etiolated growth occurred after the single harvest at the end of the first two-week growth period in darkness, indicating that stored reserves had been totally exhausted within 14 days. With the 10 October sampling, however, all strains produced some etiolated growth during a second two-week growth period, and most growth during that interval was produced by the northernmost-adapted strains (Figure 3). Additionally, both pumpelly brome and Polar produced a very small amount during the third two-week growth period but none was produced

by Manchar and Achenbach.

At the time of the final (10 October) digging of plants, foliage of pumpelly bromegrass was becoming somewhat bleached and chlorotic, a characteristic noted near freeze-up in numerous other experimental studies (Figure 4) with that native species at this location. In contrast, foliage of the other three bromes remained dark green until killed by frost.

The foliar bleaching noted in pumpelly brome in the growth period prior to freeze-up parallels that phenomenon noted here in northernmost-adapted strains within other grass species (while the foliage of more southern-adapted strains in each species remained green); those species include red fescue (Klebesadel et al. 1964), reed canary grass (Klebesadel and Dofing 1990), and slender wheatgrass (Klebesadel 1991).

Mean winter survival of plants of both pumpelly brome and Polar that were left in the field was 100%, significantly better than Manchar (94%) and Achenbach (12%) (Table 1). Moreover, the few surviving plants of



Figure 4. Photo of a bromegrass nursery not a part of the present study, but presented to show contrasting growth form and herbage appearance on individual plants of two bromegrass species near the end of the growing season. Growth shown here on 5 October was produced since first-cutting topgrowth removal in July. Foreground: Individual plants of native Alaskan pumpelly bromegrass (*B. pumpellianus*) consisting predominantly of leaves with virtually no elongated culms. Rear: Scattered five-plant lots of taller growth of smooth bromegrass (*B. inermis*) consisting mostly of elongated culms. Foliage of pumpelly bromegrass was becoming bleached and chlorotic by this date, in contrast to the dark green leaves of the more southern-adapted smooth bromegrass.

Achenbach showed severe winter injury and the surviving Manchur plants showed more evidence of winter injury than those of Polar or pumpelly brome. These results agree with those of many other tests wherein pumpelly brome and Polar have been consistently more winter hardy than introduced, more southern-adapted smooth bromegrasses when differential winter survival has occurred (Klebesadel 1970, 1971a, 1984; Klebesadel and Helm 1992; Wilton et al. 1966).

These differential patterns of expression of stored reserves as etiolated growth provide the basis for speculation as to a state of rest or dormancy assumed by 10 October in pumpelly brome and Polar, and the relationship of this phenomenon to winter survival.

The total amount of reserves stored by 10 October (after termination of the growing season), presented as

two-year means of etiolated growth, was highest with Polar (243 mg/g), which survived both winters at 100%, and lowest with Achenbach (179 mg/g) which averaged only 12% survival. However, total reserves in the very winter-hardy pumpelly brome (192 mg/g) were little more than in non-hardy Achenbach, hardly a difference sufficient to be causally related to their widely disparate levels of actual winter survival (100% vs. 12%).

One characteristic of stored food reserves that seemed correlated with winter survival, however, was the rate at which stored reserves were expressed as etiolated growth, leading to different amounts expressed after the first two-week period (Figure 2). For the four bromegrasses, means of stored reserves (mg/g) expressed after the first two-week growth period in

darkness were 39, 33, 18, and 3% for pumpelly brome, Polar, Manchar, and Achenbach, respectively, which survived the winters in the same order at 100, 100, 94, and 12%. The similarity of these two numerical patterns suggests a good association between that facet of food-reserve measurement and winter survival.

It could be contended that pumpelly brome required the full four to six weeks to produce 192 mg etiolated growth per g of crown + rhizome tissue at a normal rate of reserve expression in darkness. Yet the closely related Polar was able to produce 210 mg/g in only the first 14-day growth period in darkness. Therefore, it is interpreted that the slow expression of food reserves by pumpelly brome is evidence of an assumed state of dormancy that is not well understood, but which apparently is associated with superior winterhardiness in Alaska. Similar relationships have been noted in other species at this location (Klebesadel 1971b, 1985a; Klebesadel and Helm 1986).

