

Effects of Daily Photoperiod/Nyctoperiod and Temperature on Autumn Development of Crown Buds and Dormancy, Freeze Tolerance, and Storage of Food Reserves in Latitudinal Ecotypes of Biennial White Sweetclover

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SUMMARY

The investigation consisted of both an outdoor experiment and one conducted indoors in controlled-environment chambers. Objectives were to determine during late-summer/autumn the effects of (a) the changing daily light/dark (photoperiod/nyctoperiod) pattern, and (b) seasonally lowering temperatures on pre-winter behavior of sweetclover. Measurements were made of certain morphological characteristics and various facets of the winter-hardening process. Both experiments were conducted at the University of Alaska's Palmer Research Center (61.6°N) in southcentral Alaska.

ECOTYPES

Three strains of biennial white sweetclover (*Melilotus alba* Desr.) – adapted to widely diverse latitudes – were compared for responses to treatments. The northernmost-adapted ecotype, 'Matanuska white,' is an introduced strain that has undergone adaptive modification through natural selection during many years at 61.6°N in Alaska's Matanuska Valley. The intermediate-latitude ecotype was the Canadian cultivar 'Arctic' adapted at 50° to 56°N; and the southernmost-adapted ecotype was the U.S. cultivar 'Spanish,' adapted at 35° to 50°N.

OUTDOOR EXPERIMENT

All three were broadcast-seeded in field plots 28 June and subjected to four different patterns of photoperiods/nyctoperiods after 25 August. Some plants were dug on 20 September and more on 12 October (the latter near the time of seasonal soil freeze-up) to measure treatment effects. Experimental photoperiod/nyctoperiod patterns compared were (a) normally prevailing for 61.6°N (photoperiods decreasing gradually from 15 to 10.5 hours), (b) normally shortening photoperiods but with nyctoperiods interrupted for 90 minutes near midpoint, (c) constant, long (15-hour) photoperiods, and (d) photoperiods gradually shortened artificially during the same calendar period (from 10.5 to 9 hours), simulating conditions prior to onset of winter conditions at more southern latitudes.

- Ecotypes differed under normal photoperiods and temperatures; subarctic-adapted Matanuska white produced the most and largest crown buds and developed highest level of freeze tolerance. Spanish, the most southern-adapted, produced the fewest and smallest crown buds, had lowest dry-matter concentration in overwintering tissues, and developed least freeze tolerance. Arctic, of intermediate latitudinal adaptation, was intermediate in all respects.

- Artificially shortened photoperiods caused the two introduced cultivars to develop the most and largest macroscopic crown buds, smaller mainstem diameters, highest levels of both dry-matter concentration and stored food reserves in overwintering tissues, and greatest tolerance to freeze stress.

- Interrupted nyctoperiods, at the other extreme, resulted in lowest dry-matter concentration in overwintering tissues and greatest injury from freeze stress in all three strains. Moreover, interrupted nyctoperiods caused poorest development of crown buds and lowest levels of stored reserves in Arctic and Spanish. These results confirm that the integrity and the duration of nyctoperiods are critical environmental stimuli for inducing plants to prepare adequately for winter.

- Crown-bud development and food-reserve storage of Matanuska white were generally less affected by photoperiod treatments than occurred with Arctic and Spanish.

GROWTH-CHAMBER EXPERIMENT

- With progressively shortened photoperiods (12 to 6 hours) in growth chambers, widely alternating but gradually lowering temperatures (daily minimum lowered from 40° to 32°F) resulted in vastly-increased freeze tolerance and greater food-reserve storage than were engendered by continuous warm temperatures.

- In contrast to the considerable influences of light/dark treatments on development of crown buds and dry-matter concentration in overwintering tissues in the outdoor experiment, lowering temperatures in the indoor experiment did not affect those characteristics in any of the three sweetclover strains.

GENERAL

- The most favorable growth-chamber treatment for high levels of stored food reserves and maximum development of freeze tolerance (strongly alternating but consistently and regularly lowering temperatures with shortening photoperiods) was markedly more effective with all ecotypes than the most favorable treatment in the outdoor experiment (shortened photoperiods). The considerable difference between the most favorable treatment in each of the two experiments in the levels of food-reserve storage and freeze tolerance achieved is believed attributable to less effective temperature stimulus in the outdoor experiment; randomly fluctuating temperatures with relatively warm minima occurred during much of the growth period outdoors prior to freeze-tolerance tests.

- The pre-winter dormancy detected in sweetclovers in this and other studies at this location was confirmed to be a response to shortening photoperiods/lengthening nyctoperiods, rather than lowering temperatures.

- These results confirm that adaptation to subarctic photoperiod/nyctoperiod pattern during the time of lowering temperatures in late summer and autumn is essential for attainment of optimum morphological and physiological changes associated with typical biennial habit of sweetclover and best winter survival at this latitude. Additionally, however, appropriately lowering temperatures also contribute vitally to high levels of food-reserve storage and to maximum development of freeze tolerance. Low temperatures alone

apparently propel freeze-tolerance development to maximum levels that are achieved some weeks after the foliage-receptor of the photoperiod/nyctoperiod stimulus has been destroyed by killing frost.

- These findings also provide insights into the effects of natural selection over many years on Matanuska white—an introduced, naturalized sweetclover strain.

- Finally, these results provide a better understanding of why northern-adapted plants tend to survive winters well at this latitude and why those adapted at more southern latitudes generally are not adequately stimulated in this subarctic area to undergo fully the physiologic alterations that contribute to good winter survival.

INTRODUCTION

Few introduced forage legumes survive winters dependably under cropland use in this northern area (Hodgson and Bula 1956; Klebesadel 1971b, 1980, 1985, 1992b). Of the legumes utilized for forage, biennial sweetclovers (*Melilotus* spp.) are among the most winter-hardy (Bula and Smith 1954; Klebesadel 1971b, 1980; Ouellet 1976).

Biennial white sweetclover (*M. alba* Desr.) represents a crop with well-established forage potential, within which northern-adapted cultivars and selections give evidence of near-adequate winterhardiness for use in Alaska (Hodgson and Bula 1956; Klebesadel 1971b, 1980, 1992b).

ENVIRONMENTAL STIMULI

Little is known about the specific environmental requirements of sweetclover for optimum cold-hardiness development and for food-reserve storage; both are prerequisites to good winter survival at subarctic latitudes. Numerous studies have shown that the seasonally shortening duration of daylight (photoperiods), with simultaneously lengthening duration of daily dark periods or nights (nyctoperiods) and decreasing growing temperatures prior to onset of winter conditions are vital to promoting the storage of food reserves and development of freeze tolerance (Devlin and Witham 1983; Hodgson 1964; Moschkov 1935; Pohjakallio 1961; Smith 1942; Steponkus 1978; Tysdal 1933).

There has not been complete agreement, however, concerning the relative importance and specific influences of light and temperature upon the several individual plant alterations and physiologic changes that together comprise the complex winter-hardening process. Moreover, few studies of this nature have been reported for biennial sweetclover, and none are known for sweetclover at subarctic latitudes.

TERMINOLOGY

The terminology associated with winter hardening and winterhardiness research tends to be somewhat ambiguous and confusing, with different terms sometimes used to describe similar phenomena. In this report, the ability of plants to tolerate freeze stress is termed "freeze tolerance." Others have referred to this same phenomenon as "cold resistance." That terminology is somewhat misleading. Inasmuch as no insulation or other protection from cold is involved in hardiness development, plants do not truly "resist" the freeze stress (Levitt 1956). Instead, through beneficial physiologic changes, overwintering tissues are able to "tolerate" freezing; therefore, tolerance and hardiness are utilized here as the descriptive terms of

choice. Others have used "frost" hardiness in the same context but, inasmuch as the term frost as commonly utilized involves crystallized water on a visible surface, the choice of the words freeze and freezing seem more appropriate than frost to describe the internalized low-temperature stress.

FREEZE TOLERANCE

The specific intracellular changes that permit plant tissues to tolerate freeze stresses without lethal injury have been studied and conjectured by many investigators and reviewed by some (Devlin and Witham 1983; Dexter 1956; Smith 1964a, 1964b; Steponkus 1978). Dexter et al. (1932) developed a procedure useful for measuring the extent of plant tissue injury, as variously influenced by (a) environmental factors that induce freeze tolerance (Hodgson 1964), (b) genetic capacity of different strains or ecotypes to develop freeze tolerance (Bula and Smith 1954; Bula et al. 1956; Hodgson and Bula 1956; Klebesadel 1971b, 1992a, 1992b; Klebesadel and Helm 1986), (c) different temperatures of artificially imposed freeze stress (Klebesadel and Helm 1986; Klebesadel 1993), and (d) the progression of freeze-tolerance development during the hardening period (several of the above references).

CROWN BUDS AND MAINSTEM DIAMETERS

Development of buds at the cotyledonary node, or crown, of biennial sweetclover occurs near the end of the seedling growing season (Kasperbauer et al. 1962, 1963; Klebesadel 1992a, 1992b; Smith 1942). Those buds, from which growth originates during the following growing season, become numerous and large just prior to winter on biennial sweetclover grown in its area of adaptation. Kasperbauer et al. (1963) reported that sweetclover crown-bud initiation and development are controlled by diurnal dark periods (nyctoperiods) during autumn.

Thus, development of several, large crown buds per plant during late summer and autumn provides a conspicuous morphological indication of harmonious adaptation of biennial sweetclover to late-season diurnal light/dark pattern. Mid-temperate-adapted biennial sweetclovers grown in the subarctic typically produce only a few small crown buds resembling annual sweetclover (Klebesadel 1992a, 1992b).

Another morphological indicator of adaptation is both the diameter and height of the mainstem during the seedling year. When grown at mid-temperate latitudes, biennial sweetclovers produce a modest amount of top growth on a main-stem that, at the end of the seedling growing season, is much smaller in diameter than the taproot just below the cotyledonary node (Klebesadel 1992b, Fig. 4; Smith et al. 1986, Fig. 14.1). When grown under the unusual photoclimate of this

northern area, however, biennial sweetclovers produce much taller growth on mainstems that at their base are much larger in diameter, resembling stems produced by annual sweetclover (Klebesadel 1992b).

DRY-MATTER CONCENTRATION IN OVERWINTERING TISSUES

Upper taproot and basal stem tissues, including the crown area, are the principal tissues concerned in sweetclover's successful winter survival. Survival in areas with long, cold winters is dependent upon the development of extreme freeze tolerance within those overwintering tissues. Physiological changes during development of freeze tolerance involve disappearance of free water and increased osmotic concentration of cell sap (Levitt 1956; Smith 1964a, 1964b; Steponkus 1978). These changes are accompanied by a simultaneous increase in dry-matter concentration in the tissues involved. Earlier reports (Greathouse and Stuart 1936; Li and Weiser 1971; Metcalf et al. 1970; Sjoseth 1964) have noted that, within a species, freeze tolerance was positively correlated with dry-matter concentration in overwintering tissues.

PRE-WINTER STORAGE OF FOOD RESERVES

High levels of food-reserve storage prior to winter are necessary as a source of energy for metabolic changes concerned with developing and maintaining coldhardiness, for living over winter, and for initiating growth the following spring (Bula and Smith 1954; Graber et al. 1927; Greathouse and Stuart 1936; Smith 1964b; Steponkus 1978; Tysdal 1933). High levels of reserves do not assure a high level of winterhardiness (Bula et al. 1956; Hodgson and Bula 1956), but plants with low levels of food reserves cannot develop high levels of hardiness (Smith 1964b).

