Morphological, Physiological, and Winterhardiness Comparisons Among Latitudinal Ecotypes of Biennial Sweetclover (*Melilotus* species) in Subarctic Alaska

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SUMMARY

Objectives of this study were to compare, within two species of biennial sweetclover, several morphological and physiological characteristics of strains adapted to a wide range of latitudes and to relate those characteristics to winter survival and forage production in subarctic Alaska. All experiments were conducted at the University of Alaska's Matanuska Research Farm (61.6°N) near Palmer in southcentral Alaska.

Strains included in the most extensive comparisons were two long-resident, introduced roadside populations at 61.6°N in Alaska's Matanuska Valley; both were suspected of having undergone some degree of genetic modification toward subarctic adaptation as a result of natural-selection pressures during many generations in the Matanuska Valley. One of those was grown from seed gathered from a colony of biennial white sweetclover (*Melilotus alba* Desr.), called "Matanuska white" in this report, and the other was from a stand of biennial yellow sweetclover (*M. officinalis* [L.] Lam.), referred to as "Palmer yellow."

Also compared were two cultivars of intermediate latitudinal origin, adapted at 50° to 56°N in Canada; these were Arctic (white) and Erector (yellow). Two cultivars of still more southern adaptation (35° to 50°N in the conterminous United States) were Spanish (white) and Madrid (yellow). Hubam, a cultivar of annual white sweetclover (*M. alba* var. *annua* Coe) was included, also.

Others compared for winter hardiness only were biennial white cultivars Cumino and Polara from Saskatchewan and Denta from Wisconsin, and biennial yellow cultivars Yukon from Saskatchewan and Goldtop from Wisconsin. Additionally, experimental lines derived from certain of the above-mentioned strains, through seed harvest from surviving plants following one to several generational cycles of natural selection for winter survival, were also compared for progress toward increased winter hardiness.

- All strains flowered profusely during the seeding year, a phenomenon not seen in biennial sweetclovers when grown at more southern latitudes. This and other nontypical behavioral responses of introduced biennial cultivars are attributable to growth under unaccustomed patterns of long daily mid-summer photoperiods/short nyctoperiods at subarctic latitudes, far north of the historic global areas of sweetclover culture.

- The southernmost-adapted biennials exhibited growth characteristics more like the annual cultivar Hubam than like biennials in the seedling year. They produced high forage yields from tall, large-diameter stems, formed few and small crown buds, stored low levels of food reserves, and developed inadequate levels of freeze tolerance; thus their winter survival was very poor.

- The cultivars Hubam, Spanish, and Madrid, that produce tall growth in the seeding year with early planting, and, by inference, newer low-coumarin biennial cultivars adapted at 30° to 50°N, should be considered useful for annual forage production in Alaska.

- Contrasted with recently introduced cultivars, the long-resident subarctic strains produced lower seeding-year forage yields from shorter plants with smaller-diameter stems, formed more and larger crown buds, stored high levels of food reserves, achieved high levels of freeze tolerance and dormancy, and survived winters well when cold stress was not extreme. Except for flowering, the two subarctic strains generally exhibited developmental and physiologic characteristics during the seedling growing season typical of biennial sweetclover when grown where it is adapted.

- The Canadian cultivars tended to be intermediate in most characteristics between the southernmost-adapted and the subarctic strains. Arctic, and a related strain (AK-Syn.1) selected in Alaska from Arctic for enhanced winter survival, tended to survive better than the roadside strains during extremely cold winters, presumably because field-selection for winter hardiness selects for greater tolerance to certain winter-stress facets than occur in the snow-insulated roadside habitats where the subarctic strains evolved.

- Matanuska white initiated significant storage of food reserves earlier than the three other biennial white strains, Arctic, AK-Syn.1, and Spanish. Palmer yellow stored higher levels of food reserves than the Canadian cultivar Erector which in turn surpassed the more southern-adapted U.S. cultivar Madrid. The annual Hubam stored no measurable food reserves.

- The very slow expression of stored food reserves as etiolated growth by Matanuska white, and to a lesser extent by AK-Syn.1, is interpreted to indicate a state of dormancy that could be broken in two ways: (a) when autumn-dug Matanuska white plants were subjected to three freeze-thaw cycles before placement in the warm, dark chamber, most of total stored food reserves were released to grow to exhaustion in only 6 weeks in contrast to 18 weeks required without the freeze-thaw treatment, and (b) when winter-surviving plants of Matanuska white and AK-Syn.1 were dug in early May, dormancy was no longer evident.

- The somewhat inconsistent ranking of the most winter-hardy strains in percent winter survival from year to year suggests that the dominant stresses operative in killing of plants differ from winter to winter. For example, it is believed that the subarctic-adapted roadside strains that evolved locally over many years and possess conspicuous pre-winter dormancy survive winters best when the dominant stress is freeze-thaw temperature oscillations. In contrast, experimental lines derived during a shorter period of natural selection in more exposed field nurseries apparently survive winters better than the roadside strains when the dominant stress is low temperature.

- Selection for improved winter survival by harvesting seed from surviving second-year plants in stands of biennials severely decimated by winter kill showed promise on the modest scale pursued. The increase in winter hardiness through this avenue tended to be more rapid and effective in a relatively nonhardy cultivar than in more winter-hardy strains. This strategy should be expanded in the future with low-coumarin strains toward developing subarctic-adapted cultivars free of the disadvantages inherent in high-coumarin sweetclovers.

- These results provide a better understanding of the relationships of freeze-tolerance development, food-reserve storage, and dormancy to winter survival of biennial sweetclover in the Subarctic. They also demonstrate why only northern-adapted crop strains can be depended upon for satisfactory winter survival in northern agricultural areas.

- The obligate biennialism of both sweetclover species facilitates rapid generational cycling which in turn accelerates gene sorting through natural-selection pressures in a new environment. Natural selection over many generations in this subarctic environment apparently is adapting the introduced roadside populations to the unusual photoperiod/nyctoperiod regimen characteristic of this high latitude and thereby shifting them back toward their original biennial habit. This adaptive modification is occurring at a latitude where biennial sweetclovers newly introduced from more southern latitudes initially are ill-suited to function as biennials, due to the northern growing season's unique and unaccustomed diurnal photoperiod/nyctoperiod pattern.

- These documented instances of adaptive modification toward harmony with subarctic climatic influences are informative in appreciating the breadth and plasticity inherent within a species gene pool and how environmental influences can act through natural selection to shift the genetic constitution of a population toward more successful survival.

INTRODUCTION

Sweetclovers belong to the genus *Melilotus* and originated in temperate regions of Europe and Asia. Twenty species are recognized worldwide; 11 are annuals and nine are biennials (Smith and Gorz 1965). The two sweetclover species cultivated most extensively in the northern states and Canadian provinces are biennials; one is white-flowered (*M. alba* Desr.) and the other yellow-flowered (*M. officinalis* [L.] Lam.).

Sweetclover Utilization

Sweetclovers are grown for pasturage, harvested forage, and for soil improvement (Smith et al. 1986). As with other legume crops, sweetclover is valued for its ability to incorporate atmospheric nitrogen into its plant parts, resulting in high-protein herbage and nitrogen-rich organic matter when the crop is plowed down for soil improvement. The crop is valued also as "bee pasture" for honey production.

A disadvantage of sweetclover is the high content of the chemical compound coumarin in herbage of the older cultivars and regional strains (Smith et al. 1986). Coumarin reduces palatability and also is objectionable because feeding spoiled sweetclover hay may cause "bleeding disease" in livestock. Emphasis in sweetclover improvement during recent years has been directed toward developing cultivars low in coumarin (Smith and Gorz 1965).

Sweetclover is less tolerant of soil acidity than many other forage legumes (Smith et al. 1986). This characteristic suits the crop to Alaska soils ranging from pH 6.0 to 7.5; considerable acreages in this range exist in the Matanuska Valley as well as in the Tanana Valley of the Interior. More acidic soils, as occur extensively on the Kenai Peninsula, preclude sweetclover culture without liming to lessen soil acidity.

The potential for sweetclover culture on Alaska farms is a question not resolved at present. Considerable agronomic research emphasis in Alaska has been directed toward identifying or developing dependable biennial or perennial forage legumes to incorporate their valuable attributes into our northern cropping systems (Bula et al. 1956; Hodgson 1964; Hodgson and Bula 1956; Irwin 1945; Klebesadel 1971b, 1985b, 1980).

Earlier Trials in Alaska

Early attempts (1913 to 1941) to grow biennial sweetclover in subarctic Alaska were few and generally disappointing due to poor winter survival (Irwin 1945). Those results were somewhat surprising because biennial sweetclovers are considered to be among the most winter-hardy of the forage legumes (Ouellet 1976; Smith et al. 1986).

Hodgson and Bula (1956) grew 11 cultivars from Canadian and conterminous U.S. sources in Alaska to study levels and changes of several chemical constituents in overwintering tissues during the hardening period, and relationships of those to cold resistance and winter survival. Winter survival was generally poor, but they noted significant positive correlations between pre-winter levels of carbohydrate reserves, nitrogen fractions, and tolerance to artificially imposed freeze stress.

Naturalized Alaska Roadside Populations

The aforementioned observations of marginal to poor winter survival of introduced cultivars of biennial sweetclovers in experimental trials in Alaska (Hodgson and Bula 1956; Irwin 1945; also unpublished information, Alaska Agricultural and Forestry Experiment Station) contrast sharply with good winter survival of biennial sweetclovers along local roadsides. Those roadside patches of sweetclover have escaped from earlier attempts at field culture (Fig. 1).

It is recognized that unharvested plants in roadside habitats are not subjected to utilization pressure; moreover, with abundant insulating snow cover they experience milder winter stresses than harvested plants in open croplands. Such differences could account for considerable disparity in winter survival in the dissimilar habitats.

Additionally, however, closer examination indicated that plants in the roadside populations were shorter and possessed better crown-bud development in autumn of the seedling year than occurred in introduced cultivars grown in experimental tests. Despite the short, biennial life cycle of the sweetclovers, plants that survive the first winter produce copious seed crops during the second year of growth for ongoing propagation.

To facilitate closer comparisons between the roadside populations and Canadian and U.S. cultivars, seed was collected from local roadside stands of biennial white and biennial yellow sweetclovers to grow experimentally. A collection from an extensive stand of *M. alba*, located about two miles ENE of the former village of Matanuska, was designated "Matanuska white," and seed from a stand of *M. officinalis* about one-half mile SSW of Palmer was tested as "Palmer yellow."

The roadside populations have been persistent over a considerable period, and observations over time indicate such stands are spreading. No known records document when these sweetclover stands became established. The road along which both grow was constructed about 1916. A local resident recalls that the Matanuska white population was growing in the present site as early as 1929.

Figure 1. Patches of introduced biennial white and yellow sweetclover are common along Alaska roads, especially in areas of agricultural development. Once established, these patches tend to expand due to the abundant production of seed by plants during their second year of growth. The roadside habitat is less stressful than agricultural fields since roadside plants are not subjected to harvest, and insulating snow cover persists better along roadsides than in wind-swept farm fields.

Factors That Influence Winter Hardiness

Numerous studies have determined qualitative and quantitative changes in overwintering tissues of biennial and perennial plants prior to and during winter and have related those changes to cold hardiness or actual winter survival in the field. Earlier reports on this subject have been reviewed extensively elsewhere (Dexter 1941; Smith 1964a, 1964b; Steponkus 1978).

In general, plants with the capacity to develop cold hardiness respond to lowering temperatures and shortening photoperiods (lengthening nyctoperiods) of late summer and autumn by storing carbohydrate food reserves and by undergoing protoplasmic alterations that render overwintering tissues cold tolerant (Devlin and Witham 1983; Dexter 1956; Hodgson 1964; Hodgson and Bula 1956; Smith 1964a, 1964b; Smith 1942; Steponkus 1978; Tysdal 1933).