Smith (1964) stated that stored carbohydrate food reserves provide the energy used by plants to develop hardiness in preparation for winter, as well as providing for the plant's needs in living through the winter and initiating new growth in spring. Although plants with high levels of carbohydrate reserves do not invariably develop a high degree of cold hardiness (Bula et al. 1956), plants low in reserves cannot develop a high level of winterhardiness (Smith 1964).

Stored food reserves, as related to winter-hardening behavior in forages, have been determined by chemical analyses for nonstructural carbohydrates in storage tissues (Bula et al. 1956; Graber et al. 1927; Hodgson 1964; Hodgson and Bula 1956; MacLeod 1965), and by weighing the amount of etiolated growth produced after placing plants in a warm, dark chamber until exhaustion of reserves (Graber et al. 1927; Klebesadel 1971b, 1985a; Klebesadel and Helm 1986; MacLeod 1965; Wright et al. 1967).

The latter technique of measuring stored reserves has a long history (Graber et al. 1927). However, as commonly employed elsewhere (a) uniform-size soil cores are taken from a forage stand, or (b) unweighed plants grown in uniform-size pots are used, or (c) etiolated growth is reported simply on a per-plant basis. Such sampling techniques necessarily and erroneously assume equiponderance of plant tissue among samples as a basis for comparison of etiolated growth produced therefrom. The technique employed in this and in earlier studies in Alaska (Klebesadel 1971b, 1985a; Klebesadel and Helm 1986) circumvents those weaknesses by relating mass of etiolated growth directly to the mass of plant storage tissues that produced the growth.

Chemical determination of stored reserves as carbohydrates is performed necessarily on dead plant

tissue. Therefore, unlike the etiolated-growth technique which utilized living plants, chemical analysis precludes detection of any indications of dormancy or rest status in plants that may be associated with winter survival.

MacLeod (1965), using potted plants in indoor culture, determined food reserves in storage organs of four forage species by etiolated-growth technique and by chemical analyses for total available carbohydrates. He found good correlation between the two techniques in orchardgrass (*Dactylis glomerata* L.), alfalfa (*Medicago sativa* L.), and timothy (*Phleum pratense* L.), but not in brome grass.

Etiolated-growth determinations of stored food reserves necessarily are conservative in that no measure is taken of reserves consumed through breakdown of carbohydrates and released as respiratory by-products during the term that etiolated growth is produced. Reserves in plants requiring long periods to reach exhaustion in darkness obviously would be more conservatively estimated than plants reaching exhaustion quickly. Graber et al. (1927) calculated that five-month-old alfalfa plants taken from the field in late November, and producing etiolated growth in darkness for four weeks until exhaustion of reserves, lost 36% of plant dry weight as unmeasured respiration products.

Tolerance to Freezing

Relative injury ranking of the four brome grasses in response to freeze stress was similar at all levels of freezing in the order Achenbach > Manchar > Polar > pumpelly brome (Figure 5). The extremely winter-hardy pumpelly brome was least injured at each temperature. Polar, representing hybridity between pumpelly and northern-type smooth bromes, was only slightly more injured than pumpelly brome at each temperature, and differences were not statistically significant (5% level). Relative freezing injury of the four brome grasses paralleled their latitudinal adaptation. The southernmost-adapted Achenbach was consistently most injured at each temperature, while Manchar, of intermediate latitudinal adaptation, was always intermediate in injury between Achenbach and subarctic-adapted Polar and pumpelly brome.

At the mildest level of freeze stress (-12°C), injury to Manchar was significantly but, in magnitude, not much greater than that of Polar and pumpelly brome. In contrast, injury to the southernmost-adapted Achenbach at -12°C was considerably greater than the others. All four bromes sustained much greater injury at -24°C than at -12°C. However, the injury of Manchar relative to the other bromes shifted considerably from -12°C to -24°C. At the latter temperature, injury to Manchar was similar to that of Achenbach and much