Rapid pre-winter storage of food reserves in overwintering plant tissues occurs simultaneous with physiologic changes that increase freeze tolerance in those tissues. Both phenomena are induced to occur in plants by seasonally shortening daily photoperiods (lengthening nyctoperiods) and lowering temperatures (Hodgson 1964; Hodgson and Bula 1956; Smith 1964a, 1964b; Steponkus 1978; Tysdal 1933).

DORMANCY

In addition to the above measurable manifestations of changes in several forage crops prior to winter, detection of a pre-winter dormancy condition has been noted in biennial sweetclover. Observations of its onset and disappearance, and speculation as to its acquisition, role, and importance have been reported (Klebesadel 1992b).

THESE EXPERIMENTS

This study was undertaken to compare the responses of three ecotypes of biennial white sweetclover of diverse latitudinal adaptation to four photoperiodic patterns imposed for seven weeks prior to onset of winter in an outdoor study, and to decreasing vs. constant warm temperature during a period of simulated-autumnal shortening photoperiods in controlled-environment chambers. Plant responses were measured by (a) number and size of crown buds developed, (b) effect on dry-matter concentration in overwintering tissues, (c) extent of injury resulting from artificially-imposed freeze stress, (d) levels of stored food reserves, and (e) occurrence of dormancy. Both experiments were conducted at the University of Alaska's Palmer Research Center (61.6°N) in southcentral Alaska.

EXPERIMENTAL PROCEDURE

OUTDOOR EXPERIMENT: Plots measuring 4 x 4 feet were broadcast-seeded on 28 June in Bodenburg silt loam (Aquic Cryochrept). Fertilizer disks in the seedbed prior to planting supplied N, phosphorus (as P₂O₅), and potassium (as K₂O) at 32, 148, and 64 lb/acre, respectively. Duplicate plots of each of three sweetclover strains were located randomly within each of four adjacent areas, each of which was utilized for a separate photoperiod treatment.

Sweetclover strains used were (a) Matanuska white, a mass selection drawn from a population (of uncertain original derivation before its introduction into Alaska) that has undergone adaptive change in an untended roadside habitat in the local Matanuska Valley for 30 to 50 years (Klebesadel 1992b), (b) the Canadian cultivar Arctic, adapted at 50° to 56°N, and (c) the cultivar Spanish, adapted at 35° to 50°N in the conterminous U.S. All strains were seeded at 10 lb/acre following admixture of a commercial *Rhizobium* bacterial inoculant with the seed. Frequent hand weeding kept the plots weed-free.

Photoperiod treatments were initiated on 25 August and continued until 12 October. Facilities and procedures employed to provide photoperiod/nyctoperiod treatments were described and pictured earlier (Klebesadel 1971a, 1973). A graphic depiction of photoperiodic patterns imposed during the seven-week period is shown in Figure 1.

On 30 September and 12 October plants were dug from each plot for several determinations. Aerial growth beyond one inch above the cotyledonary node was severed immediately to arrest transpirational moisture loss and was discarded. Taproot growth beyond four inches below the cotyledonary node and all branch roots were severed and discarded, also. Roots were wrapped in moist toweling and, as rapidly

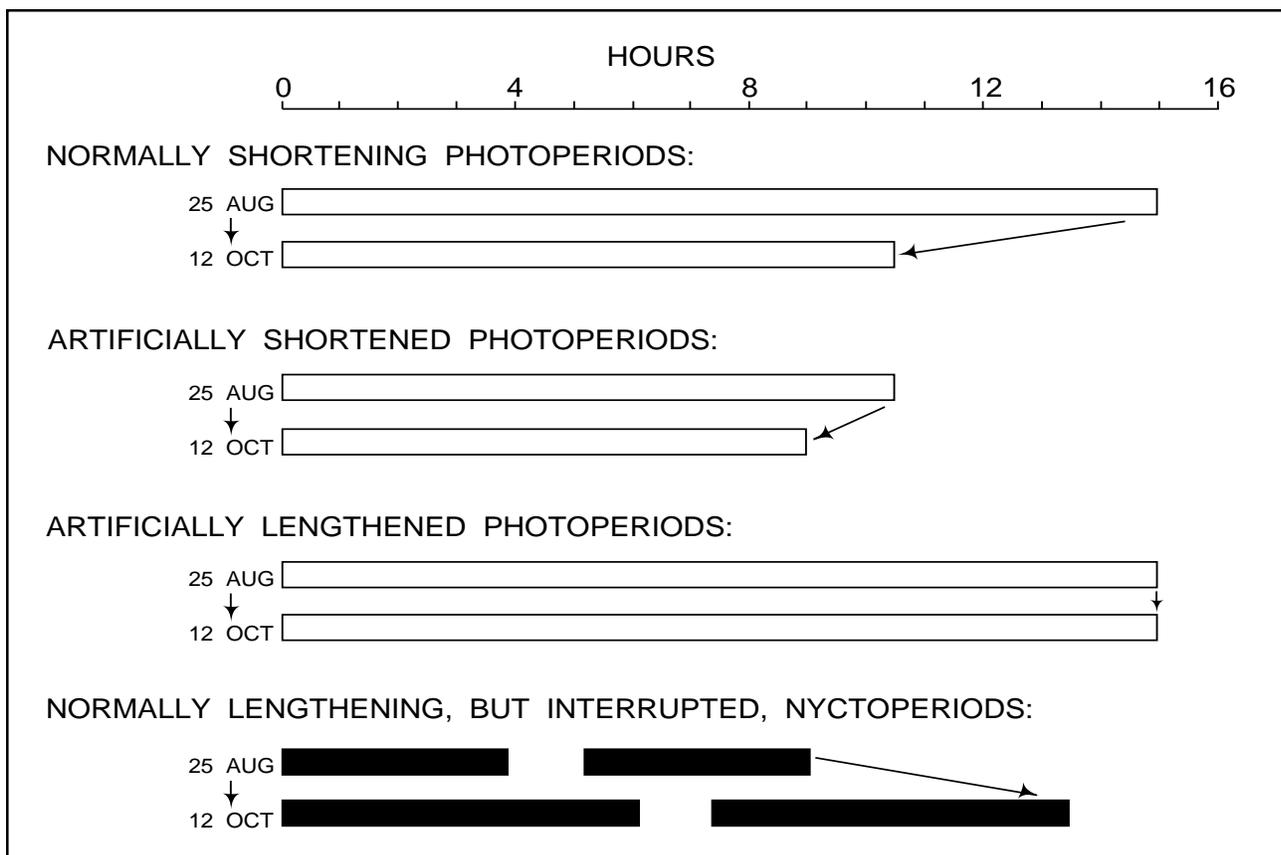


Figure 1. Graphic depiction of experimental photoperiods/nyctoperiods imposed on sweetclover strains in outdoor experiment during the seven-week period, 25 August to 12 October.

as possible, samples were withdrawn for determinations of tissue dry-matter concentration, freeze tolerance, crown-bud numbers and sizes, and stored food reserves. Crown buds were counted, and each was measured to the nearest mm, on 25 plants per plot. Percent dry-matter in stem-base/ crown/ taproot segments and stored food-reserve measurements in the same tissues (as weighed etiolated growth produced in darkness) were accomplished as follows: Soil was washed from stem-base/ crown/ taproot segments (plants) with a cold-water spray to remove all traces of soil and plant debris; they were then surface-dried with absorbent toweling. A random sample of each strain of plants was withdrawn, weighed, dried to constant weight at 140°F, and reweighed. Percent dry matter derived thusly was used to calculate extrapolated dry weights of plants used for stored food-reserve determinations. Twenty plants of each strain were potted on each sampling date.

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 (65°+2°F) dark chamber with the base of pots im-

mersed in ¼ to ½ 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 plants until no more appeared; this point represented exhaustion of food reserves as plants died shortly thereafter. The first harvest of etiolated growth was delayed until the distal portions of the growth began to wilt; subsequent harvests were then taken at equal intervals thereafter: three-week intervals for the outdoor experiment and two-week intervals for chamber-grown plants. 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 140°F and stored food reserves are reported as milligrams (mg) oven-dry etiolated growth per oven-dry gram (g) of plant storage tissue potted.

To determine tolerance of stem-base/ crown/ taproot segments to artificially imposed freeze stress, those segments were prepared as described above for food-reserve determinations. Then they were rinsed three times in distilled water, dried of superficial moisture, and placed into large (1- x 8-inch) stoppered test tubes. Freeze tolerance was determined by the electri-

cal 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 the test tubes for 20 hours at +14°F. Then 50 ml of distilled water were added to each test tube and all were stored for 20 hours at 40°F to permit diffusion of cell electrolytes from injured tissues. Solutions were then decanted, brought to 77°F, and specific conductivities determined for each. Solutions were returned to appropriate original tubes containing plants and all were heated to boiling to destroy intact cells. Solutions were again decanted, brought to 77°F, and a second specific conductivity determined for each.

Percent injury from freezing was calculated using the specific conductance ($\times 10^{-6}$) following freezing (=partial tissue injury) as a percent of specific conductance ($\times 10^{-6}$) following boiling (=total tissue injury).

CHAMBER EXPERIMENT: The same three strains were each seeded in 20, two-gallon stone crocks in a 2:1 silt-sand mixture on 14 July. Commercial fertilizer was added to approximate (on a soil-volume basis) that applied in the outdoor plot study. Seedlings were thinned to 10 per crock and grown in a glasshouse until

20 September when the 20 crocks of each strain were divided randomly into two equal sets. Each set was placed into a controlled-environment chamber operating with 12-hour photoperiod, 12-hour nyctoperiod.

Beginning 13 October, daily photoperiods were shortened (and nyctoperiods commensurately lengthened) equally and simultaneously in the two chambers by approximately 7.5 minutes per day (Fig. 2). (This slightly exceeded the approximately 6 minutes/day normal change in photoperiod/nyctoperiod near the autumnal equinox at this latitude.) Photoperiods were shortened until they reached 6 hours on 1 December and were maintained at that duration until plants were removed from the chambers on 13 December.

During the 12-weeks, from 20 September to 13 December, temperature in one of the chambers ("warm") was maintained between 62° and 68°F. Maxima during the photoperiod reached 68°F and minima during the nyctoperiod reached 62°F (Fig. 2). Temperature in the other ("chilling") chamber was decreased gradually over the 12-week growing period. There were wide daily oscillations of temperature, with maxima during the photoperiod and minima during the nyctoperiod, respectively, of 68° and 40°F at the start and of 50° and 32°F at termination of the experiment. Controls that regulated temperature caused minor, regular, and frequent oscillations of 2°

