DISSERTATION

DEEP UNDERCOOLING AND WINTER HARDINESS LIMITATIONS IN CONIFERS AND CONIFER MISTLETOE PARASITES

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MICHAEL R. BECWAR ENTITLED DEEP UNDERCOOLING AND WINTER HARDINESS LIMITATIONS IN CONIFERS AND CONIFER MISTLETOE PARASITES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS OF THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

DEEP UNDERCOOLING AND WINTER HARDINESS
LIMITATIONS IN CONIFERS AND CONIFER MISTLETOE PARASITES

This study examines the occurrence and ecological significance of deep undercooling in conifers and conifer mistletoe parasites. Differential thermal analysis was used to detect low temperature exotherms (LTE's) that result from the freezing of deep undercooled water.

LTE's near -40°C were found in winter collected stem and leaf tissue of the dominant timberline species of the Colorado Rocky Mountains, Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*), and in numerous other woody species having an elevational limit at or below timberline. Based on a 5°C/1000 m temperature lapse rate, -40°C temperatures are predicted at timberline elevation in Colorado. Therefore, the winter hardiness limitation of deep undercooling may be a factor determining the abrupt and uniform cessation of upright tree growth in this region.

The occurrence and variation of LTE's were studied in provenance collections of three boreal forest conifer species, white spruce (*P. glauca*), black spruce (*P. mariana*), and jack pine (*Pinus banksiana*). LTE's were found in the leaf tissue of all 71 acquisitions of white and black spruce between -43 and -49°C, and in 6 out of 20 acquisitions of jack pine between -45 and -47°C. LTE's were not found in

the stem tissue of any acquisition of the three species. The four most northern acquisitions of white spruce showed less variability in the LTE temperature (-47.8 $\pm$ 0.5°C) than the four most southern acquisitions (-45.8 $\pm$ 2.6°C). This suggests natural selection pressures in the north have favored trees with LTE's in leaf tissue at lower temperatures.

Seeds of two conifer dwarf mistletoe parasites collected in Colorado; *Arceuthobium americanum* and *A. cyanocarpum*, avoid freezing in winter by deep undercooling all freezable tissue water to near -33°C. Seeds of *A. americanum* cooled to -40°C, below the LTE temperature, had 0% germination. In contrast, seeds cooled to -30°C, above the LTE temperature, had 52% germination, similar to 54% for control seeds held at -2°C. Winter injury temperatures for the vegetative tissue of six conifer dwarf mistletoe parasites and *Phoradendron juniperinum* collected in Colorado and Arizona ranged from -25 to <-70°C. The vegetative tissue of the mistletoe species studied do not avoid freezing by deep undercooling and, therefore, may have maximum winter hardiness levels even lower than those determined.

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TABLE OF CONTENTS

CHAPTER		Page
I	FREEZING AVOIDANCE TO -40°C IN PLANTS	1
	ABSTRACT	1
	INTRODUCTION	2
	WINTER SURVIVAL MECHANISM	3
	DETERMINATION OF FREEZING POINTS	6
	DEEP UNDERCOOLING OF WATER	12
	THE OCCURRENCE OF DEEP UNDERCOOLING IN PLANTS	17
	ECOLOGICAL IMPLICATIONS OF DEEP UNDERCOOLING IN PLANTS	26
	CONCLUSIONS	52
	LITERATURE CITED	55
II	DEEP UNDERCOOLING OF TISSUE WATER AND WINTER HARDINESS LIMITATIONS IN TIMBERLINE FLORA	61
	ABSTRACT	61
	INTRODUCTION	62
	MATERIALS AND METHODS	64
	RESULTS	66
	DTA and Winter Hardiness Determination	66
	Timberline Temperatures	75
	DISCUSSION	77
	LITERATURE CITED	79
III	DEEP UNDERCOOLING AND WINTER HARDINESS IN PROVENANCE COLLECTIONS OF WHITE SPRUCE, BLACK SPRUCE, AND JACK PINE	81
	ABSTRACT	81
	INTRODUCTION	83
	MATERIALS AND METHODS	85
	RESULTS AND DISCUSSION	87
	CONCLUSIONS	106
	LITERATURE CITED	108

CHAPTER		Page
IV	FREEZING AVOIDANCE AND WINTER HARDINESS IN SEEDS OF CONIFER DWARF MISTLETOE (*Arceuthobium*)	110
	ABSTRACT	110
	INTRODUCTION	111
	MATERIALS AND METHODS	112
	RESULTS	114
	DISCUSSION	121
	LITERATURE CITED	124
V	WINTER HARDINESS IN VEGETATIVE TISSUE OF CONIFER MISTLETOES, *Arceuthobium* AND *Phoradendron*	126
	ABSTRACT	126
	LITERATURE CITED	133

APPENDIX		
I	LOW TEMPERAURE EXTREMES; THEIR EFFECT ON PLANTS AT TIMBERLINE	135
	ABSTRACT	135
	INTRODUCTION	136
	TIMBERLINE	138
	TIMBERLINE LOW TEMPERATURE EXTREMES . .	142
	TIMBERLINE FLORA WINTER HARDINESS . . .	143
	SUMMARY.	147
	LITERATURE CITED	148
II	EFFECTS OF SOLAR AND SUPPLEMENTAL 280 to 320 nm UV IRRADIANCE ON HERBACEOUS CROP PLANTS	149
	ABSTRACT	149
	INTRODUCTION	151
	MATERIALS AND METHODS	153
	Exclusion Study	154
	Field Lamp Study	154
	Greenhouse Lamp Study	160
	RESULTS AND DISCUSSION	161
	Exclusion Study	161
	Field Lamp Study	167
	Greenhouse Lamp Study	170
	LITERATURE CITED	173

STATEMENT OF PURPOSE

The purpose of this research was to study plant responses to specific environmental stresses. A major portion of the study was devoted to the occurrence and ecological implications of freezing avoidance by deep undercooling in conifers and conifer mistletoe parasites. A separate study was conducted to determine the effect of high levels of UV radiation on four herbaceous plant species.

The dissertation is divided into five chapters and two appendices. The chapters and appendix I pertain to the winter hardiness studies and appendix II to the UV study.

CHAPTER I

FREEZING AVOIDANCE TO -40°C IN PLANTS

ABSTRACT

In winter, some plant tissues survive low temperature extremes by maintaining their water in a liquid state to near -40°C. If temperatures drop below this lower limit of freezing avoidance, injury results. This article reviews literature on freezing avoidance by deep undercooling in plant tissue and examines ecological implications of this winter hardiness limitation.

INTRODUCTION

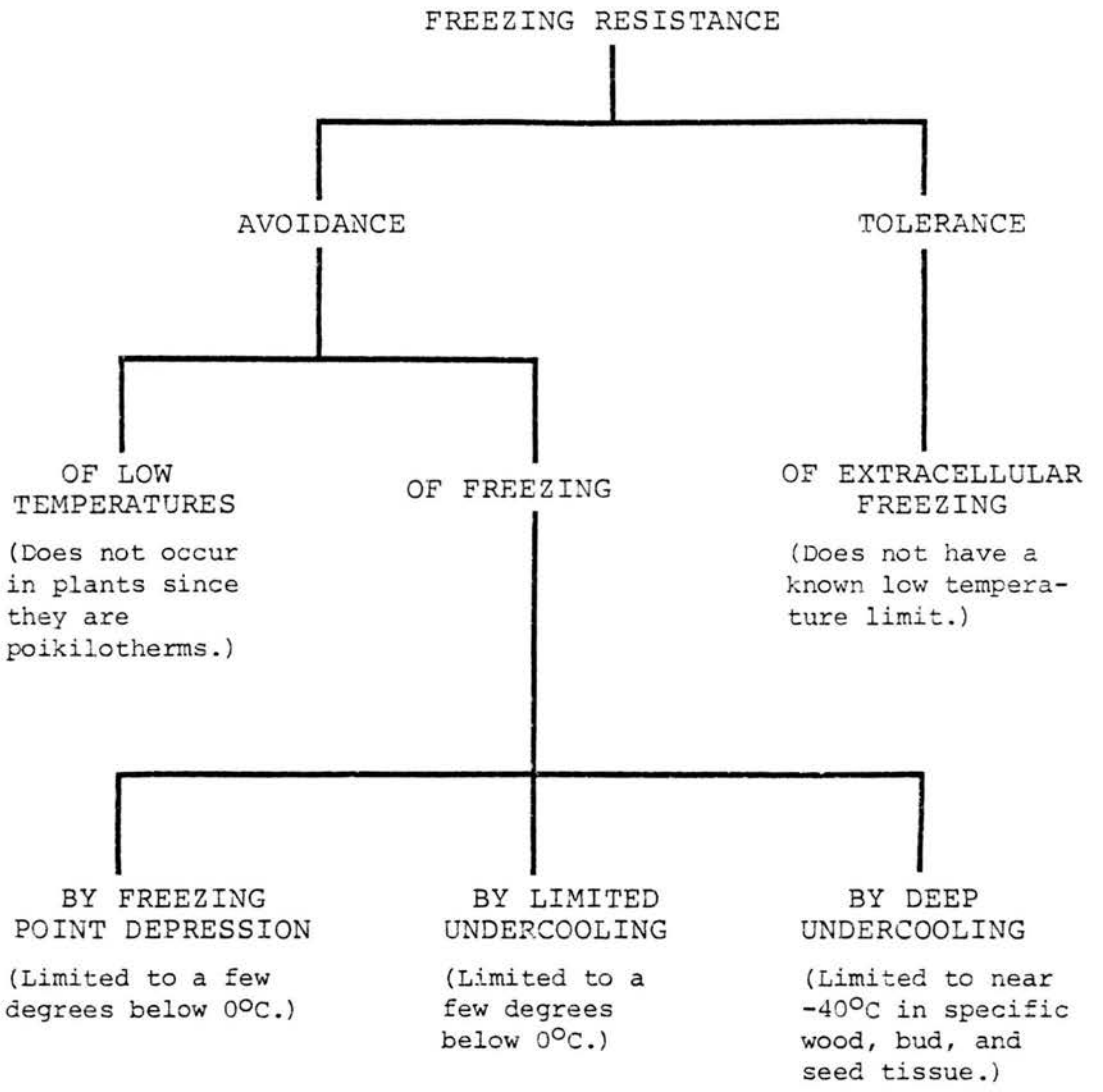
Plants, unlike warm-blooded animals, assume the temperature of their environment, even in winter (39). Biologists refer to plants as poikilotherms and temperature regulating animals as homeotherms. Thus, the tissue temperature of the living wood of an oak tree twig in midwinter is essentially the same as the ambient winter air temperature (37). In contrast, the internal core temperature of a caribou in the Yukon on a severe winter day is +38°C, far above typical ambient temperature extremes near -51°C (20). The caribou avoids the low temperature by internal heat production and insulation. The living wood tissue of the oak tree equilibrates with the low temperature. Because of this basic difference between plants and warm-blooded animals, unique survival mechanisms have evolved in plants to cope with subfreezing temperatures. Some plant tissues in winter have the ability to avoid freezing to near -40°C by deep undercooling cellular water (11, 22). Freezing avoidance by undercooling has also been found in insects, which are also poikilotherms (60). This article is about deep undercooling in plant tissue and the ecological implications of this -40°C winter hardiness limitation.

WINTER SURVIVAL MECHANISMS

Living organisms survive subfreezing temperatures by either avoiding the low temperature itself or by avoiding or tolerating freezing (39). As indicated, warm-blooded animals avoid low temperature. Most cold-blooded animals also avoid low temperature. Exceptions, as certain arctic fish, have been found (62). These fish equilibrate with the surrounding water temperature of -1 to -2°C by avoiding freezing (16, 31). Such freezing avoidance, by freezing point depression and/or slight undercooling of body fluids, is limited to a few degrees below 0°C. Since plants do not expend heat to avoid low temperature, their winter survival mechanisms are limited to tolerance or avoidance of freezing (Table I).

In winter some plant tissues tolerate freezing under conditions of slow cooling in nature. All the freezable tissue water in a paper birch tree in the Yukon is indeed frozen in midwinter (25). Freezing in the birch tree occurs between cells, *extracellularly*, rather than within cells, *intracellularly* (69). In general intracellular freezing is lethal (39). A scenario of the freezing process in a birch tree is as follows: Ice initially forms in extracellular regions (69) because the water located there is more pure than cellular water. In winter acclimated tissue, the plasma membrane serves as a barrier to ice growth into the cell (38, 40). Since the chemical

Table I. Types of freezing resistance in plant tissue, based on the convention of Jacob Levitt (39). A description of each type is given in parenthesis.



potential of ice is more negative than the chemical potential of cellular water, water moves from within cells to extracellular freezing sites. As extracellular ice accumulates, the cells dehydrate. In this dehydrated state, the dormant living tissue of the birch tree, or other very hardy trees, can survive even the lowest recorded temperature in North America, -63°C (20).

Winter survival of plant tissue by avoidance of freezing is accomplished by freezing point depression and/or undercooling (39). In plant tissue, freezing point depression is limited to about -4°C (39), and in itself would offer insufficient protection in regions where winter temperatures fall below -4°C. Undercooling refers to cooling of water "under" or below its normal freezing point without ice formation (28). Deep undercooling is undercooling to near -40°C, and occurs in selective tissue of some winter dormant plants, reproductive and vegetative buds (22, 54), wood tissue (8, 22), conifer leaves (8), and seeds (6, 35, 64).