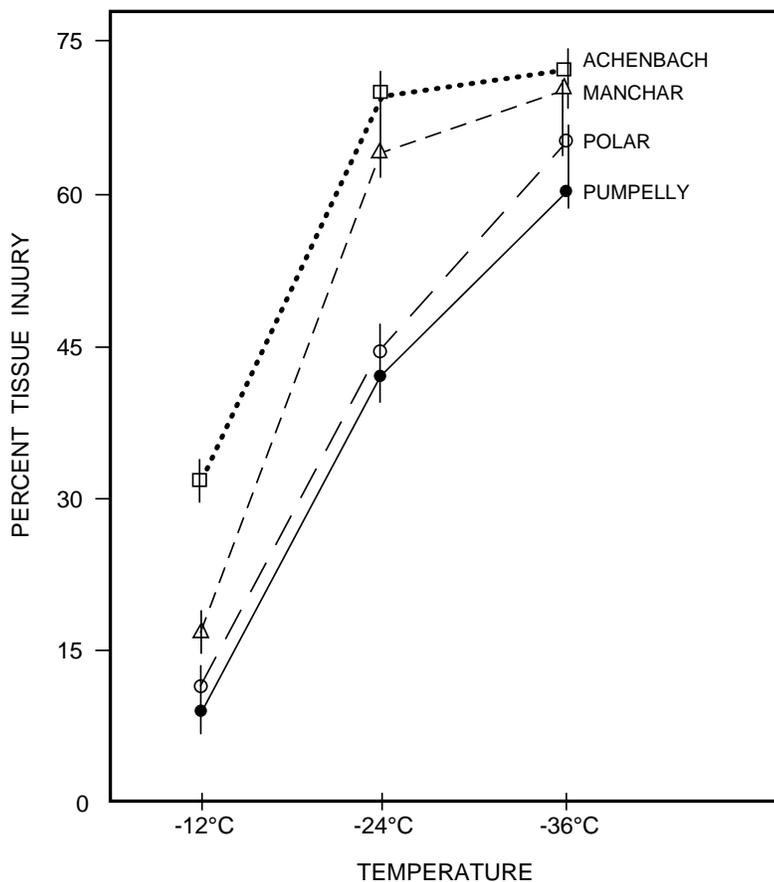


Figure 5. Percent injury in overwintering rhizome tissues of four bromegrass strains of diverse latitudinal adaptation, when frozen for 20 hours at three different temperatures. Test conducted in late October, after termination of the growing season and just prior to soil freeze-up, on plants seeded 9 May of the same year. At each temperature, means not connected by the same vertical line are significantly different (5% level) using Duncan's Multiple Range Test.

greater than the relatively similar injury sustained by Polar and pumpelly brome.

Differences among the four bromes were less at -36°C than at -24°C or -12°C . Injury of Achenbach and Manchar was not much greater at -36°C than at -24°C , indicating that both were at near maximum injury at both temperatures. In contrast, the more winter-hardy Polar and pumpelly brome sustained much more injury at -36°C than at -24°C . Yet the trend lines and injury levels in Figure 5 infer that freezing at -36°C may not have inflicted maximum injury. At that temperature more than at the other two, however, the widest gap was seen between Polar and pumpelly bromegrass in extent of injury sustained. The difference though was not statistically significant.

A puzzling aspect of these data was the failure of the percent injury values for Achenbach to be nearer 100% when frozen at -24°C and -36°C when its rhizomes sustained what is believed to be near maximum

tissue injury from freezing. One reason may be that inadequate time was allowed for total diffusion of electrolytes from freeze-injured cells to the surrounding water; another may be simply structural impedance by rhizome tissues preventing total outflow of electrolytes from freeze-injured cells in inner rhizome tissues. The electrolyte outflow logically would have been more rapid following the greater injury and cellular disruption occasioned by boiling. Similar results were noted earlier with timothy (Klebesadel and Helm 1986).

These relationships in comparative low-temperature injury among the four bromegrasses closely parallel winter survival differences observed over three decades in numerous field tests. Achenbach and other southern-type cultivars almost invariably sustain significant winter injury or succumb totally during typical winters here (Klebesadel 1970, 1971a; Klebesadel and Helm 1992).

Following mild-to-moderately stressful winters, Manchar survives with no apparent injury and produces first-cutting forage yields equivalent to the more winter-hardy Polar and pumpelly brome (Klebesadel and Helm 1992). Such winters probably impose cold stresses somewhat similar to, or less stressful than, the -12°C treatment, where Manchar differed little from Polar and native brome. During more severe winters, the intermediately hardy Manchar sustains greater

injury, leading to considerably reduced first-cutting forage yields (Klebesadel and Helm 1992). Injury during such winters causes Manchar and other intermediately hardy cultivars to be severely injured, more like southern-type bromes and similar to the rhizome freeze-injury pattern of the strains noted at -24°C (Figure 5).

