Extreme Northern Acclimatization in Biennial Yellow Sweetclover *(Melilotus officinalis*) at the Arctic Circle

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SUMMARY

A population of yellow-flowered biennial sweetclover *(Melilotus officinalis* [L.] Lam.) persisted unattended for about 15 years in Fort Yukon, Alaska (66.6°N), just north of the Arctic Circle, before seed was collected and subsequently increased for testing at the Matanuska Research Farm (61.6°N) near Palmer, Alaska. This Arctic-Circle strain showed marked morphological and behavioral differences from more southern-adapted cultivars within the same species.

- With spring seeding, the Arctic-Circle strain produced shorter growth on smaller-diameter main stems by autumn of the seeding year, yielded less forage, and possessed more and larger crown buds than more southern-adapted cultivars.

- The Arctic-Circle strain initiated storage of food reserves earlier and, in late October, over-wintering root-crown-stem tissues were higher in dry-matter concentration, were less injured by freeze stress, and had assumed a greater degree of dormancy than the cultivars of more southern adaptation.

- The Arctic-Circle strain was vastly superior in winter survival to biennial yellow sweetclover from Canada (cultivars Yukon and Erector) and the conterminous states (cultivars Goldtop and Madrid).

- The several characteristics exhibited by the strain that evolved at Fort Yukon resemble those of typical biennial sweetclover when grown where it is adapted. In contrast, the behavior of mid-temperate-adapted biennial cultivars, when grown in Alaska far north of their latitude of adaptation, resembled annual sweetclover.

- The obligate biennialism and therefore rapid generational cycling of this species apparently facilitated rapid selective modification toward subarctic adaptation. That adaptation, involving a shift toward enhanced winter hardiness, permitted seed maturation during the second year of growth, thus perpetuating the adventive population for continued selection.

- Natural selection within the introduced population enabled it to regain biennial habit in an area of extremely long summer photoperiods (short nyctoperiods), relatively early termination of growing season, and very low winter temperatures.

- The Arctic-Circle strain of biennial yellow sweetclover may serve as the basis for a subarctic-adapted cultivar useful in Alaska and in other northern areas of the world.

- These results contribute to a better understanding of natural selection effects in the adaptive modification of crop plants and in the evolution of latitudinal ecotypes.

- These findings also provide insights into mechanisms that condition winter survival of plants in the Subarctic.

INTRODUCTION

Biennial sweetclovers (*Melilotus* spp.) are among the most winter-hardy of the legumes used as forage crops (Bula and Smith 1954; Gorz and Smith 1973; Hodgson and Bula 1956; Klebesadel 1971b, 1980). Common strains and cultivars of both yellow-flowered *(M. officinalis* [L.] Lam.*)* and white-flowered *(M. alba* Desr.*)* species are grown extensively in the Middle West and Great Plains areas of the United States (Gorz and Smith 1973), and the prairie provinces of Canada (Greenshields 1957). However, due to inadequate winter hardiness in Alaska of strains currently available (Hodgson and Bula 1956; Irwin 1945; Klebesadel 1971b, 1980), sweetclovers from other areas are not dependable for use as biennials in southcentral Alaska.

Adaptive Modification

Several accounts from more southern latitudes (Brand 1908; Smith 1958, 1964; Smith et al. 1986; Waldron 1912) report genetic modification conferring enhanced winter hardiness in alfalfa *(Medicago sativa* L.*)* through natural selection when relatively non-hardy strains were grown for a time in areas of more severe winter stresses. Considerable progress also has been made in adapting alfalfa to a subarctic environment (Klebesadel 1971b).

Little is known concerning the potential for modifying temperate-adapted sweetclover toward improved winter hardiness in subarctic regions. Goplen (1971) , working near 53.4°N in Saskatchewan, described selective modification within the more southern-adapted biennial yellow cultivar Madrid for increased winter survival there. Madrid originated from near 40°N in Spain, and has been grown within a few degrees of that latitude for over 50 years in the United States. However, initial plantings in Saskatchewan, considerably north of its accustomed latitudes, sustained severe winter kill (Goplen 1971). Seed produced by surviving plants resulted in a more winter-hardy, "naturalized" strain of Madrid, well adapted to western Canada. That strain was released as the cultivar Yukon in 1970 (Goplen 1971). Yukon averaged 6% winter kill over six station years in uniform regional sweetclover tests in western Canada when cultivars Erector and Madrid sustained 13% and 41% winter kill, respectively.

Discovery of Far-Northern Sweetclover Population

A gardener living in the community of Fort Yukon, Alaska (66.6°N) grew biennial yellow sweetclover as a green manure crop until his death in 1948. Thereafter, the garden was left untended and the sweetclover persisted without further disturbance (Fig. 1). Seed collected from the stand in 1963 was planted for evaluation at the Matanuska Research Farm (61.6°N) near Palmer in southcentral Alaska (Fig. 2). Evidence of superior winter survival in this material prompted two additional seed collections at Fort Yukon and subsequent seed increases at the Matanuska Research Farm.

Fort Yukon, located about six miles north of the Arctic Circle (Fig. 2), is 445 feet above sea level and is situated at the confluence of the Yukon and Porcupine Rivers (Fig. 3). Although only about 240 miles from the Arctic Ocean, the area experiences some of the warmest summer temperatures in the state (record high is 100°F), as well as the coldest in winter (record low is -78°F) (USDA 1941). July average is 61.2°F; January average is -21.6°F. Average date of last 32°F in spring is 26 May; first occurrence in late summer is 24 August, for a normal frost-free period of only 90 days. Extremely long mid-summer photoperiods compensate considerably for the short growing season; sunlight is continuous there for approximately 30 days (7 June to 7 July).

Fort Yukon is an isolated community not connected to the road system of Alaska (Fig. 3). It is situated near the center of a large basin consisting of interior forest and other native vegetation but with no agricultural development. Therefore, the garden plot of sweetclover was totally isolated from any possibility of interpollination with other sweetclover populations.

Precise origin of the *M. officinalis* seed lot used by the Fort Yukon gardener cannot be determined. His widow believed that it was ordered from a seed catalog, which suggests that it was common yellow stock from a source in the conterminous states.

This Investigation

The objective of this study was to determine the extent of differences in morphology, hardening behavior, winter hardiness, and other agronomic characteristics between the sweetclover from Fort Yukon (hereinafter called "Arctic-Circle strain") and other available cultivars within the same species from Canada and the conterminous United States. All tests reported here were conducted at the University of Alaska's Matanuska Research Farm (61.6°N) in the Matanuska Valley of southcentral Alaska.

EXPERIMENTAL PROCEDURES

All plantings were made in the field without companion crops in Knik silt loam (coarse-silty over sandy or sandy-skeletal, mixed, nonacid Typic Cryochrept). All sites had good surface drainage, and pH range was 5.8 to 6.2. Commercial fertilizer disked into each seedbed supplied nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) at 32, 128, and 64 lbs/acre, respectively. *Rhizobium* bacterial inoculant was mixed with each seed lot just prior to planting.

All plantings utilized randomized complete block experimental designs with two to six replications; plantings were made on dates indicated in Table 1 or stated elsewhere. All row seedings were 18 inches apart and rows were from 22 to 36 feet long in various experiments. When seedlings in rows were 1 to 2 inches tall, they were thinned by hand to leave individual plants 4 to 6 inches apart. Broadcast-seeded plots measured 5 x 16 feet. Winter survival counts were made in spring when living plants had initiated growth. In broadcast-seeded plots, a steel-rod grid measuring 3 x 8 feet, divided into one-foot squares, was centered in each plot and all enclosed living and dead plants were counted.

With all forage harvests from broadcast-seeded plots, yields were derived from a swath 2.5 feet wide mowed from the centerline of each plot after a 1.25-foot strip was mowed and discarded from both ends of all plots to remove border effects. Mowing was done with a sickle-equipped plot mower leaving approximately a two-inch stubble. With all harvests of forage and etiolated growth, samples were dried to constant weight at 140°F. All yields are reported on the oven-dry basis.

On 16 October 1971, typical plants of the Arctic-Circle strain, Erector, Yukon, and Madrid were dug from plots seeded 16 June 1971. All aerial growth one inch above the cotyledonary node, all root growth two inches below that node, and all remaining lateral roots were severed and discarded. Remaining stem-crown-taproot segments were washed and photographed to show morphological differences.