The freezing process in an oak tree twig, which has specific tissue that avoids freezing by deep undercooling (25) occurs as follows: Ice formation initiates in the extracellular region of the cortex (69). There, interconnected regions between cells allow for formation of large aggregates of extracellular ice. Unlike the freezing process in the birch tree, not all of the cells freeze extracellularly and dehydrate. Instead, the cells of the

parenchyma tissue in the wood (xylem) remain hydrated, and their cellular water undercools (23, 25). In general, freezing does not occur in these cells unless the temperature drops below -40°C, the lower limit of this freezing avoidance mechanism. The freezing at -40°C occurs intracellularly and is lethal (23, 39). Thus, the oak tree and numerous other hardwoods have a -40°C winter hardiness limit (25). Many other trees, including birch, which do not have tissue that survive by freezing avoidance, withstand much lower temperatures (58).

DETERMINATION OF FREEZING POINTS

Water is a homogeneous molecular substance always present in living tissue, and usually in relatively large quantities. This makes detection of structural transitions in tissue water easier than detection of such transitions in other less abundant molecular substances, e.g. lipids and proteins. For water, the liquid to ice change is a heat releasing or exothermic reaction. A solid to liquid change is a heat absorbing or endothermic reaction. Methods outlined here to study freezing in plants all relate to the measurement of the heat

released during the freezing (liquid to ice transition) of tissue water.

A simple measurement of the temperature of the sample during a controlled freezing test can be very effective in determining at what temperature freezing occurs. It also provides a qualitative indication of the amount of heat released during the freezing process. This technique is referred to as thermal analysis (70). One plots the sample temperature vs. time, and a freezing profile of the sample is obtained. Such a profile shows distinct peaks or exotherms indicating the precise freezing points of the tissue water (Figure 1). Thermal analysis has limited sensitivity and can not detect the freezing of small quantities of tissue water. To improve sensitivity, differential thermal analysis (DTA) is used (50). It provides increased sensitivity enabling the detection of individual freezing events that account for very small quantities of tissue water (5). The DTA method measures the temperature difference between a living tissue sample and a dry reference material of the same relative thermal characteristics in which no freezing occurs (Figures 1 and 2). During a controlled freezing test this temperature difference is monitored on a chart recorder. The freezing of water in the sample results in a temporary temperature difference between the sample and the reference and a corresponding peak or exotherm on the chart recorder. DTA freezing profiles

Figure 1. Comparison of thermal analysis and differential thermal analysis (DTA). The peaks or exotherms indicate freezing points. (From Burke et al., 1976)

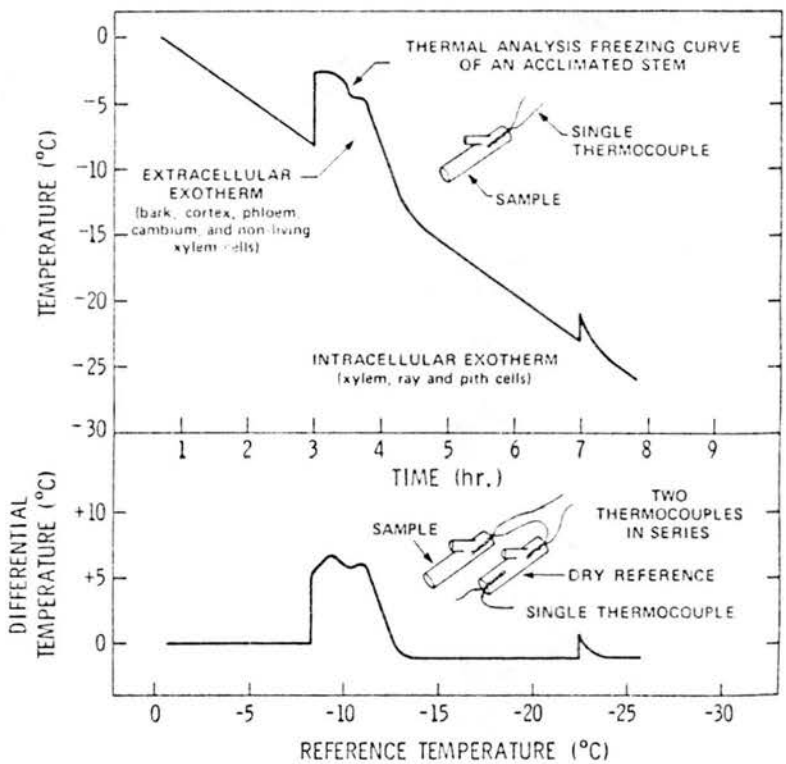
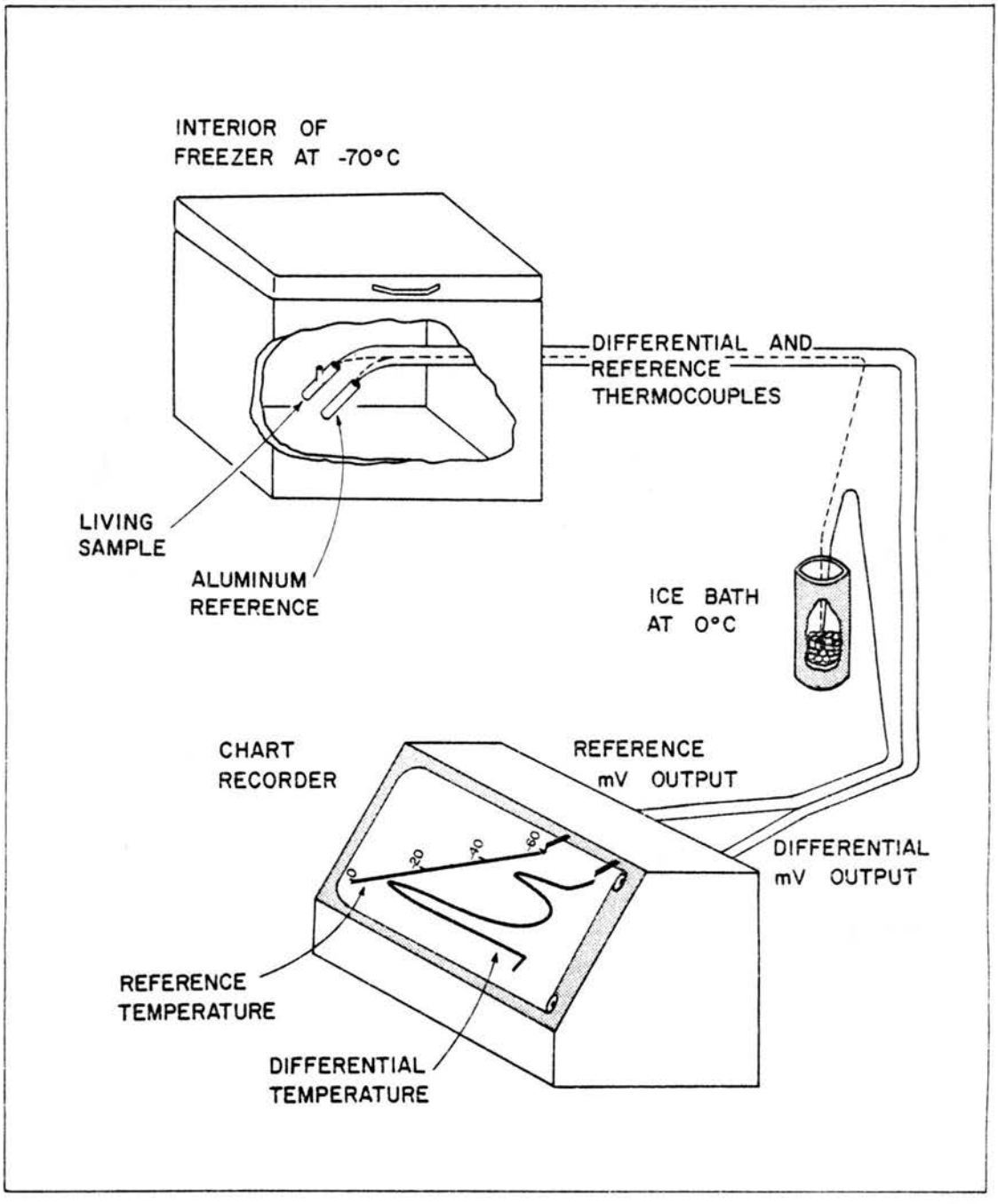

THERMAL ANALYSIS FREEZING CURVE
OF AN ACCLIMATED STEM
SINGLE
THERMOCOUPLE
SAMPLE
EXTRACELLULAR
EXOTHERM
(bark, cortex, phloem,
cambium, and non-living
xylem cells)
INTRACELLULAR EXOTHERM
(xylem, ray and pith cells)
TEMPERATURE (°C)
0
-5
-10
-15
-20
-25
-30
1
2
3
4
5
6
7
8
9
TIME (hr.)
DIFFERENTIAL
TEMPERATURE (°C)
+10
+5
0
SAMPLE
TWO
THERMOCOUPLES
IN SERIES
DRY REFERENCE
SINGLE THERMOCOUPLE
0
-5
-10
-15
-20
-25
-30
REFERENCE TEMPERATURE (°C)

Figure 2. DTA equipment and technique used to study the freezing process in plant tissue. A living sample and a dry reference material, e.g. aluminum, are placed in a freezer preset at -70°C. Thermocouples wired in series are inserted in each. Thermocouples in series detect small temperature differences. The thermocouple in the reference material also monitors its temperature. An ice bath at 0°C serves as a temperature standard for the thermocouples. As the temperature of the sample and reference decrease, the millivolt output of the thermocouples is recorded on a chart recorder. Freezing of water in the tissue releases heat and results in a temperature difference between the sample and reference, which in turn results in a millivolt output recorded as a peak or exotherm on the chart recorder. A typical freezing curve of an acclimated red oak stem is shown on the chart recorder.


INTERIOR OF
FREEZER AT -70°C
DIFFERENTIAL AND
REFERENCE
THERMOCOUPLES
LIVING
SAMPLE
ALUMINUM
REFERENCE
ICE BATH
AT 0°C
CHART
RECORDER
REFERENCE
mV OUTPUT
DIFFERENTIAL
mV OUTPUT
0
20
40
60
REFERENCE
TEMPERATURE
DIFFERENTIAL
TEMPERATURE

are usually plotted as the difference temperature vs. one of the following: the reference temperature, sample temperature, or time.

DEEP UNDERCOOLING OF WATER

If one places a beaker of tap water in a freezer it will freeze slightly below 0°C. The freezing of such bulk water is due to nucleation by foreign surface and suspended insoluble particles. These are called heterogeneous nucleators (19). Even very pure bulk water will freeze slightly below 0°C due to heterogeneous nucleation (Figure 3A). Only one ice nucleator is necessary for initiating freezing and it is exceedingly difficult to remove all ice nucleators from a large sample of water. Therefore, one might ask how water can be made to deep undercool under experimental conditions since heterogeneous nucleators are so ubiquitous. Furthermore, how can some plant tissues, which would seem to contain numerous heterogeneous nucleators, contain deep undercooled cellular water? The answer to both questions relates to the isolation of water into small quantities. Experimentally, water can be made to deep undercool by dispersing the water into