Tolerance to Freezing

If biennial or perennial forage plants grown in north-temperate or subarctic regions were subjected abruptly in mid-summer to the extreme cold stresses they endure without injury during winter, all would be killed. Therefore, the ability of plants to tolerate the freezing temperatures of winter requires environment-stimulated changes during late summer and autumn (referred to as "winter-hardening") that prepare plant cells of overwintering tissues to tolerate sub-freezing temperatures without injury.

In woody species, those changes must be effected throughout all parts of the above-ground growth. In contrast, aerial tissues of herbaceous plants, such as forage legumes and grasses, are routinely killed back to the plant crown region each autumn and only the tissues of the plants' overwintering structures (crown and roots) undergo protective winter-hardening changes.

In sweetclover, the principal overwintering structures include the stem base, crown buds near the soil surface, and the taproot. Dexter et al. (1932) devised the electrical conductance technique for measuring injury to plants after subjecting them to artifically imposed freeze stress under carefully controlled conditions in the laboratory.

This procedure is useful in comparing the gradual development of increasing freeze tolerance during late summer and autumn within crop strains of differing genetic constitutions by withdrawing plants from the field at various intervals for such freeze-tolerance tests (Bula et al. 1956; Hodgson 1964; Hodgson and Bula 1956). The technique is useful also in measuring differential injury when plants are frozen at different temperatures (Klebesadel and Helm 1986) or to ascertain the effects of various factors that influence the hardening process (Hodgson 1964).

Storage of Food Reserves

Stored food reserves in the form of readily available carbohydrates are vital to many plant functions. They provide the energy required for various metabolic processes involved in the development and maintenance of cold hardiness (Smith 1964b; Steponkus 1978), and for developing new growth in spring and after forage harvests (Smith 1964a, 1964b).

Accurate measurement of stored food reserves is useful in comparing various cultivars and strains as well as for assessing crop response to various management practices. Additionally, knowledge of food-reserve storage also contributes to better understanding of cold hardiness development and adaptation. High levels of stored food reserves are not invariably associated with high levels of cold hardiness, for nonhardy plants may store high levels of carbohydrates (Bula et al. 1956). However, plants with low levels of carbohydrate storage cannot develop high levels of cold hardiness (Dexter 1941; Smith 1964a, 1964b).

Carbohydrate reserves in forage crops can be measured chemically utilizing enzyme or acid hydrolysis (Smith 1981). Those techniques provide a precise measure of total nonstructural carbohydrate (TNC) levels present; however, those determinations are derived using killed plant tissue and therefore cannot supply any indication of a rest condition or dormancy present within overwintering plant tissues.

Another technique for measuring stored food reserves involves growing plants to exhaustion in a warm, dark environment and weighing the quantity of etiolated growth produced in the dark as a measure of stored food reserves (Graber et al. 1927). A possible source of imprecision in this technique, as it has been employed in some other studies (Baker and Jung 1968; Burton and Jackson 1962; MacLeod 1965; Sprague et al. 1962), is the assumption of equal plant tissue mass with plants grown in pots, or within equiponderant or equal-sized soil cores extracted from the field. This weakness was circumvented in the results presented here and earlier (Klebesadel 1971b, 1985a; Klebesadel and Helm 1986) by utilizing only plant tissue washed free of soil, and only the principal storage tissues of the plants studied. Stored food reserves expressed could then be related directly to mass of storage tissues utilized.

The etiolated-growth technique provides a conservative estimate of stored food reserves because it does not provide a measure of that portion of total reserves that are volatilized as respiration products during the time required for the test (Graber et al. 1927). With plants that have entered a state of dormancy, the time required for total exhaustion of reserves may be considerable, ranging up to 14 to 16 weeks with periodic harvests of etiolated growth at biweekly or triweekly intervals. Moreover, when plants are compared that remain alive for dissimilar terms in the dark environment, the differential loss of reserves as unmeasured gaseous respiration products introduces some distortion in the comparative results obtained via etiolated growth.

Stout (1984) believes that the etiolated-growth technique and chemical determination of TNC do not measure the same pool of food reserves, but that both measurements may provide useful, but different, information. MacLeod (1965) found a significant positive correlation between food reserves determined by the etiolated growth technique and percent total available carbohydrates (TAC) determined chemically in alfalfa (*Medicago sativa* L.).

This report summarizes a series of experimental investigations with sweetclover conducted over several years. Objectives of these investigations were to learn more about sweetclover adaptation and performance in this high-latitude area, to measure the extent to which adaptive modification may have progressed in long-resident roadside populations, and to better assess the potential usefulness of this legume in Alaskan agriculture. All experiments reported here were conducted at the University of Alaska's Matanuska Research Farm (61.6°N) in southcentral Alaska.

EXPERIMENTAL PROCEDURES

General field procedures: All field experiments were planted in Knik silt loam (Typic Cryochrept) with good surface drainage. Preplant commercial fertilizer disked into each plowed seedbed supplied nitrogen (N), phosphorus (as P_2O_5), and potassium (as K_2O) at 28, 112, and 56 lb / acre, respectively. Commercial *Rhizobium* bacterial inoculant was mixed with each seed lot immediately before planting.

At each forage harvest, each row or broadcast-seeded plot was harvested after a 1.25-foot strip was clipped and discarded from both ends of all rows or plots to remove border effects. Harvests of broadcast-seeded plots were accomplished by clipping and weighing a 2.5-foot-wide swath from the centerline of each plot. All clipping was done with a sickle-equipped mower that left about a two-inch stubble.

An herbage sample was taken from each row or plot for dry-matter determination (dried to constant weight at 60°C) and was ground finely for Kjeldahl nitrogen analysis. All forage yields and crude protein (N x 6.25) data are reported on the oven-dry basis. To determine leaf and stem proportions of forage, a second larger sample was withdrawn from row harvests and dried to constant weight at 60°C. All leaves were stripped from the stems, then leaves and stems were weighed separately and each portion was ground finely for Kjeldahl N analysis.

Comparison cultivars from more southern latitudes: (In referring to numbered experiments hereinafter, Experiment is abbreviated to Exp.) To determine the extent of morphological uniqueness (if any) of the two Matanuska Valley roadside populations (Matanuska white and Palmer yellow), a total of 96 different strains in the two species was obtained from numerous world sources (Exp. 1).

To permit more detailed comparisons of morphological and physiological characteristics in several experiments described below, two representative cultivars adapted to more southern latitudes were obtained within each of the two species. These were 'Arctic' white and 'Erector' yellow, of intermediate latitudinal adaptation (50° to 56°N) in Canada, and 'Spanish' white and 'Madrid' yellow of more southern adaptation (35° to 50°N) in the conterminous United States. Hubam, a cultivar of annual white sweetclover (*M. alba* var. *annua* Coe), and AK-Syn.1, a biennial white selection, also were included in the tests. AK-Syn.1 represents three successive generations of natural selection at the University of Alaska's Matanuska Research Farm (61.6°N) in Southcentral Alaska for winter-surviving plants primarily out of the cultivar Arctic.

Other cultivars included in row and broadcast field plantings (described in Table 3) for winter hardiness comparisons included the biennial white cultivars 'Cumino' and 'Polara' from Saskatchewan and 'Denta' from Wisconsin; biennial yellow cultivars were 'Yukon' from Saskatchewan and 'Goldtop' from Wisconsin.

World comparisons (Experiment 1): Seed was obtained of many other cultivars, strains, and accessions of the two species from numerous world sources to plant for morphological comparison with Matanuska white and Palmer yellow. Within *M. alba* this included 8 cultivars and common white from the conterminous states, 3 cultivars from Canada, and 24 numbered accessions from 10 nations on five continents. Within *M. officinalis* there were three cultivars, common yellow, and four numbered accessions from the United States; one cultivar and four numbered accessions from Canada; and 48 numbered accessions from five countries in Asia and eight countries in Europe. All were seeded in rows 4 feet long and 18 inches apart in two replications on 21 May. All were dug from the soil on 29 October of the same year after killing frost, and size and abundance of crown buds were rated visually for each.

Crown buds; root/stem diameter (Exps. 2 and 3): Broadcast-seeded plots of eight sweetclover strains listed in Table 1 were planted without a companion crop in consecutive years on 22 May (Exp. 2) and on 30 May (Exp. 3). Randomized complete block experimental designs were used with two replications. Plants were dug from plots on 23 October (Exp. 2) and on 19 October (Exp. 3) and washed free of soil. The number and length of macroscopic crown buds were recorded for 20 plants from each plot. On the same plants, measurements were made of stem diameter one inch above the cotyledonary node, and taproot diameter one inch below the same node; these data were used to calculate ratios of root/stem diameter (Table 1).

Seeding-year plant heights and forage yields (Exps. 4 and 5): Drilled rows of eight sweetclover strains listed in Tables 1 and 2 were seeded in two consecutive years (Exps. 4 and 5). In Exp. 4, rows planted on 20 May were 20 feet long and 24 inches apart; in Exp. 5, rows planted on 29 May were 20 feet long and 18 inches apart. Randomized complete block experimental designs were used with six replications. Seeding-year plant height was measured near the end of the growing season (8 October in Exp. 4, 5 October in Exp. 5) by recording the height of the tallest plants at five evenly spaced points along each row (Table 1). Forage harvests that followed shortly thereafter were on 8 October in Exp. 4 and 9 October in Exp. 5 (Table 2).

Freeze tolerance (Exps. 6 and 7): Plants were dug from the same plots described above as Exps. 2 and 3 for determination of tolerance of overwintering tissues of the eight strains to artificially imposed freeze stress. Plants were dug from Exp. 2 in the seedling year on 26 September and 23 October (Exp. 6) and from Exp. 3 on 24 September and 19 October (Exp. 7). Upon removal from the soil, most aerial growth was severed immediately to arrest transpirational water loss. Plants of uniform size were washed in cold water and all lateral roots were excised from the taproots. Stems were then severed one inch above and taproots four inches below the cotyledonary node as shown in Fig. 2. All traces of dead plant tissue and soil were removed, and stem-base/crown/taproot segments (plants) were rinsed three times in distilled water, dried of superficial mois-

Table 1. *Two-year means of morphological characteristics of Melilotus strains of diverse latitudinal adaptation when grown in southcentral Alaska at 61.6 °N. Sampling dates are means of two years.*

		7 Oct.	21 October		
Species and strain	Latitude of adaptation	Plant height [1]	Crown buds per plant	Length per bud	Root/stem diameter
	°N	inches	no.	mm	ratio
Biennial white (*M. alba*):					
Matanuska white	61-62	31 c	5.2 a [2]	4.4 a	2.41 a
AK-Syn.1	61-62	48 b	4.0 b	3.3 b	1.55 b
Arctic	50-56	52 b	3.3 bc	3.3 b	1.53 b
Spanish	35-50	54 ab	1.9 de	2.3 c	1.36 bc
Annual white (*M. alba* var. *annua*):					
Hubam	30-50	69 a	0.7 f	0.5 e	1.16 c
Biennial yellow (*M. officinalis*):					
Palmer yellow	61-62	44 bc	3.4 bc	2.8 bc	1.68 b
Erector	50-56	50 b	2.7 cd	2.6 bc	1.65 b
Madrid	35-50	57 ab	1.5 de	1.4 d	1.38 bc

[1] These measurements from plants in drilled rows (Exp. 4 and 5); other data in table are from broadcast-seeded plots (Exps. 2 and 3).

[2] Within each column, means not followed by a common letter are significantly different (5% level) using Duncan's Multiple Range Test.

ture, and placed into large (1- x 8-inch) test-tubes. Freeze tolerance was determined by the electrical conductance method (Dexter et al. 1932). Measurements were made on triplicate, 10-gram samples from each plot, each sample consisting of five plants. Exact weights were achieved by plant sorting and slight trimming of taproots. Samples were then frozen in test-tubes for 20 hours at -10°C (= +14° F) and later boiled. Calculations of percent injury from freezing, as related to total tissue destruction from subsequent boiling, were calculated as described earlier (Hodgson 1964). Mean sampling dates and two-year mean values for percent injury are presented in Figure 5.