Stored food reserves, dry-matter concentration in over-wintering tissues, and tolerance to artificial freeze stress were determined in late summer and autumn of 1964 and 1965 with plants from plots broadcast-seeded 30 May 1964 and 16 June 1965. Plants dug 6 October and 19 October in 1964, and 1 October and 19 October 1965, were prepared for freeze-tolerance treatments as described earlier (Bula et al. 1956). Electro-conductivity readings were made on the liquid, in which plants were immersed, both after freezing (14°F for 20 hours)

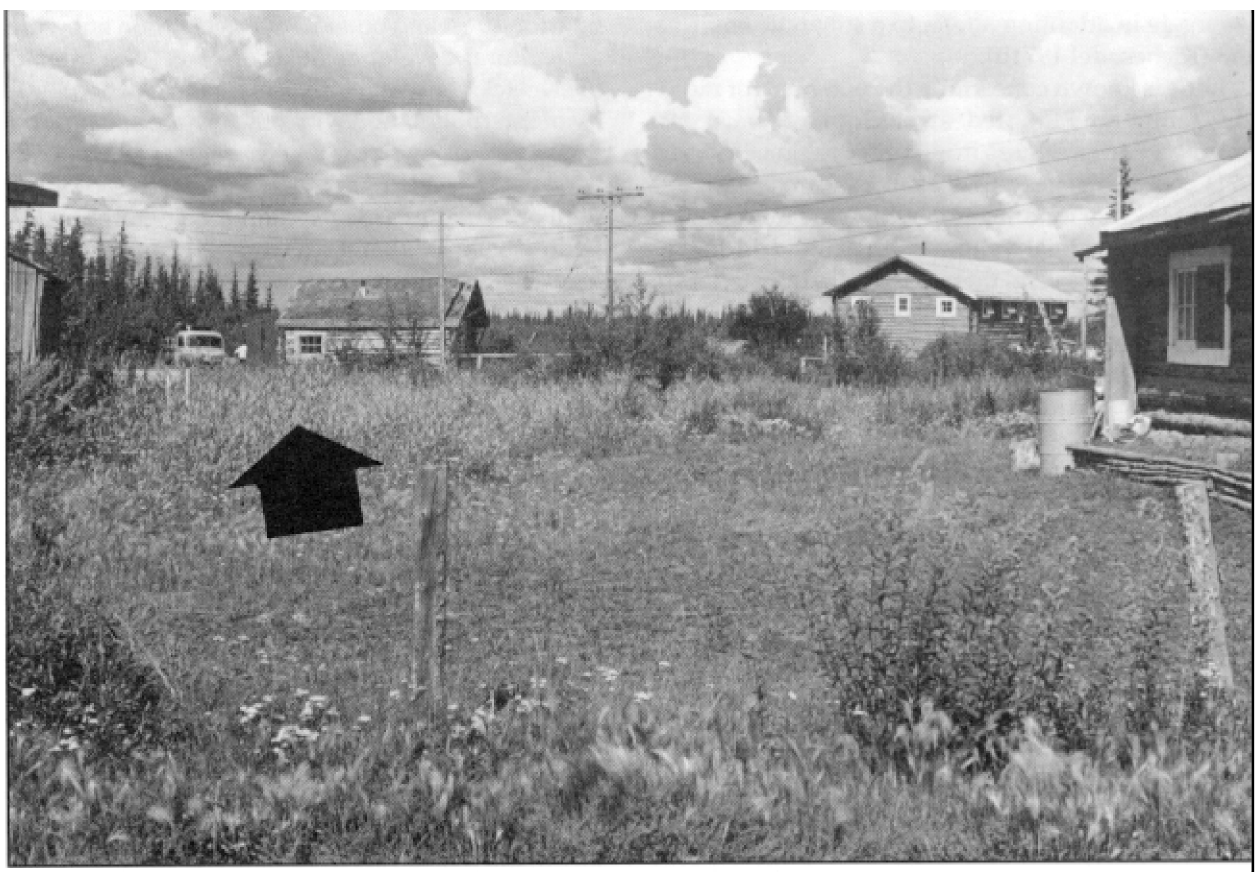

Figure 1. *Home yard in the community of Fort Yukon, AK, just north of the Arctic Circle, showing stand of biennial yellow sweetclover (arrow) that persisted unattended for about 15 years before seed was collected for experimental study described in this report.*

and after boiling, to permit calculation (from determinations of specific conductance x 10^{-6}) of freezing injury as a percent of total injury possible to plant tissues as induced by boiling (Dexter 1956; Hodgson 1964).

To measure stored food reserves (expressed as etiolated growth in darkness, Graber et al. 1927), and to determine dry-matter concentration in overwintering tissues, plants were dug 19 August and 20 October in 1964, and 27 August and 19 October in 1965. Plant preparation, potting, and etiolated-growth harvests were done as described earlier for alfalfa (Klebesadel 1971b), except that harvests of sweetclover etiolated growth were made at three-week intervals.

Results and Discussion

Diurnal light/dark influences on sweetclover behavior and morphology are discussed here only in terms of the daylight component, or photoperiod, since that has been the custom followed in most earlier reports and discussion is therefore simplified. It is recognized, however, that the concomitant duration of daily darkness, or nyctoperiod (Klebesadel 1971a), is, more often than recognized, the dominant influence (Kasperbauer et al. 1962, 1963).

Field tests conducted over several years revealed that there were considerable differences between the Arctic-Circle strain and other cultivars of biennial yellow sweetclover when grown here.

Winter Survival

Winter survival of the Arctic-Circle strain from Fort Yukon was consistently and markedly superior to that of cultivars of the same species adapted to more southern latitudes in numerous field tests (Tables 1 and 2; Figs. 4 and 5). In eight field tests involving broadcast seedings or spaced plants in rows, the Arctic-Circle strain averaged 72% winter survival, while Erector and Madrid averaged 6% and 1%, respectively (Table 1). Field sites differ in exposure of plants to winter stresses, and severity of individual winters in the Matanuska Valley differs markedly (Klebesadel 1974). Winter survival of the Arctic-Circle strain in the eight tests ranged from 38% to 96%. The cultivars Yukon and Goldtop were not included in all tests, but their winter survival was poor and generally similar to that of Erector and Madrid. The superior winter hardiness here of the Arctic-Circle strain is of considerable significance because of the paucity of forage legumes adequately winter-hardy for use in this subarctic area

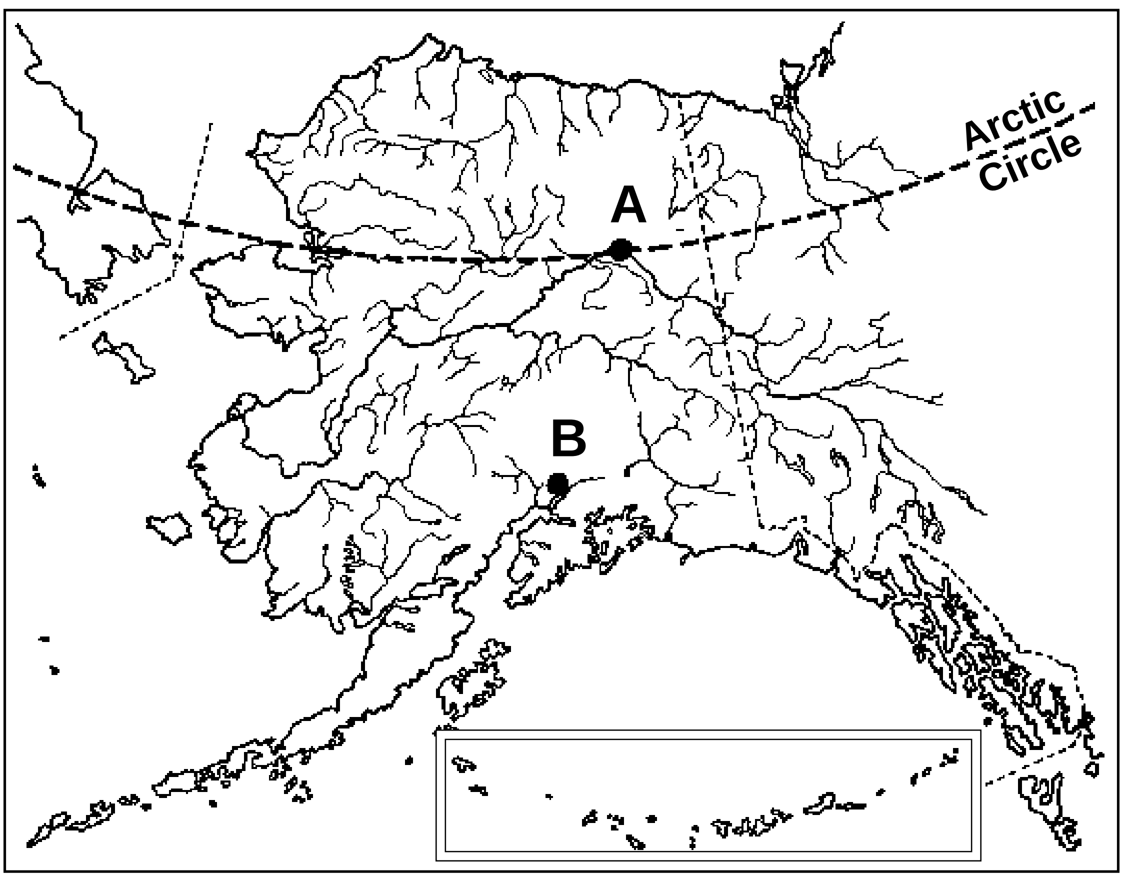

Figure 2. Map of Alaska showing locations of (A) Fort Yukon (66°34′ N) and (B) the Matanuska Research Farm (61°34′ N) where field tests were conducted; locations are approximately 365 miles apart.