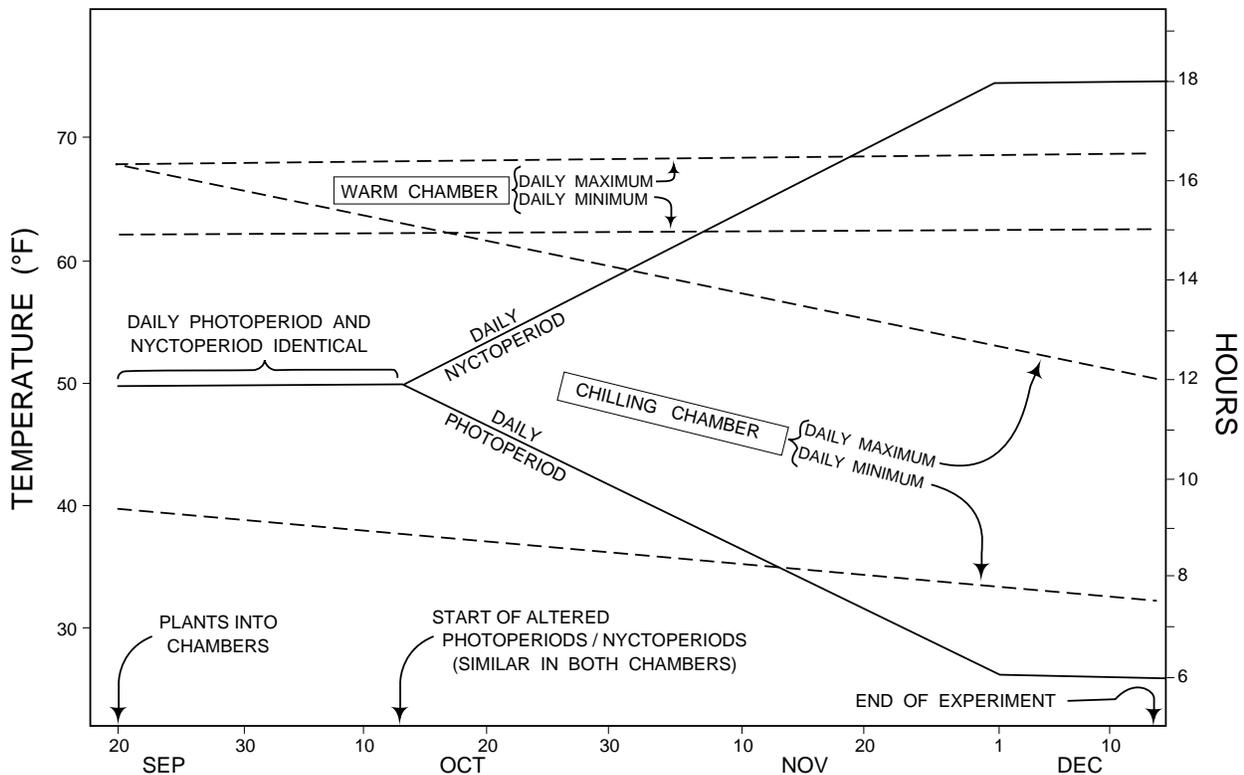


Figure 2. Daily photoperiod/nyctoperiod pattern in controlled-environment chambers (similar in both), and different temperature regimes employed in each.

Table 1. Summary of dates, activities performed, and elapsed time in days from seeding to specific events in both the outdoor and the indoor controlled-environment chamber experiments.

Date	Activity	Elapsed time (days) from seeding
Outdoor experiment:		
28 June	Plots seeded	
25 August	Start of nyctoperiod treatments	58
30 September	First sampling of plants	94
12 October	Final sampling of plants	106
Chamber experiment:		
14 July	Crocks seeded in glasshouse	
20 September	Crocks to chambers + start of lowering temperatures in chilling chamber	68
13 October	Start of lengthening nyctoperiods	91
14 December	End of experiment	152

to 4°F below the maximum and above the minimum temperatures reported here.

All seedlings were withdrawn from the crocks on 13 + 14 December, aerial growth was severed immediately, and stem-base/crown/taproot segments were prepared as in the outdoor study. Determinations of tissue dry-matter concentration, stored food reserves, and injury from artificial freeze stress were accomplished as in the outdoor study.

Table 1 summarizes activities, dates, and seedling ages for both the outdoor field experiment and the indoor controlled-environment chamber experiment. All references to statistical significance in the discussion of results are based on 95% confidence limits.

RESULTS AND DISCUSSION

In the following discussion of results, it is recognized that with 24-hour days, lengthening or shortening the photoperiod has an opposite effect on the nyctoperiod. To simplify discussion, and to follow prior standard terminology and discourse on light/dark studies in the scientific literature, reference in this report will also emphasize the duration of light or photoperiod. However, with mention of a “shortened photoperiod” effect on plants, it should be appreciated that while the effect on plants may actually be from a shortened photoperiod, it may instead be due to a simultaneously lengthened nyctoperiod, or the combined influence of both phenomena.

It should be recognized also that the treatment in

the outdoor experiment that provided light for 90 minutes near the middle of each dark period actually represented a three-fold influence: (a) it interrupted the continuity of the nyctoperiod and essentially created two each night; (b) it shortened each nyctoperiod by 90 minutes; and (c) it added 90 minutes of light to each 24-hour period. Results described in this report are discussed principally in terms of (a), but the other two influences were also inherent in that treatment.

Photoperiodic treatments for seven weeks prior to 12 October, under naturally occurring outdoor temperatures, markedly influenced crown-bud size and number, development of freeze tolerance, and dry-matter concentration and storage of food reserves in overwintering tissues of sweetclover ecotypes. Two temperature regimes imposed during progressively shortening photoperiods in controlled-environment chambers also exerted considerably different influences on development of freeze tolerance, on food-reserve storage, and on buildup of dormancy.

CROWN BUDS AND MAINSTEM DIAMETERS

OUTDOOR EXPERIMENT: Sweetclover ecotypes differed significantly in both number and size of crown buds produced by 12 October under the normal sub-arctic pattern of decreasing late-summer/autumn photoperiods (Fig. 3, Table 2). Matanuska white, possessing best adaptation to the local pattern of seasonal photoperiodic changes, produced the most and largest crown buds. Spanish, grown farthest from its accustomed pattern of autumn photoperiods, produced the fewest

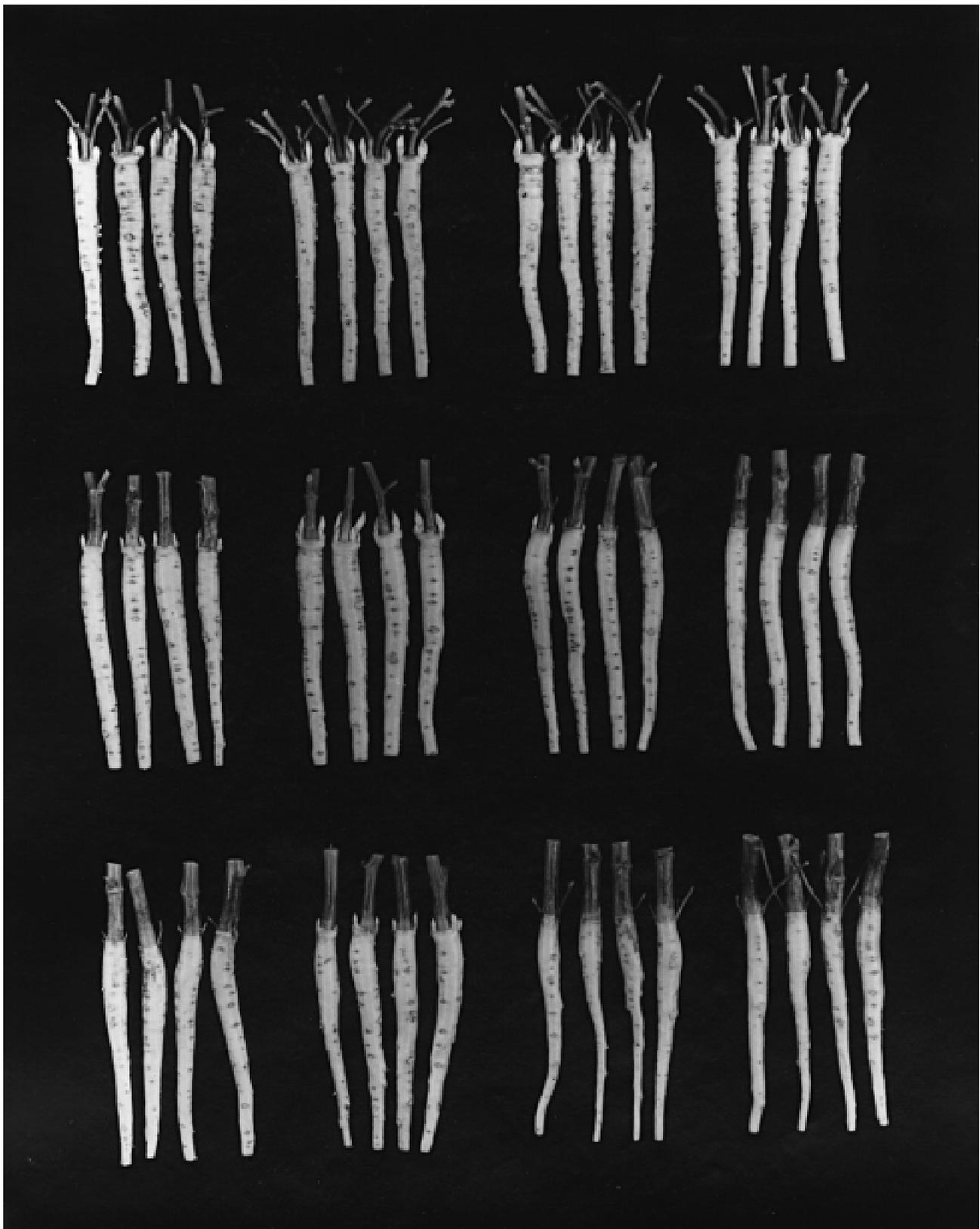


Figure 3. Typical four-plant groups of stem-base/crown/taproot segments of three field-grown sweetclover strains of diverse latitudinal adaptation seeded 28 June and photographed in mid-October showing morphological development as influenced by photoperiod/nyctoperiod treatments during the seven weeks prior to photo. (Top to bottom): Matanuska white, Arctic, Spanish. Photoperiod treatments (left to right): Normal, shortened, and lengthened photoperiods; interrupted nyctoperiods.

and smallest crown buds. Arctic, of intermediate latitudinal adaptation, produced crown bud numbers and sizes that were intermediate between the other two strains.

Artificially lengthened photoperiods resulted in about the same number and size of crown buds in all three strains as occurred under normal photoperiods (Table 2). Interrupted nyctoperiods resulted in the fewest and smallest buds on all three strains.

Shortened photoperiods seven weeks prior to sampling resulted in significantly more and larger crown buds with both Arctic and Spanish but not with Matanuska white (Table 2, Fig. 3). This treatment, most closely simulating the autumn light/dark pattern where the two introduced cultivars are adapted, resulted in best crown-bud development with both Arctic and Spanish. The combination of more and larger

crown buds resulted in markedly greater total bud length per plant in Arctic and Spanish (Table 2).

Shortened photoperiods also tended to result in smaller mainstem diameters just above the crown in Arctic and Spanish (Fig. 3). Small mainstem diameter in the seedling year is characteristic of normal biennial habit (Kasperbauer et al. 1962; Klebesadel 1992b; Smith et al. 1986). In contrast, large mainstem diameters are characteristic of annual sweetclover (Klebesadel 1992b), or biennial seedlings grown under unaccustomed long photoperiods (short nyctoperiods) (see Spanish grown under normal and lengthened photoperiods, also interrupted nyctoperiods, in Fig. 3).

CHAMBER EXPERIMENT: In the controlled-environment chambers, both temperature treatments were under the same light/dark pattern. Although the

Table 2. Influence of four photoperiod treatments during the previous seven weeks on characteristics of macroscopic crown buds in mid-October on seedling plants of three sweetclover strains of diverse latitudinal adaptation grown at Palmer, Alaska.