Stored food reserves (Exps. 8 and 9): Broadcast-seeded plots of eight sweetclover strains identified in Figure 6 were planted without a companion crop in consecutive years on 22 May (Exp. 8) and on 30 May (Exp. 9). Plants were dug from plots in the seedling year on 23 October in Exp. 8 and on 19 October in Exp. 9. After soil was washed from roots with a cold-water spray, all lateral roots, all taproot growth beyond four inches below the cotyledonary node, and all stem growth beyond one inch above the same node were severed and discarded.

After trimming, all stem-base/crown/taproot segments (plants) were washed to remove all traces of soil and plant debris and surface-dried with absorbent toweling. A random sample of plants of each strain was withdrawn, weighed, dried to constant weight at 60°C, and reweighed. Percent dry matter derived thusly was used to calculate extrapolated dry weights of plants used for stored food-reserve determinations. Twenty-five plants of each strain were potted on each sampling date.

Table 2. *Two-year means of seeding-year forage yields of sweetclover strains grown in rows, leaf vs. stem proportions of forage, and crude protein concentrations of leaves and stems; and two-year means of winter survival, second-year first-cutting forage yields, and crude protein concentration of the same strains grown in broadcast plots that were established in the seeding year with a barley companion crop. All data on oven-dry basis (140°F).*

	Seeding year (Exps. 4 and 5; rows)					Second year (Exps. 12 and 13; broadcast-seeded plots)		
		Proportion of forage[1]		Crude protein[1]				
Species and strain	Forage yield	leaves	stems	leaves	stems	Winter survival	Forage yield	Crude protein[1]
	Tons/acre	%	%	%	%	%	Tons/acre	%
Biennial white (*M. alba*):								
Matanuska white	1.06 b[2]	46 a	54 e	17.0 de	4.9 b	61 a	1.67 a	19.1
AK-Syn.1	1.94 ab	32 cde	68 abc	17.5 cde	4.3 c	66 a	1.55 a	18.9
Arctic	2.56 ab	33 cde	67 abc	16.2 e	4.3 c	59 a	1.02 b	18.0
Spanish	3.20 a	31 de	69 ab	19.7 b	5.7 a	1 c	0.02 d	—
Annual white (*M. alba* var. *annua*):								
Hubam	3.67 a	28 e	72 a	23.2 a	5.8 a	- [3]	- [3]	- [3]
Biennial yellow (*M. officinalis*):								
Palmer yellow	2.34 ab	43 a	57 e	18.9 bc	5.4 ab	27 b	0.89 b	16.2
Erector	2.81 ab	39 abc	61 cde	18.0 b-e	5.1 b	11 c	0.43 c	16.6
Madrid	3.99 a	35 bcd	65 bcd	18.5 bcd	5.3 ab	1 c	0.03 d	—

[1] Data derived for one year only.

[2] Within each column, means not followed by a common letter differ significantly (5% level) using Duncan's Multiple Range Test.

[3] An annual; not included in 2-year experiments.

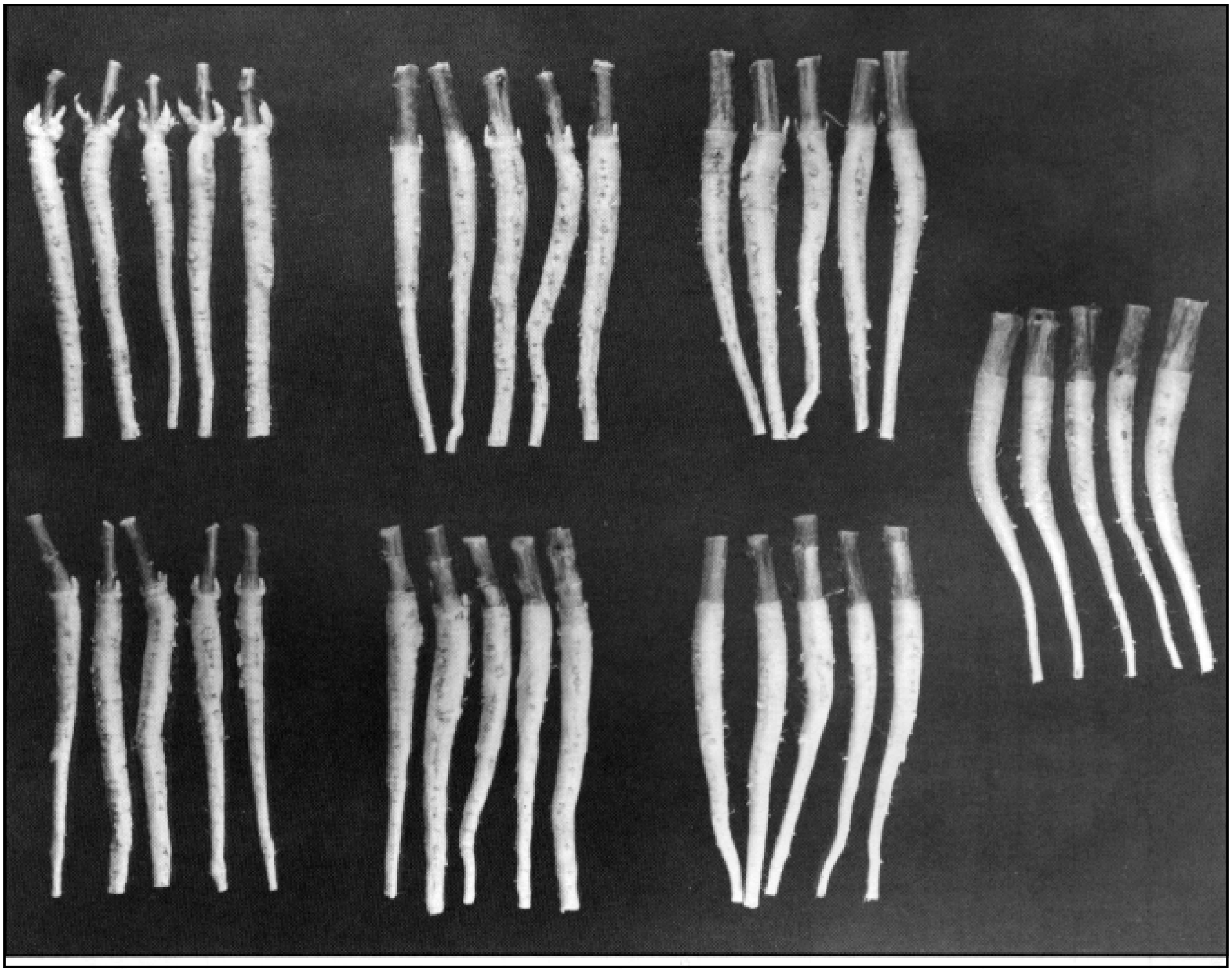

Figure 2. *Five-plant groups of typical stem-base/crown/taproot segments (lateral roots excised) showing differences among sweetclover strains in stem diameter and crown-bud development near end of the first year of growth (planted 22 May; photo 29 September). Note more and larger crown buds, and decrease in stem diameter, in plant groups from right to left (= strains of progressively more northern adaptation).* ***Top row (left to right):*** *Biennial white strains Matanuska white, Arctic, Spanish;* ***bottom row (left to right):*** *Biennial yellow strains Palmer yellow, Erector, Madrid;* ***far right:*** *The annual white cultivar Hubam.*

Plants were weighed individually and embedded vertically in moist vermiculite in plastic pots. Eight or nine plants were spaced evenly in each pot and embedded so that the cotyledonary nodes were just above the vermiculite surface. Pots were then placed into a warm (18° ± 1.5°C) dark chamber with the base of pots immersed in one-quarter to one-half inch of water. Fungicide (PCNB) in water spray was applied to plants as needed, usually about three times weekly, to prevent mold development.

Etiolated growth was harvested from seedlings at successive three-week intervals until no more appeared; this point represented exhaustion of food reserves as plants died shortly thereafter. Etiolated growth was harvested back only to the lowermost node to ensure the presence of regenerative bud sites throughout the test period. Harvested etiolated growth was dried at 60°C and stored food reserves are reported as milligrams (mg) oven-dry etiolated growth per oven-dry gram (g) of plant storage tissue potted.

Temperature effects on dormancy (Exp. 10): Matanuska white sweetclover was broadcast-seeded on 30 May and seedling plants were dug 19 October when soil had begun to freeze. Roots were prepared for stored food-reserve measurements as described in Exps. 8 and 9. After a sample of plants was withdrawn to determine dry-matter concentration, the remaining plants were divided randomly into four 25-plant lots, each lot to receive a different temperature treatment before potting. Plants were kept wrapped in water-

Table 3. *Comparative winter survival of biennial white and yellow sweetclover cultivars and strains in twelve separate experiments involving nine different winters at the Matanuska Research Farm (61.6°N).*

Species and strain or cultivar	Latitude of origin or adaptation	Years of individual experiments[1]											
		1963-64	1964-65	1965-66	1966-67	1969-70(a)	1969-70(b)	1969-70(c)	1971-72(a)	1971-72(b)	1973-74	1974-75	1980-81
	°N	Percent winter survival											
Biennial white (*M. alba*):													
Mat. white (orig.)	61.6	91	37	87	89	56	33	72	—[2]	9	10	68	12
Mat. white II[3]	61.6	—	—	—	—	61	46	—	—	36	—	—	—
Mat. white III[4]	61.6	—	—	—	—	—	—	—	88	28	—	—	22
Mat. white IV[5]	61.6	—	—	—	—	—	—	—	—	—	—	94	—
AK-Syn.1	61.6	49	83	63	86	64	56	51	64	13	59	90	45
Arctic	50-56	51	66	43	73	63	46	29	—	6	20	85	—
Cumino (orig.)	52.1	—	—	—	7	6	4	—	1	0	0	13	1
Cumino II[6]	61.6	—	—	—	—	24	12	—	—	—	21	—	—
Cumino III[7]	61.6	—	—	—	—	—	—	—	50	5	62	57	22
Cumino IV[8]	61.6	—	—	—	—	—	—	—	—	—	71	—	—
Polara	52.1	—	—	—	—	—	—	—	2	0	0	29	2
Spanish	35-50	0	1	0	0	0	1	—	—	0	—	—	—
Denta	43.1	—	—	—	0	—	—	—	—	—	0	0	—
Biennial yellow (*M. officinalis*):													
Palmer yellow	61.6	17	36[9]	—	—	—	—	—	—	—	—	—	—
Yukon	52.1	—	—	—	—	—	—	—	2	0	0	40	1
Erector	49.8	9	12	—	6	7	3	—	6	0	0	27	0
Goldtop	43.1	—	—	—	0	—	—	—	—	—	0	0	0
Madrid	35-50	0	0	—	4	1	0	—	1	0	0	4	0

[1] 1963-64: Planted 22 May 1963, 5 x 10 ft. broadcast-seeded plots in barley companion crop (tall stubble left over winter), two replicates.
1964-65: Planted 28 May 1964, 5 x 10 ft. broadcast-seeded plots in barley companion crop (tall stubble left over winter), two replicates.
1965-66: Planted 18 May 1965, drilled rows 16 ft. long, 18 in. apart, four replicates.
1966-67: Planted 9 June 1966, drilled rows 16 ft. long, 24 in. apart, four replicates.
1969-70: Planted 2 June 1969, spaced plants in rows 22 ft. long, 18 in. apart, six replicates in each of two tests: (a) = moderate winter-exposure field site; (b) = maximum winter-exposure field site.

(*Table 3 continued on page 9*)

(Table 3 continued from page 8)

1969-70(c): Planted 6 June 1969, 5 x 15 ft. broadcast-seeded plots, three replicates, maximum winter-exposure field site.
1971-72(a): Planted 16 June 1971, 5 x 16 ft. broadcast-seeded plots, three replicates, moderate winter-exposure field site.
1971-72(b): Planted 17 June 1971, 5 x 15 ft. broadcast-seeded plots, four replicates, maximum winter-exposure field site.
1973-74: Planted 28 June 1973, spaced plants in rows 36 ft. long, 18 in. apart, five replicates.
1974-75: Planted 10 June 1974, spaced plants in rows 36 ft. long, 18 in. apart, two replicates.
1980-81: Planted 20 June 1980, spaced plants in rows 35 ft. long, 18 in. apart, four replicates.