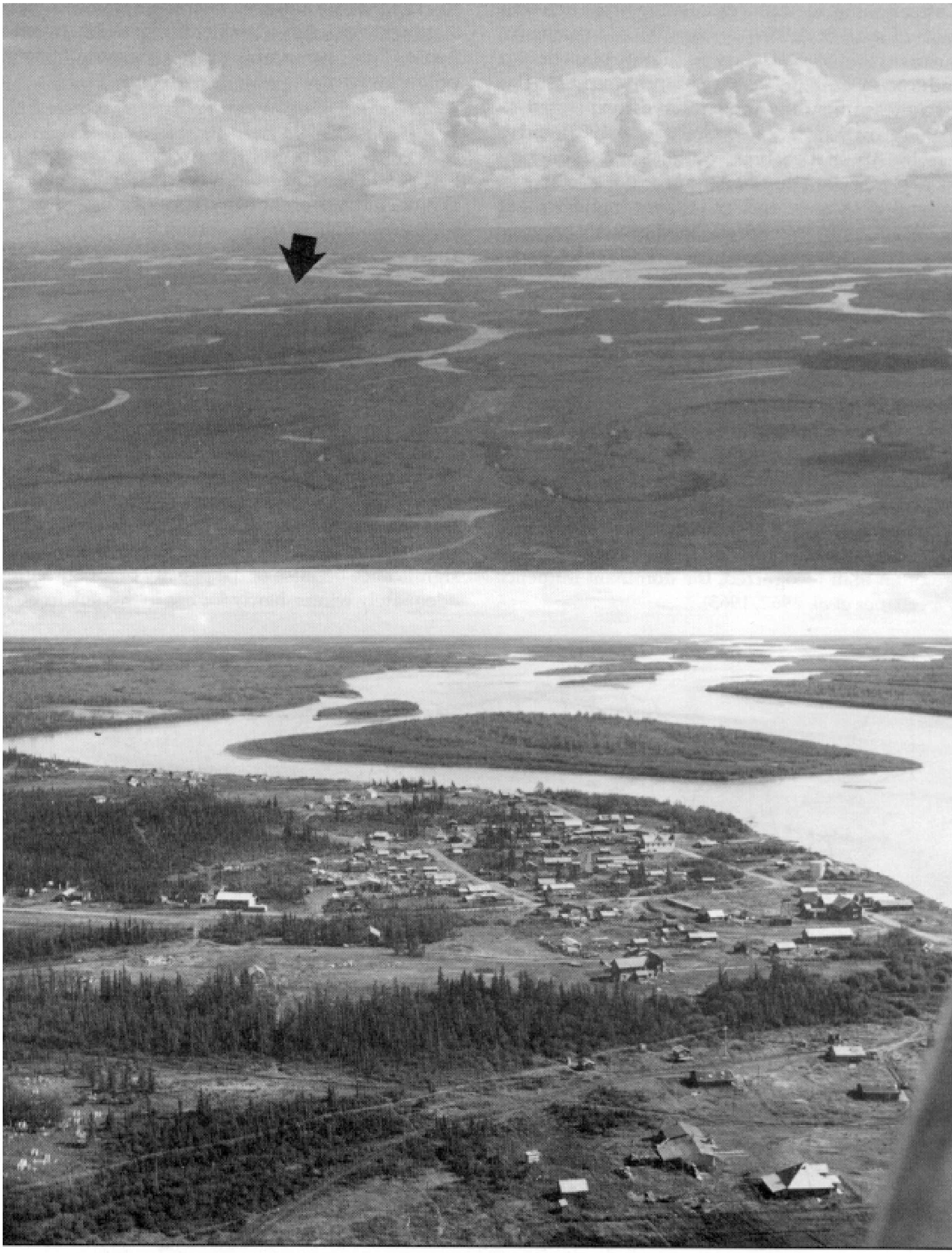

Figure 3. *(Upper) Aerial view of location of Fort Yukon at confluence of Porcupine and Yukon Rivers, showing remoteness of the non-agricultural community which is accessible only by aircraft or river travel. (Lower) Close-up aerial view of Fort Yukon and Yukon River; sweetclover stand is among homes near center of photo.*

Table 1. *Comparative winter survival of Arctic-Circle strain and other North American cultivars of biennial yellow sweetclover in eight experiments that involved six different winters at the Matanuska Research Farm (61.6 °N).*

		Years of tests [1]							
Origin and strain	Latitude of origin	1966-67	1969-70	1971-72(a)	1971-72(b)	1973-74	1974-75	1980-81	Mean
	°N. Lat.	- - - - - -	- - - - - -	- - - - - -	- - - - - -	Percent winter survival	- - - - - -	- - - - - -	- - - - - -
ALASKA:									
Arctic-Circle strain	66.6	78	65	94	45	87	96	38	72
CANADA:									
Yukon	52.1	—[2]	—	2	0	0	40	1	#[3]
Erector	49.8	6	5	6	0	0	27	0	6
CONTERMINOUS U.S.:									
Goldtop	43.1	0	—	—	—	0	0	0	#
Madrid	40.5	4	1	1	0	0	4	0	1

[1] 1966-67: Drilled rows 16 ft. long, four replicates, planted 9 June 1966.
1969-70: Spaced plants in rows 22 ft. long, means of two tests, six replicates per test, planted 2 June 1969.
1971-72(a): Broadcast plots 5 x 15 ft., intermediate winter exposure, three replicates, planted 16 June 1971.
1971-72(b): Broadcast plots 5 x 15 ft., maximum winter exposure, four replicates, planted 17 June 1971.
1973-74: Spaced plants in rows 36 ft. long, five replicates, planted 28 June 1973.
1974-75: Spaced plants in rows 36 ft. long, two replicates, planted 10 June 1974.
1980-81: Spaced plants in rows 35 ft. long, four replicates, planted 20 June, 1980.

[2] Not included in test.

[3] Not planted in all tests so mean not calculated.

Table 2. *Two-test means of seeding-year forage yields and subsequent winter survival of four biennial yellow sweetclover strains of diverse latitudinal origin in broadcast-seeded plots without companion crop at the Matanuska Research Farm (61.6 °N). Mean planting date 16 June, mean date of seeding-year height measurements and forage harvest 8 October; values shown are means of three and four-replicate tests. Forage yields on oven-dry basis.*

Strain or cultivar	Latitude of origin	Plant height	Seeding-year forage yield	Winter survival	Second-year first-cutting forage yield[1]
	°N. Lat.	Inches	Tons/acre	Percent	Tons/acre
Arctic-Circle strain	66.6	12-16	0.46	70	1.17
Yukon	52.1	28-32	1.69	1	0.03
Erector	49.8	24-30	1.22	3	0.07
Madrid	40.5	30-36	1.84	1	0.02

[1]Data from one test; in other test the three introduced cultivars winter-killed 100% so Arctic-Circle strain was left for seed production.

(Hodgson 1964; Hodgson and Bula 1956; Irwin 1945; Klebesadel 1971b, 1980).

Winter temperatures in North Dakota, Minnesota, and Wisconsin (USDA 1941) where cultivars such as Madrid and Goldtop survive winters well, are as severe or more so than temperatures in southcentral Alaska where those cultivars do not survive. Evidence from other studies at this location (Hodgson 1964; Klebesadel 1971a, 1985b) and elsewhere (Moschkov 1935; Pohjakallio 1961) indicates that failure of the more southern-adapted cultivars to survive winters at northern latitudes is due in considerable measure to unaccustomed climatic influences prior to onset of winter, precluding adequate preparation for winter, rather than to the effects of winter stresses alone.