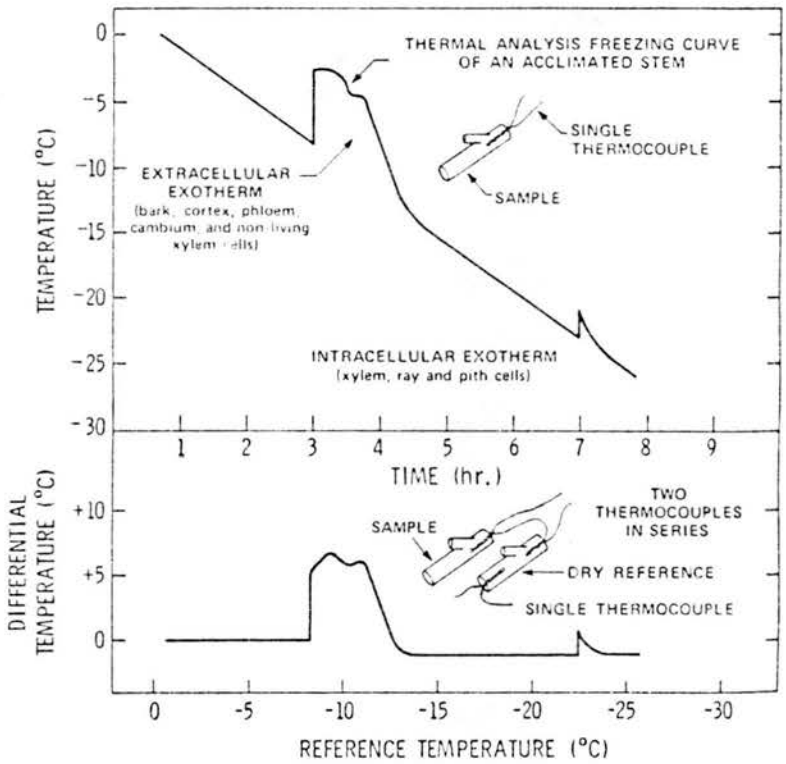
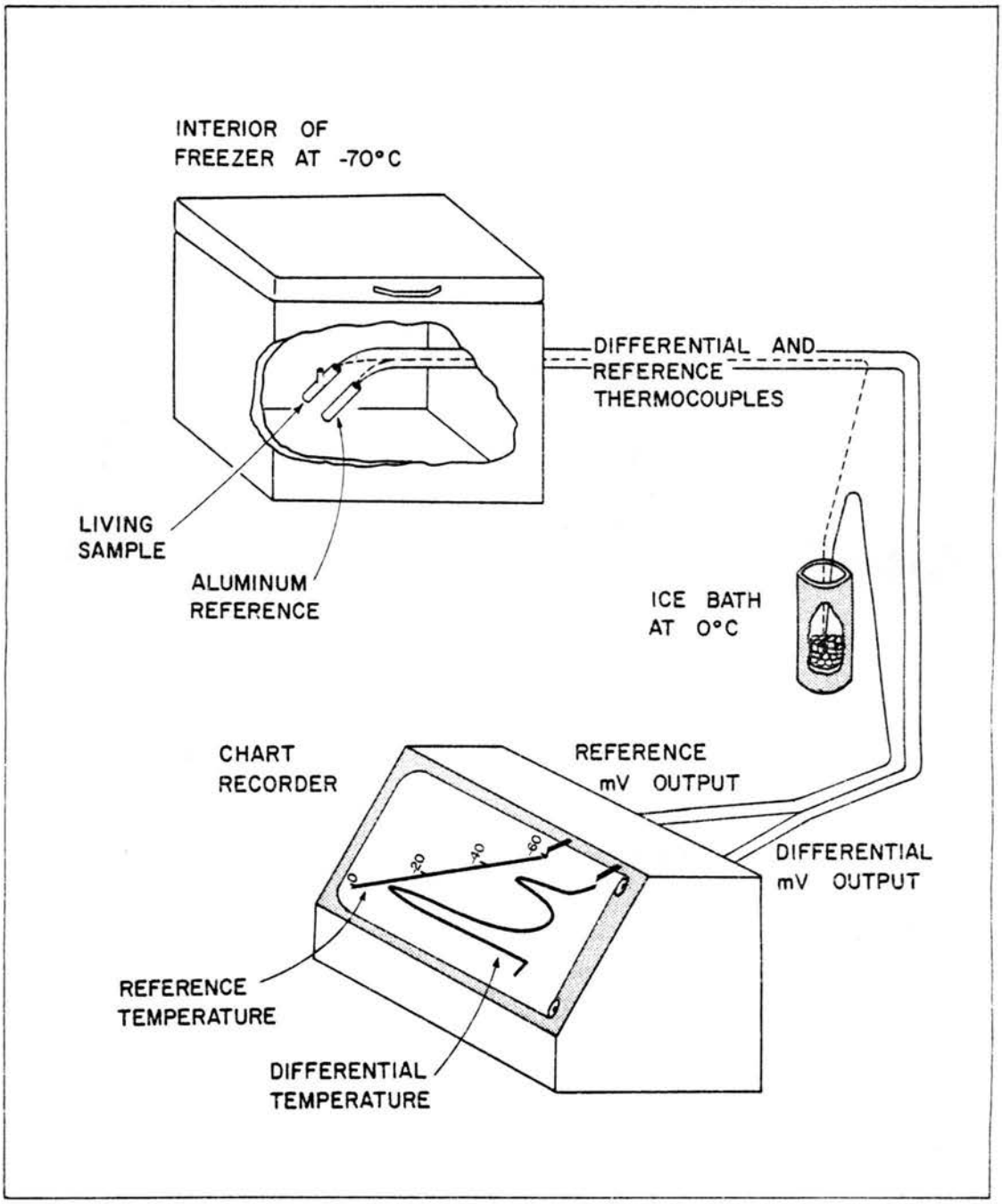

THERMAL ANALYSIS FREEZING CURVE
OF AN ACCLIMATED STEM
SINGLE
THERMOCOUPLE
SAMPLE
EXTRACELLULAR
EXOTHERM
(bark, cortex, phloem,
cambium, and non-living
xylem cells)
INTRACELLULAR EXOTHERM
(xylem, ray and pith cells)
TEMPERATURE (°C)
0
-5
-10
-15
-20
-25
-30
1
2
3
4
5
6
7
8
9
TIME (hr.)
DIFFERENTIAL
TEMPERATURE (°C)
+10
+5
0
SAMPLE
TWO
THERMOCOUPLES
IN SERIES
DRY REFERENCE
SINGLE THERMOCOUPLE
0
-5
-10
-15
-20
-25
-30
REFERENCE TEMPERATURE (°C)

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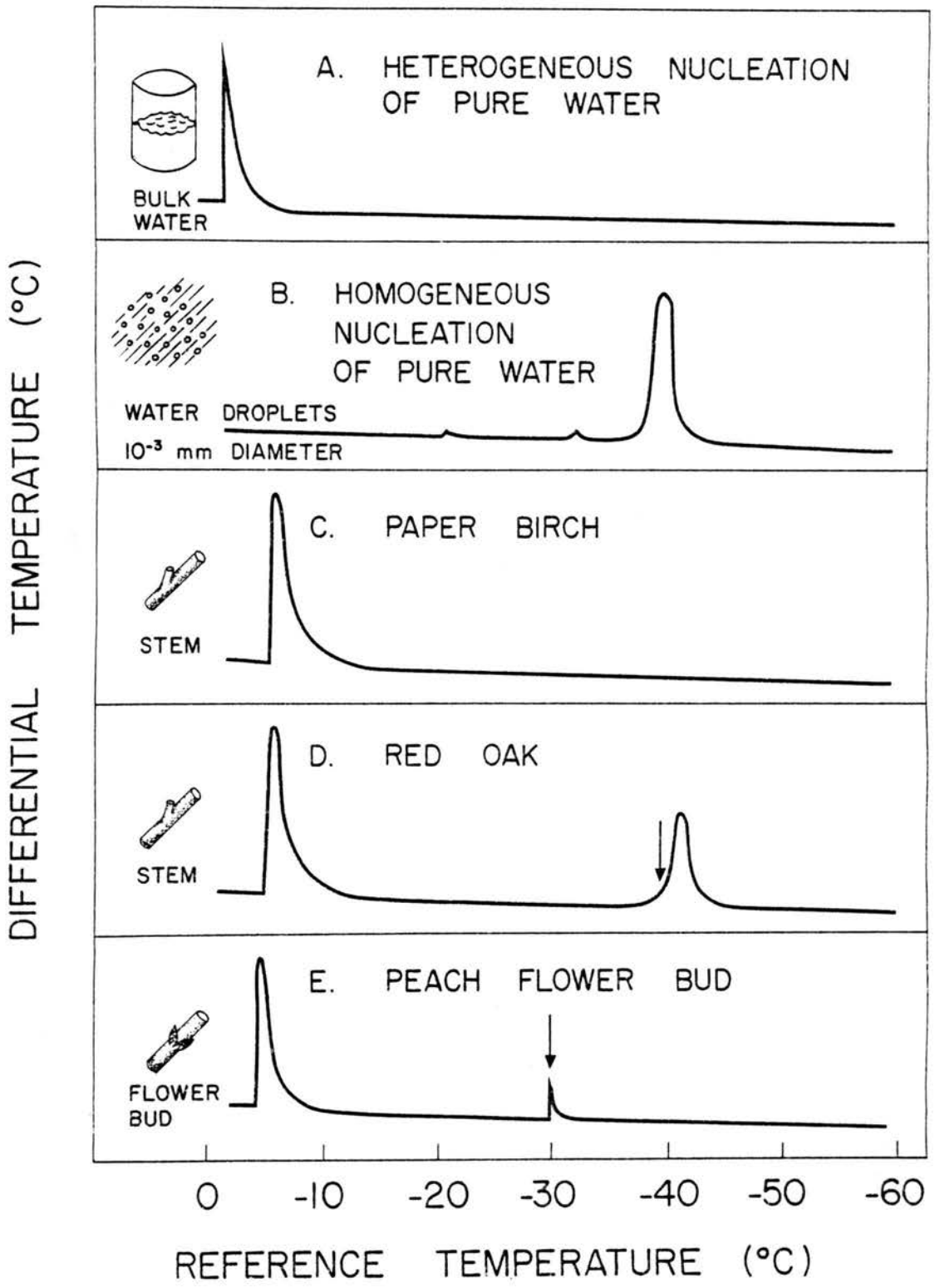

INTERIOR OF
FREEZER AT -70°C
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Figure 3. DTA freezing profiles of water and winter collected stem and bud tissue of deciduous trees. A: Pure bulk water freezes slightly below 0°C. B: Pure water dispersed in fine droplets (diameter $\leq 10^{-3}$ mm) freezes at -38°C. C: All of the freezable water in a paper birch stem freezes similar to bulk water, slightly below 0°C. D: Some of the freezable water in a red oak stem freezes like bulk water, but about one-third deep undercools and freezes at -40°C. E: The freezable water in the bud scales and axis of a peach flower bud freezes slightly below 0°C, whereas the primordia deep undercools and freezes at -30°C. The arrows indicate the temperature at which injury occurs. (From Burke et al., 1976)


A. HETEROGENEOUS NUCLEATION OF PURE WATER
BULK WATER
B. HOMOGENEOUS NUCLEATION OF PURE WATER
WATER DROPLETS
10^{-3} mm DIAMETER
C. PAPER BIRCH
STEM
D. RED OAK
STEM
E. PEACH FLOWER BUD
FLOWER BUD
DIFFERENTIAL TEMPERATURE (°C)
0 -10 -20 -30 -40 -50 -60
REFERENCE TEMPERATURE (°C)

droplets as in a cloud or more commonly in a hydrocarbon medium (53, Figure 3B). As the size of the droplets decreases, the probability that each droplet will contain heterogeneous nucleators also decreases (19). Therefore, the likelihood of maintaining water in an undercooled state increases as the droplet size decreases. As will be seen, plants which deep undercool isolate tissue water in a similar fashion.

The low temperature limit of deep undercooling is the homogeneous nucleation temperature of water, T_n (36, 53). Experimental determinations of T_n, by the method just described, indicate a value of -38°C for pure water (53). The following theoretical description, based on nucleation theory (21), explains how water can remain in a liquid state below its normal freezing point and why ice spontaneously forms at T_n. Here the water is assumed to be dispersed into very small droplets. At temperatures below the normal freezing point and above T_n, ice-like clusters of water molecules are constantly forming, breaking up, and reforming. For ice crystalization to occur, a critical cluster size must be reached. The critical cluster size decreases with decreasing temperature. Therefore, the probability of forming an ice-like cluster of critical size increases with decreasing temperature, since fewer molecules must unite to form the cluster. As long as the temperature remains above T_n the probability of forming the critical cluster size

is low and the water will remain in a liquid state for relatively long periods of time. Upon passing through T_n, the number of ice-like clusters increases rapidly while simultaneously the critical cluster size required to form ice decreases rapidly (19, 21). This combined effect leads to the spontaneous formation of ice.

The presence of solutes in the water further decreases the T_n. For dilute aqueous solutions, T_n is related to the melting point depression, T_m, as follows (53):

$$T_n = -38°C + (1.8 \pm 0.4)T_m.$$

As mentioned earlier the T_m of plant tissue is usually -4°C or less. The above equation predicts the lowest T_n for plant tissue to be -47°C. In plant tissue studied thus far the lowest freezing points observed in winter dormant tissue have been -47°C in woody stems and -49°C in conifer leaves (5, 22). Thus, within the limits of experimental error, the observed freezing points agree with the predicted T_n for such tissue.

THE OCCURRENCE OF DEEP UNDERCOOLING IN PLANTS

In 1906, K. Weigand at the Botany Department of Cornell University was among the first researchers to report that water in living plant tissue does not always freeze at its normal freezing point (69). He found that winter dormant bud tissue of several hardwood trees did not freeze until cooled below -26°C. One of the first researchers to explicitly implicate undercooling of water as a mechanism of freezing avoidance in living organisms was R.W. Salt at Lethbridge, Alberta, Canada (59). In 1950, he reported that some insects avoid freezing in winter by undercooling. In the field of plant science, the Russian physiologists I.I. Tumanov and O.A. Krasavtsev, at Moscow, were the first to propose that freezing avoidance by undercooling was a winter survival mechanism in some woody plants (66).