Winters of extreme stress, as occurred locally in 1956-57 and 1961-62 (Klebesadel 1974), can virtually eliminate such intermediately winter-hardy strains as Manchar, Carlton, and Canadian commercial, while stands of the more winter-hardy Polar and native pumpelly brome sustain little injury (Wilton et al. 1966). The pattern that associates winter survival of bromegrasses with latitudinal origin or adaptation agrees with other ecotypic studies (Wilsie 1962) and parallels results derived with other species in Alaska (Bula et al. 1956; Hodgson and Bula 1956; Klebesadel 1970, 1971b, 1985a, 1991; Klebesadel et al. 1964; Klebesadel and Dofing 1990; Klebesadel and Helm 1986).

CONCLUSIONS

The ability of overwintering plant tissues to tolerate sub-freezing temperatures is a vital characteristic of perennial plants that permits successful winter survival (Smith 1964; Steponkus 1978). The present results show that Achenbach, the southernmost-adapted cultivar compared, and, to a lesser extent, the somewhat more northern-adapted Manchar, do not develop freeze tolerance, at least not in this northern environment, to the extents observed in subarctic-adapted Polar and pumpelly brome. The relative ranking of freeze injury sustained by rhizomes of the four bromegrasses paralleled differences in latitudinal adaptation as well as observed comparative differences of winter survival of plants in the field.

In another study (Klebesadel 1971a, 1985b) at the same location, winter survival of a bromegrass cultivar similar to Achenbach in latitudinal adaptation was enhanced markedly by shortening daily photoperiods for seven weeks prior to onset of winter conditions, thus approximating autumn photoperiods that occur prior to growth cessation at its latitude of adaptation. This demonstrated that southern-adapted bromegrasses are not induced to undergo adequate winter-hardening changes due to disharmony with the unaccustomed, longer daily photoperiods they are exposed to prior to onset of winter conditions in Alaska.

The very poor winter survival of Achenbach in the present study, far divorced from its latitude of adaptation, was associated with greater susceptibility to freeze injury, significantly lower dry-matter concentration in overwintering tissues, and somewhat lower pre-winter storage of food reserves than in the more winter-hardy strains. All of these represent shortcomings in preparation for stresses routinely imposed during winter.

Slow expression of stored food reserves as etiolated growth, in plants sampled just prior to onset of winter, may be associated with superior winter survival of bromegrass in this subarctic area. The relatively slow expression of stored food reserves may be an indication of dormancy, which may serve in some manner to protect plants from critical mid-winter dehardening (Dexter 1941) that could occur during thawing and refreezing temperature fluctuations common here during winter (Klebesadel 1974). Fall dormancy, associated with superior winter survival, has been observed here also in far-northern-adapted strains of alfalfa (Klebesadel 1971b), timothy (Klebesadel and Helm 1986), and sweetclover (Klebesadel 1992).

One perplexing behavioral characteristic was the lateness with which pumpelly brome initiated food-reserve storage, as measured by production of etio-

lated growth (Figure 2), contrasted with Polar, Manchar, and Achenbach. It would seem logical that the subarctic-adapted grass would begin food-reserve storage earlier instead of later than the less winter-hardy, introduced strains. However, once initial evidence of stored reserves was detected in pumpelly brome in late August, it proceeded rapidly to surpass Manchar and Achenbach by late September. The very rapid accumulation of food-reserve storage in overwintering tissues of pumpelly brome was coincident with the appearance of foliar bleaching and yellowing.

Inasmuch as foliage on the three other strains of bromegrass remained undamaged from frost during the onset of foliar bleaching in pumpelly brome, this suggests that the phenomenon may be photoperiod-induced rather than tissue injury from low temperatures. The interrelationships of environmental stimuli, foliar bleaching, and the rapid rate of food-reserve buildup in pumpelly brome should be an interesting and informative area if investigated more comprehensively in future research.

The present results provide new insights into plant winter-hardening behavior in this subarctic area, and assist in understanding why differences occur in winter survival of bromegrasses from different latitudinal origins when grown in Alaska. Additional investigations that monitor biochemical constituents and their dynamics and alterations in plants, as they relate to winter hardening and to criteria measured in this study, should provide improved understanding of underlying phenomena that influence winter survival of plants in northern regions.

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