Strain and latitudinal adaptation	Photoperiod	Buds per plant	Length per bud	Total bud length per plant	Elongated buds per plant
		no.	mm	mm	no.
Matanuska white (61° to 62°N)	Normal	4.9 b ¹	4.5 a	22.0 ab	1.5 ab
	Shortened	4.1 bc	4.7 a	19.5 bc	1.3 abc
	Lengthened	5.1 b	4.7 a	24.0 ab	1.1 bc
	Int. nyct. ²	3.2 cd	4.1 a	13.2 cd	1.7 a
Arctic (50° to 56°N)	Normal	3.3 cd	2.9 b	10.1 de	0.0
	Shortened	6.4 a	4.4 a	27.9 a	0.0
	Lengthened	2.5 de	2.5 bc	6.2 ef	0.1 e
	Int. nyct.	2.1 ef	1.9 bc	4.1 ef	0.0
Spanish (35° to 50°N)	Normal	0.9 g	1.7 c	1.7 f	0.9 cd
	Shortened	2.3 de	2.9 b	6.7 def	0.6 de
	Lengthened	1.1 fg	2.2 bc	2.5 f	0.8 cd
	Int. nyct.	1.0 g	1.8 c	1.9 f	0.9 cd

¹Within each column, means not followed by a common letter are significantly different (5% level) using Duncan's Multiple Range Test.
²Interrupted nyctoperiod.

same differences in crown buds were seen among ecotypes as occurred under normal photoperiods/nyctoperiods in the outdoor study, temperature treatments influenced neither abundance nor size of crown buds on the three sweetclover strains; these findings affirm that the daily light/dark pattern, not temperature, serves as the causal stimulus for crown-bud development.

DISCUSSION

These results agree with those of Kasperbauer et al. (1963) that diurnal nyctoperiods are the determinative stimulus for crown-bud development. It is believed that the differences in crown-bud abundance, as influenced by the different photoperiodic treatments, would have been greater with a term of treatments longer than seven weeks, that is, if treatments had been initiated earlier than the 25 August date used in this study.

Moreover, the shortened photoperiods might have caused greater crown-bud development, especially in Arctic and Spanish, if those cultivars had not previously been exposed to the very long photoperiods (short nyctoperiods) of midsummer at this latitude that tend to induce annual-type development in those cultivars (Klebesadel 1992b).

The normal behavior of biennial sweetclover crown buds, formed late during the seedling year, is to remain unelongated during the first-growing season, then to elongate to produce the aerial growth of the plant during the second-growing season (Smith et al. 1986). All crown buds ordinarily do remain unelongated during the seedling year under short autumn photoperiods in the U.S. Midwest (Smith 1942). However, with artificially extended photoperiods there, analogous to normal Alaska conditions, some crown buds elongated into shoots (Smith 1942). In the present outdoor experiment, elongation of one to two buds per plant was common in Matanuska white, less so in Spanish, and virtually non-existent in Arctic (Table 2, Fig. 3).

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). Arctic has been grown since in Canada at 50° to 56°N. That relatively northern origin and continued northerly culture of Arctic may account for that cultivar's typical biennial behavior with no seedling-year crown-bud elongation when grown somewhat farther north in Alaska.

In contrast, the 20th-century introduction that evolved to become Matanuska white is believed to have been brought a greater distance north to Alaska from more southern latitudes in the conterminous U.S. Although the population from which Matanuska white

was selected has grown at 61.6°N for 30 to 50 years, it is probable that it traces in antiquity to more southern-adapted germplasm than Arctic; that may account for Matanuska white's greater tendency for elongation of some crown buds in the seeding year.

DRY-MATTER CONCENTRATION IN OVERWINTERING TISSUES

OUTDOOR EXPERIMENT: Photoperiod treatments in the outdoor study influenced dry-matter concentration considerably in overwintering tissues of the three ecotypes (Fig. 4). Under normal photoperiods, stem-base/crown/taproot segments of Matanuska white contained approximately 30% dry matter; Arctic from intermediate latitudes averaged about 27.5%, and the more southern-adapted, less winter-hardy Spanish (Klebesadel 1992b) averaged only 23%.

Shortened photoperiods had no effect on dry-matter concentration in Matanuska white. In contrast, that treatment increased dry-matter concentration slightly in Arctic, and markedly more and to a significant extent in the less-hardy Spanish. Dry-matter concentration of Spanish with shortened photoperiods

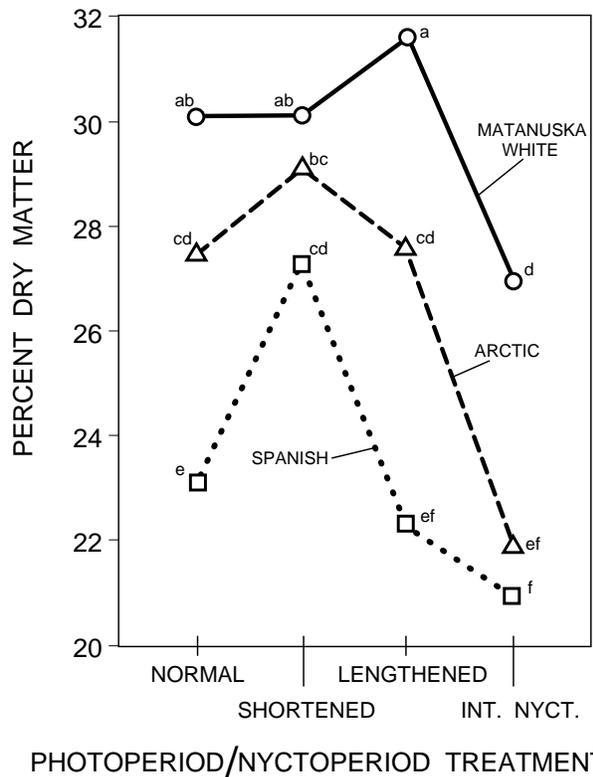


Figure 4. Dry-matter concentration on 12 October in stem-base/crown/taproot segments of three sweetclover strains as influenced by four photoperiod/nyctoperiod treatments during the previous seven weeks in outdoor experiment. Points not accompanied by a common letter are significantly different (5% level) using Duncan's Multiple Range Test.

Table 3. Influence of constant warm vs. decreasing (“chilling”) temperature in controlled-environment chambers on dry-matter concentration of stem-base/crown/taproot segments of three sweetclover strains of diverse latitudinal adaptation. Decreasing-temperature treatment in chilling chamber was imposed gradually over twelve weeks prior to dry-matter determination. Diurnal photoperiods were shortened gradually (from 12 to 6 hours) in both chambers over a seven-week period during the 12 weeks that plants were in the chambers (Fig. 2).

Sweetclover strain	Latitude of adaptation	Chamber temperature	Dry-matter concentration
			%
Matanuska white	61° to 62°N	Warm	32.8 a ¹
		Chilling	31.6 a
Arctic	50° to 56°N	Warm	29.8 a
		Chilling	29.0 a
Spanish	35° to 50°N	Warm	30.1 a
		Chilling	30.9 a

¹ Within column, means not followed by a common letter are significantly different (5% level) using Duncan’s Multiple Range Test.

approximated that of Arctic under normal subarctic photoperiods.

Compared with normal photoperiods, lengthened photoperiods tended to increase dry-matter concentration slightly in Matanuska white, did not affect Arctic, and tended to cause a slight decrease in Spanish; however, none of the differences were statistically significant.

Interrupted nyctoperiods resulted in significantly lower dry-matter concentration than did normal dark periods in all three strains. Inasmuch as overwintering tissues normally increase in dry-matter concentration during hardiness development, it is obvious from these results, and earlier findings with perennial grasses (Klebesadel 1971a, 1973, 1985), that interruption of the diurnal dark period with light inhibits normal preparation of plants for winter. In the grass study, interrupted nyctoperiods predisposed several grasses to severe winter injury or total winterkill.

CHAMBER EXPERIMENT: Differences among ecotypes in dry-matter concentration were small in the controlled-environment chambers where temperature

was the only experimental variable (Table 3). No differences in dry-matter concentration were apparent in any of the three strains as influenced by warm vs. lowered temperature.

DISCUSSION

Results from the two experiments indicate that diurnal light/dark effects (as compared in the outdoor experiment), and not lowering temperatures (as were evaluated in the growth-chamber experiment), were responsible for differences in dry-matter concentration among and within sweetclover ecotypes in the outdoor test. These results contrast with those of Paulsen (1968) who found temperature to be much more effective than photoperiod in increasing dry-matter concentration during cold-hardening in seedlings of winter wheat (*Triticum aestivum* L.).

Additional comments concerning dry-matter concentration appear at the beginning of the discussion of freeze tolerance.

FREEZE TOLERANCE

OUTDOOR EXPERIMENT: When sampled on 30 September, five weeks after start of photoperiod treat-

ments, and also on 12 October, southernmost-adapted Spanish sustained greater injury from artificially imposed freeze stress, over all treatments, than did Arctic or Matanuska white (Fig. 5). During the two-week interval between samplings, Spanish increased relatively little in freeze tolerance, regardless of treatments.

Arctic was injured more by freezing than Matanuska white over all treatments except shortened photoperiods where injury was equal in both strains on both sampling dates. That treatment, most closely simulating late-season photoperiods in its area of origin, was most beneficial to freeze-tolerance development in Arctic. On the final sampling date, Arctic sustained 57% injury with normal subarctic photoperiods, but only 46% with shortened photoperiods.

In contrast to Arctic, Matanuska white on 30 September sustained markedly more injury from freezing where photoperiods had been shortened than occurred with normal photoperiods. However, by 12 October, the effects of those two photoperiod treatments on Matanuska white were reversed, with that strain (like Arctic) showing a marked increase in freeze tolerance and least injury where photoperiods had been shortened.

The shortened-photoperiod treatment, initiated 25 August, involved an abrupt shortening of daily

photoperiods from the normally occurring 15 hours on that date to 10.5 hours, followed by modest daily decreases thereafter (Fig. 1). Where exposed to the normal progression of shortening photoperiods, identical to those under which it has evolved, Matanuska white developed considerably higher freeze tolerance by 30 September than under artificially shortened photoperiods (where it was deprived of all photoperiod durations between 15 and 10.5 hours).

Because onset of winter conditions at this latitude is both early and relatively abrupt (Klebesadel 1992b-Table 4), there undoubtedly has been natural selection in Matanuska white for early initiation of cold-hardiness development under longer photoperiods than would be necessary for the other cultivars when grown at their more southern latitudes. Natural selection within an introduced population of biennial yellow sweetclover (*M. officinalis* (L.) Lam.) at the Arctic Circle resulted in a strain that developed freeze tolerance earlier and to a greater extent than Canadian or U.S. cultivars in that species (Klebesadel 1992a).