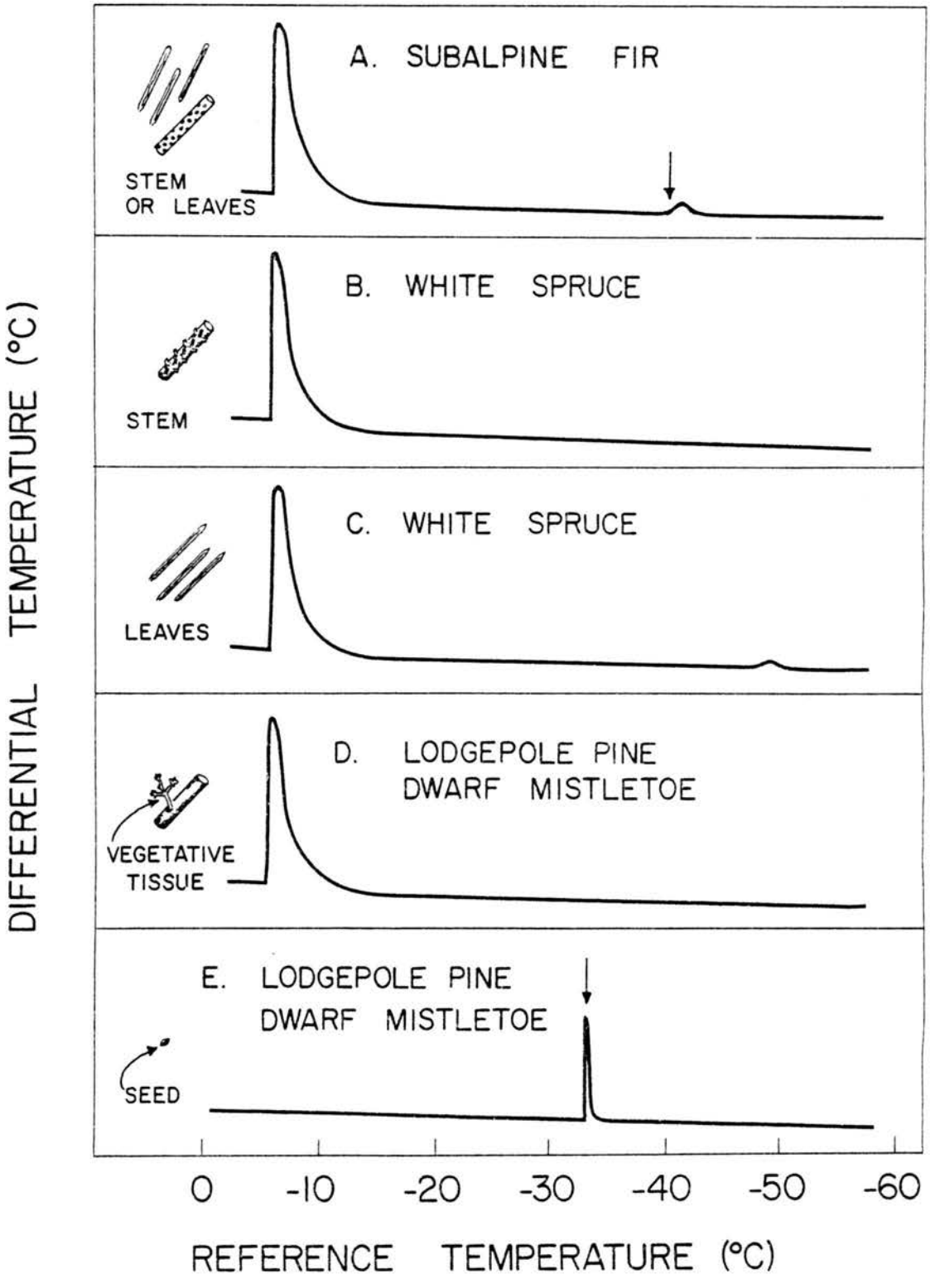
In the late 1960's major advances were made in the understanding of the freezing process in woody plants by studies conducted by C.J. Weiser and colleagues at the University of Minnesota, Laboratory of Plant Hardiness, in St. Paul (70). They used the thermal analysis method to study the freezing process in woody plant tissues. Thermal analysis freezing profiles of some acclimated stems exhibited two distinct freezing events (Figure 1). The first occurred slightly below 0°C and

was often composed of two closely positioned peaks or exotherms. In winter, injury was not associated with the first freezing event (70). The second distinct freezing event occurred at a much lower temperature, and injury was associated with this low temperature exotherm. A freezing profile of a stem of the same plant collected in summer exhibited only the high temperature freezing event slightly below 0°C, but not the low temperature exotherm found in winter collected samples (50). In summer, injury was associated with the high temperature freezing event.

The use of thermal analysis methods on woody plants marked the beginning of an era of research that greatly advanced the understanding of both the biophysical changes that occur during acclimation and deacclimation and the mechanism of injury near -40°C in woody plants. For decades it had been known that some plants can acclimate to survive sub-zero temperatures (39). Little was known about the specific changes that occur enabling a tree to withstand -35°C in winter, but be killed by the slightest frost in summer. Studies by Weiser and others (50, 70) at Minnesota and by Tumanov, et al. (66, 67) in Russia clearly showed that the freezing process itself changed with the time of season and that there appeared to be a causal relationship between low temperature freezing near -40°C and injury.

Soon the list of woody plant species with wood or bud tissue which avoid freezing to near -40°C in winter began to grow (25, 26, 27, 29, 30, 44, 45, 46, 48, 49, 51). This accumulating evidence suggested that the mechanism of freezing avoidance was indeed a common and important winter survival mechanism in temperate zone woody plants. Although DTA studies showed that injury to the living wood tissue of apple in winter was associated with a low temperature exotherm (51), there was only circumstantial evidence that the exotherm resulted from the freezing of deep undercooled cellular water. The first studies that quantitatively showed low temperature exotherms in plants were caused by the freezing of deep undercooled tissue water were conducted by M.F. George, M.J. Burke, and C.J. Weiser (26) on azalea flower buds and by George and Burke on shagbark hickory wood (23). Their work on freezing avoidance in flower buds will be discussed later. The hickory tree served as an ideal model system to study deep undercooling because in midwinter all of the freezable water in the wood tissue undercooled to near -40°C. Therefore, the DTA freezing profile of acclimated hickory wood exhibited only one exotherm near -40°C. Only a small fraction of the wood tissue water undercools in most other hardwoods and conifers which avoid freezing to near -40°C (8, 25, Figures 3 and 4).

Figure 4. DTA freezing profiles of various winter collected conifer and conifer mistletoe parasite tissues. A: Approximately 3% of the tissue water in a subalpine fir stem or leaf deep undercools and freezes at -40°C. B: All of the freezable water in a white spruce stem freezes slightly below 0°C. C: Less than 1% of the freezable water in a white spruce leaf deep undercools and freezes at -47°C. D: All of the freezable water in the vegetative tissue of lodgepole pine dwarf mistletoe freezes slightly below 0°C. E: All of the freezable water in a lodgepole pine dwarf mistletoe seed deep undercools and freezes at -35°C. The arrows indicate the temperature at which injury occurs. (For evidence, see Chapters II - V.)


A. SUBALPINE FIR
STEM OR LEAVES
B. WHITE SPRUCE
STEM
C. WHITE SPRUCE
LEAVES
D. LODGEPOLE PINE DWARF MISTLETOE
VEGETATIVE TISSUE
E. LODGEPOLE PINE DWARF MISTLETOE
SEED
DIFFERENTIAL TEMPERATURE (°C)
0 -10 -20 -30 -40 -50 -60
REFERENCE TEMPERATURE (°C)

George and Burke provided considerable evidence to show that the low temperature exotherm of hickory resulted from the freezing of cellular water in a manner predicted for an undercooled dilute aqueous solution (23). They employed various techniques, including nuclear magnetic resonance spectroscopy, differential scanning calorimetry, and low temperature light microscopy to establish that the low temperature freezing occurred intracellularly in the living parenchyma cells of the wood. These parenchyma cells occur in individual groups or bundles referred to as rays (18). In a hickory stem typical rays can be 0.5 mm in height (43). When stem tissue is sectioned to 0.1 mm in thickness, to allow for microscopic observations of freezing, the deep undercooling ability of the parenchyma tissue is destroyed. Thus, the structural integrity of the ray is necessary for deep undercooling.

In an intact hickory stem, at temperatures slightly above -40°C, the tissue water in individual rays avoids freezing while simultaneously surrounding tissue, e.g. the vascular cambium and cortex, freeze extracellularly. How can the undercooled water in ray cells remain in equilibrium with the ice in adjacent tissues without sublimating to the extracellular freezing sites? George and Burke (23) reasoned that either a barrier existed, impermeable to water movement, separating the undercooled tissue water from the ice, or that the water potential of

the ray parenchyma cells is reduced to that of ice. Experiments showed that there was no greater barrier to the exchange of D_2O with tissue water in hickory wood than in red osier dogwood stem which has little capacity to resist dehydration and does not deep undercool. This leads to the conclusion that the water potential of the parenchyma cells is reduced to that of the ice. At -40°C the water potential of the undercooled cells would have to be about -400 bars to maintain an equilibrium. Ray parenchyma cells do have thick, highly lignified, and rigid cell walls (18) and it is suspected that they can withstand compression stress equivalent to -400 bars without collapsing (43). If the cell walls surrounding each ray have capillaries of 100Å or less in diameter an equilibrium between the undercooled cellular water and ice in adjacent tissue would be maintained (1) as long as vapor cavitation is prevented.

In summary, these studies suggested that isolation of water in the ray parenchyma tissue is a key factor in allowing for deep undercooling of tissue water to near -40°C. The thick cell walls in these tissues prevent cell collapse and do not allow for the extracellular freezing and cell dehydration process to occur. Therefore, deep undercooling in wood tissue of some species is likely to be related to specific structural or morphological features of that tissue. This supposition is strongly supported by the fact that the deep undercooling

ability of the tissue is not lost after killing the tissue by extremely high or low temperatures (51). Such pretreatment invariably destroys the integrity of the protoplasmic constituents and the plasma membrane, but the general structural integrity of the cell wall should remain intact.

DTA freezing profiles of many temperate zone woody stems exhibit a broad low temperature exotherm in winter, typically over a 5 to 10°C temperature range (25, Figure 3D). In contrast, DTA freezing profiles of many temperate zone flower primordia in winter exhibit a sharply spiked low temperature exotherm (12, 25, Figure 3E). Calorimetry studies show that the broad exotherm of the stem is comprised of a number of individual freezing events. Each corresponds to a separate nucleation event that occurs in an isolated group of ray parenchyma cells (23). The spiked low temperature exotherm in flower primordia indicates that it is caused by a single nucleation event and that ice growth through the primordia is extremely rapid and explosive in nature (24). Primordia are killed by such low temperature freezing (26, 47, 70).

As indicated earlier, water can be made to undercool to near -38°C if it is dispersed into very small droplets. George, Burke, and Weiser (26) have proposed the following mechanism to explain how isolated tissue such as flower primordia can avoid freezing by

undercooling to similar temperatures. In *Rhododendron* flower primorida the cells are small (a typical cellular volume is 10^{-8} cm^3), tightly packed, and have thin cell walls (61). In contrast to stems, there are no large extracellular spaces to accommodate extracellular freezing. It is known that ice will not form in a capillary of 10^{-7} cm diameter above -30°C (40). If individual primordia cells were isolated by cell walls with capilaries of 10^{-7} cm diameter, ice growth could not spread from cell to cell until the temperature dropped below -30°C. Above -30°C heterogeneous ice nucleation would have to occur within each cell for the whole primordium to freeze. A key factor in this explanation of how flower primordia avoid freezing is whether or not individual primordia cells contain heterogeneous nucleators.

Studies by D. Rasmussen and colleagues at the Cryobiology Research Institute at Madison, Wisconsin showed that living cells do not necessarily provide effective sites for heterogeneous nucleation (52). They showed that individual yeast cells, contained in small water droplets dispersed in oil, deep undercool to 30°C without freezing. They concluded that if the cells contained effective heterogeneous nucleators, freezing would have initiated above the homogeneous nucleation temperature, -38°C. Although the cells could be cooled to -35°C and rewarmed without injury, they could not be held at -35°C for extended periods of time without injury. This

suggests that a secondary process within the cells generates substrates, e.g. heterogeneous nucleators, on which ice can eventually form. Besides the relevance of this study to the occurrence of freezing avoidance in flower primorida just cited, determining rates of freezing due to heterogeneous nucleation is of great practical importance in the field of cryopreservation.

ECOLOGICAL IMPLICATIONS OF DEEP UNDERCOOLING IN PLANTS

It is logical to reason that low temperature, in some way, limits the distribution of native plants. It is more difficult to ascribe a specific aspect of low temperature that is limiting. Are distributions limited by extreme or average minimum temperatures? Is duration of exposure to one or both of the above important? Furthermore, what physiological response of the plant to the key limiting factor of low temperature ultimately prevents it from growing in colder regions? The freezing of deep undercooled tissue water near -40°C is an example of a plant response to lower temperature that is known to limit plant hardiness (22). The subject of this section is how this freezing avoidance mechanism is associated with native plant distributions.