[2] Not included in experiment.

[3] Field selection; seed harvested from few winter-surviving plants in field plot seeded with "original" Matanuska white (=Mat. white-orig.).

[4] Field selection; seed from two successive generations of field-surviving plants; first generation derived from Mat. white-orig. (Mat. white-II not in lineage).

[5] Field selection; bulk of seed from plants of Mat. white-II and Mat. white-III with 32% mean winter survival.

[6] Field selection; seed produced in greenhouse isolation from 18 transplants surviving of several thousand in field plot seeded from "original" Cumino.

[7] Field selection; seed from surviving plants of Cumino II in rows with 18% mean winter survival.

[8] Field selection; seed produced in greenhouse isolation from transplants with 5% winter survival in plots seeded with Cumino III.

[9] Palmer yellow was excluded from future tests. After 1965 a more winterhardy naturalized subarctic strain of biennial yellow sweetclover was included in tests. Its performance is discussed in a separate report (Klebesadel 1992).

Table 4. *Average number of days during late summer/autumn between time that shortening photoperiods reach a specific duration and that location's mean date of first killing frost (-2.2℃ = 28℉).*

	Days after each photoperiod until killing frost		
Photoperiod (hours)	Palmer, AK 61.6°N.	Fargo, ND 46.9°N.	Springfield, IL 39.8°N.
15	24	64	117
14	14	42	74
13	4	24	49
12	0[1]	5	27

[1] Mean killing-frost date precedes 12-hour photoperiod by 7 days.

saturated toweling prior to and during treatments.

Temperature treatments involved storage in a refrigerator (+5°C) and in a freezer (-2.5° ± 1.5°C) as follows: (A) potted same day as dug (check treatment), (B) 14 days in refrigerator, (C) 14 days in refrigerator + 2 days in freezer, and (D) same as C but additionally 1 week in refrigerator + 1 week in freezer + 1 week in refrigerator + 1 week in freezer. Plants of each treatment were potted immediately after temperature treatments were concluded and etiolated growth was harvested until exhaustion of plants as described for Exps. 8 and 9.

Dormancy in autumn vs. spring (Exp. 11): Plots of Arctic, AK-Syn.1, and Matanuska white were broadcast-seeded on 16 June. Some plants were removed from the field on 27 August and 19 October of the seeding year; plants that survived the winter were dug also on 6 May of the following spring. All roots were prepared, potted, and harvested for etiolated growth as in Experiments 8, 9, and 10. Twenty-five plants of each strain were used in the August and October samplings. Thirty-three plants of Matanuska white were used from the 6 May sampling but only 13 plants of AK-Syn.1 were found alive to represent that strain. No plants of Arctic survived the winter to measure stored food-reserve status of that cultivar in spring.

Winter survival and second-year forage yields (Exps. 12 and 13): Broadcast-seeded plots of four biennial white and three biennial yellow sweetclover strains (listed in Table 2) were established with 'Edda' barley (*Hordeum vulgare* L.) companion crops in randomized complete block experimental designs with two replications. Plantings were in late May in two consecutive years (Exps. 12 and 13); individual plot size was 5 x 15 feet. Barley was sowed at 70 lb/acre in drilled rows 6 inches apart, and sweetclovers were seeded at 20 lb/acre. The immature barley companion crop was removed near mid-July, leaving a 10- to 12-inch stubble to retain a protective layer of snow in place against the evacuation force of strong winter winds (Dale 1956; Klebesadel 1974). The stubble was clipped and removed the following spring before new growth appeared on sweetclovers.

Commercial fertilizer supplying P_2O_5 and K_2O at 114 and 60 lb/acre, respectively, was applied as a uniform topdressing on 20 April (Exp. 12) and on 25 March (Exp. 13) before initiation of second-year growth. After spring growth of sweetclovers had started, counts of living and dead plants were made in each plot and winter survival percentages were calculated. Counts were made by recording all plants within a 1- x 2-foot quadrat placed at six random locations along the centerline of each plot.

Seed recovery from winter-surviving plants (Exp. 14): During the course of numerous field experiments, including those summarized in Table 3 and others, seed was sometimes recovered from plants that survived winters, especially if winter kill was severe and survivors few. The objective was to later plant that seed to compare with the progenitor strain to determine the extent of progress toward improved winter hardiness that might be achieved by that avenue of selection. If surviving plants were very few, they were transplanted to a greenhouse with screened openings to contain captive honeybees for pollination in isolation. When plants were left in the field for seed production, other plants of the same species were prevented from flowering within several hundred feet of the seed producers. Table 3 summarizes the selection and performance of lines derived from Matanuska white and the Canadian cultivar Cumino.

RESULTS AND DISCUSSION

Flowering

All of the sweetclovers flowered during the seedling year in these spring-planted experiments (Fig. 3). This behavior differs from normal behavior of biennial sweetclover at the more southern latitudes where both species typically are grown. Biennial sweetclover plants normally do not flower during the first season of growth because of longer summer nyctoperiods in those more southern areas (Kasperbauer et al. 1963b).

Investigators at mid-temperate latitudes (Kasperbauer et al. 1963b; Smith 1942) have induced flowering in seedling-year biennial sweetclover by using artificial lighting to provide extended photoperiods that left diurnal nyctoperiods of seven hours or less, durations shorter than normal at those latitudes. The fact that flowering occurs during the seedling year in biennial sweetclovers at this latitude is therefore understandable because shortest diurnal nyctoperiods in June are 4.5 hours; moreover, that period is not total darkness but is considered to be twilight owing to the sun's relatively shallow distance below the horizon.

Morphological Characteristics

World Comparisons—Visual: Crown-bud development of Matanuska white and Palmer yellow were compared visually with 36 other strains of *M. alba* and 61 of *M. officinalis* from various world sources. Matanuska white was clearly superior to all others in number and size of crown buds in late October of the seedling year. Moreover, none of the 97 introduced sweetclovers surpassed Palmer yellow in bud size and number. However, four accessions of *M. alba* and six of *M. officinalis* were rated as having some plants within the row that approximately equalled Palmer yellow in size and abundance of crown buds. Surprisingly, Man-

Figure 3. Drilled rows of sweetclover strains seeded 29 May and photographed 22 September of the same year, showing abundant flowering on tall growth. In more southern regions of sweetclover culture (southern Canada and the conterminous states), biennial strains produce less top growth and generally do not flower during the seeding year.

churia was the only relatively northern-source country; other near-comparable accessions were from lower latitudes that included the countries of Turkey, Iran, Algeria, Afghanistan, Spain, and Cyprus.

Measurements of Crown Characteristics: Exposure of the biennial sweetclovers introduced from Canada and the conterminous United States to unaccustomed photoclimate at this high latitude resulted not only in profuse flowering, but also in strikingly atypical morphological development in the stem-base/crown area, causing them to resemble the annual Hubam more than typical biennial growth (Fig. 2).

Biennial sweetclovers, when grown at latitudes where they are adapted, produce a limited amount of aerial growth on a stem of relatively small diameter, and during autumn they develop numerous crown buds at the cotyledonary node (Fig. 4) while the taproot undergoes rapid growth and enlargement (Kasperbauer et al. 1963a; Smith et al. 1986; Smith 1942). As a result of those growth characteristics, plants near the end of the first season of growth possess a well developed crown with the diameter of the tap root immediately below the crown considerably larger than the diameter of the stem just above the crown (Fig. 4).

Precise measurements of morphological characteristics were made of the two Alaska roadside ecotypes and selected North American cultivars representing diverse latitudinal origins (Table 1). Greater differences were noted in all morphological comparisons between Matanuska white vs. the other white strains than between Palmer yellow vs. other yellow strains. Matanuska white had significantly more and larger crown buds than any of the other strains (Table 1, Fig. 2).

Kasperbauer et al. (1963a) noted that crown bud formation in autumn on biennial sweetclovers is induced by shortening photoperiods. All of the strains included in these tests were exposed to the same shortening photoperiods during autumn, yet only Matanuska white developed crown buds approximating typical biennial sweetclover in near-normal size and in greatest abundance. (It should be recognized, however, that even though superior to the other strains, Matanuska white did not produce as many crown buds as is typical of biennial sweetclovers at more southern latitudes [compare Figs. 2 and 4; also photos in Kasperbauer et al. 1963a; Smith et al. 1986]).

Two possible reasons are suggested for failure of the introduced cultivars to form crown buds in size and numbers typical of biennial sweetclovers at more southern latitudes. One is that the introduced sweetclovers were not exposed to sufficiently short photoperiods for an adequate number of days before termination of the growing season. Normal date of first frost (0°C = 32°F) at the Matanuska Research Farm is 9 September, when photoperiods have shortened to 13.6 hours, and normal date of first killing frost (-2.2°C = 28°F) is 19 September, when photoperiods have

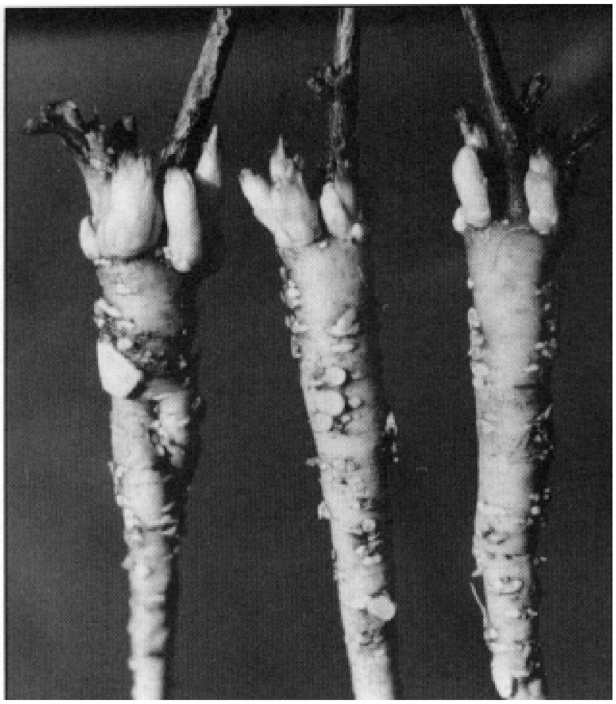

Figure 4. Typical stem-base/crown/taproot development (lateral roots excised) of biennial white sweetclover by mid-November of the seedling year when grown in its area of adaptation. Note small-diameter stems and abundance of crown buds on these plants photographed on 14 November in southern Wisconsin where plants are not subjected to extremely long diurnal photoperiods/short nyctoperiods in mid-summer as occur in Alaska.

shortened to 12.6 hours. Therefore, introduced cultivars are divorced from their accustomed, much longer term of short photoperiods that occur before termination of the growing season at their latitude of origin (Table 4).

Another possible reason for the failure of the introduced cultivars to form typical numbers and size of crown buds may lie in their having been caused to develop growth resembling annuals by the unaccustomed long photoperiods/short nyctoperiods of Alaska's midsummer. Once caused to develop toward annual habit with tall, large-diameter stems, the introduced cultivars may have largely lost the ability to respond to subsequent shorter photoperiods. Alternatively, their failure to develop normal size and numbers of crown buds may involve a combination of the foregoing reasons as well as other unrealized factors.

Although the Palmer yellow strain tended to differ from Erector and Madrid in having shorter plant height and smaller-diameter stems, those differences were not statistically significant at 95% confidence limits (Table 1). Palmer yellow had significantly more and larger crown buds than Madrid, but the differences between Palmer yellow and Erector were not significant.

The reason for lesser differences between the Palmer yellow strain vs. the introduced yellow cultivars than occurred between Matanuska white and the other white strains is not clearly understood. Both roadside populations probably derived from near-similar latitudes in the conterminous United States. One possibility is that the white roadside population may have been in place longer than the yellow one. This would cause it to be subjected to natural-selection pressures for a longer period which in turn would result in greater adaptive modification than occurred with the yellow population.