The impaired early development of freeze tolerance in Matanuska white (as indicated at the 30 September sampling) parallels a somewhat similar interference with seed-head production in subarctic-adapted pumpelly bromegrass (*Bromus pumpellianus* Scribn.) when that grass similarly was deprived of pre-

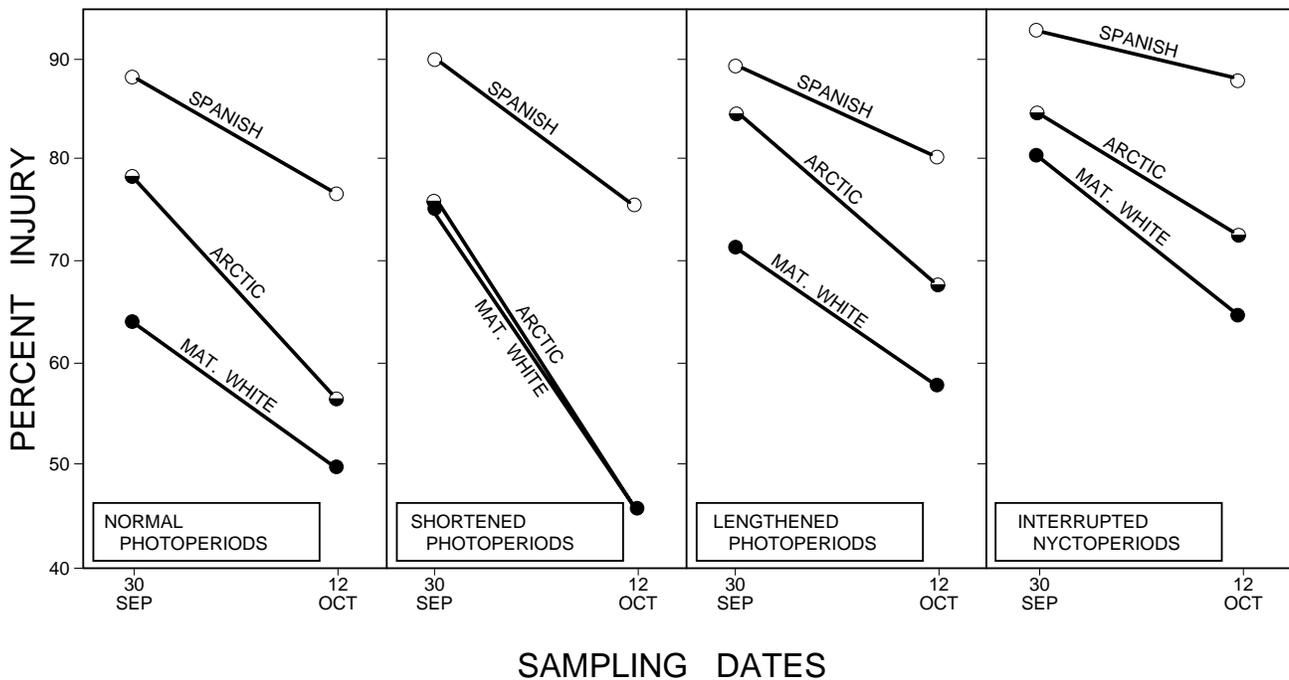


Figure 5. Percent injury from freezing on two sampling dates in overwintering tissues of three latitudinal ecotypes of sweetclover as influenced by four photoperiod/nyctoperiod treatments in outdoor experiment. Treatments were initiated 25 August, plants sampled 30 September and 12 October; percent injury was determined after artificial freeze stress at 14 °F for 20 hours.

winter exposure to accustomed critical photoperiods (Klebesadel 1971a, 1973).

The effects of the different photoperiod treatments in influencing differential tolerance to freeze stress were much greater in Matanuska white and Arctic than in the relatively nonhardy Spanish (Fig. 5). Interrupted nyctoperiods were detrimental to development of freeze tolerance in all three sweetclovers. The importance of uninterrupted nyctoperiods for cold hardiness/winter survival has been reported earlier (Klebesadel 1971a, 1973; Kramer 1937).

Lengthened photoperiods resulted in less freezing injury in all ecotypes than interrupted dark periods, but more injury than with either normal or shortened photoperiods; however, the latter differences were not statistically significant in the nonhardy Spanish. Compared with the normally prevailing daily light/dark pattern, shortened photoperiods did not increase freeze tolerance significantly in Spanish. Arctic, in contrast, with a greater genetic potential for cold-hardiness development (Klebesadel 1992b), sustained markedly less freeze injury when grown under artificially shortened than under the normally shortening subarctic photoperiods.

CHAMBER EXPERIMENT: At the only sampling of plants grown in the warm chamber, percent injury from freezing was significantly different for the three ecotypes, ranging from 80% for Spanish to 54% for Matanuska white, with Arctic intermediate at 63% (Fig. 6). Although all three sustained considerable injury from freezing, the differences noted among ecotypes probably were engendered by differential response to the changing photoperiod/nyctoperiod regime. This contrasts with the growth-chamber results of Paulsen (1968) who found no hardiness development in winter wheat seedlings without lowering temperatures.

Gradually lowered temperatures over 12 weeks, during which photoperiods were gradually shortened from 12 to 6 hours, resulted in markedly greater tolerance to freezing in all three ecotypes than where temperatures remained warm (Fig. 6). The dramatically increased freeze tolerance resulting from lowered temperatures, compared with warm, occurred to approximately the same extent with all three strains. Although Matanuska white was less injured by freezing than Arctic where preconditioning temperatures were warm, they sustained about equal injury where chamber temperatures had been lowered. The nonhardy Spanish was injured more than the other two strains under both treatments.

DISCUSSION

Earlier reports (Greathouse and Stuart 1936; Li and Weiser 1971; Metcalf et al. 1970; Paulsen 1968;

Sjoseth 1964; Smith 1964a; Steponkus 1978) have noted a close relationship between dry-matter concentration (or, conversely, moisture content) in overwintering tissues and tolerance to freeze stress. Some of the present results are in agreement with those reports, others are not. The different levels of dry-matter concentration in stem-base/crown/tap-root segments (Fig. 4), as influenced by photoperiod treatments in the outdoor study, were related in general to patterns of injury from freeze stress (Fig. 5). For example, with all three ecotypes, interrupted nyctoperiods resulted invariably in lowest dry-matter concentration and most injury from freezing.

However, dry-matter concentration in overwintering tissues was not invariably correlated with

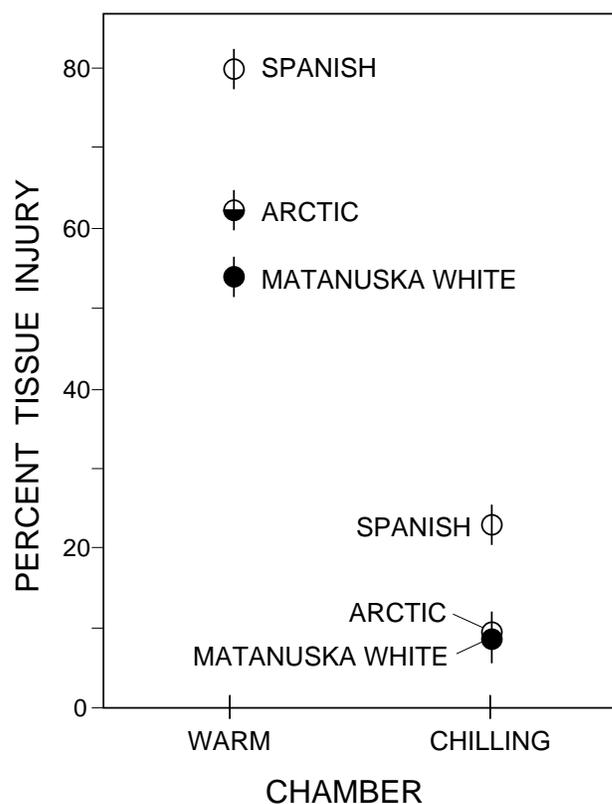


Figure 6. Percent injury from artificial freeze stress in overwintering tissues of three latitudinal ecotypes of sweetclover as influenced by two dissimilar temperature treatments imposed during 12 weeks of growth in controlled-environment chambers. One chamber (WARM) was maintained at 62° to 68°F; in the other chamber (CHILLING), temperatures alternated widely (warm during photoperiod, cool during nyctoperiod) but with gradually lowering daily minima from 40° to 32°F (see Fig. 2). Photoperiods were gradually shortened from 12 to six hours in both chambers; percent injury was determined after artificial freeze stress at 14°F for 20 hours. Means not joined by a vertical line differ significantly (5% level).

freeze tolerance. Examples in the outdoor study were: (a) Matanuska white showed highest dry-matter concentration under lengthened photoperiods, yet this treatment resulted in greater freeze injury than either normal or shortened photoperiods, (b) shortened photoperiods with Spanish resulted in markedly higher dry-matter concentration than normal photoperiods but essentially no better freeze tolerance. Further, in the chamber study, lowered temperatures did not affect dry-matter concentration in overwintering tissues, yet that treatment resulted in strikingly increased freeze tolerance.

The relatively winter hardy Arctic (Hodgson and Bula 1956; Klebesadel 1992b), as well as the less hardy Spanish, developed much higher levels of freeze tolerance in the chilling chamber (Fig. 6) than resulted from the most favorable treatment (shortened photoperiods) in the outdoor experiment (Fig. 5). Percent tissue injury at the final outdoor sampling (with the most favorable treatment) vs. the most effective chamber treatment, respectively, were Spanish (76% vs. 23%), Arctic (46% vs. 10%), and Matanuska white (46% vs. 9%).

Inasmuch as (a) the artificial freeze-stress test was identical in its evaluation of plant injury from both the outdoor and the chamber experiments, and (b) photoperiod/nyctoperiod pattern was closely controlled in both indoor and outdoor experiments, the considerable differences in freezing injury logically must be due to other factors. Three factors that seem most obvious will be discussed separately; these are (a) duration of exposure of plants to treatments, (b) different planting dates, and (c) differences in low-temperature patterns.

(a) Duration of exposure of plants to treatments: Plants in the growth-chamber experiment were exposed to treatment effects for about two weeks longer than in the outdoor experiment (Table 1). This difference could have a contributory effect but is not believed to account for very much of the marked differences in freeze tolerance noted between the two experiments.

(b) Different planting dates: Although both experiments were seeded after the summer solstice and when natural photoperiods had begun to shorten (Table 1), plants for the chamber experiment were seeded more than two weeks later than those for the outdoor experiment. The significance of different planting dates lies in the fact that it is known that temperate-latitude-adapted biennial sweetclovers spring-seeded in subarctic Alaska are disadvantaged by early growth during the very long photoperiods/short nyctoperiods that have their greatest expression during late June (Klebesadel 1985). Those conditions tilt unadapted biennial sweetclovers toward annual habit, producing (a) tall,

thick mainstem growth, (b) abundant flowering, and (c) few and small crown buds, all three being uncharacteristic of normal seeding-year growth of biennial sweetclovers when grown where they are adapted (Klebesadel 1992b; Smith 1942).

Those abnormal growth phenomena are associated with, and probably help cause, deficient pre-winter development of freeze tolerance and curtailed storage of food reserves below their inherent genetic capacity to do both. Having been "tricked" by the unaccustomed subarctic light/dark balance in summer toward behaving more like annuals than biennials, they fail to make adequate preparation for winter. Therefore, the somewhat earlier seeding of the outdoor experiment might have tended to expose plants more to the disadvantageous mid-summer light/dark conditions here than occurred with the chamber plants.

(c) Differences in low-temperature patterns: It is believed that this factor was dominant in influencing the differences in freeze tolerance achieved by plants in the two experiments. The chilling effect in the chamber was more regular and sustained (Fig. 2) than the brief onset of low minimum temperatures outdoors during late September/early October (Fig. 7). With the outdoor experiment, minimum temperatures during most of September remained generally above 40°F (Fig. 7). One early low of 30°F was recorded on 25 August. Except for that occurrence, only during late September and October were daily minimum temperatures near or below freezing.

Thus it is believed that the naturally occurring temperatures in the outdoor test, with random fluctuations and relatively high minima during most of August and September, were much less effectual in stimulating development of freeze tolerance to the genetic potential of plants than were the controlled temperatures indoors.

It is noteworthy that a very marked increase in freeze tolerance occurred during the brief 12-day period between the first and second samplings in the outdoor experiment (Fig. 5). It is believed that the rapidly lowering outdoor temperatures during late September and October assisted materially in fostering that rapid increase in freeze tolerance. However, shortening photoperiods (=lengthening nyctoperiods) with their contributory and inseparable influence, were operating prior to and coincident with the dramatic temperature change; therefore, the rapid increases in freeze tolerance undoubtedly were engendered by the combined effects of the temperature/light stimuli, and the relative influence of each can only be conjectured.