Temperate-adapted forages are unable to develop maximum, timely winter hardening under the relatively longer photoperiods occurring during the weeks prior to freeze-up here; hence they fail to survive the winters (Hodgson 1964; Klebesadel 1971a, 1985b). Smith (1942) found that by subjecting sweetclover grown at mid-temperate latitudes of Ohio to lengthened photoperiods prior to winter, almost 100% of the plants failed to survive the winter (paralleling field experience in Alaska). In contrast, no winter killing was evident in Ohio in plants exposed to normally occurring autumn photoperiods there. Conversely, several studies conducted in the Subarctic have provided artifically shortened photoperiods during several weeks prior to the onset of winter to create conditions resembling those in the area of origin of mid-temperate-adapted plants. This causes plants grown far north of their accustomed environment to develop greater freeze tolerance (Hodgson 1964) and to survive northern winters markedly better (Klebesadel 1971a; Moschkov 1935; Pohjakallio 1961).

Natural selection within the introduced yellow sweetclover population at Fort Yukon undoubtedly favored genotypes that initiate earlier preparation for winter under longer photoperiods. The few plants that survive, when winter kill of exotic cultivars is extensive in northern latitudes, provide the base for the initiation of such selection.

After the superior winter hardiness in the Arctic-Circle strain was recognized, further tests were conducted to attempt to relate hardiness differences to certain morphological and physiological characteristics and to latitude of adaptation. Although only a few cultivars of *M. officinalis* exist, they originate from a range of different latitudes (Table 1).

Plant Height and Forage Yields

Seeding-year growth of the Arctic-Circle strain was considerably shorter than the more southern-adapted cultivars (Fig. 5; Tables 2 and 3) and, consequently, it produced the lowest seeding-year forage yields. Conversely, the southernmost-adapted Goldtop and Madrid, farthest-removed from accustomed photoperiodic conditions, grew tallest and produced the highest forage yields. Yukon and Erector, of intermediate latitudinal adaptation, were generally intermediate in plant height and forage yield.

Kasperbauer et al. (1962) and Smith (1942), using supplemental lighting on seedling plants at Midwest locations, found that biennial sweetclovers grew tall and behaved as annuals with 16- to 20-hour photoperiods. Maximum photoperiods at Palmer, AK, in June are 19.5 hours (when nyctoperiods are 4.5 hours including considerable twilight). These are conditions that foster tall growth and high seeding-year forage yields in mid-temperate-adapted cultivars grown here.

In contrast to low seeding-year forage yields, the

Table 3. *Two-year means of seeding-year forage yields of four biennial yellow sweetclover strains of diverse latitudinal adaptation, seeded in rows at the Matanuska Research Farm. Mean planting date 1 June, mean harvest date 25 September. Forage yields on oven-dry basis.*

Strain or cultivar	Latitude of origin	Plant height [1]	Forage yield
	°N Lat.	Inches	Tons/acre
Arctic-Circle strain	66.6	42-44	1.51
Erector	49.8	52-54	1.61
Goldtop	43.1	52-54	2.49
Madrid	40.5	54-56	2.48

[1] Heights recorded one year only.

Arctic-Circle strain always greatly surpassed the introduced cultivars in second-year forage yields. This was due to its superior winter survival compared to the sparse to non-existent second-year stands of the non-hardy introduced cultivars (Tables 1 and 2; Figs. 4 and 5).

Crown Morphology

In October of the seeding year, the more southern-adapted Madrid, Yukon, and Erector had large-diameter main stems, poorly developed crowns, and fewer and much smaller crown buds (Fig. 6), all characteristics resembling annual sweetclover (Klebesadel 1992;

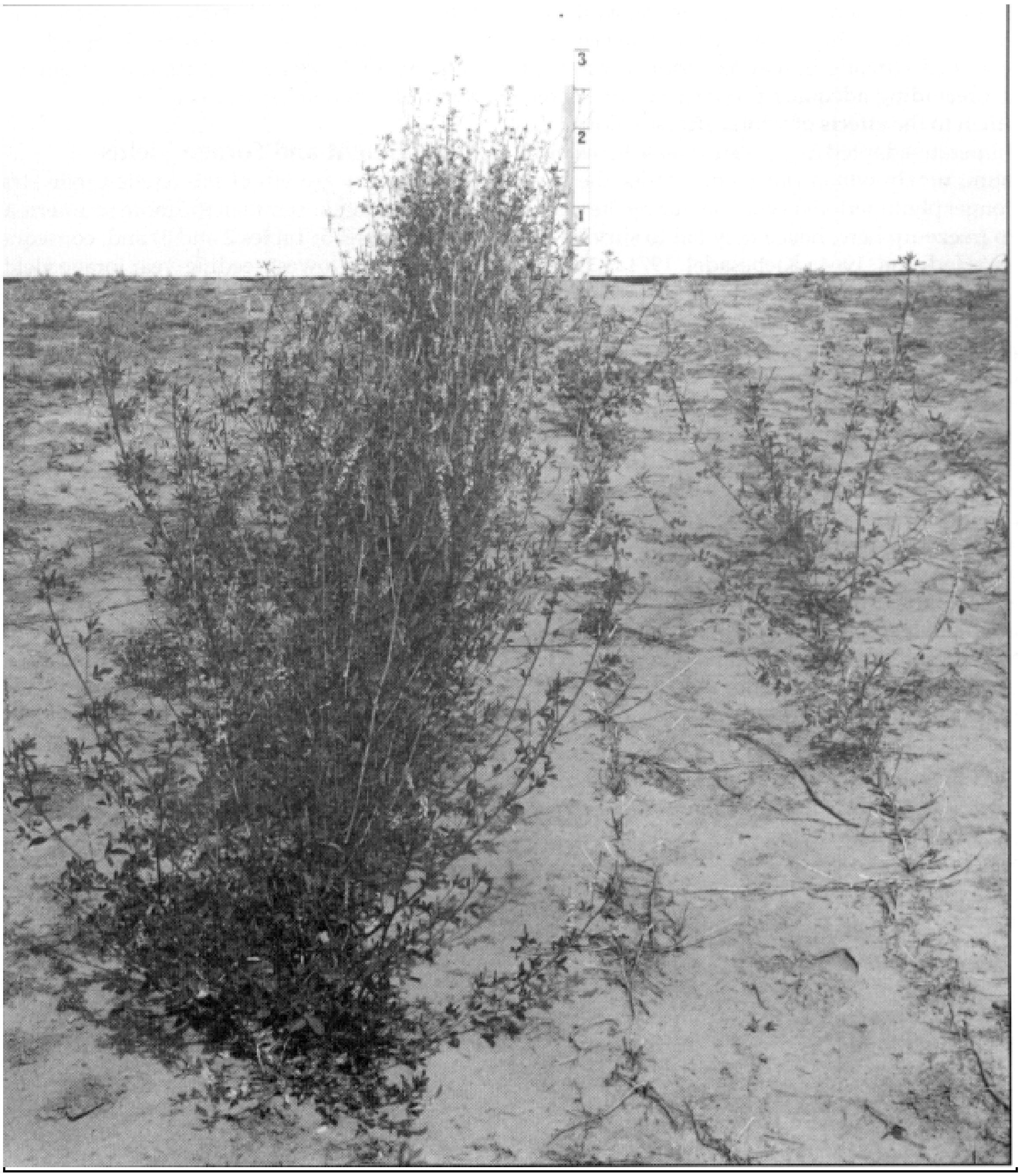

Figure 4. Comparative winter survival of three strains of biennial yellow sweetclover as spaced plants in rows (left to right): Arctic-Circle strain, Madrid, Erector (winter survival in test: 63%, 1%, 7%, respectively). Numbered stake at end of Madrid row is three feet tall.