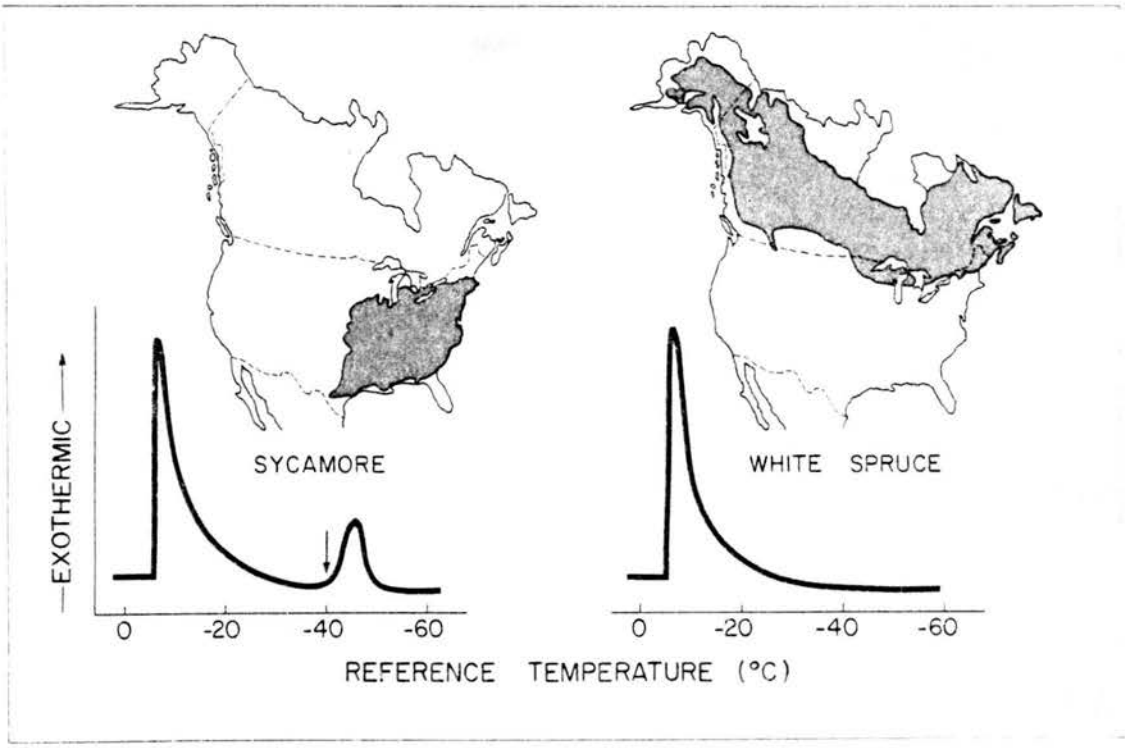
Studies by George et al. (25) at the Laboratory of Plant Hardiness at the University of Minnesota showed that hardwood trees which survive by deep undercooling to -40°C are not native to regions where -40°C temperatures occur regularly. Specifically; their northern distribution coincides with the -40°C average annual extreme minimum temperature isotherm (Figures 5 and 6). Trees with low temperature exotherms resulting from the freezing of deep undercooled tissue water are native to the Eastern Deciduous Forest of the eastern United States. Trees native to the more northern Boreal Forest of Canada and Alaska do not have low temperature exotherms and they survive lower temperatures (25, Figure 6). In all, 49 woody plants species native to North America were surveyed. Twenty-five of the 32 species native to the Eastern Deciduous Forest (78%) had low temperature exotherms between -41 and -47°C. None of the 17 species native to the Boreal Forest of Canada had low temperature exotherms.

Deep undercooling also occurs in the wood tissue of numerous horticulturally important trees and shrubs (12, 44, 46, 49, 50). Apple and pear are examples that have wood ray parenchyma tissue that deep undercools to near -40°C and is injured by exposure to lower temperatures (46, 51). The relationship between deep undercooling and the northern limit of apple and pear production is the same as that found in hardwoods native

Figure 5. Plant hardiness zone map of North America. The map is divided in three hardiness regions. Region A has average annual minimum temperatures below -40°C and much lower extreme minimum temperatures. Region B has a finite probability of -40°C temperatures but not on a yearly basis. Region C has no probabilit of -40°C temperatures. The dotted line between regions A and B is the -40°C average annual minimum temperature isotherm. (From Burke et al., 1975)


HUDSON BAY
A
B
C

Figure 6. DTA freezing profiles of winter collected stem tissue and native tree distributions. A sycamore tree native to the Eastern Deciduous Forest has a low temperature exotherm in the stem near -40°C resulting from the freezing of deep undercooled tissue water. Its distribution is restricted to a region south of the -40°C isotherm where temperatures rarely, if ever, reach -40°C. (Compare its distribution to the -40°C isotherm in Figure 5.) A white spruce tree native to the Boreal Forest of Canada does not deep undercool its stem tissue water and is native to regions where temperatures regularly drop below -40°C. (From George et al., 1974)


SYCAMORE
WHITE SPRUCE
—EXOTHERMIC—
0
-20
-40
-60
0
-20
-40
-60
REFERENCE TEMPERATURE (°C)

to the Eastern Deciduous Forest. Namely, apple and pear production does not occur in regions where -40°C winter temperatures occur regularly (46). The wood ray parenchyma tissue injured at -40°C is involved in lateral transport of water and nutrients, but is not considered to be of vital importance for tree survival (18). The close relationship between the northern limit of trees in which injury to wood ray cells occurs at -40°C and the -40°C temperature isotherm suggests that they are of ultimate importance in tree survival (Figure 6).

Blackheart is a winter related injury that occurs in apple and pear trees grown in marginal northern production regions with severe winter temperatures (13). The symptom of this winter injury is characteristically the same as observed in apple and pear wood frozen below -40°C, i.e. browning of the ray parenchyma tissue (17, 50). Blackheart injury weakens the tree, and secondary wood rotting organisms often eventually result in death of the tree (13). Based on this evidence, it seems certain that the initial cause of blackheart injury is the freezing of deep undercooled water in the living ray parenchyma cells of the wood. Hardwoods having the same freezing avoidance mechanism as apple and pear are also likely to be eventually killed by secondary wood rotting organisms if their ray parenchyma cells are initially injury by exposure to -40°C.

As stated previously, the mechanism of freezing avoidance by deep undercooling also occurs in dormant flower buds of numerous temperate zone woody plants. Although the native distribution of some woody species may be restricted by the freezing of deep undercooled tissue water in dormant bud tissue (54, 55), this does not always appear to be the case. In an extensive study of numerous *Prunus* species, Burke and Stushnoff (12) found that pin cherry and chokecherry are both native to the Boreal Forest region of Canada, and yet pin cherry flower buds have a low temperature exotherm and chokecherry flower buds do not. The wood tissue of these *Prunus* species does not have a low temperature exotherm (12).

How can pin cherry, with flower buds that deep undercool in winter, be native to regions of Canada where winter temperatures routinely drop below the lower limit of undercooling (42)? Apparently, the answer relates to a basic difference between the properties of deep undercooled water in wood and flower buds. Exposure to subfreezing temperatures for extended periods of time affects the position and size of the low temperature exotherm in some flower buds but not in wood tissue (12, 51). When flower buds of pin cherry were held at subfreezing temperatures (above the primordia exotherm temperature) for several hours, the primordia exotherm moved to lower temperatures and became smaller. If the buds were held at the prefreezing temperatures for

extended prefreezing treatments no injury occurred in flower primordia cooled to as low as -80°C (12). Such rapid acclimation may explain how flower buds of pin cherry survive without injury in northern regions.

Although freezing avoidance by deep undercooling has not been found in the stem tissue of any Boreal Forest tree species (12, 25), it has been found in the leaf tissue of Boreal Forest conifer trees. In studies on provenance collections of white spruce, black spruce, and jack pine, Becwar (5) found low temperature exotherms in winter acclimated leaf tissue between -43 and -49°C. Such exotherms accounted for less than one percent of the freezable tissue water. (A provenance collection is composed of trees grown at one site from seeds collected throughout the native distribution of the species). In contrast to the low temperature exotherm in pin cherry flower buds, the low temperature exotherm of white spruce leaf tissue does not change in size or position after similar prefreezing for extended periods of time. Viability tests of white spruce did not detect injury associated with the small amount of low temperature freezing in the leaves, and most samples survived -70°C without injury (5). These results suggest that the leaves of some Boreal Forest conifer species tolerate the injury associated with a small amount of low temperature freezing, since they are native to regions where temperatures often drop below -50°C (42).

The following three conclusions summarize the ecological implications of deep undercooling to the northern distribution of woody plants. First, low temperature exotherms that result from the freezing of deep undercooled tissue water in stem tissue of hardwoods cause injury to specific tissues, and this may be the most important factor limiting their northern distribution (10, 25). Second, flower buds of some trees lose their low temperature exotherm by prolonged exposure to subfreezing temperatures and survive much lower temperatures (12). Therefore, it does not seem likely that freezing avoidance by deep undercooling in the flower buds of such trees limits their distribution. Lastly, small low temperature exotherms in some Boreal Forest conifer leaves are not associated with detectable injury symptoms and probably do not play a role in limiting the northern distribution of such trees (5).

The transition from the Deciduous Forest to the Boreal Forest is associated with the -40°C average annual minimum temperature isotherm. A majority of the native hardwoods south of the isotherm have a -40°C hardiness limit, whereas trees native to regions north of this isotherm survive much lower temperatures (25, Figure 6). Since minimum temperatures decrease with increasing elevation (3, 65), one might ask if a similar transition occurs in the woody flora of mountainous

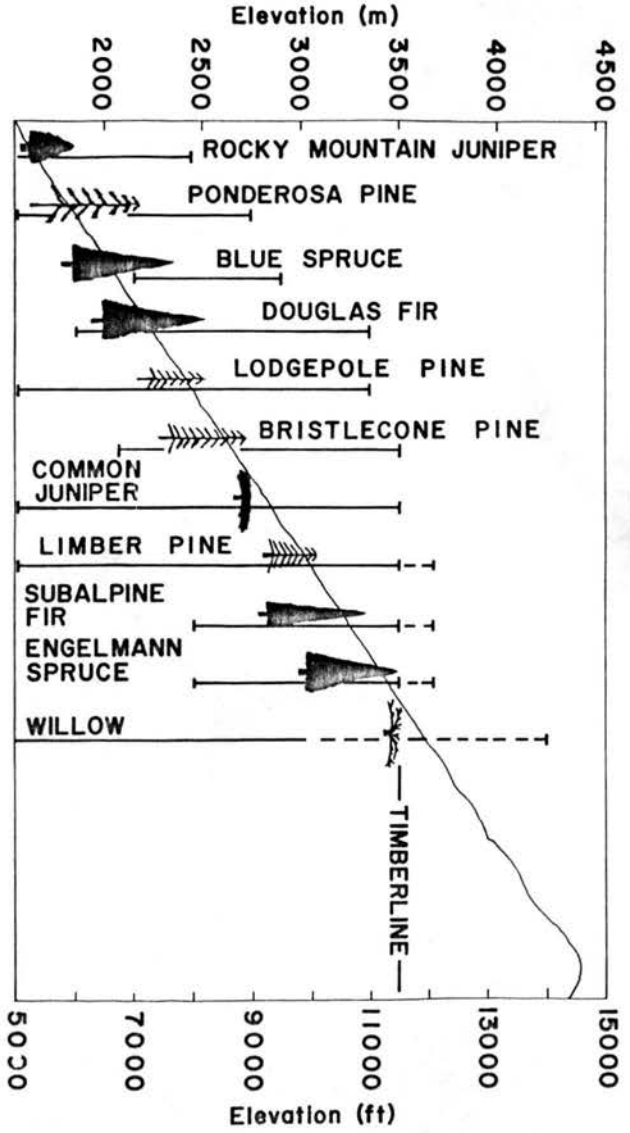
regions at an elevation corresponding to the -40°C isotherm. That is, could there be an upper elevational limit of trees having a -40°C hardiness limitation in mountainous regions, just as there is a northern limit imposed by the same hardiness limitation?

The most prominent transition in mountainous flora is timberline (41, Figure 7). It marks the upper elevational limit of upright tree growth (2, Figure 8). Above this elevation only low growing woody shrubs and herbaceous alpine plants survive. The woody flora at timberline and lower elevations is dominated by coniferous trees with hardwood shrubs as understory vegetation (2). Recently, Becwar et al. (8) conducted a study to examine the winter hardiness of the timberline flora native to the Colorado Rocky Mountains and to determine the minimum temperatures that occur at the elevation of timberline. They found that a majority of the flora, including dominant timberline conifer species (Figure 4A), have a -40°C winter hardiness limit. This is due to the presence of a deep undercooled fraction of tissue water. Furthermore, based on temperature lapse rates of 5°C cooling per 1000 m elevation gain in mountainous regions (3, 65), -40°C low temperature extremes are predicted at the elevation of timberline. Lower temperature extremes are predicted above timberline in Colorado (2). These results suggest that from a winter hardiness point of view, the timberline limit is analogous to the

Figure 7. Timberline in the Colorado Rocky Moutains is an abrupt transition from the coniferous forest to open alpine tundra. The area shown is near Berthoud Pass, Colorado, where timberline occurs at 3440 m.