Unusually warm temperatures during the period of normal hardiness development in perennial plants, that would tend to slow the process, can adversely

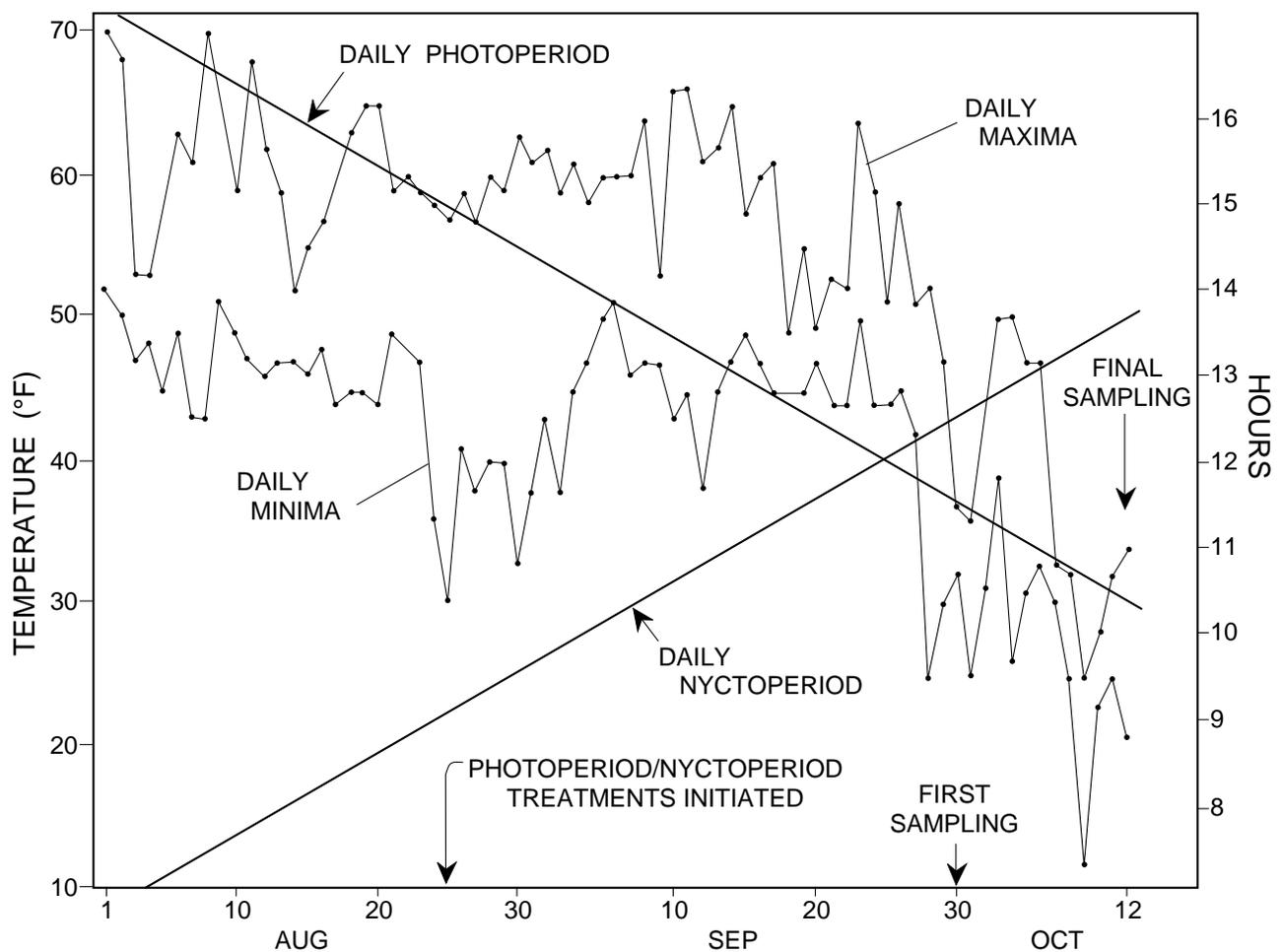


Figure 7. Daily maximum and minimum temperatures and trend lines for naturally occurring daily photoperiod and nyctoperiod for 10 weeks prior to final sampling of sweetclover ecotypes on 12 October in outdoor experiment. Experimentally altered photoperiod and nyctoperiod treatments are depicted in Figure 1.

affect winter survival. The relatively warm period during hardiness development in the outdoor experiment was somewhat similar to a term of atypically warm days in mid-October of 1975. That warm period was followed by a rapid and unusually severe plunge of temperatures that remained low for about two weeks in late October and early November, resulting in unusual and extensive winterkill locally of both herbaceous and woody plants (Klebesadel 1977).

Roadside sweetclovers were among the plants noted the following spring to have sustained very high rates of winterkill. That combination of weather effects represents conditions that can be highly effective in natural selection, eliminating plants poorly disposed to early winterhardening. Surviving plants would then interpollinate and tend to increase their better-adapted genetic/physiologic disposition toward early development of freeze tolerance within the ensuing populations.

THE INTERRELATIONSHIPS OF LATITUDE, PHOTOPERIOD/NYCTOPERIOD, AND TEMPERATURE AS THEY AFFECT ANNUAL DEVELOPMENT OF FREEZE TOLERANCE

Tysdal (1933) reported that development of freeze tolerance in winter-hardy strains of alfalfa was favored most by (a) short (7-to 8-hour) photoperiods, (b) alternating temperatures (68°F during the photoperiod and 32° to 41°F during the nyctoperiod), and (c) light intensity adequate for a high rate of photosynthesis. In the present study, the high levels of freeze tolerance attained by Arctic and Matanuska white in the chilling growth chamber were attained in general agreement with the favorable conditioning criteria described by Tysdal. The only significant divergences were a somewhat lower temperature during the photoperiod in much of the present study, and slightly shorter photoperiods at termination of the experiment.

Hodgson (1964), in an outdoor study at this location, found that artificially shortened daily photoperiods (resembling pre-winter conditions in its area of origin) favored development of freeze tolerance in temperate-adapted Ranger alfalfa (*Medicago sativa* L.), compared to exposure to normally shortening photoperiods. The practical significance of enhanced freeze tolerance should be manifested in better winter survival, and some reports confirm that linkage. In Finland, Pohjakallio (1961) found that shortened pre-winter photoperiods promoted better winter survival of red clover (*Trifolium pratense* L.) from more southern latitudes. Similarly, in Alaska, Klebesadel (1971a, 1973; 1985) reported markedly-improved winter survival of introduced, temperate-zone grasses with provision of pre-winter photoperiod/nyctoperiod regime resembling that at their latitude of origin.

Pertinent to these findings, Moschkov (1935) in Russia stated: "...many species (ecotypes) of southern latitudes survive very low temperatures without damage (in their area of origin) but freeze to death in more northern latitudes during periods of less severe temperatures... shortening the periods of daylight... in northern latitudes can produce the photoperiodic conditions of southern latitudes and, thus, produce a maximum of resistance (tolerance) to below-freezing temperatures."

Those earlier findings, and the markedly enhanced freeze tolerance fostered in Arctic sweetclover by shortened photoperiods in both outdoor and chamber experiments in the present study, illustrate clearly why ecotypes from more southern latitudes, where winter cold is often more severe than occurs in southcentral Alaska (Klebesadel 1985), often fail to survive winters here (Hodgson and Bula 1956; Klebesadel 1971a, 1971b, 1973, 1980, 1985, 1992b; Klebesadel and Helm 1986).

During the pre-winter period of lowering temperatures at more southern, mid-temperate latitudes, relatively short photoperiods (long nyctoperiods) that promote food-reserve storage and development of freeze tolerance prevail for several weeks prior to onset of winter conditions. In contrast, equally short photoperiods/long nyctoperiods prevail for a markedly shorter period prior to plant growth cessation and soil freezing in subarctic Alaska (Klebesadel 1992b-Table 4). Normal first autumn occurrence of 28°F at Palmer is 28 September, only one week after the autumnal equinox when photoperiods have shortened to less than 12 hours. Average first occurrence of 24°F is 8 October, only about two weeks after the equinox.

Bula and Smith (1954) and Smith (1964b) have noted that the winter-hardening period for herbaceous legumes in southern Wisconsin (ca. 43°N) begins near the autumnal equinox and continues for 8 to 10 weeks; during much of that time the leaves remain actively functional because the hardening period pre-

cedes killing frosts. Maximum development of freeze tolerance there is reached shortly after permanent soil freezing in late November or early December. Therefore, temperate-latitude-adapted ecotypes, attuned to developing winterhardiness during several weeks of short photoperiods, would be poorly adapted to initiating freeze tolerance to their full genetic capacity during the relatively brief interval of short photoperiods preceding growth cessation and onset of lethal freezing temperatures at subarctic latitudes.

It should be recognized that the September and October determinations of freeze-tolerance levels in the outdoor experiment represent only two assessments of plant hardiness up to the time of the soil freezing. A progression of increasing freeze-tolerance development in overwintering legumes continues well into November and December before reaching maximum levels (Bula et al. 1956). The trend lines of percent injury in Figure 5, especially with Matanuska white and Arctic, suggest that freeze-tolerance development was actively continuing. Therefore, those values represent pre-winter comparisons and thus are only broadly predictive of the final levels of freeze tolerance that would be achieved by those ecotypes.

While the results of the present study and others (Hodgson 1964; Tysdal 1933) affirm that the combination of shortening photoperiods and lowering temperatures are responsible for the several plant changes concerned in hardiness development, the relative influences of those two environmental influences logically change during the total course of those physiologic alterations.

Functional leaves are required for the photosynthetic activity responsible for buildup of stored food reserves, and must serve also as the logical receptor mechanism (Fuchigami et al. 1971; Loehwing 1938) for stimulating onset of dormancy and any other photoperiodic/nyctoperiodic "messaging" influences important to initiating development of freeze tolerance. However, the normal autumn occurrences of killing frosts in mid-to-late October in this area destroy all above-ground plant tissues, especially the leaves, well before midpoint in the total course of freeze-tolerance development. Therefore, the continued progression of freeze-tolerance development beyond the time of foliage destruction logically is solely a temperature-driven activity.

A report on factors inducing cold-hardiness development in the woody redosier dogwood (*Cornus stolonifera*) by van Huystee et al. (1967) may describe a pattern somewhat parallel to that which occurs in forage crops. They indicate that cold hardening in dogwood proceeds in two distinct phases. The first is induced by short photoperiods (long nyctoperiods), a process initiated by the triggering action of the light/