AK-Syn.1, selected predominantly from Arctic, did not differ significantly from Arctic in measured morphological characteristics (Table 1). The greatest tendency toward a difference was in reduced plant height of AK-Syn.1. The southernmost-adapted Spanish and Madrid did not differ significantly from the annual Hubam in root/stem diameter or in plant height, but both biennials had significantly more and larger crown buds than Hubam.

Seeding-Year Forage Yields

Seeding-year forage yields of Matanuska white, the shortest-growing, finest-stemmed strain, were lowest of the eight sweetclover strains compared (Table 2), although not significantly different from four of the other strains. Spanish and Madrid, the two southernmost-adapted cultivars, tended to be somewhat shorter than the annual Hubam (Table 1) but produced seeding-year forage yields equivalent to Hubam (Table 2). Though differences were not always significant, there was a clear and consistent gradient toward taller growth (Table 1) and heavier forage yields (Table 2) within each species as strains were progressively further removed from their latitude of adaptation.

The subarctic ecotypes (Matanuska white and Palmer yellow) tended to be shortest in seeding-year growth (Table 1) and produced the highest proportion of leaves in the forage (Table 2). The tallest-growing Hubam tended to be lowest in proportion of leaves. However, crude protein (N x 6.25) concentration in the leaves of this annual cultivar that develops very little tolerance to freeze stress was significantly higher than in the leaves of the biennial cultivars.

Hodgson and Bula (1956) found overwintering crown/root tissues of the most winter-hardy sweetclover cultivars to be highest in various nitrogenous fractions. Similarly, Smith (1964a) summarized numerous investigations that reported increases in soluble protein nitrogen, and the quantity of water-binding proteins, in overwintering tissues of various plant species during the winter-hardening process.

This suggests that foliar N in the biennial strains may be mobilized for incorporation into N compounds important in the development of cold hardiness in overwintering tissues. In contrast, failure of the annual Hubam to undergo hardiness development, and therefore not drawing heavily upon foliar N, would leave that foliage with the high concentration found in this study (Table 2). Further support for this thesis is seen in the significantly higher crude protein concentration in leaves of the nonhardy Spanish contrasted with the other three more winter-hardy biennial white strains.

Kirk (1926) in Saskatchewan found seedling-year aerial growth of Arctic was 63.3% leaves; in contrast, it was only 33% leaves at this latitude where it was induced to produce larger, taller stems that comprised the more dominant portion of the forage.

Freeze Tolerance

Overwintering tissues of the several sweetclovers showed a considerable range of injury to artificially imposed freeze stress (Fig. 5). All biennial strains were injured less in late October than in late September, indicating the progression in cold-hardiness development during autumn.

During the 26 days between the tests, Palmer yellow, Arctic, Matanuska white, and AK-Syn.1 developed tolerance to freeze stress more rapidly than intermediately winter-hardy Erector or the nonhardy Spanish and Madrid (Tables 2 and 3; Fig. 6). As a result, there was a much greater range of injury among strains in late October than at the earlier sampling. The annual Hubam increased only slightly in tolerance to freezing between the two autumn dates.

Arctic was clearly and significantly the least injured by cold stress of the introduced cultivars. Hodgson and Bula (1956) earlier found Arctic to be the most winter hardy in field plots of 11 introduced strains at this location. Arctic was selected at Saskatoon, Saskatchewan (52°N) from plants grown from seed collected near Semipalatinsk, Russia (ca. 50° to 51°N) in 1913 (Hansen 1927). That relatively northern origin apparently contributes to Arctic a greater genetic capacity for cold hardiness development and a more appropriate winter-hardening response to northern seasonal patterns than occurs in strains that trace to more southern origins.

Adaptation to the unique interrelationship of photoperiodic pattern and growing season at northern latitudes is important for successful winter survival (Hodgson 1964; Klebesadel 1971a, 1985b; Klebesadel and Helm 1986; Moschkov 1935; Pohjakallio 1961). The complex changes at the cellular level that contribute to freeze tolerance (Smith 1964a; Steponkus 1978) are initiated during the latter part of the growing season by accustomed seasonal patterns of photoperiodic and temperature stimuli (Hodgson 1964; Moschkov 1935; Pohjakallio 1961; Smith 1964b; Tysdal 1933).

A clear difference was seen between the two Canadian cultivars in the superior cold tolerance of Arctic over Erector. Although both are grown successfully in Canada, it is very likely that Arctic's progenitor germplasm derived from a more northern ancestral origin which then would account for the superior performance of Arctic over Erector when grown at this latitude.

Spanish and Madrid, cultivars adequately winter hardy for use as biennials in the U.S. Midwest, are nonhardy at this latitude and very poorly adapted for use as biennials in Alaska (Tables 2 and 3; Fig. 6). Those southernmost-adapted cultivars, and Erector also, when grown at this latitude are too far removed from accustomed environmental stimuli prior to onset of winter to develop high levels of cold tolerance. Or, perhaps of equal or greater significance, the short Alaskan nyctoperiods of midsummer may have induced such a tendency toward annual habit that adequate physiologic preparation for winter was no longer possible when the advent of hardening conditions occurred in Alaska.

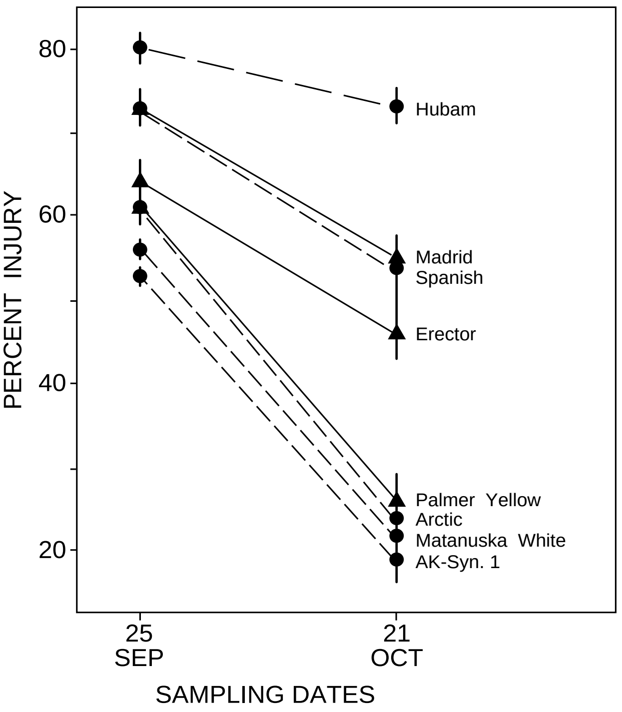

Figure 5. *Two-year means of percent injury to overwintering tissues of sweetclover strains of diverse latitudinal adaptation when frozen 20 hours at -10°C (= +14°F) on two dates during the winterhardening period. Means not joined by a common vertical line are significantly different (5% level) using Duncan's Multiple Range Test. (Exps. 6 and 7)*

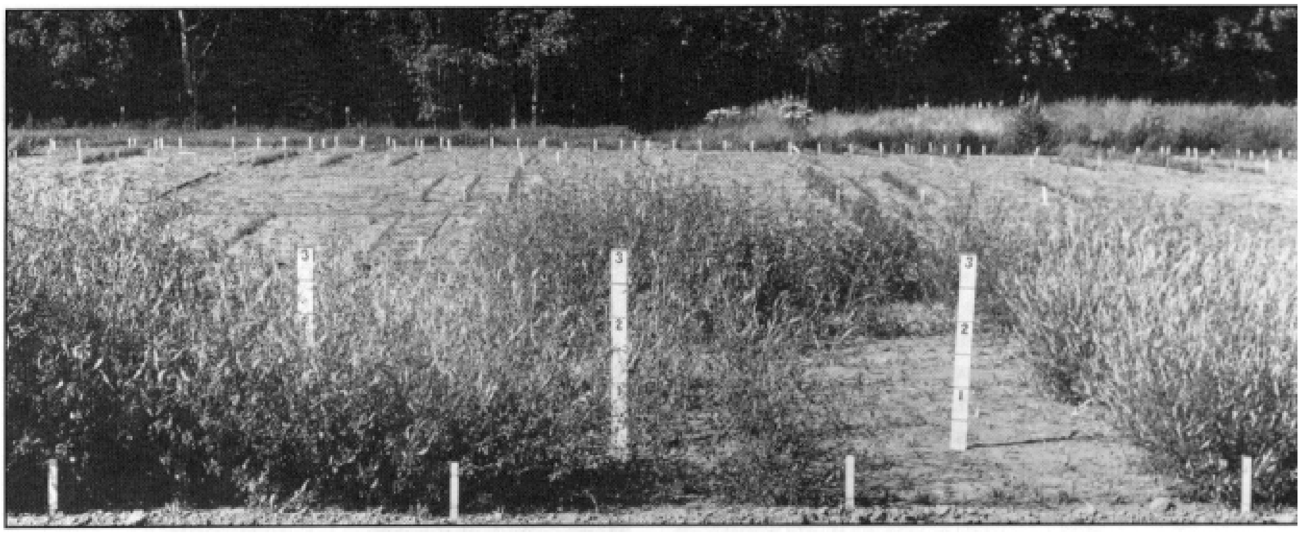

***Figure 6.** Comparative winter survival and second-year growth of biennial yellow and white sweetclover strains in broadcast-seeded plots (mean percent winter survival of each strain appears in parentheses after strain identity). Three-foot-tall white stakes are in centers of plots of (left to right) Palmer yellow (17%), Erector (9%), and Madrid (0%). Plot on extreme left is AK-Syn.1 (49%); plot on extreme right is Matanuska white (91%).*

Stored Food Reserves

Considerable differences were found among the eight sweetclover strains in amount of stored food reserves determined as mg etiolated growth per g of root-crown storage tissues (Fig. 7).

The annual white cultivar Hubam produced virtually no evidence of stored food reserves (Figs. 7 and 8). Spanish white and Madrid yellow, adapted at 35° to 50°N in the United States and farthest removed from their latitude of adaptation, produced low amounts of etiolated growth (Fig. 7).

It is likely that an inadequate period of critically short photoperiods prior to termination of the growing season in Alaska (Table 4) precludes storage of high levels of food reserves in Spanish and Madrid; consequently, their low energy status precludes development of adequate levels of cold hardiness (Fig. 5; Smith 1964b). Moreover, the midsummer diurnal photoperiod/nyctoperiod pattern that shifted their growth behavior toward annual status very likely also diminished their capacity to respond appropriately (for biennials) to the interrelationship of late summer and autumn temperature and photoperiod/nyctoperiod that stimulate physiologic preparation for winter.

Arctic white and Erector yellow, both from Canada and adapted at 50° to 56°N, are more northern-adapted than Spanish and Madrid and, when grown in Alaska, are not as far divorced from their latitude of adaptation as the U.S. cultivars. Not surprisingly, then, Arctic and Erector produced much higher levels of food reserves than Spanish and Madrid (Fig. 7). Those higher levels of stored food reserves in the Canadian cultivars parallel higher levels of freeze tolerance (Fig. 5) and winter survival (Fig. 6; Table 2) in Alaska than occurred with the more southern-adapted Spanish and Madrid.

Palmer yellow, a strain that has undergone some modification through natural selection toward subarctic acclimatization, gave evidence of higher levels of stored food reserves than Erector or Madrid (Fig. 7). This parallels Palmer yellow's superior tolerance to artificial freeze stress (Fig. 5) and better winter survival in the field (Fig. 6; Table 2) than the yellow cultivars Erector and Madrid.

AK-Syn.1, an Alaskan selection primarily from Arctic and representing three successive generations of natural selection in the field for improved winter survival, gave evidence of somewhat higher stored food reserves than Arctic (Fig. 7). This higher level of food reserves parallels the tendency toward better tolerance to artificial freeze stress (Fig. 5) and generally superior winter survival of AK-Syn.1 over Arctic (Table 3).