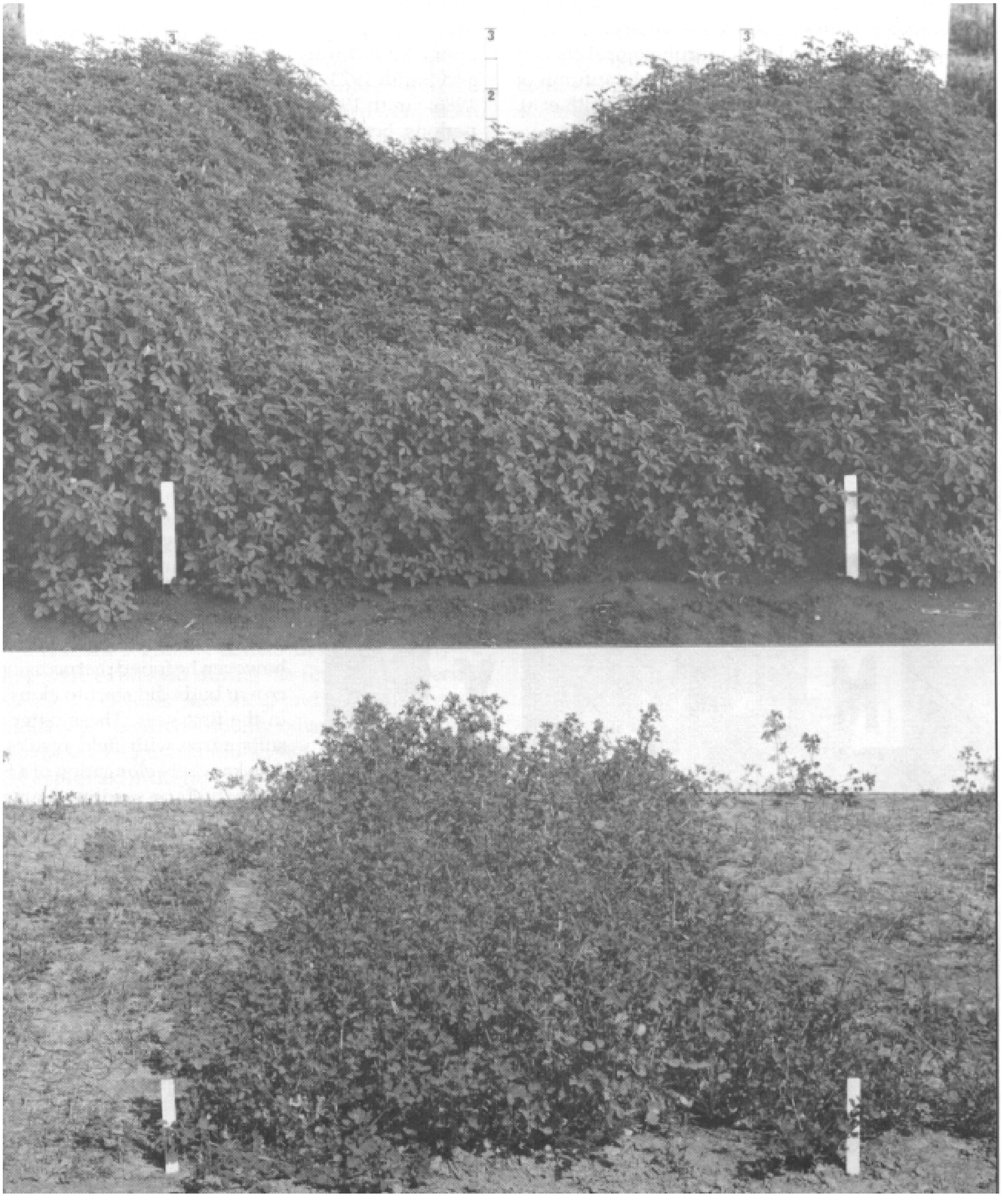

Figure 5. *(Upper) Seeding-year growth in broadcast-seeded plots of three biennial yellow sweetclover strains at the Matanuska Research Farm (left to right) Yukon, Arctic-Circle strain, Erector. Planted 16 June, photo 23 September, plots were harvested 8 October. Numbered stakes are 3 feet tall. (Lower) The same plots the following spring with winter survival (left to right) of 4%, 96%, and 6%.*

Smith 1942). In contrast, the Arctic-Circle strain had small-diameter main stems, well developed crowns, and several large crown buds, morphological characteristics typical of biennial sweetclover in autumn of the seeding year (Kasperbauer et al. 1962; Smith et al. 1986; Smith 1942).

Development of crown buds in autumn of the seeding year is important in biennial sweetclover, as they overwinter and elongate to become the second-year aerial stems (Smith et al. 1986; Smith 1942; Smith and Gorz 1965). Under field conditions in the U.S. Midwest, biennial sweetclover plants produce several crown buds during autumn of the seeding year (Gorz and Smith 1973; Kasperbauer et al. 1962; Smith et al. 1986; Smith 1942). Under artificially extended photoperiods, however, Smith (1942) found that no crown buds, or only one, developed per plant. Kasperbauer et al. (1962) determined that the shortening photoperiods during autumn were responsible for the development of crown buds. Exposure of the mid-temperate-adapted *M. officinalis* cultivars to the very long mid-summer photoperiods at this latitude apparently promoted non-typical, tall aerial growth on large-diameter stems in the seeding year and suppressed crown bud development, causing those cultivars to develop much like annuals.

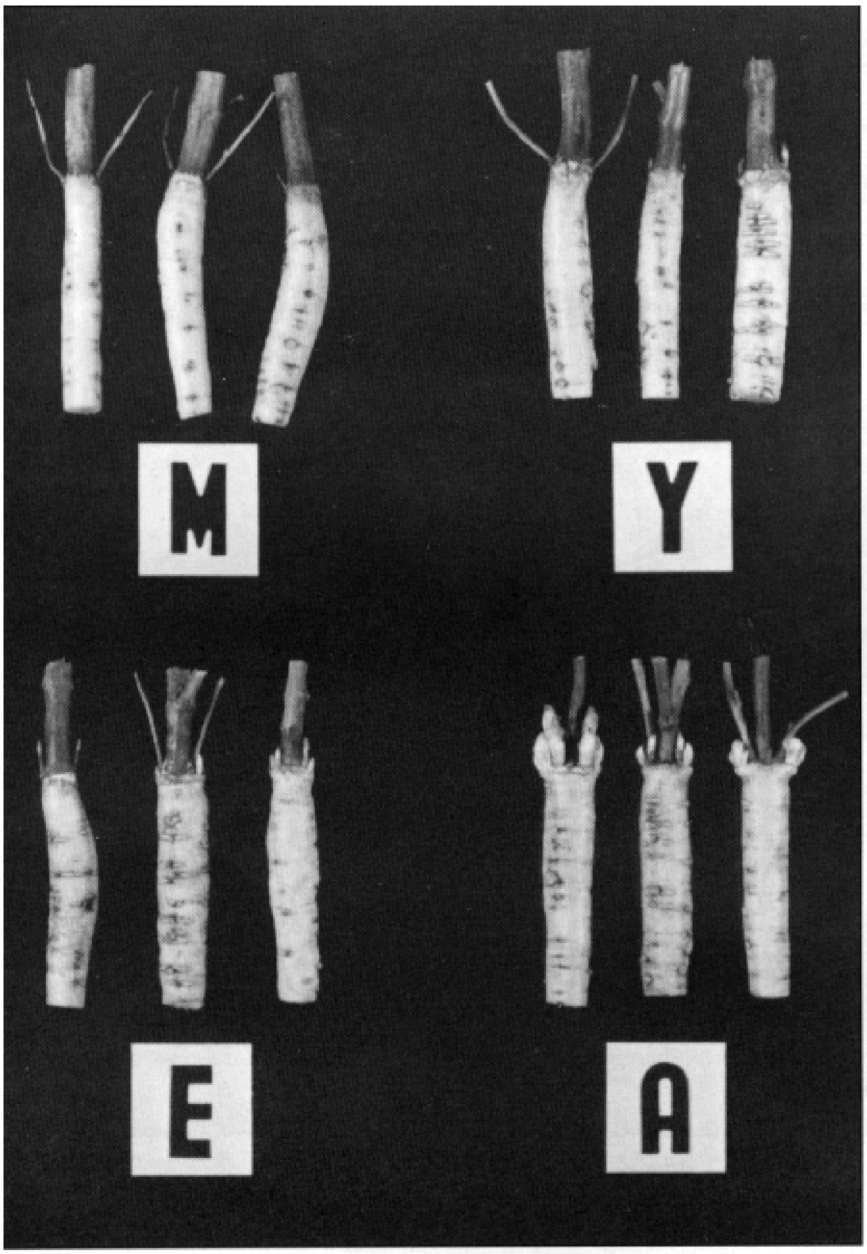

Figure 6. *Typical stem-crown-taproot segments (lateral roots excised) of four biennial yellow sweetclover strains seeded 16 June at the Matanuska Research Farm and photographed 16 October. M = Madrid, Y = Yukon, E = Erector, A = Arctic-Circle strain. Note mainstem:root diameters, development of crown buds.*

Smith (1942) in the Midwest noted that sweetclover crown buds rarely start elongation growth during the seeding year; mid-summer photoperiods there are 16 to 17 hours long. With artificially extended photoperiods, however, he found that occasional crown buds did start to elongate in the first year. These latter results agree with field results in Alaska where elongation of a few crown buds on seedling plants of sweetclover is common (Fig. 6) under subarctic conditions of naturally occurring, relatively long summer photoperiods. Where tall, dense, aerial growth of plants imposes heavy shading, these buds elongate into non-vigorous tillers that frequently shrivel and die (Fig. 6, M). Where plants are open-grown or where the aerial canopy is less dense, those branches remain healthy, but less vigorous than the primary stem (Fig. 6, A). The practical significance of crown-bud elongation during the seeding year is that it eliminates potential growth sites that ordinarily would not become active until the following year. This could be especially disadvantageous for plants that develop very few crown buds.