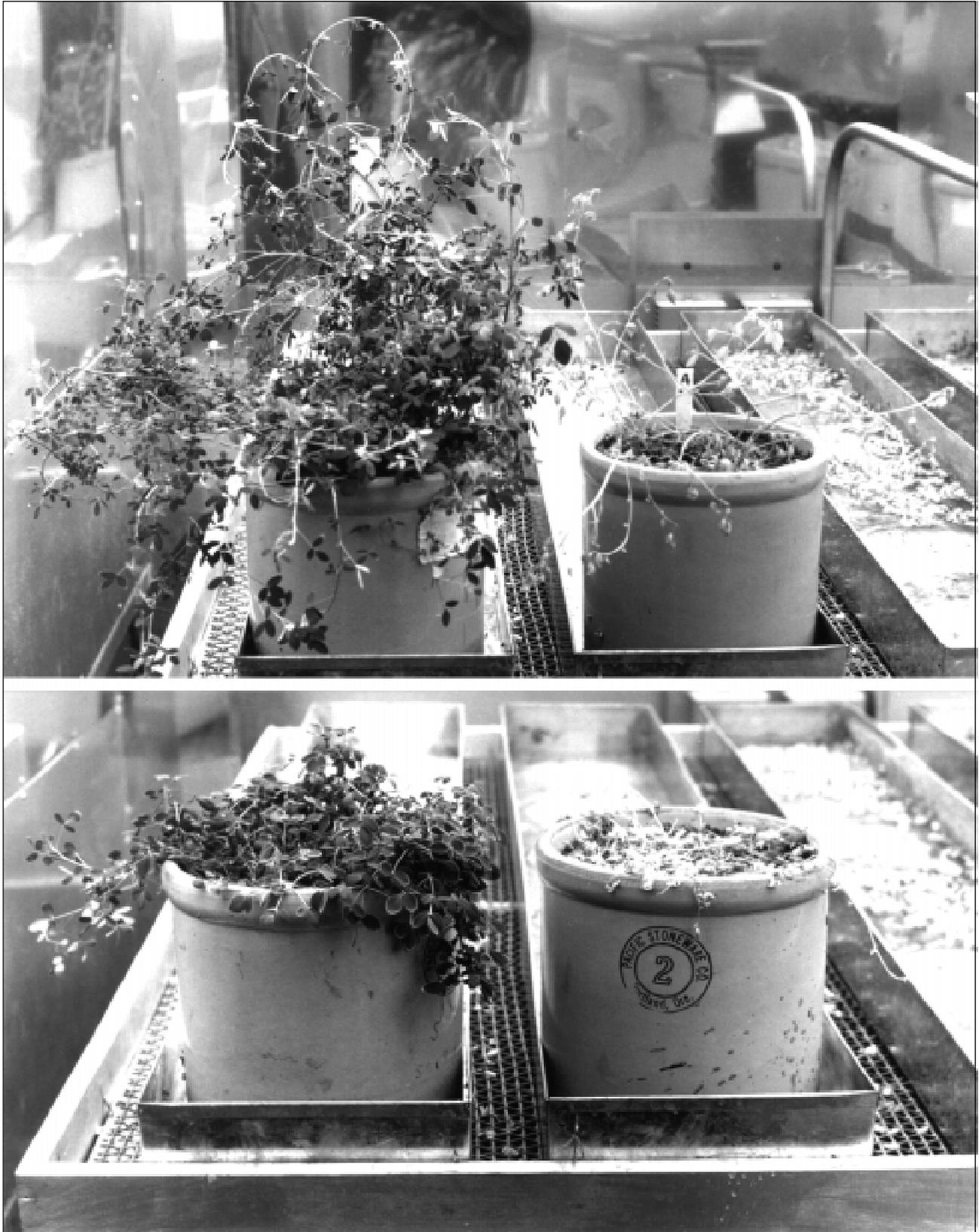


Figure 8. Appearance of sweetclover plants at termination of indoor growth-chamber experiment. Top photo shows plants of Arctic, lower photo those of Matanuska white. In both photos, left container is from warm chamber and right container is from chilling chamber.

dark stimulus on foliage as the receptor; the second phase is induced by freezing temperatures.

If similar in herbaceous forages, the cold-driven second phase would continue the process of freeze-tolerance development with low temperatures alone acting upon the overwintering tissues. The dual-action scenario of freeze-tolerance development being initiated by photoperiod/nyctoperiod influences, followed by low-temperature-driven continuation of the process considerably beyond the time of foliage destruction (Bula et al. 1956), may rationalize the respective contributions of the two stimulus effects upon the total course of freeze-tolerance development. Low temperatures obviously are important throughout the process, while the photoperiod/nyctoperiod influence can contribute only up to the time of foliage destruction by killing frost.

Paulsen (1968) reported that development of freeze tolerance in winter wheat seedlings occurred in controlled-environment chambers only when temperatures were lowered. Unlike many other reports, however, he found development of freeze tolerance was greater under constant, long (18-hour) photoperiods than under decreasing photoperiods. Citing conflicting results of several investigators, Paulsen concluded that shortening photoperiods were more effective in hardiness development in outdoor studies than in controlled-environment chambers.

Shih et al. (1967) in West Virginia measured development of freeze tolerance in alfalfa under outdoor conditions vs. after exposure to four photoperiod/temperature combinations for 10 weeks in controlled-environment chambers. Their results were the reverse of the present results with sweetclovers in that they found greatest development of freeze tolerance under outdoor conditions. However, those opposite results are believed caused by the outdoor temperatures being more favorable, and their chamber conditions less favorable, for promoting development of freeze tolerance than occurred in the present study.

Their outdoor temperatures showed many minima near freezing, with considerable diurnal amplitude, such that many daily maxima ranged from 50° to 60°F. Those temperature conditions approximate the realm of optimum for development of freeze tolerance according to Tysdal (1933), and more favorable than occurred in the present outdoor experiment (Fig. 7).

In their chamber experiment, Shih et al. (1967) found that greatest freeze tolerance developed with an unvarying short photoperiod of eight hours (16-hour nyctoperiod) in combination with cold temperatures (44.6°F during the photoperiod, 35.6°F during the nyctoperiod). That their most favorable combination of chamber stimuli was inferior to outdoor conditions

may have been due to the alternating light/dark temperature range of only 9°F (inadequate diurnal amplitude?) and a temperature of only 44.6°F during the photoperiod, considerably below the optimum photoperiod temperature of 68°F reported by Tysdal (1933).

It should be noted that, in the present study, not only temperatures (especially minima) but also photoperiod/nyctoperiod patterns differed between the outdoor and indoor experiments (Figs. 1, 2). Photoperiods in the outdoor experiment were artificially shortened from 10.5 to nine hours during the course of treatments while, in the growth chambers, photoperiods underwent a greater change (shortened from 12 to six hours).

At termination of the growth-chamber experiment, foliage on the sweetclovers remained green in the warm chamber, but had become dried and non-functional near the end of the treatment period in the chilling chamber (Fig. 8). Inasmuch as the lowest temperatures recorded in the chilling chamber reached but did not go below 32°F, the drying of the stems and foliage in the chilling chamber is believed due to senescence induced by the combination of very short photoperiods and low nyctoperiod temperatures, rather than actual freeze injury sustained by the aerial growth.

STORED FOOD RESERVES AND DORMANCY

OUTDOOR EXPERIMENT: At the 30 September sampling, Spanish showed least food-reserve storage, Arctic the most, and Matanuska white was intermediate (Fig. 9). Interrupted nyctoperiods was the least favorable treatment for food-reserve storage in Spanish and Arctic but, strangely, tended to be favorable in Matanuska white. Shortened photoperiods, a pre-winter regime more like their area of origin, clearly favored reserve storage in Spanish and Arctic.

Two weeks later, food-reserve levels were considerably higher in all three ecotypes and the ranking remained similar (Fig. 9). However, during the initial growth period in darkness there was a slower release of reserves as etiolated growth in Matanuska white than in Arctic and Spanish. This is interpreted as evidence of greater dormancy (Klebesadel 1992b). Similar behavior was noted in an introduced strain of biennial yellow sweetclover that, through natural selection over several generations, has undergone adaptive modification toward subarctic adaptation in Alaska (Klebesadel 1992a). The slower release of reserves in Matanuska white contributed to a longer term of reserve expression before exhaustion. This implies that significant reserves in that ecotype were volatilized as respiration products and thus were not measured as etiolated growth by this technique (Graber et al. 1927; Klebesadel 1992b).

In food-reserve storage, as in earlier comparisons in the present study, Matanuska white displayed less response to photoperiodic treatments than the introduced cultivars. Shortened photoperiods, creating a diurnal light / dark pattern more similar to pre-winter conditions in their areas of origin, clearly enhanced food-reserve storage in Arctic and Spanish, but was no more effective than lengthened photoperiods in Matanuska white.

Lengthened photoperiods, an environmental condition that would be encountered by growing crops at a more northern location than Palmer, resulted in slightly lower food-reserve levels than the normal subarctic light / dark pattern with Arctic and Spanish. In contrast, that treatment resulted in a somewhat increased level of reserves in Matanuska white. Interrupted nyctoperiods, compared with normal, resulted in markedly lower food-reserve storage in Arctic, somewhat less in Spanish, but did not affect total storage in Matanuska white. Moreover, interrupted nyctoperiods

tended to prevent onset of dormancy more than the other treatments in Spanish and Arctic, but did not affect dormancy in Matanuska white.

CHAMBER EXPERIMENT: Considerably higher levels of food-reserve storage were evident in all three ecotypes where temperatures had been lowered during the shortening photoperiods (Fig. 10). As with the markedly improved freeze-tolerance displayed by Spanish after growing in the chilling chamber (Fig. 6), that cultivar also stored much higher levels of food reserves in the chilling chamber than in the warm chamber (Fig. 10). Spanish surpassed Matanuska white in total food reserves measured; however, Matanuska white showed evidence of greater dormancy, especially in terms of etiolated growth produced during the first two-week period.

All three strains under both treatments, except Arctic from the chilling chamber, displayed consider-

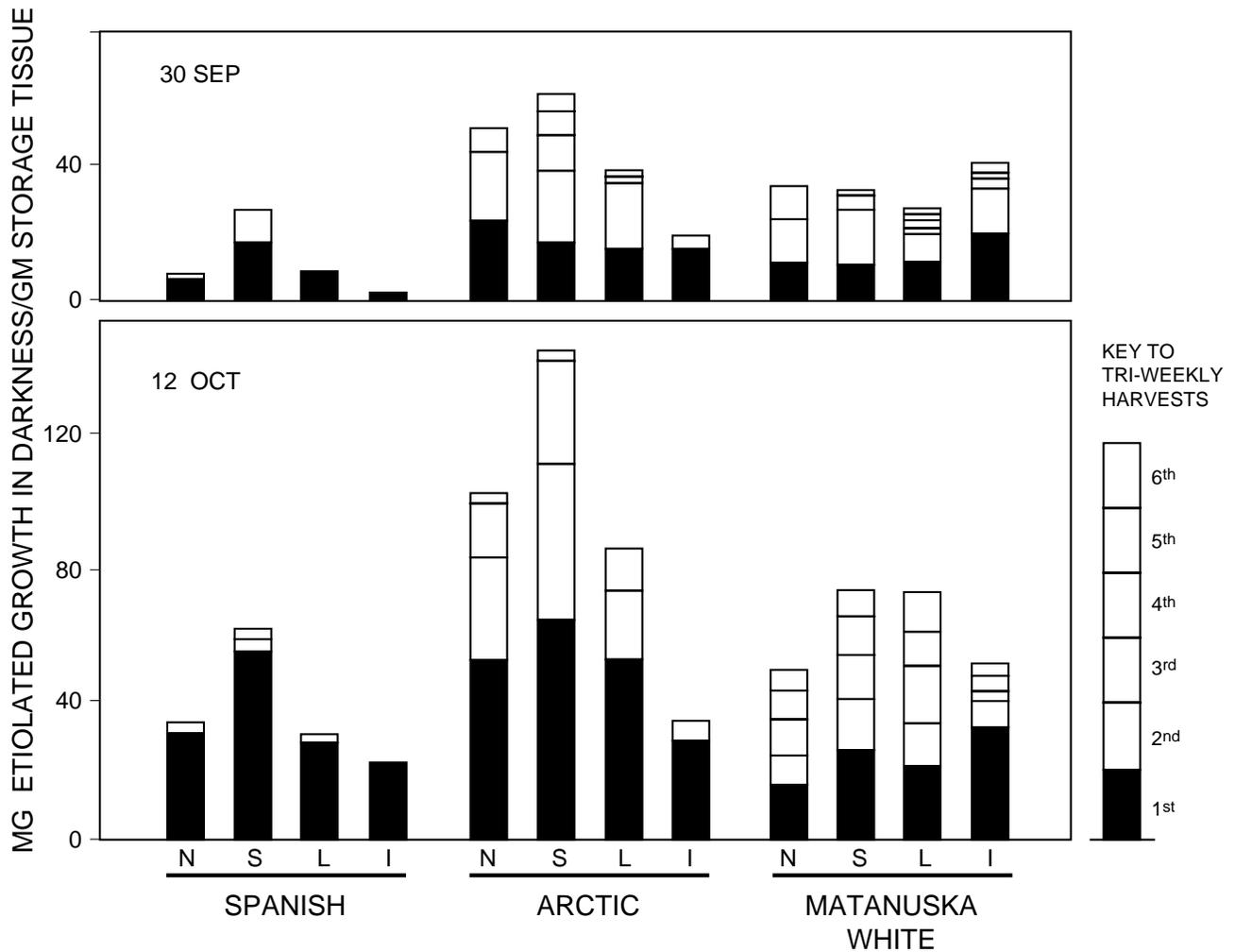


Figure 9. Levels of stored food reserves in three latitudinal ecotypes of sweetclover on two sampling dates and as influenced by four photoperiod treatments initiated 25 August in outdoor experiment. Photoperiod/ nyctoperiod treatment abbreviations: N = normal, S = shortened photoperiods, L = lengthened photoperiods, I = interrupted nyctoperiods.