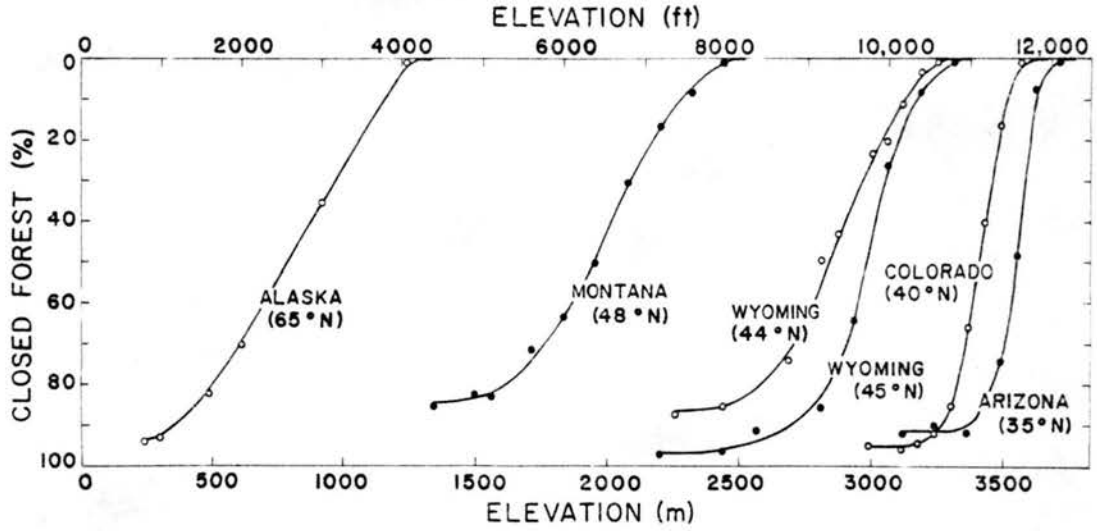
Figure 8. Dominant native woody flora in the Colorado Rocky Mountains. In this region, 40° north latitude, timberline occurs at 3400 m. The dominant trees at timberline are Engelmann spruce and subalpine fir. Solid vertical bars by each species indicate its elevational distribution as an upright tree. Dashed extensions of bars indicate that the species extends to higher elevations as a compact, low growing, shrub. (From Becwar et al., 1980)


Elevation (m)
2000
2500
3000
3500
4000
4500
ROCKY MOUNTAIN JUNIPER
PONDEROSA PINE
BLUE SPRUCE
DOUGLAS FIR
LODGEPOLE PINE
BRISTLECONE PINE
COMMON JUNIPER
LIMBER PINE
SUBALPINE FIR
ENGELMANN SPRUCE
WILLOW
TIMBERLINE
5000
7000
9000
11000
13000
15000
Elevation (ft)

northern limit of the Eastern Deciduous Forest. Above timberline -40°C temperatures occur with increasing probability preventing survival of trees which avoid freezing to -40°C, just as hardwoods with the same hardiness limit are not native to northern regions where -40°C temperatures occur regularly. The following desciption of the physiographic features of timberline further suggests that the cause of this transition in Colorado is an abrupt plant stress response, e.g. the freezing of deep undercooled tissue water at -40°C.

Timberline, as used here, refers to 'alpine' timberline of mountainous regions in the temperate zone and not to the northern limit of tree growth, 'boreal' timberline. In the temperate zone of North America, timberline occurs as far south as 35° north latitude (2). There, the elevation of the 50% transition from closed forest to alpine tundra occurs at 3600 m elevation (Figure 9). Sixteen hundred kilometers to the north in Glacier National Park, Montana, timberline occurs at 2000 m (Figure 9). In general, timberline elevation in the Rocky Mountains decreased about 125 m per degree latitude in moving from south to north (15). The environmental factor responsible for the cessation of tree growth in this region must therefore have a similar slope with change in latitude. The elevation of low temperature isotherms do in fact decrease with increasing latitude in a fashion similar to the

Figure 9. The timberline transition from closed forest to open alpine tundra of six mountainous regions in the western United States. The open and closed circles are percent closed forest values calculated at various elevations on contour lines of U.S.G.S. topographical maps of each region. Note that the timberline transition occurs at higher elevations and is very abrupt at southern latitudes, whereas the transition at northern latitudes occurs at lower elevations and is more gradual. The sharp transition in the southern latitudes suggests that an abrupt plant stress response limits tree growth in relation to a gradually worsening environment at higher elevations.


ELEVATION (ft)
0
2000
4000
6000
8000
10,000
12,000
CLOSED FOREST (%)
0
20
40
60
80
100
ALASKA
(65°N)
MONTANA
(48°N)
WYOMING
(44°N)
WYOMING
(45°N)
COLORADO
(40°N)
ARIZONA
(35°N)
0
500
1000
1500
2000
2500
3000
3500
ELEVATION (m)

elevation of timberline in this region (15). The timberline transition also becomes less abrupt in moving from south to north (Figure 9). In Rocky Mountain National Park, Colorado, 80% of the transition occurs over a 200 m change in elevation. In contrast, in McKinley National Park, Alaska, 80% of the transition occurs over a 800 m change in elevation.

The elevation at which timberline occurs in Rocky Mountain National Park, Colorado, is essentially independent of exposure. To determine this, measurements were made on U.S.G.S topographical maps of this region. The elevation of timberline was recorded at 1.6 km (one mile) intervals along the line forming the upper limit of the closed forest. The total distance covered in this manner was 177 km. These data were averaged to give the following results:

3339 $\pm$ 113 m, all exposures included
3327 $\pm$ 94 m, north " "
3366 $\pm$ 85 m, south " "
3328 $\pm$ 120 m, east " "
3319 $\pm$ 135 m, west " "

It is unlikely that one environmental factor would cause timberline to be at 3328 m on east-facing slopes and that a different factor would cause it to be at a nearly identical elevation, 3327 m, on north-facing slopes.

In summary, the sharpness of the timberline transition in Colorado and its uniformity of elevation over a large region independent of exposure suggest that one environmental factor is ultimately limiting tree growth. Such a sharp transition in this region is indicative of an abrupt plant stress response in the flora. The more gradual timberline transition of northern regions suggests that a number of factors are involved in limiting tree growth. Although the -40°C winter hardiness limit may be the one factor limiting tree growth at timberline in Colorado, it can not explain alpine timberline in northern sub-arctic regions, e.g. Alaska. Nor can it explain the northern most limit of tree growth, boreal timberline. There, the dominant timberline flora does not have a -40°C winter hardiness limit and survives much lower temperatures (5, 8).

Freezing avoidance by undercooling to between -15 and -20°C has been found in hydrated seeds of lettuce, rice, and wheat (35, 64). Recently, freezing avoidance by deep undercooling all freezable water to near -35°C (Figure 4E) was found in the seeds of two dwarf mistletoe species (*Arceuthobium*) (6). Dwarf mistletoe is an angiosperm parasite of conifers (34, Figure 10). This winter hardiness limit appears to be of ecological significance in limiting the elevational and/or latitudinal distribution of some dwarf mistletoe species.

Figure 10. Dwarf mistletoe parasite (*Arceuthobium vaginatum*) on ponderosa pine. (From Hawksworth and Wiens, 1972)



In the Colorado Rocky Mountains *A. americanum* does not extend to the upper elevational limit of its principal host, lodgepole pine (32, Figure 11). At its upper elevational limit, 3050 m, low temperature extremes are near -35°C (14). Lower average minimum temperatures occur at higher elevations (4). Whereas the seeds of *A. americanum* are killed at -35°C, the vegetative tissue survives much lower temperatures (7). In fact, studies have shown that *A. americanum* can survive vegetatively if inoculated onto its host at higher elevations (33). Thus, the upper elevational limit of this species may be determined by the -35°C winter hardiness limit in the seeds. There is other strong evidence in support of the contention that low temperature extremes near -35°C limit the distribution of *A. americanum* on lodgepole pine. Seeds of *A. americanum* inoculated onto lodgeple pine in the fall of the year in British Columbia, Canada, had 0% germination and infection after the 1970-71 winter, when the lowest recorded temperature reached -39°C (63). In comparison there was 38% germination and infection of inoculated seeds after the following 1971-1972 winter, when the lowest recorded temperature only reached -32°C (63).

In light of these studies on dwarf mistletoe seed winter hardiness, it is interesting to observe the northern limit of eastern dwarf mistletoe, *A. pusillum*, which parasitizes black and white spruce (34, Figure 12).

Figure 11. Elevational distributions of three conifer species and their dwarf mistletoe parasites in the Colorado Rocky Mountains (40° north latitude). Solid vertical bars indicate the elevational distribution of each species. Shaded bars indicate the elevational distribution of the principal dwarf mistletoe parasite on each species. Whereas ponderosa pine dwarf mistletoe extends to the upper elevational limit of its host, lodgepole and limber pine dwarf mistletoe do not. (From Hawksworth, 1956 and 1979)

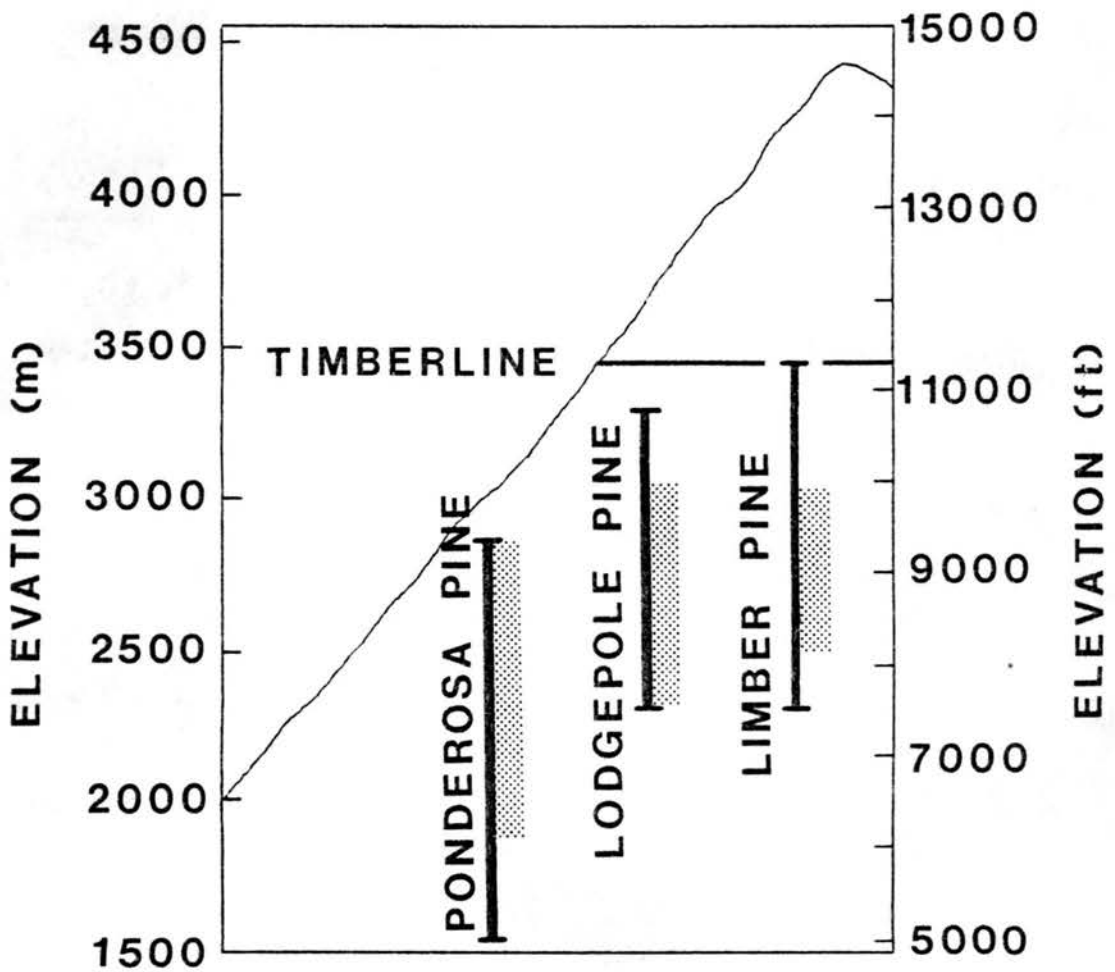
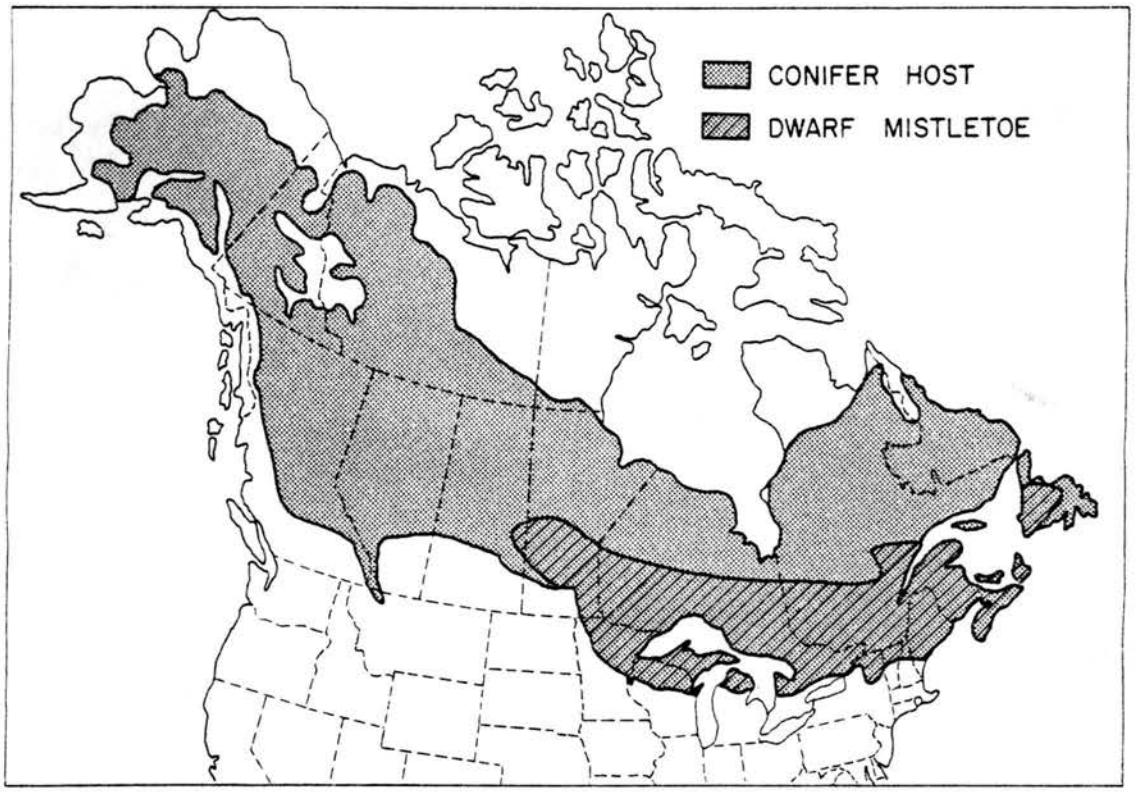