Stored Food Reserves

On 23 August, the earliest date of sampling, the Arctic-Circle strain showed much higher levels of stored food reserves than the more southern-adapted Erector and Madrid (Fig. 7). The latter two were about equal, and their stored food reserves were less than one-fourth those of the Arctic-Circle strain. At the final sampling two months later (after killing frost and about the time of soil freeze-up), Erector, adapted to intermediate latitudes, had markedly increased food reserves, apparently about equaling the Arctic-Circle strain. Stored reserves in the southernmost-adapted Madrid nearly doubled between the two sampling dates, but were only about one-third those of Erector and the Alaska strain.

Between the late August and late October samplings, reserve food storage in the Arctic-Circle strain apparently increased only about 25%. However, a considerable change was noted in the rate of expression of those reserves. Only two three-week periods in darkness were required to totally exhaust the food reserves after the first sampling in late August. During the first three-week growth period in darkness in late August, the Alaska strain expressed 54 oven-dry milligrams (mg) etiolated growth per oven-dry gram (g) of root-crown segments potted. In contrast, only 32 mg/g were expressed during the first growth period after the 21 October sampling, and four three-week periods were required to totally exhaust food reserves. With the late August sampling, 98% of stored reserves were expressed in the first growth period and 2% in the second. With the October sampling, percents of total reserves expressed in the four growth periods were 46%, 23%, 19%, and 12%. Comparative percents of reserves expressed by the more southern-adapted cultivars during the same growth periods followed different patterns; for Erector, percents were 61%, 24%, 10%, and 5%, and for Madrid, they were 78%, 18%, and 4%.

In an earlier study (Klebesadel 1971b) with alfalfas at this location, a subarctic strain that expressed food reserves slowly on a 78%, 16%, 5%, 1% pattern over four growth periods in darkness averaged 68% winter survival, while temperate-adapted Vernal, which expressed reserves more rapidly on a 91%, 9% pattern, survived at only 49%. In the same report, subarctic-adapted *M. falcata* L., that expressed reserves relatively slowly during six consecutive and equal growth periods on a pattern of 74%, 15%, 7%, 2%, 1%, 1%, averaged 83% winter survival, while two alfalfa strains that expressed reserves more rapidly, on a pattern of 82%, 13%, 4%, 1%, averaged only 14% winter survival.

The considerably altered pattern of slower expression of reserves by the Arctic-Circle strain following sampling in October may indicate onset of a state of dormancy in that strain, a characteristic contributing to superior adaptation and enhanced winter survival in the subarctic. That dormancy, or state of rest, precluding rapid expression of stored food reserves, becomes evident in subarctic-adapted strains just prior to onset of winter; that dormancy state can be erased by exposure of plants to a sequence of artificially applied freeze-thaw treatments and it disappears routinely during the course of winter (Klebesadel 1992).

The total levels of stored food reserves expressed as etiolated growth by the Arctic-Circle strain appear to be not greatly different in the 23 August and 21 October samplings (Fig. 7). However, Graber et al. (1927) recognized that the etiolated-growth technique for determining stored food reserves fails to measure significant amounts of gaseous respiration products lost during the test periods. Respiratory breakdown of stored food reserves probably proceeds rapidly under the warm conditions provided for expression of etiolated growth. Therefore the total levels of stored food reserves measured in October, which required 84 days to reach exhaustion (in the Arctic-Circle strain and Erector), undoubtedly were more conservative measures than those made in August; in the August determination, most stored food reserves were expressed in

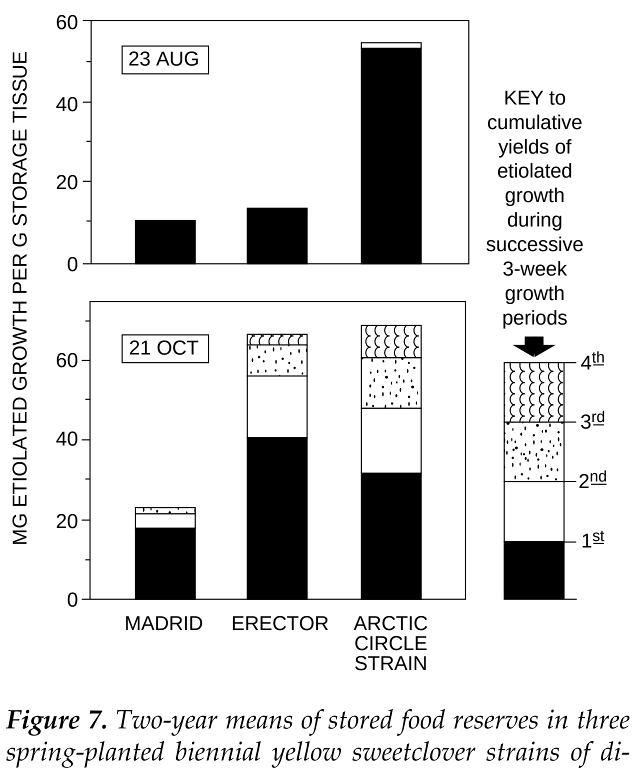

Figure 7. Two-year means of stored food reserves in three spring-planted biennial yellow sweetclover strains of diverse latitudinal adaptation, as measured by etiolated growth in darkness harvested at three-week intervals until exhaustion, from root-crown segments removed from field (upper) in late August and (lower) in late October; all weights on oven-dry basis.

only 21 days, permitting much less time for loss of unmeasured respiratory products.

Smith (1964) and Smith et al. (1986) note that carbohydrates stored in over-wintering plant parts provide the energy used by the plant in developing hardiness, in living over winter, and in beginning growth in spring. Plants with high carbohydrate reserves do not necessarily develop a high degree of hardiness, but plants low in reserves cannot develop a high level of hardiness.

Smith (1942) found that roots of July-seeded *M. officinalis* contained 30.4% starch (51.6% total carbohydrates) in late October when grown under Ohio's normally shortening photoperiods (average 12.6 hours), but only 11.9% starch (27.2% total carbohydrates) when grown for the 12 weeks prior to 23 October under artificially extended photoperiods of 16 hours. This indicates that exposure to an accustomed pattern of shortening photoperiods is necessary for storage of desirable high levels of carbohydrate reserves prior to winter. In the Ohio study, plants that were exposed to extended photoperiods and failed to store high levels of carbohydrates winter-killed at a rate of virtually 100%, while no winter killing was evident in plants that had grown under normal Ohio photoperiods and stored high levels of carbohydrates.

In contrast to the present results with sweetclover, Bula et al. (1956) in Alaska found that chemically determined total available carbohydrate (TAC) levels were higher in late August, and throughout the subsequent winter-hardening period, in non-hardy "Arizona Common" alfalfa than in hardier Ranger or in very winter-hardy, subarctic-adapted *M. falcata.* In comparing several biennial sweetclover strains here, Hodgson and Bula (1956) found some inconsistencies between late-October levels of TAC and subsequent winter survival. Nonetheless, the non-hardy Madrid was consistently lower in TAC than the more winter-hardy, more northern-adapted Erector, paralleling present results of reserves measured by etiolated growth.

Dry-Matter Concentration in Over-wintering Tissues

Root-crown tissues in each of the three biennial yellow sweetclover strains compared were below 20% dry matter at the first sampling on 23 August (Fig. 8). Thereafter, dry-matter concentration in overwintering tissues increased rapidly in all three strains from late August to 21 October. The increases were much greater with all three strains during the 31-day period between the first two sampling dates than during the 28-day period between the last two sampling dates. Total increase in dry-matter concentration between first and last sampling dates were: Arctic-Circle strain 82%, Erector 56%, and Madrid 48%.

Numerous investigators have noted that winter injury is greater in plants with high levels of tissue moisture (Steponkus 1978). Metcalf et al. (1970) found a close relationship between moisture content in winter cereal plant crowns and injury from freezing at different temperatures; greatest injury occurred with highest crown moisture (lowest dry-matter concentration). They found also that small differences in crown moisture percent at a specific freezing temperature resulted in large differences in freezing injury.

Results with sweetclover in the present study parallel the above findings. The Arctic-Circle strain, superior in winter survival, had the highest dry-matter concentration in overwintering tissues at the final sampling date (21 October), which was about the time of soil freeze-up and near onset of winter. The non-hardy Madrid was lowest in dry-matter concentration while Erector, generally poor but intermediate in winter survival, was intermediate in dry-matter concentration in root-crown tissues.