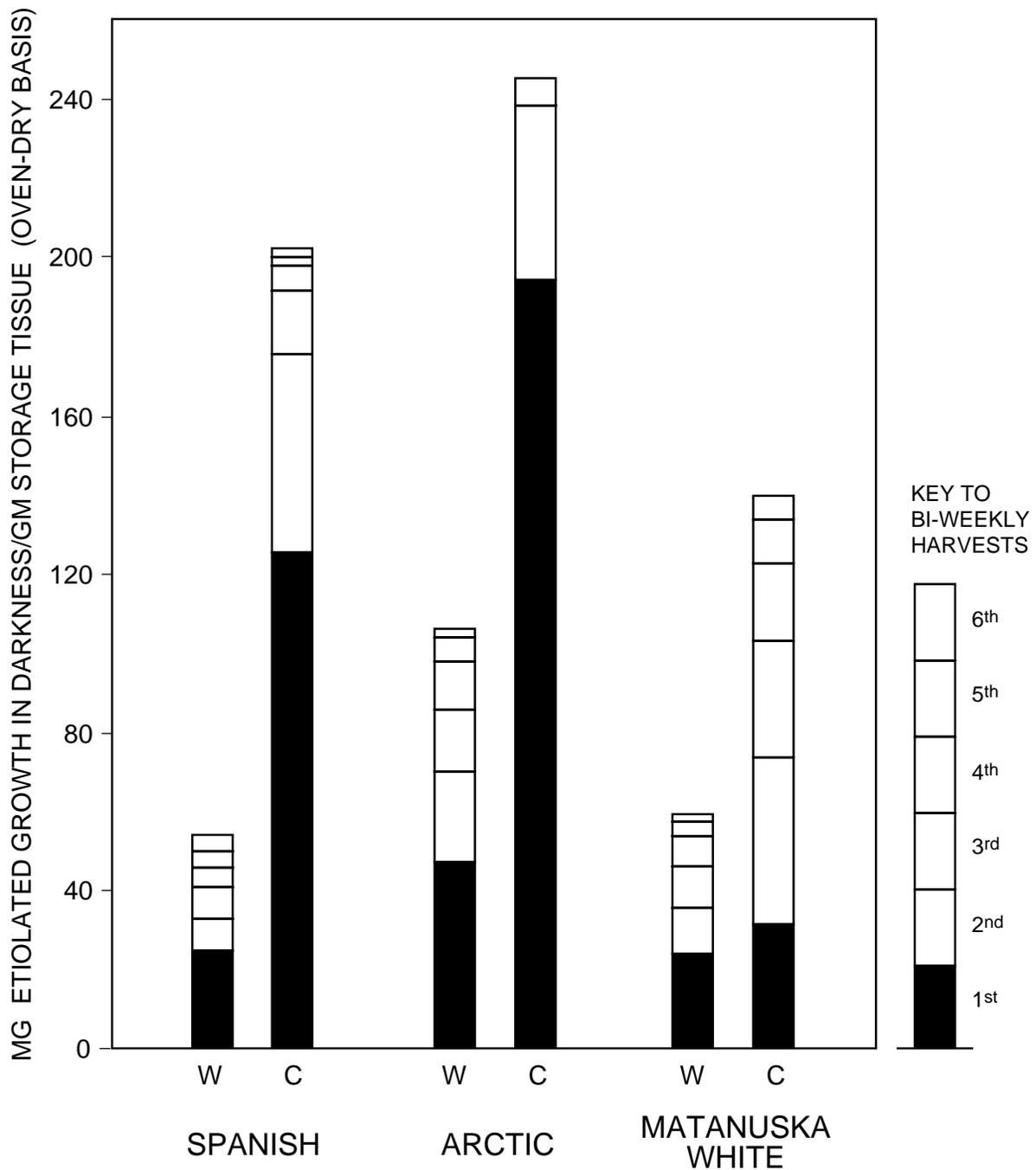


Figure 10. Levels of stored food reserves in three latitudinal ecotypes of sweetclover as influenced by growth in two controlled-environment chambers with different temperature patterns during which photoperiods were shortened from 12 to six hours in both chambers. W = constant warm chamber, C = chilling chamber with daily minima lowered from 40° to 32°F over 12 weeks (see Fig. 2).

able evidence of dormancy (i.e., slow expression of stored reserves as etiolated growth). That slow release of reserves, requiring 12 weeks (six harvests) for total exhaustion, suggested that onset of the dormancy characteristic was favored by shortening photoperiods/lengthening nyctoperiods rather than by temperature effects. That suspicion was initially unsettling

because it was believed that low temperatures would be a more logical causal stimulus for onset of dormancy.

DISCUSSION

An additional clue that light/dark effects, rather than temperature, caused onset of dormancy was noted in comparison of the two experiments. In the chamber

experiment where photoperiods were reduced to six hours (nyctoperiods lengthened to 18 hours) prior to termination of the experiment, the dormancy effect was much more pronounced, especially in Arctic and Spanish, than in the outdoor experiment where photoperiods were not shortened as much. Corroboratory evidence that light/dark effects rather than temperature serve to effectuate the dormancy was found in investigations by Coville (1920) and others since (Taylorson and Hendricks 1976; Wareing and Saunders 1971). Coville found that onset of dormancy occurred late in the growing season (when plants were exposed to short photoperiods) even when temperatures were no lower than 55° to 70°F.

The dormancy noted by Coville precluded normal growth until it was eliminated by artificial chilling of the plants (as would occur normally during the course of winter). In our earlier work with Matanuska white sweetclover, the dormancy detected, strongly evident near onset of winter, was effectively erased (a) by subjecting plants to a series of alternate freeze/thaw temperature oscillations or (b) by low temperature effects during the course of winter (Klebesadel 1992b).

Cornforth et al. (1965, 1966) identified a "dormin" that regulated bud growth in certain woody perennials. That compound was found to occur in several other plant species, also, including potato and cabbage. This plant hormone was found to increase markedly with change from long-day to short-day conditions and to stop buds from growing. That evidence parallels the present findings in sweetclover. Whether this or other similar-acting plant hormone(s) operate in sweetclover bud dormancy remains to be determined.

A more accurate assessment of absolute levels of food-reserve storage in the chamber-grown plants could have been achieved by breaking the dormancy effect (via a series of freeze/thaw exposures) before reserves were measured (Klebesadel 1992b).

The greater evidence of dormancy in Matanuska white in this and in earlier studies (Klebesadel 1992b) may be an adaptive characteristic, selectively acquired in the Matanuska Valley area where this strain evolved. This area, transitional between coastal and interior climates (Dale 1956), is randomly subjected during winter to the inflow, through broad valleys, of warm air masses from the Gulf of Alaska and cold air from interior sources. These result in highly variable winter temperatures (Klebesadel 1974), imposing considerable stresses on overwintering plants.

Dexter (1941) reported that warm temperatures during winter tend to dehardening plants, resulting in increased injury from subsequent freeze stress. It is possible that the strongly evident dormancy in Matanuska white may be a protective characteristic, acquired through natural selection in this area of strongly

alternating winter temperatures, that assist in preventing mid-winter dehardening of plants. Alternatively, that phenomenon may be merely a localized suppressive influence that serves to prevent crown bud elongation until the second year. Further work may clarify this question.

A parallel is seen between the two experiments in the treatments that resulted in (a) the highest levels of freeze tolerance (lowest percent tissue injury) and (b) the highest levels of food-reserve storage. With both responses, however, the most favorable treatment in growth chambers (chilling) surpassed the most favorable treatment (shortened photoperiods) in the outdoor experiment (compare Figs. 5 and 6 for freeze tolerance, Figs. 9 and 10 for stored food reserves).

The generally-lower nyctoperiod temperatures in the chilling chamber (than occurred in the outdoor experiment) may have had a greater suppressive effect on dark respiration, resulting in greater accumulation of food reserves in the chilling chamber. Those higher levels of food reserves, coupled with the stimulatory influences of shortening photoperiods and lowering temperatures in the chilling chamber, probably all contributed cumulatively to the higher levels of freeze tolerance manifested in all three sweetclovers by that treatment.

CONCLUSIONS

These results provide new insights concerning high-latitude adaptation, and the interrelationships of environmental stimuli and biennial sweetclover responses involved with the winter-hardening process, responses that relate directly to winter survival at high latitudes.

Provision of shortened photoperiods (lengthened nyctoperiods) for several weeks prior to freeze-up simulated a diurnal light/dark pattern, coincident with lowering temperatures, more like the areas of origin of Arctic and Spanish than occur normally at this northern latitude. With artificially-shortened photoperiods, the introduced cultivars from more southern sources (a) produced more and larger crown buds, (b) achieved higher dry-matter concentration in overwintering tissues, (c) stored higher levels of food reserves, and (d) sustained less freeze injury (marginally true with the non-hardy Spanish) than those cultivars grown with the normally-prevailing local pattern of photoperiods/nyctoperiods during autumn.

In contrast, subarctic-adapted Matanuska white appeared relatively neutral to modified photoperiods. Only in lesser injury from freezing did the northernmost ecotype respond advantageously to shortened photoperiods.

Of the four light/dark treatments compared, in-

errupted nyctoperiods were consistently the most detrimental to all three ecotypes in all characteristics evaluated (sole exception was food-reserve storage in Matanuska white). These results reinforce other evidence indicating the importance of the integrity of the diurnal dark period in plant-response phenomena.

Other conclusions include: (a) a high level of stored food reserves is a prerequisite to achieving a high level of freeze tolerance, and low nyctoperiod temperatures foster storage of highest levels of reserves, (b) shortening photoperiods/lengthening nyctoperiods acting upon leaves as the receptors apparently are important along with low temperatures in initiating the development of freeze tolerance, although (c) low temperatures are the driving stimulus that propels freeze-tolerance development to its ultimate limits well after the time of frost-killing of foliage, and (d) the detected pre-winter dormancy, effectuated by shortening photoperiods/lengthening nyctoperiods, may merely preclude elongation of crown buds during autumn or, additionally, may assist in promoting better winter survival, possibly by preventing dehardening during winter thaw periods common in this area. More study of this phenomenon is needed to fully identify its characteristics and role.

In view of all results, autumn development of crown buds and onset of dormancy were seen to be induced by shortening photoperiods/lengthening nyctoperiods rather than lowering temperatures.

The relatively northern origin in Russia of the progenitor germplasm of Arctic sweetclover, and its continued culture at relatively northern latitudes in Canada, renders understandable the generally superior winterhardiness of that cultivar over several other, more southern-adapted, introduced strains in Alaska (Hodgson and Bula 1956; Klebesadel 1992b). That northern-adaptational history also explains its better response to light/dark and temperature treatments toward high levels of food-reserve storage and freeze tolerance in this study than the more southern-adapted Spanish.

These results suggest that additional investigations should prove informative wherein the interrelationships of latitudinal ecotypes and environmental variables are assessed in terms of biochemical alterations in addition to the responses measured in this study.

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