4500
4000
3500
3000
2500
2000
1500
ELEVATION (m)
15000
13000
11000
9000
7000
5000
ELEVATION (ft)
TIMBERLINE
PONDEROSA PINE
LODGEPOLE PINE
LIMBER PINE

Figure 12. Native distribution of eastern dwarf mistletoe and its principal hosts, black spruce and white spruce in North America. Compare the northern limit of this dwarf mistletoe to the hardiness zones in Figure 5. Note that the northern limit of eastern dwarf mistletoe closely parallels the -40°C average annual minimum temperature isotherm, whereas its hosts extend far north of the isotherm. (From Hawksworth and Wiens, 1972)


CONIFER HOST
DWARF MISTLETOE

Recall that the northern limit of trees having a -40°C winter hardiness limit closely parallels the -40°C average annual extreme minimum temperature isotherm (Figures 5 and 6). Note that the same holds true for eastern dwarf mistletoe (Figure 12), although its host extends further north and survives much lower temperatures (5). It is likely that the seeds of this dwarf mistletoe species also have a -40°C winter hardiness limit.

CONCLUSIONS

Deep undercooling is a common and important winter survival mechanism in some temperate zone plants. In such plants, its occurrence limits winter hardiness to near -40°C. This hardiness barrier has ecological implications to plant distribution and is associated with both northern and upper elevational limits of numerous plants. In contrast to freezing avoidance by deep undercooling, winter survival by tolerance of extracellular freezing does not have a low temperature limit. Therefore, from an evolutionary or winter hardiness point of view, freezing avoidance by deep undercooling is an undesirable characteristic. Little

is known about the specific structural features associated with its occurrence, but there are some clues. In wood tissue rigid cellular structure is associated with the undercooling characteristic. In buds and seeds that deep undercool, isolation of specific tissues appears to be important. The following areas of research are likely to be of importance in gaining a better understanding of deep undercooling in plants; the role of the cell wall in undercooling to the homogeneous nucleation temperature, and how changes in heterogeneous nucleators within cells may relate to changes during acclimation and deacclimation. Just as our present understanding of the freezing process in plants is in part based on fundamental principles of physical science, further research in this area will require a continued interplay between plant biology and physical science.

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CHAPTER II

DEEP UNDERCOOLING OF TISSUE WATER AND WINTER HARDINESS LIMITATIONS IN TIMBERLINE FLORA

ABSTRACT

Deep undercooled tissue water, which froze near -40°C, was found in winter collected stem and leaf tissue of the dominant timberline species of the Colorado Rocky Mountains, Englemann spruce (*Picea engelmanni* Parry) and subalpine fir [*Abies lasiocarpa* (Hook.) Nutt.], and in numerous other woody species having an elevational limit at or below timberline. Based on a 5°C/1000 m temperature lapse rate, climatological investigations predict -40°C temperatures occur at timberline elevation in Colorado and other continental mountainous regions of the western U.S. These results suggest that the winter hardiness limitation of deep undercooling may be a factor determining timberline, the cessation of upright tree growth in this region.

INTRODUCTION

Many woody angiosperm species survive winter temperatures by deep undercooling of tissue solutions to near -40°C (7). Deep undercooling refers to undercooling to temperatures as low as the homogeneous nucleation temperature of the aqueous solution; about -40°C for the plant solutions studied previously (4, 19). The freezing of this undercooled fraction of water can be observed as an exotherm during cooling at a constant rate by differential thermal analysis (DTA). In shagbark hickory (*Carya ovata* L.) such low temperature exotherms arise from the freezing of undercooled water in the xylem ray parenchyma cells (8). Injury to the xylem and pith tissue is associated with the occurrence of exotherms when the stem is cooled below the temperature of the low temperature exotherm, about -40°C (16, 17). Deep undercooling is also the mode of freezing resistance in flower buds of azalea (10) and numerous *Prunus* species (14), and shoot buds of grape (13).

The -40°C hardiness limit, associated with the presence of low temperature exotherms, is found in much of the woody angiosperm flora of the Eastern Deciduous Forest (9) and in most cultivated angiosperm trees of the temperate zone; e.g. apple, stone fruits, and grape (13, 18). In terms of ecological significance, Eastern Deciduous Forest trees with a low temperature exotherm near

-40°C have a northern distribution limit closely paralleling the -40°C average annual minimum isotherm (9). Likewise, apple and pear production is restricted to latitudes where there is low or no probability of -40°C temperatures (15). In contrast, boreal forest trees of more northern latitudes, where -40°C winter temperatures occur with regularity, do not have low temperature exotherms and survive lower temperatures (9).

In mountainous regions of western North America a prominent transition occurs in the flora at timberline. Cessation of upright tree growth occurs at the elevation of timberline. It marks the transition from the subalpine forest to low growing woody shrubs and the alpine tundra (1, 5). In general, the environment in mountainous regions becomes more severe with increasing elevation (2, 3). The limited number of temperature records in winter at timberline elevation in Colorado show that minimum temperatures at that elevation are near -40°C (3, 21). This study reports the occurrence of deep undercooling in conifers and other woody flora native to the Colorado Rocky Mountains, and examines the importance of deep undercooling in relation to the elevational limit of this forest, timberline.

MATERIALS AND METHODS

Cuttings of the trees and shrubs studied were collected from the Roosevelt National Forest in northern Colorado, except white fir samples which were collected from Pike National Forest in central Colorado. See Table I for scientific names of species included in the study. All angiosperm samples were collected in December. All gymnosperm samples were collected in November and December. In addition, Engelmann spruce and subalpine fir were also collected at various times from January through June. Engelmann spruce and subalpine fir were collected at 3200 m just below the local timberline elevation. Other species were collected at elevations ranging from 1500 to 3000 m. All samples were taken from branches located above the snow pack, placed in plastic bags, and returned to the laboratory for use. Conifer samples were held at +2°C and angiosperm samples were held at -10°C until use.

DTA studies, as described elsewhere (10), were conducted on stem sections (approximately 2 cm long, 0.4 cm diameter) of the most recent years growth and on conifer leaves to determine if low temperature exotherms resulting from the freezing of deep undercooled water occurred. A cooling rate of 1°C/min was used in all DTA experiments. Previous studies have shown that the temperature at which the low temperature exotherm occurs is essentially unaffected by the cooling rate (17).

Viability tests were conducted on 6 cm stem cuttings from the most recent year's growth. With conifers, stems with intact leaves were used. Samples were stressed to temperatures between -5 and -60°C, at 5°C intervals, using a temperature controlled methanol bath refrigeration system. The cooling rate was 5°C/hr. Samples were wrapped in moist cotton gauze, cooled to -5°C, and held at that temperature for 12 hours to insure that freezing of the tissue was initiated uniformly in all samples before cooling to lower temperatures. Samples contained in closed test tubes were removed from the bath at the desired test temperature, placed in a dewar containing methanol at the same temperature, and allowed to warm at 4 to 5°C/hr to above freezing. Thereafter, samples were held at 25°C and 100% RH for three to six days before determining the extent of injury. The extent of oxidative browning of dissected stem tissue was used as a criterion for rating injury visually. The injury temperature is the highest temperature at which greater than 50% injury occurred relative to the -5°C sample. Measurement of relative electrolyte loss from the stressed samples, as described elsewhere (6), was also made within three to six days after exposure to the test temperatures to determine at what temperature injury occurred.

RESULTS

DTA AND WINTER HARDINESS DETERMINATIONS

In Figure 1, DTA profiles of stems from gymnosperms and angiosperms native to the Colorado Rocky Mountains show low temperature exotherms near -40°C. The temperature at which visual injury occurs (solid circles, Figure 1) is associated with the low temperature exotherm. In shagbark hickory, such low temperature exotherms result from the freezing of deep undercooled water in the xylem ray parenchyma cells (8). Although it has not been determined which specific cells of the conifer stem deep undercool, separation of the bark (all tissue outside of the xylem) from the xylem and pith revealed the presence of low temperature exotherms in the xylem and pith tissue. A large high temperature exotherm is also found between -10 and -13°C (Figure 1). However, this exotherm is caused by freezing which is not injurious to these tissues during winter (16).

The species included in the study are listed in Table I. The gymnosperms and angiosperms are each ranked according to their upper elevational limit in Colorado (11). Engelmann spruce and subalpine fir are the dominant trees at timberline (1) which, in Colorado, occurs as high as 3660 m (5, 11). The results in Table I show that the dominant timberline species have low temperature exotherms resulting from the freezing of deep undercooled water.

Figure 1. DTA profiles of stems of species native to the Colorado Rocky Mountains with low temperature exotherms that indicate freezing of deep undercooled tissue water. a: gymnosperms. b: angiosperms. DTA profiles are plotted as the relative temperature difference between the sample and the reference on the ordinate vs. the reference temperature on the abscissa. The large exotherm in the Engelmann spruce DTA profile indicates approximately a 3°C temperature difference between sample and reference. Solid circles on the temperature scale indicate the visual injury temperature as determined in separate viability experiments on stems collected at the same time. The DTA curve shown for each species is one representative DTA freezing profile of a single stem section.

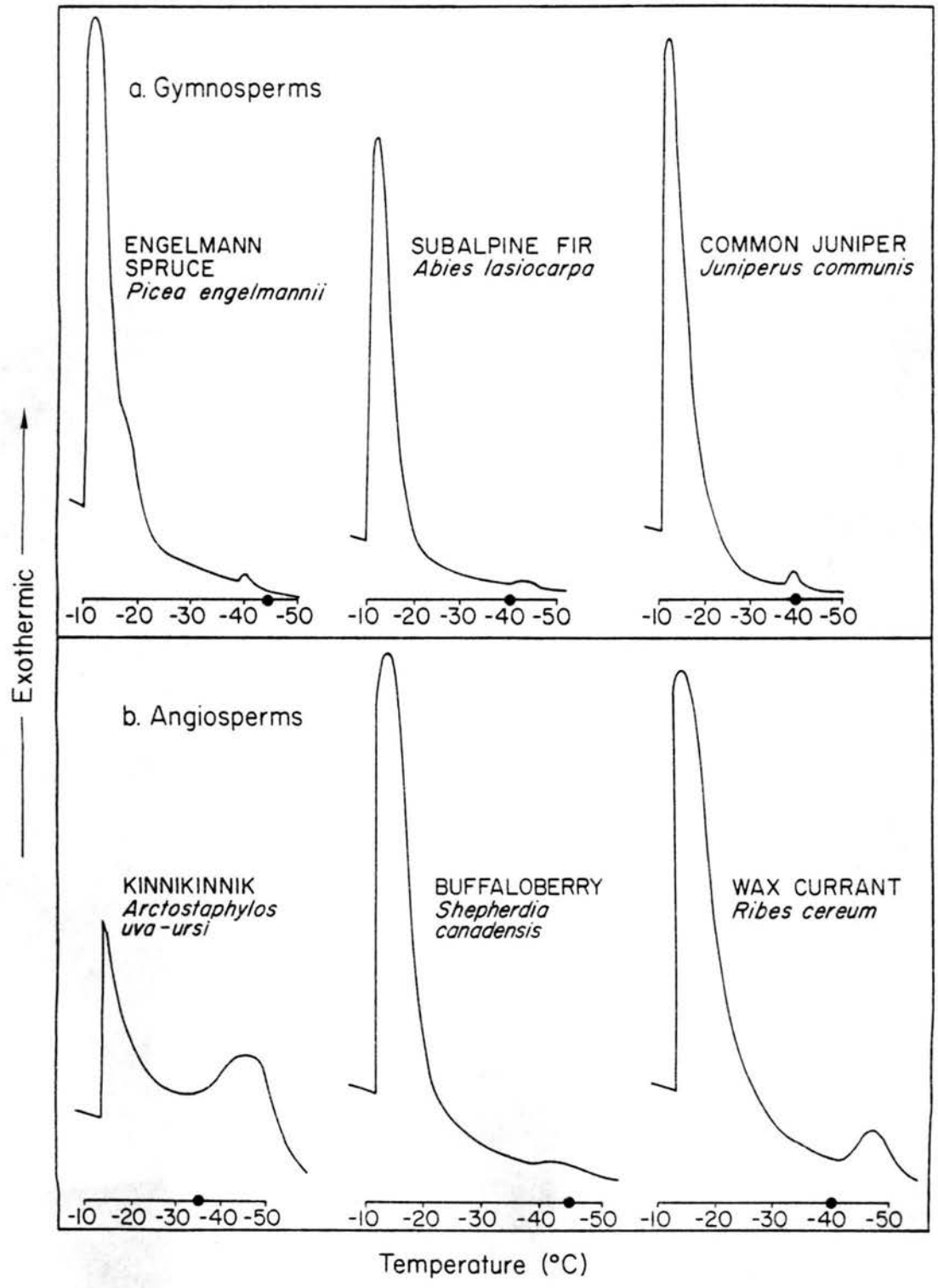

a. Gymnosperms
ENGELMANN SPRUCE
Picea engelmannii
SUBALPINE FIR
Abies lasiocarpa
COMMON JUNIPER
Juniperus communis
-10 -20 -30 -40 -50
b. Angiosperms
KINNIKINNIK
Arctostaphylos uva-ursi
BUFFALOBERRY
Shepherdia canadensis
WAX CURRANT
Ribes cereum
Exothermic
Temperature (°C)