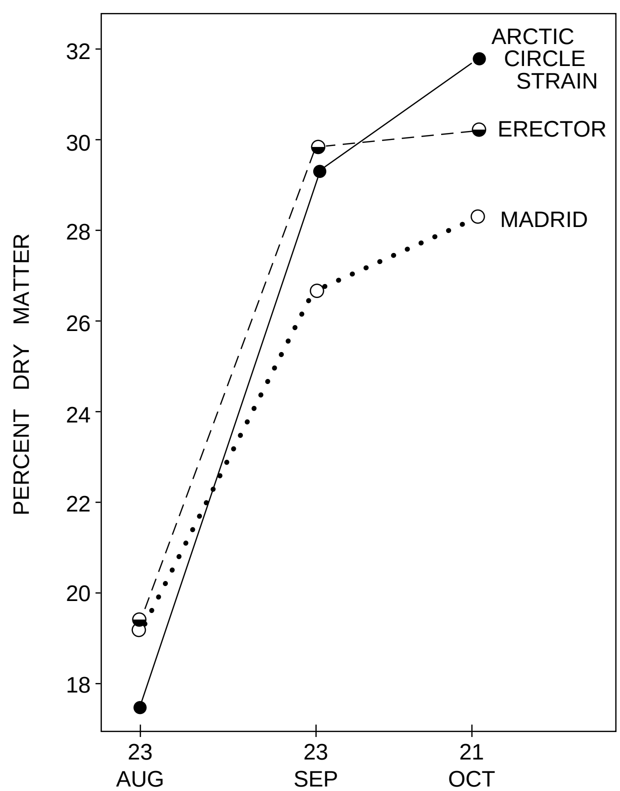

Figure 8. *Two-year means of changes in dry-matter concentration in over-wintering root-crown tissues of three biennial yellow sweetclover strains on three mean sampling dates during the winter-hardening period at the Matanuska Research Farm.*

Tolerance to Freezing

Two-year means of comparative injury in overwintering tissues of the Arctic-Circle strain, and two biennial yellow sweetclover cultivars of more southern adaptation, when frozen artificially on two sampling dates in October are shown in Fig. 9. In one of the years when the three strains were frozen on 18 August (data not shown), prior to initiation of appreciable cold hardiness, all were severely injured (Madrid 88%, Erector 92%, Arctic-Circle strain 86%). By 4 October (Fig. 9), all three strains showed less injury than in August, but they differed in extent of injury. The southernmost-adapted Madrid was most injured by freeze stress, the Arctic-Circle strain was least injured, and Erector, from intermediate latitudinal origin, was intermediate in injury sustained.

During the 15 days between the two October samplings, all three strains increased in tolerance to freeze stress; however, Madrid increased least and the Arctic-Circle strain most, so that the magnitude of the differences among the strains increased also. The 4 October sampling date was 16 days after normal occurrence of first killing frost at this location; the 19 October sampling was near the time that soil normally freezes.

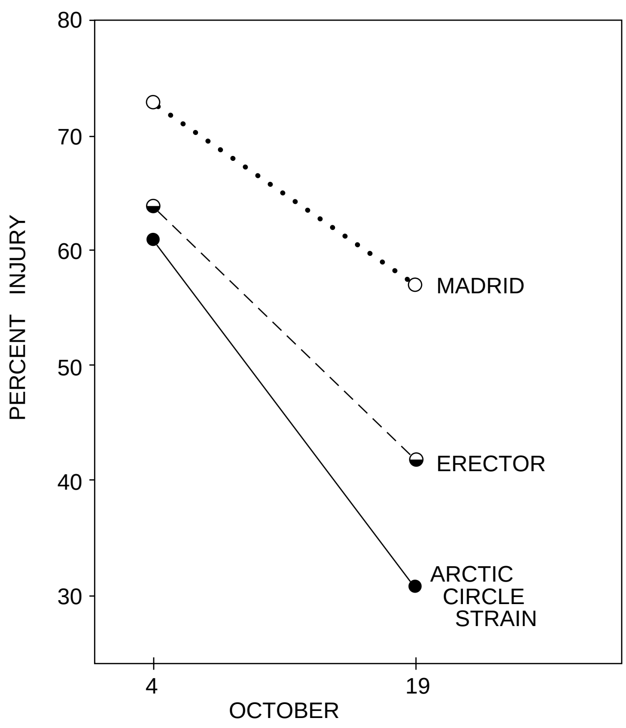

Figure 9. *Two-year means of comparative injury in overwintering taproot-crown tissues of three spring-planted biennial yellow sweetclover strains of diverse latitudinal adaptation when frozen artificially (14°F for 20 hours) on two dates in October during the winter-hardening period.*

Tysdal (1933) reported that short photoperiods and low temperatures, both of which occur normally in autumn, each contribute to development of the winter-hardening process in plants, but plants winter-harden best with the combination of both stimuli. For shortening photoperiods to contribute maximum benefit to the development of cold hardiness, the receptor mechanism (leaves) must be intact and functional. Tysdal noted that a hardening period of at least two weeks is necessary for maximum hardiness development. Thus, short photoperiods that stimulate initiation of cold-hardiness development logically should prevail for at least two weeks prior to occurrence of killing frost which ends the functional life of the leaves.

Normal occurrence of first killing frost (28°F) at Fort Yukon is 7 September when photoperiods exceed 14 hours (Fig. 10). At Palmer, normal first killing frost occurs 19 September when photoperiods exceed 12.5 hours. In contrast, a considerable duration of short photoperiods (less than 12 hours) prevails prior to killing frost at latitudes where the cultivars Madrid and Goldtop are adapted. For example, first killing frost where Goldtop originated (Madison, WI) normally occurs 31 October when photoperiods are less than 10.5 hours. Accordingly, Goldtop or Madrid grown at Madison normally would be exposed to photoperiods shorter than 12 hours for about five weeks before occurrence of killing frost. Growing Madrid at Saskatoon (52.1°N), considerably north of its latitude of adaptation, normally would preclude exposure of plants to the beneficial effects of photoperiods less than about 12 hours. The poor winter survival of Madrid grown at Saskatoon (Goplen 1971) therefore is not surprising. An exaggeration of this plant/photoperiodic maladjustment occurs when sweetclover cultivars adapted to the midwestern states or Canadian provinces are grown in subarctic Alaska.

Conclusions

Natural selection toward adaptation to subarctic climatic influences proceeded rapidly in the garden plot of biennial yellow sweetclover left unattended for about 15 years near the Arctic Circle. The markedly enhanced winter survival in the Arctic-Circle strain apparently is related to natural selection pressures shifting that population toward a genotypic constitution that retains biennial habit. This is in contrast to a strong tendency toward annual habit exhibited by more southern-adapted cultivars when grown under Alaska's unaccustomed seasonal photoperiodic pattern.

Biennial characteristics of the Arctic-Circle strain, apparent near the end of the seedling year growing season and in contrast to introduced cultivars, were reduced aerial growth (less total herbage, shorter stature, smaller-diameter stems) and better developed crowns that possessed more and larger crown buds. The Arctic-Circle strain surpassed others in earlier storage of food reserves and, in late October near onset of winter, it showed greater dormancy, higher dry-matter concentration in overwintering tissues, and less injury from freeze stress. These characteristics undoubtedly contributed to its vastly superior winter survival. Generally similar characteristics have been noted near onset of winter in subarctic-adapted cultivars of grasses that displayed superior winter survival at this location compared with more southern-adapted cultivars (Klebesadel 1985a; Klebesadel and Helm 1986).