Stored food reserves in Matanuska white were expressed as etiolated growth in a quite different sequence of amounts at the successive triweekly harvests than occurred with the other hardiest white strains (Fig. 7). Arctic and AK-Syn.1 produced 64% and 68% of their total etiolated growth in the first three weeks in darkness; in contrast, Matanuska white produced only 27% of its total etiolated growth in the first three weeks (Figs. 7 and 8). Moreover, Arctic and AK-Syn.1 expressed virtually all of their stored food reserves within 9 weeks, while Matanuska white required 18 weeks for total expression.

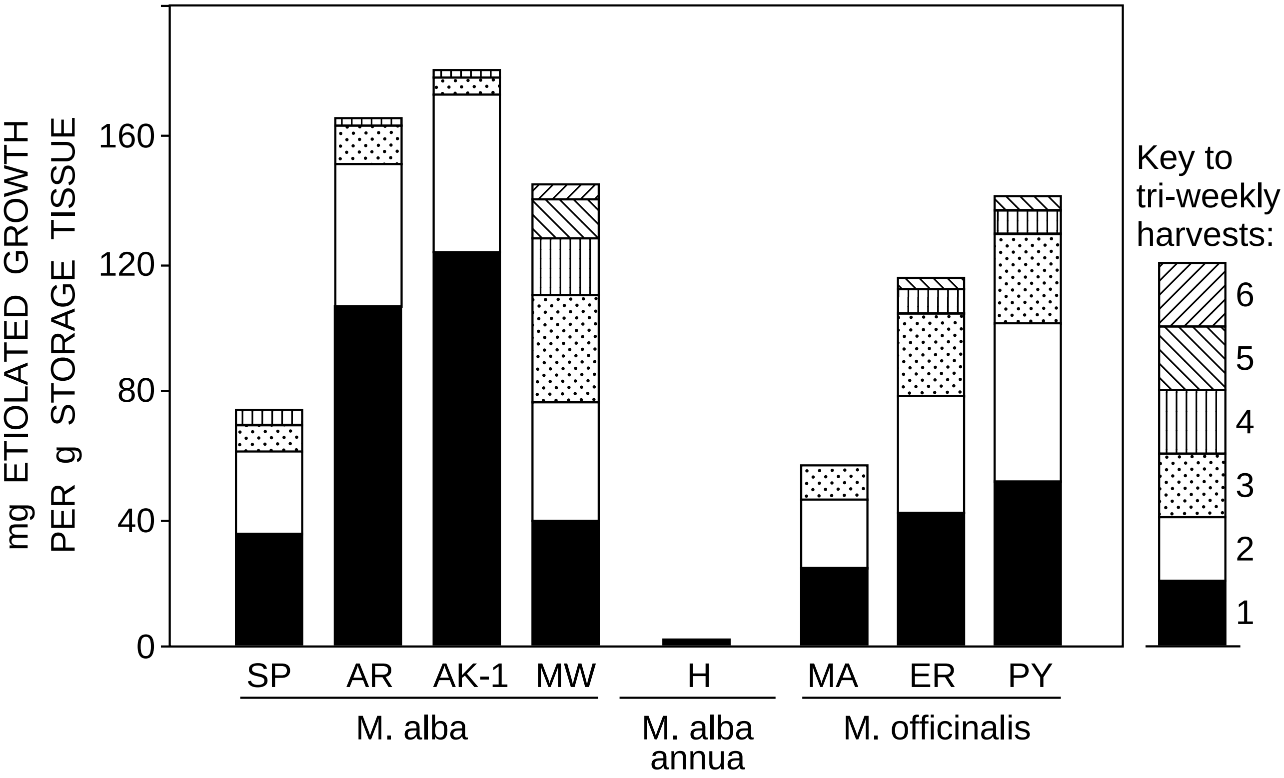

Figure 7. *Two-year means of stored food reserves expressed as etiolated growth from root-crown segments of four biennial white (M. alba), one annual white (M. alba annua), and three biennial yellow (M. officinalis) sweetclover strains of diverse latitudinal adaptation. Seeding-year plants dug from field on 21 October (mean date); all weights on oven-dry basis (60° C). Key to strains: SP = Spanish, AR = Arctic, AK-1 = AK-Syn.1, MW = Matanuska white, H = Hubam, MA = Madrid, ER = Erector, and PY = Palmer yellow. (Exps. 8 and 9)*

During the time that etiolated growth is produced in the warm, dark chamber, active respiratory activity of the plants results in a failure to measure that portion of stored food reserves volatilized as gaseous respiration products (Graber et al. 1927). This means that the bar denoting food reserves for Matanuska white in Figure 7 errs somewhat in being a more conservative estimate of actual food reserves than the bars for Arctic and AK-Syn.1; this is because Matanuska white required a considerably longer period of time (18 weeks) than did the other two white strains for total expression of stored food reserves as etiolated growth. The markedly dissimilar rates of expression of stored food reserves by Arctic and AK-Syn.1 versus Matanuska white is believed to indicate the presence of some manner of dormancy in the latter strain.

Lesser evidence of this dormancy effect in Palmer yellow than in Matanuska white (Fig. 7) may be evidence of a shorter term of subarctic residence and hence a less extensive period for selective modification in the yellow strain.

Temperature Effects on Dormancy

Repeated freeze-thaw cycles were effective in breaking much of the bud dormancy in Matanuska white. Seedlings dug on 19 October and potted and placed into the warm, dark chamber immediately (Treatment A) required 18 weeks for total expression of stored food reserves as etiolated growth (Fig. 9). When held for 14 days at 5°C (Treatment B), or if subjected additionally to a subsequent 2-day light freeze (-2.5°C) (Treatment C), gradually more of stored reserves were expressed during the earlier growth periods in darkness, and a lesser period of 15 weeks was required for total expression of stored food reserves. When the plant segments were subjected to three freeze-thaw cycles before placement in the dark chamber (Treatment D), only nine weeks were required for total expression of reserves (Figs. 9 and 10).

As the dormancy effect disappeared in treatments A through D, the percentages of total stored food reserves expressed in the first three-week growth period in darkness for each treatment were 27, 32, 35, and 79, respectively. The percentages of total reserves expressed as etiolated growth in the sequence of tri-weekly harvests from beginning to exhaustion for treatment A were 27, 21, 20, 15, 13, and 4. In contrast, percentages for treatment D were 79, 19, and 2. That

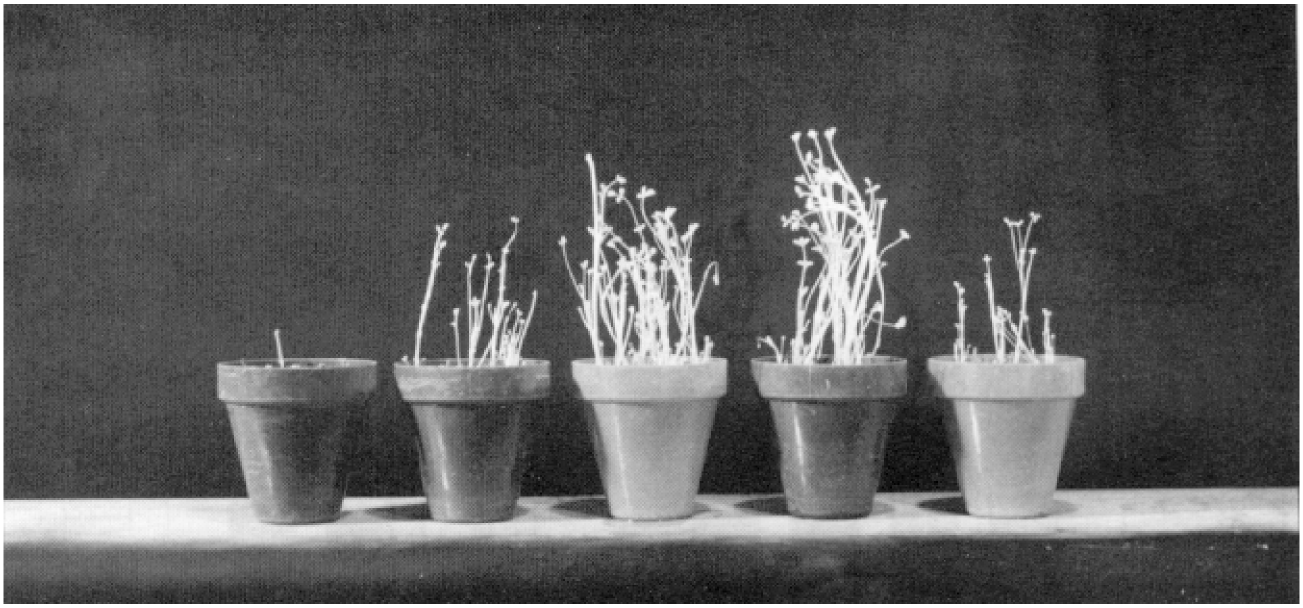

Figure 8. *Pots of five different white sweetclover strains (1 annual, 4 biennial) showing representative amounts of etiolated growth produced during first three-week period in warm, dark chamber. Left to right: Hubam, Spanish, Arctic, AK-Syn.1, Matanuska white. All seeded 31 May, dug 19 October and potted 20 October, photo 11 November. Note virtual absence of evidence of stored reserves in the annual Hubam, increasing amounts in Spanish through AK-Syn.1, but indication of apparently modest level of stored reserves in Matanuska white with dormancy restricting growth (compare with Figure 10).*

98% of stored food reserves in treatment D were expressed in only six weeks suggests that much of the bud dormancy evident in treatment A was dissipated by the three freeze-thaw cycles of treatment D (compare Figs. 8 and 10).

The difference in height of the graph bars between treatments A and D in Figure 9 illustrates the amount of unmeasured stored food reserves lost as respiration products when 18 weeks are required for total exhaustion of stored food reserves (Treatment A) versus only 9 weeks (Treatment D).

Dormancy in Autumn vs. Spring

Further evidence of the effects of cold or freeze-thaw effects in breaking bud dormancy was found when stored food reserves were determined in *M. alba* strains in both autumn and the following spring (Fig. 11). Plants of Arctic, AK-Syn.1, and Matanuska white dug on 27 August, 19 October, and after winter on 6 May displayed markedly different levels of stored food reserves and rates of expression as etiolated growth. Following the 27 August digging, total levels of stored food reserves (mg etiolated growth per g storage tissue) for Arctic, AK-Syn.1, and Matanuska white were 16, 24, and 54, respectively, and only three or six weeks were required for total expression of reserves (Fig. 11). These data indicate that Matanuska white was more active in early storage of food reserves than the other two strains.

This point is reinforced by results of an earlier sampling on 18 August in Exp. 11 (data not graphed); following that sampling, Arctic, AK-Syn.1, and Matanuska white produced etiolated growth totals of 0, 5, and 44 mg/g, respectively. This evidence of earlier initiation of food-reserve storage in Matanuska white probably indicates a behavioral response acquired through natural selection that confers a more timely preparation for the relatively earlier termination of the growing season and onset of winter in this northern area than at more southern latitudes (Klebesadel 1985b). This behavior suggests that Matanuska white initiates food-reserve storage in response to longer photoperiods (or shorter nyctoperiods) than cause the process to be initiated in more southern-origin ecotypes adapted where growing seasons are longer.