The relative timing of food-reserve storage and protoplasmic alterations that permit over-wintering tissues to tolerate freezing, and the interrelationships of these two phenomena in the winter-hardening process in forages, are not yet fully understood (Smith 1964; Steponkus 1978). However, it is clear that storage

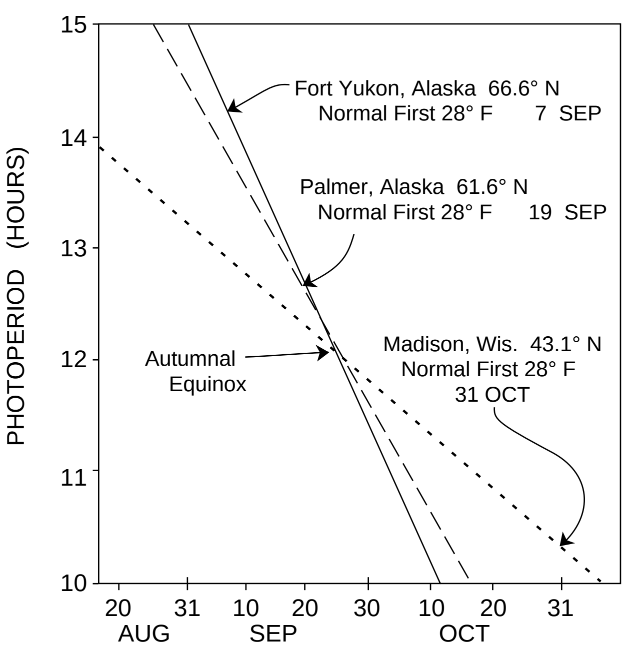

Figure 10. *Interrelationship of changing daily photoperiods during August, September, and October at three locations of diverse latitude, and normal first occurrence of killing frost (28°F) during those months at each location. Fort Yukon = location of natural selection for Arctic Circle strain; Palmer = location of tests reported here; Madison = origin of cultivar Goldtop, and representative of area of cultivar Madrid adaptation. Normal first killing frost at Fort Yukon, Palmer and Madison occurs at photoperiods of 14.2, 12.6, and 10.3 hours, respectively; arrow from each location name touches photoperiod line at point where normal killing frost occurs.*

carbohydrates are derived from photosynthetic activity and that storage must be accomplished prior to frost-killing of foliage where the manufacture occurs. The very winter-hardy Arctic-Circle strain, attuned to initiating storage of reserves as early as mid-to-late August (Fig. 7), and much earlier than the non-hardy cultivars, was nonetheless as injured by freeze stress in late August as those non-hardy cultivars (data not shown in graph; see discussion of "Tolerance to Freezing"). This infers that for ideal adaptation and winter survival, reserve-food storage is initiated well before cold-hardiness development, and must be completed before frost kills the foliage.

Earlier reports (Bula et al. 1956; Hodgson 1964) and these results (Fig. 9) reveal that development of cold hardiness continues well past the normal time of killing frost. In fact, maximum freeze tolerance in forage legumes was not achieved until mid-winter (January and February) in Wisconsin (Bula and Smith 1954; Jung and Smith 1961; Ruelke and Smith 1956). With development of cold hardiness initiated by the combination of shortening photoperiods (lengthening nyctoperiods) and lowering temperatures (Hodgson 1964; Tysdal 1933), the total winter-hardening process, though set in motion by both stimuli, continues to progress well beyond the time that killing frost destroys the plant foliage (Bula et al. 1956; Hodgson 1964). This implies that lowering temperatures alone carry the process to its ultimate limits.

Mechanisms That Facilitate Adaptive Modification

The greatly enhanced winter hardiness in Alaska of the Arctic-Circle strain gives rise to certain additional considerations. It is likely that further improvement in the hardiness of this strain is possible with artificial direction of the selection process. Clausen (1958) noted that although plant ecotypes adjust to specific environments through genetic recombination and natural selection, they are highly variable ecologic-genetic entities with great potentials for further adjustment. Genes control biochemical and physiological processes of plants, which adjust them to their environment, and plants can modify markedly when subjected to natural-selection effects in a different environment (Cooper 1965; Stebbins 1950; Wilsie 1962). This is possible because "genes that previously were unable to express themselves may become activated if the organism that carries them is moved to an environment that differs markedly from its natural one" (Clausen 1958). This rationalization and discussions of other instances of ecotypic evolution (Cooper 1965; Wilsie 1962) help to explain biological mechanisms facilitating the observed changes within the Fort Yukon population of sweetclover, changes which resulted

Figure 11. *Seed-production field of Arctic-Circle strain of biennial yellow sweetclover showing profusion of flowering at full bloom on 17 August of second year of growth; mature seed was combine-harvested on 14 October.*

from natural selection pressures in a latitudinal and photoperiodic environment markedly different from where the sweetclover originated.

Stebbins (1950) notes that adaptive modification or evolution is regulated both in speed and in direction by the genetic constitution of the population and the environmental influences acting upon it. He reports that alterations of the environment can be one of the causes of progressive evolution. In the case of the sweetclover population that evolved into the Arctic-Circle strain, the transfer of the original seed lot from mid-temperate to Arctic Circle latitudes imposed a markedly altered growth environment.

The normally biennial habit of *M. officinalis* is ideally suited to rapid modification toward improved winter hardiness through natural selection. All seedling plants not developing adequate hardiness are eliminated during the subsequent winter. Plants whose genetic makeup cause them to develop adequate hardiness to survive the winter produce a copious seed crop (Fig. 11) and die at the end of that second growing season. This rapid cycling of generations would tend to select more rapidly and more effectively than in a perennial species.

If the short subarctic nyctoperiods of Fort Yukon, which cause seedling-year flowering (Kasperbauer et al. 1963), were accompanied by a sufficiently long growing season to permit seed maturation, the introduced biennial sweetclover logically would behave and persist as a true annual, reseeding each year. Such conditions then would not have selected toward restoration of biennial habit. However, inadequate growing-season duration for seed maturation during the seedling year consequently imposed a premium on natural selection for genetic predisposition to winter survival to permit seed production in the second year in order to perpetuate the introduced, untended population. 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 northern latitudes.

A genetic characteristic of *M. officinalis* that may serve to facilitate rapid adaptive modification is its low chromosome number (n=16). The "hard seed" characteristic common in sweetclover would tend to slow the turnover in generations, but this obviously was a relatively minor impedance as indicated by the very considerable adaptational change effected in the population over 15 years at Fort Yukon.

Similar Cases of Adaptive Modification

Other instances of genetic/physiologic modification toward subarctic adaptation, paralleling that noted here in biennial yellow sweetclover (wherein high-latitude, natural-selection pressures acted upon a population from mid- temperate origins) have been noted in Alaska. These include biennial white sweetclover (Klebesadel 1992) and slender wheatgrass *(Agropyron trachycaulum* [Link] Malte) (Klebesadel 1991). Similarly, preliminary but substantial evidence has been reported (Klebesadel 1986) of adaptive modification toward improved winter hardiness in populations of white clover (*Trifolium repens* L.), introduced from more southern latitudes, that have persisted for many years at different locales in Alaska.

Sylven (1937) reported analogous natural selection in Sweden that conferred enhanced winter hardiness on populations of white clover that originated in Denmark and Germany, more southern areas with less stressful winters. He noted, too, that this adaptive modification occurred in two varieties that had not previously been subjected to artificial selection and which therefore possessed high levels of heterozygosity prior to transfer to Sweden. In contrast, the change in growth environment did not bring about a similar response in a more highly selected, relatively homozygous strain, indicating that natural selection can operate most effectively on a population possessing a broad genetic constitution. It is likely that the precursor sweetclover (obtained from a "seed catalog") that evolved into the Arctic-Circle ecotype was a "common" strain of the species, and therefore would have possessed a broad, unselected, and highly heterozygous genetic base.

Future Considerations

The observed genetic modification within *M. officinalis* toward improved winter survival and retention of biennial habit in the Subarctic apparently is primarily a selection for reaction to photoperiodic influences (and the seasonal interaction of photoperiodic pattern with seasonal temperature changes that determine the growing season). A theoretical consideration concerning forage improvement at northern latitudes emerges from these results. In seeking to modify temperate-adapted plant species toward subarctic adaptation, perhaps initial screening or natural selection pressure should be imposed at a latitude somewhat north of their ultimate area of use, as occurred in this instance. Such initial screening would eliminate at the outset genotypes only marginally suited to subarctic climatic influences. As noted, climatic influences at the Arctic Circle represent an exaggeration of the extent to which the climate in southcentral Alaska differs from temperate latitudes. The remarkably improved winter survival achieved via the route of development of this sweetclover lends credence to this possibility.

Turkington et al. (1978) report occurrence of adventive *M. officinalis* at 62° to 63°N in Yukon Territory and the District of Mackenzie in Canada. It may be of interest to determine the length of residence of those populations and whether adaptive modification has occurred there as well.

The present results are significant in illustrating that a valuable nitrogen-fixing, soil-improving, forage legume, adapted to mid-temperate climatological patterns, can be modified genetically toward climatic adaptation, improved winter hardiness, and therefore agricultural usefulness at subarctic latitudes. This may be of considerable significance because northern lands likely will become increasingly important for future food production if current world population trends continue.

This information on successful plant adaptational change to photoperiodic influences may be of interest to those concerned with genetic and physiological changes necessitated during historical plant migrations across global latitudes. Such natural migrations involved similar adjustments to different photoperiodic and growing-season patterns (Klebesadel 1985b).

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Explanatory Note

This report summarizes research completed several years ago. During its completion, the investigator/author assumed time-consuming research supervisory responsibilities that delayed more timely publication. It is published now because it represents heretofore unpublished information that augments Alaska's agronomic research data base.

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