When sampled 19 October, AK-Syn.1 again surpassed Arctic in total levels of reserves (81 vs. 65 mg/g), and only six weeks were required for exhaustion of Arctic vs. nine weeks for AK-Syn.1 (Fig. 11). Matanuska white, in contrast, obviously had assumed a high degree of dormancy as evidenced by a 12-week period required for total exhaustion of what appeared to be a lesser total level of reserves than measured in the same strain when sampled about 7 1/2 weeks earlier. The apparently lesser total amount of stored food reserves following the 19 October sampling is believed due to

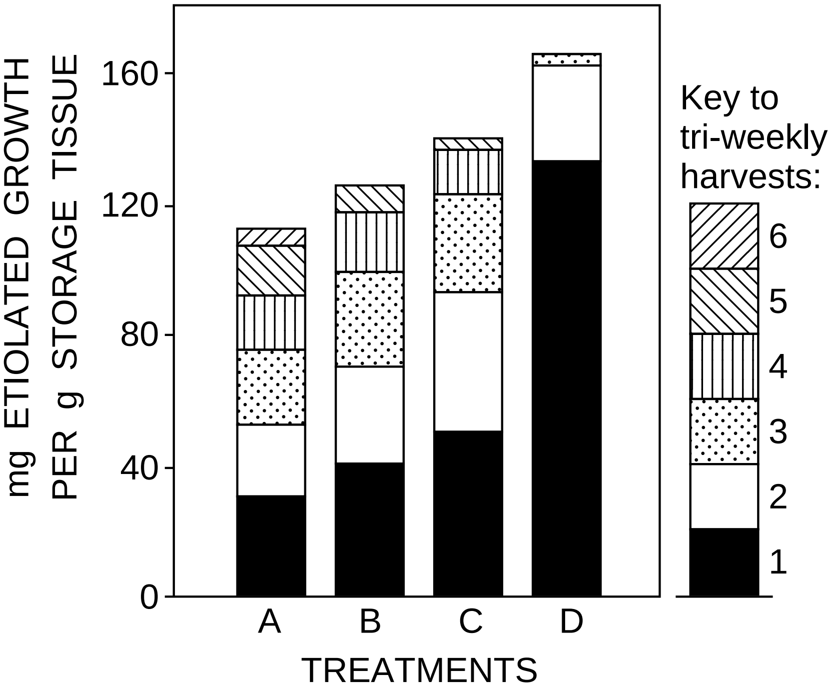

Figure 9. *Stored food reserves expressed as etiolated growth from root-crown segments of seedling-year plants of Matanuska white biennial sweetclover as influenced by three temperature treatments imposed after digging on 19 October and before potting: A = check, B = 14 days @ +5°C, C = 14 days @ +5°C, plus 2 days @ -2.5°C, D = 3 freeze-thaw cycles over 44 days. (Exp. 10)*

unmeasured gaseous respiration products lost (Graber et al. 1927) during the longer period required for food-reserve exhaustion in the second sampling.

This viewpoint is supported by the determinations of stored food reserves measured the following spring when on 6 May plants were dug before initiation of visible spring growth in the field. Plants of AK-Syn.1 and Matanuska white showed no evidence of retained dormancy in spring; virtually all of their stored food reserves were expressed as etiolated growth during the first three-week period in darkness (Fig. 11).

AK-Syn.1 showed a higher level of stored food reserves in spring than Matanuska white. No plants of Arctic survived to provide a determination of reserve levels in that cultivar. It should be recognized that stored food reserves were determined on a totally random selection of seedlings at the 27 August and 19 October samplings while only plants that survived the winter were used in the 6 May sampling; however, it is doubtful that this difference in sampling would appreciably alter the results or concepts derived from these data. Winter survival percentages were not determined in the plots but gross observations indicated that less than 10% of Matanuska white plants and less than 2% of AK-Syn.1 plants survived the relatively severe winter.

The dormancy apparent at the 19 October sampling, especially in Matanuska white, is believed to be a behavioral characteristic acquired through natural selection in the Matanuska Valley. The dormancy noted may be a relatively superficial protective mechanism that merely prevents crown buds from developing into stems until the second year. Alternatively, it may be indicative of a more encompassing condition involving more than the crown buds. Regardless, it is believed to be an acquired characteristic that may contribute to winter survival of the whole plant.

This uncertainty will require further research for resolution. The Matanuska white strain has evolved over many years in a locality where mid-winter temperature fluctuations in the freeze-thaw range are common (Dale 1956; Klebesadel 1974). The observed autumn dormancy conceivably could contribute to enhanced winter survival by assisting to protect plants against dehardening during several-day warm periods that occur commonly during winters here, typically reach 40° to 45°F (= about 5° to 8°C), and sometimes can be followed abruptly by very low temperatures in the range of -20° to -35°F (= about -29° to -37°C) (Klebesadel 1974).

Warm intervals during winter can cause harmful dehardening in plants resulting in reduced winter survival (Dexter 1941). The extent of dehardening is dependent upon the temperatures reached and the duration of warm periods. Plants were found to reharden if sufficient carbohydrate reserves were available within plants. It is not known whether the dormancy detected in sweetclovers in the present study may serve to slow or prevent dehardening during winter warm periods.

In a Wisconsin study (Jung and Smith 1960), higher energy levels (percent TAC) were maintained within alfalfa and red clover plants stored over winter at constant low temperature (28.4°F = -2°C) than plants left in the field and exposed to varying temperatures. Those results indicate that areas with strongly fluctuating winter temperatures, such as the Matanuska Valley, are more taxing on stored food reserves than areas with more constant temperatures. Morever, their

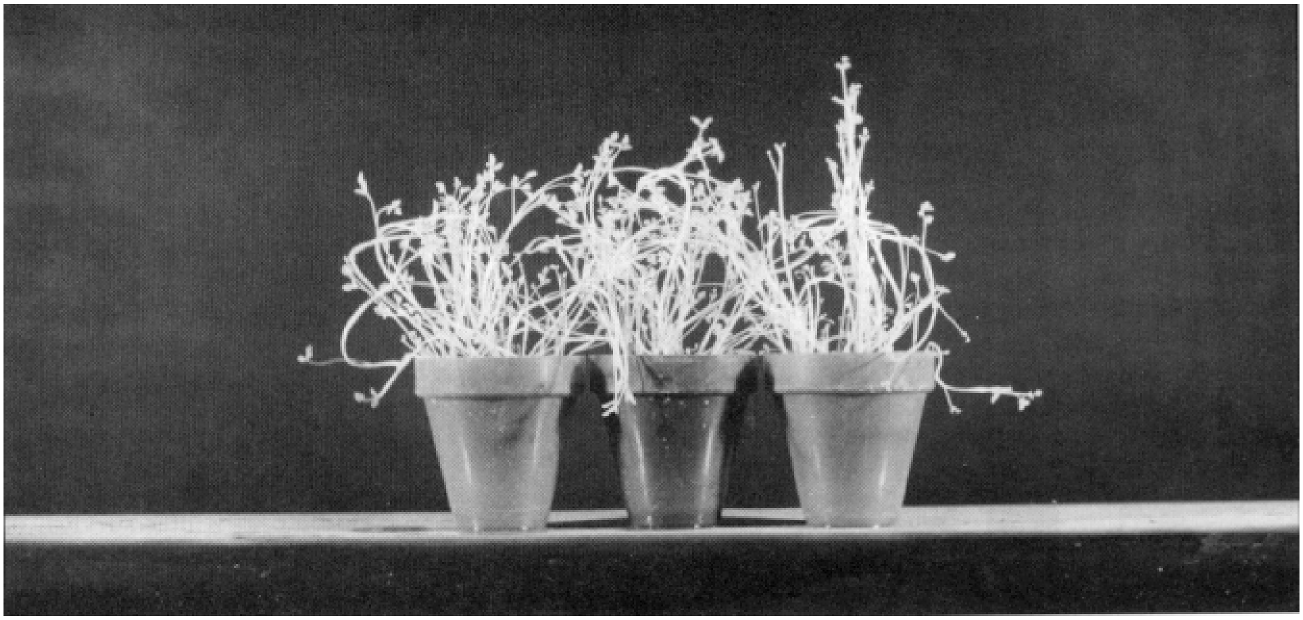

Figure 10. Three pots of Matanuska white sweetclover showing amounts of etiolated growth produced during first three-week period in warm, dark chamber after being subjected to three freeze-thaw cycles (Treatment D of Exp. 10) after digging on 19 October and before potting. Planted 31 May, photo 28 December (compare with Matanuska white in Figure 8).

results suggest that plants that have stored high levels of food reserves by the onset of winter should be better able to tolerate some loss of reserves occasioned by winter temperature fluctuations than plants entering the winter with low food reserves.

Wareing (1969) reported that dormancy is an important characteristic in adaptation of plants to cold climates; he noted also that dormancy of apical buds of many woody plants is initiated by the interaction of short photoperiods and low temperatures characteristic of autumn. Growth-inhibiting substances such as abscisic acid accumulate in the buds causing onset of dormancy. The amount of abscisic acid in dormant buds often decreased during cold treatment (Taylorson and Hendricks 1976). It is possible that abscisic acid or a similar growth inhibitor serves to enforce dormancy in sweetclover buds in autumn, and that low temperatures or freeze-thaw effects during winter dissipate that inhibitor to permit active bud growth in spring.

Matanuska white and, to a lesser extent, AK-Syn.1 assumed a state of pre-winter dormancy that precluded rapid expression of stored food reserves as etiolated growth. That dormancy was broken by exposure of plants to cold. This was accomplished by subjecting storage tissues of Matanuska white to three freeze-thaw cycles, or by leaving plants of Matanuska white and AK-Syn.1 in the field over winter. These results agree with earlier reports (Taylorson and Hendricks 1976) where dormancy in woody plant buds also was broken by exposure to cold.

Winter Survival

The relative winter survival over several years of the most winter-hardy *M. alba* strains in this area is not entirely consistent. AK-Syn.1 almost invariably survives winters somewhat better than Arctic, from which it was derived, though the difference often is not great (Tables 2 and 3). The comparative winter survival of Matanuska white vs. AK-Syn.1 and Arctic, however, has been much less consistent. In some experimental comparisons (see Tables 2 and 3), Matanuska white and AK-Syn.1 have been quite similar in winter survival. This would be expected on the basis of their very similar tolerance to artificial freeze stress (Fig. 5). In other tests, however, the two have differed markedly in survival but not in a consistent ranking (Table 3).

Table 3 summarizes numerous tests, some of which were conducted outside the limits of the present study, to illustrate this inconsistency. It is suspected that the specific habitat conditions under which the two strains evolved may contribute to these inconsistencies. In the roadside habitat where Matanuska white evolved, tall, uncut vegetation retained a protective, insulating snow cover even during severe winter winds. Natural selection for tolerance to low winter temperatures therefore would be less rigorous in the roadside habitat than in open fields where AK-Syn.1 was selected principally for low-temperature tolerance.

In croplands with short stubble, winter winds

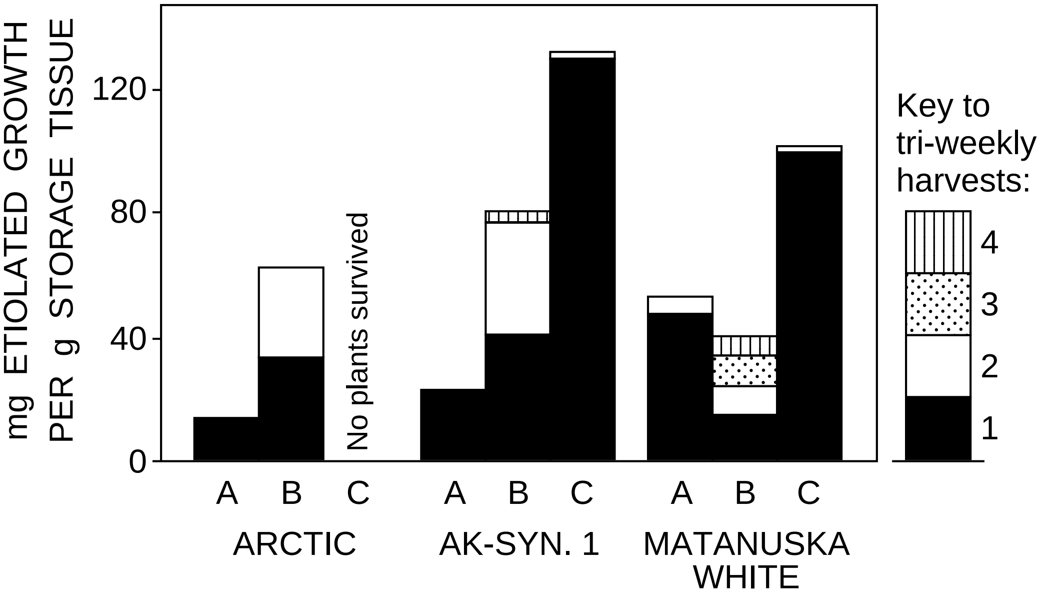

Figure 11. *Stored food reserves expressed as etiolated growth from root-crown segments of three biennial white sweetclovers planted on 16 June and sampled on three dates: A = 27 August, B = 19 October (near soil freeze-up) in the seeding year, and C = 6 May of the following spring (Exp. 11).*

remove insulating snow cover exposing plants to greater cold stress. Minimum air temperatures recorded at the Matanuska Research Farm during the winters of severe cold stress (Table 3) ranged from -35° to -38°C (-31° to -36°F) during relatively prolonged periods of extreme cold. It is believed that the Matanuska white ecotype is less able to tolerate such very low temperatures in the more exposed cropland habitat than strains such as AK-Syn.1 that were selected under field conditions.

Another possible difference between roadside-selected Matanuska white and field-selected AK-Syn.1 affecting winter survival under field conditions could be differential tolerance to dehydration. No data support this speculation but, under field conditions, the random but common occurrence of strong, cold winter winds in this area (Dale 1956; Klebesadel 1974) likely impose a considerable dehydrating effect when snow cover is absent, a recognized facet of winter stress (Steponkus 1978). Plants that have undergone natural selection to tolerate those effects, such as AK-Syn.1, may survive that element of winter stress much better than Matanuska white whose roadside habitat of tall, mixed vegetation and usual snow cover would not select for tolerance to dehydration.

Winter survival comparisons in Tables 2 and 3 illustrate clearly that only Arctic, of all cultivars compared from more southern latitudes, survived winters adequately to be considered further for possible utilization. The white cultivars Cumino, Polara, and Spanish, and yellow cultivars Yukon, Erector, Goldtop, and Madrid showed very poor winter survival.

The marked superiority of Arctic over Erector in both freeze tolerance (Fig. 5) and actual winter survival in numerous field tests (Tables 2, 3) at this location contrast with opposite rankings of winter hardiness for those two Canadian cultivars in a survey of numerous stations in Canada (Ouellet 1976).

Natural Selection in the Field for Increased Winter Hardiness

Although not a prime research objective in this study, the occasional harvest of seed from surviving plants in badly winter-decimated stands to obtain lines with enhanced winter survival tended to show a measure of success. AK-Syn.1 had been selected primarily from Arctic prior to this study. Averaged over 10 experiments where both were included (Table 3), AK-Syn.1 and Arctic averaged 61% and 48% winter survival, respectively.

In three tests where both were included, winter survival means of Matanuska white II and the original strain were 48% and 33%, respectively. Selections III and IV from Matanuska white had few direct comparisons with the original but showed generally improved survival (Table 3).

Selections II, III, and IV from the cultivar Cumino, a less winter-hardy original stock than Arctic or

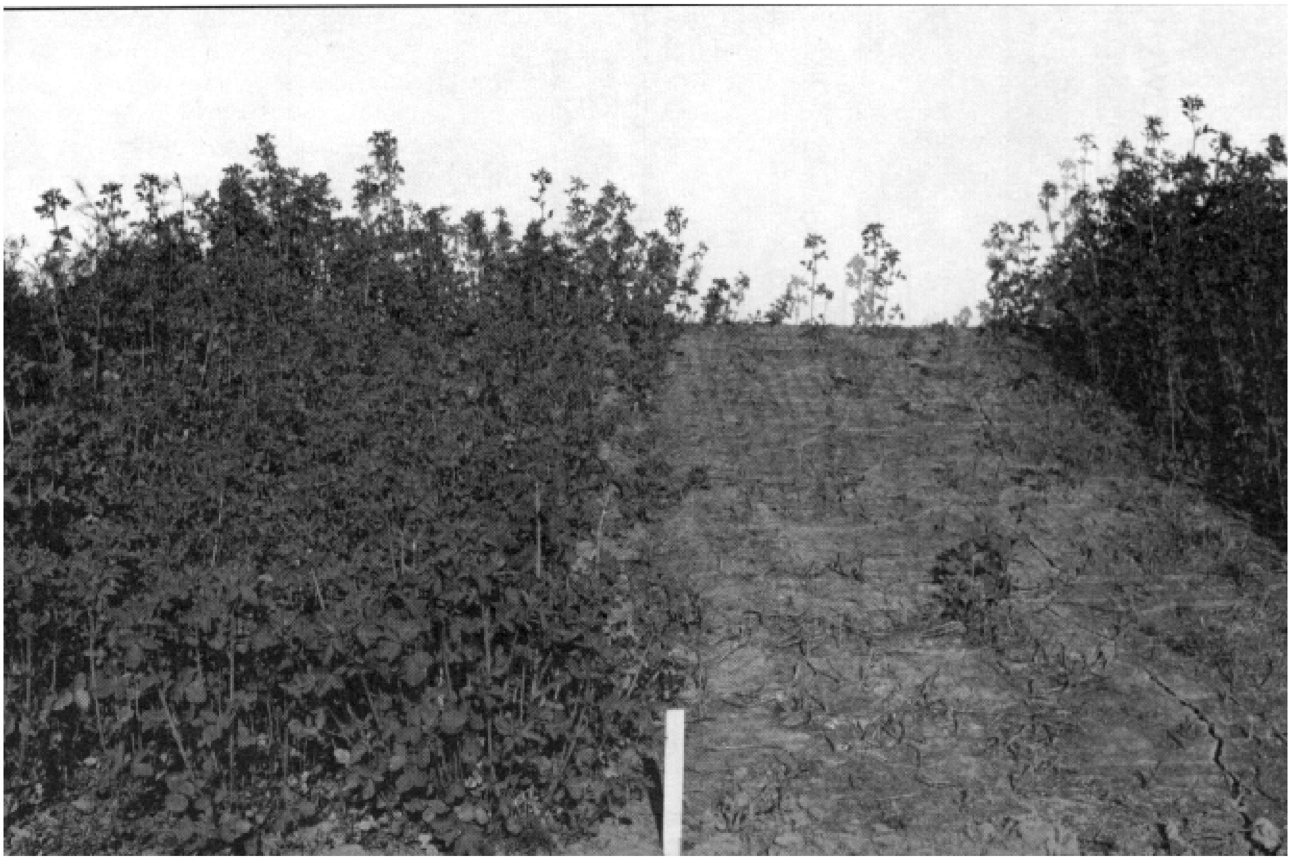

Figure 12. *Comparative winter survival of biennial white sweetclover strains illustrating effects of natural selection on winter hardiness. Left plot: Cumino III with 50% winter survival is a strain that underwent two generations of natural selection for improved winter survival from the original cultivar Cumino (plot to right of center) that had 1% winter survival in this experiment. Far right plot: Matanuska white III with 88% winter survival. Plots seeded 16 June of the previous year.*

Matanuska white, showed markedly improved winter survival. Five winter-survival comparisons of Cumino III and original Cumino (Table 3) averaged 39% and 3%, respectively (Table 3; Fig. 12).

These instances of enhanced winter survival through recovery of seed from surviving plants (= proliferation of adapted genotypes within the population coincident with elimination of poorly adapted genotypes) in sweetclover parallel the report of Goplen (1971) on the selection of the cultivar Yukon in Canada from survivors of severe winter kill in the more southern-adapted Madrid (also Klebesadel 1992). Similar improvement in winter hardiness through natural selection has been reported for alfalfa (Brand 1908; Klebesadel 1971b; Smith 1964b; Waldron 1912) and white clover (Klebesadel 1986; Sylven 1937).

Second-Year Forage Yields

Two-year means of first-cutting forage yields in the second year differed considerably among strains and much of the difference was due to differential winter survival (Table 2). Matanuska white, AK-Syn.1, and Arctic survived the winters best and produced significantly higher yields than the other strains. Yield rank of strains was Matanuska white = AK-Syn.1 > Arctic = Palmer yellow > Erector > Spanish = Madrid. The latter two, grown farthest from their latitude of adaptation, survived the winters very poorly and produced negligible yields. Mean crude protein concentration over all strains that produced appreciable yields was 17.8%.

CONCLUSIONS

Considerable adaptive modification is apparent in two long-resident biennial sweetclover populations that have persisted along Matanuska Valley roadsides; the extent of change is more pronounced in the *M. alba* than the *M. officinalis* population, perhaps due to earlier introduction and therefore a longer period of exposure to natural-selection effects. The observed modification in these populations is a shift back toward biennial habit, both morphologically and physiologically.

Current introductions of biennial sweetclover from more southern latitudes tend to behave much like annuals under the unaccustomed seasonal photoclimate at this latitude. Tall seedling-year growth followed by very poor winter survival precludes their use as biennials. However, these characteristics suit them as high producers of annual forage at this latitude.

If the short subarctic nyctoperiods that cause seedling-year flowering were accompanied by sufficiently long growing seasons to permit seed maturation, the introduced roadside biennials logically would behave as true annuals. Such conditions then would not have selected toward restoration of biennial habit. Inadequate growing-season duration for seed maturation during the seedling year consequently has placed a premium on natural selection for winter survival to permit seed production in the second year in order to perpetuate the introduced populations. These conditions therefore have selected toward a return to typical biennial habit involving plant adjustment genetically/physiologically to be in better harmony with the unusual photoperiod/nyctoperiod seasonal patterns at this northern latitude.

Other reports (Brand 1908; Cooper 1965; Goplen 1971; Klebesadel 1971b, 1986, 1992; Smith 1958, 1964b; Sylven 1937; Waldron 1912; Wilsie 1962) have noted genetic shifts or adaptive change in forage legumes as a result of natural selection pressures. Selective modification toward improved adaptation in a new environment perpetuates and proliferates within the ongoing population those genotypes best adapted (and therefore selectively favored toward reproduction) under the new environmental conditions (Mason and Stout 1954; Stebbins 1950; Wilsie 1962). The above-cited instances of natural selection occurred when the legumes were grown in a new environment that subjected them to unaccustomed seasonal climatic parameters, as with the *Melilotus* species reported here.

Cross-pollinated crops that have not been subjected to rigid artificial selection may undergo natural selection in one direction for many generations without exhausting the possibilities inherent in the supply of genetic variation present in a population (Clausen 1958; Stebbins 1950). The individual *Melilotus* introductions that gave rise to the Alaskan roadside colonies were no doubt unselected artificially prior to their introduction into this subarctic area many years ago. It is not surprising therefore that with their broad genetic base they possessed the capacity to evolve through natural selection, to the extent observed, toward adaptation to the unique aspects of the photoperiod/growing season relationships at this latitude.

The biennial habit of these *Melilotus* species results in a rapid cycling of generations which renders these species much more susceptible to natural selection in a given time than would occur with perennial species. The characteristically high percentage of hard seeds in *Melilotus* undoubtedly slows the turnover of generations to some extent, however.

It is unknown at this time whether the dormancy detected in this study, especially in the Matanuska white strain, is merely a localized growth inhibitor in the buds that prevents their development into stems until the second year. Alternatively, the dormancy may be symptomatic of a more generalized torpor of the entire overwintering system that serves to prevent dehardening during winter temperature fluctuations in the freeze-thaw range and thereby contributes to winter survival.

The fact that the dormancy is so pronounced in the Matanuska white strain that evolved where winter temperature fluctuations are very common, suggests that it may be a characteristic that contributes to overall winter hardiness. These questions should be resolved with future investigations that assess biochemical aspects of the dormancy phenomenon as it relates to winter hardiness.

The extent to which further adaptive change may be achieved in the introduced populations can be determined only by periodic future assessments. The extent of adaptive change in far northern occurrences of *Melilotus* in Canada (Turkington et al. 1978), and the length of residency of those populations, may be of interest also, and may parallel findings in this study.

Improved winter survival was achieved by harvesting seed from surviving second-year plants in stands that sustained severe winter kill. Improvement was most dramatic when pursued over several generations in a relatively southern-adapted cultivar. This strategy should be expanded to select within low-coumarin cultivars for good winter hardiness at subarctic latitudes.

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