Table I. Winter hardiness characteristics and upper elevational limits (11) of gymnosperms and woody angiosperm shrubs native to the Colorado Rocky Mountains. The exotherm temperature (low temperature exotherm) and stem injury temperature are given. Also listed in parentheses for the gymnosperms, under stem injury temperature, is the midpoint temperature in the transition from low to high relative electrolyte loss from the stem tissue.

	Exotherm[1] Temp (°C)	Stem Injury Temp (°C)	Upper Elevational Limit (m)
Gymnosperms (conifers):			
Engelmann spruce, *Picea englemannii* Parry	-39(S, L)	-45(-43)	3660
Subalpine fir, *Abies lasiocarpa* (Hook,) Nutt.	-39(S, L)	-40(-38)	3660
Limber pine, *Pinus flexilis* James	-46(L)	-45(-38)	3660
Common juniper, *Juniperus communis* L.	-38(S)	-40(-33)	3440
Lodgepole pine, *Pinus contorta* Dougl.	not found	none[2] (none[2])	3350
Rocky Mountain juniper, *Juniperus scopulorum* Sarg.	-41(S)	-45(-45)	3350
Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco	-40(S, L)	-40(-40)	3350
White fir, *Abies concolor* (Gordon) Coopes	-39(S)	not determined	3050
Blue spruce, *Picea pungens* Engelm.	not found	none[2] (-50)	2900
Ponderosa pine, *Pinus ponderosa* Laws.	not found	-45(-45)	2830
Angiosperms (woody shrubs):			
Willow, *Salix* L. spp.	not found	none[2]	4270
Kinnikinnik, *Arctostaphylos uva-ursi* L.	-37	-35	3570
Raspberry, *Rubus* L. spp.	-39	-40	3570

Table I. Continued.

	Exotherm[1] Temp (°C)	Stem Injury Temp (°C)	Upper Elevational Limit (m)
Angiosperms (woody shrubs) (cont.):			
Buffaloberry, *Shepherdia canadensis* (L). Nutt.	-40	-45	3510
Wax currant, *Ribes cereum* Dougl.	-40	-40	3470
Mountain maple, *Acer glabrum* Torr.	-39	-40	3200
Snowberry, *Symphoricarpos* Duhamel, spp.	-39	-35	3200
Sagebrush, *Artemisia tridentata* Nutt.	-34	-35	3080
Mountain mahogany, *Cercocarpus montanus* Raf.	-38	-40	3050
Nineback, *Physocarpus monogynus* (Torr.) Coult.	-40	-40	3050
Snakeweed, *Gutierrezia sarothrae* (Pursh) Britt & Rusby	-38	-35	3050
Bitterbrush, *Purshia tridentata* (Pursh) DC.	-38	-40	2740
Skunkbrush *Rhus trilobata* Nutt.	-39	-40	2740
Wild grape, *Vitis riparia* Michx.	-40	-40	2130

[1]Exotherm temperatures for stem (S) and/or leaf (L) tissue in gymnosperms and stem tissue only in angiosperms.

[2]Survived the lowest test temperature, -60°C.

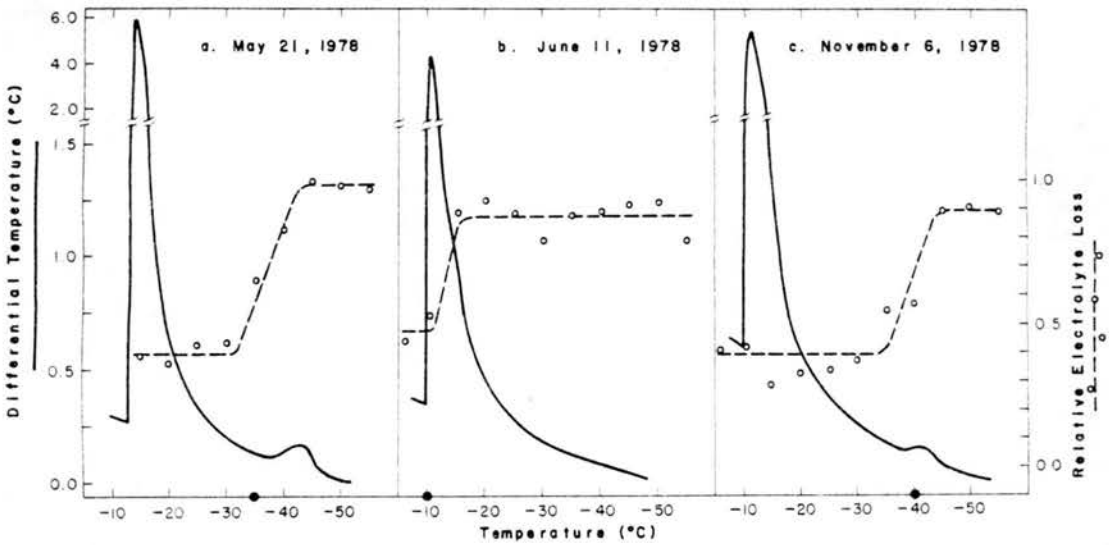
In general, most of the conifers and all of the angiosperms studied with an elevational limit at or below timberline have low temperature exotherms. Visual injury and an increase in relative electrolyte loss from the stem tissue of conifers which deep undercool are associated with the exotherm temperature (Table I). Willow, the only species studied having an upper elevational limit above timberline, where it grows as a shrub, does not have a low temperature exotherm. It survived the lowest test temperature, -60°C. Low temperature exotherms were found in the leaves of Engelmann spruce, subalpine fir, and Douglas fir at approximately the same temperature as the stem low temperature exotherm. In the other deep undercooling conifers the low temperature exotherm was found in either the stem or leaf tissue, but not both (Table I). Although stem low temperature exotherms were not found in limber pine, blue spruce, and ponderosa pine, visual injury and/or an increase in relative electrolyte loss occurred between -38 and -50°C (Table I). In contrast, stem and leaf low temperature exotherms were not found in lodgepole pine, and both tissues of this species survived the lowest test temperature, -60°C, without visual injury or an increase in relative electrolyte loss over the range of test temperatures.

The low temperature exotherms of gymnosperms given in Table I are for samples collected during November and December. Samples of Engelmann spruce and subalpine

fir were also collected at various times during winter. DTA's were run on the stem and leaf tissue of each sample to determine how consistently the low temperature exotherm was found during winter. Stem low temperature exotherms were found in Engelmann spruce in 22 out of 25 DTA runs (88%) and in subalpine fir in 17 out of 19 DTA runs (89%). Low temperature exotherms in the leaves were found in Engelmann spruce in 9 out of 11 (82%) and in subalpine fir in all 10 DTA runs.

DTA profiles of subalpine fir stems collected at three different dates are shown in Figure 2. The sample collected in May (Figure 2a), before the initiation of spring growth and bud break, had a low temperature exotherm at -38°C. The sample collected three weeks later in June (Figure 2b), after the initiation of spring growth, did not have a low temperature exotherm. Figure 2c shows that the sample collected in November, after winter acclimation, had a low temperature exotherm at -38°C as before deacclimation (Figure 2a). Associated with the stem low temperature exotherm at -38°C in the acclimated samples (Figures 2a and 2c), an increase in relative electrolyte loss from the stem tissue occurs when measured three to six days after stressing the samples. As with most other conifer species listed in Table I, this transition from low to high electrolyte loss closely agrees with the visual injury temperature (solid circles on abscissa, Figure 2) determined at the

Figure 2. DTA profiles and relative electrolyte loss of subalpine fir stems collected from the Roosevelt National Forest at 3200 m on three difference dates. a: May 21, 1978. b: June 11, 1978. c: November 6, 1978. DTA profiles are plotted as the differential temperature between the sample and the reference on the ordinate vs. the reference temperature on the abscissa. Solid circles on the abscissa indicate the injury temperature as determined in separate viability tests on stems collected at the same time. Each DTA curve shown is one DTA freezing profile of a single stem section.


a. May 21, 1978
b. June 11, 1978
c. November 6, 1978
Differential Temperature (°C)
Relative Electrolyte Loss
Temperature (°C)
6.0
4.0
2.0
1.5
1.0
0.5
0.0
-10
-20
-30
-40
-50

same time. In contrast, an increase of electrolyte loss from the sample collected in June (Figure 2b) occurs between -10 and -15°C. This suggests that deacclimation has occurred and the deep undercooling capacity with its associated low temperature exotherm is lost.

TIMBERLINE TEMPERATURES

To predict winter temperature minima at the elevation of timberline, U.S. Weather Bureau data (21) were collected from weather stations located below timberline in mountainous regions of the western U.S. (Table II) and an elevation correction was made. The elevation correction used was a 5°C temperature decrease for every 1000 m gain in elevation. This 5°C/1000 m lapse rate came from climatological data for average annual minimum temperatures in mountainous region (2).

Based on the 5°C/1000 m lapse rate of temperature, the results in Table II suggest temperature minima at timberline are near -40°C in continental mountainous locations of the western U.S. The predicted extreme January minima (Table II) at timberline in the Rocky Mountains and intermountain ranges range from -37 to -47°C with a mean of -41°C. The predicted average January minima (Table II) for the same region are somewhat higher, ranging from -29 to -38°C with a mean of -35°C. In the more coastal mountains of Oregon and Washington, predicted timberline temperatures do not approach -40°C (Table II).

Table II. Winter temperature data corrected to the local timberline elevation of mountainous regions in the western U.S. Locations having Engelmann spruce and subalpine fir (12) and at least five weather stations within 1600 m of the local timberline elevation were used. Values in parentheses are the number of local weather stations used in calculation of the predicted timberline temperatures for each location. The calculations of predicted timberline temperatures were done as follows: First, the average January minimum temperature for each station was calculated using the lowest temperature recorded in January for a given year and then taking the mean of such values for the years 1967 to 1976. Also, the extreme January minimum temperature of the station was the single lowest recorded temperature for the same 10-year period. Next, to predict the timberline average January minimum temperature and extreme January minimum temperature above each station, a lapse rate of 5°C/1000 m (2) was used. Finally, the predicted timberline temperatures within each region were averaged and standard deviation calculated.

Location	Elevation of Timberline (m)	Predicted temperature at timberline (°C)	
		Average Jan. Minimum	Extreme Jan. Minimum
Rocky Mountains of:			
New Mexico, north central (8)	3660	-33 ± 4	-41 ± 5
Colorado, central (11)	3440	-37 ± 3	-40 ± 3
Wyoming, south central (6)	3290	-33 ± 3	-43 ± 2
Wyoming, west central (6)	3110	-37 ± 3	-42 ± 5
Wyoming, north west (9)	3080	-38 ± 4	-43 ± 3
Idaho, south central (7)	2960	-35 ± 4	-40 ± 5
Montana, south west (10)	2900	-36 ± 3	-41 ± 2
Montana, north west (5)	2350	-38 ± 2	-47 ± 2
Intermountain Ranges of:			
Arizona, central (8)	3440	-29 ± 3	-38 ± 4
Utah, south central (7)	3410	-31 ± 3	-37 ± 3
Cascade Mountains of:			
Oregon, southern (5)	2590	-25 ± 3	-29 ± 4
Washington, central (5)	1980	-21 ± 4	-27 ± 4

In Colorado, the predicted temperatures closely agree with the limited number of existing records of winter temperature minima near timberline elevation. In a fourteen year climatological study located on the front range of the Colorado Rocky Mountains, Barry (3) found the extreme minimum temperature to be -37°C at 3050 m. The extreme minimum temperature for a ten-year period (1967-1976) at U.S. Weather Bureau stations (3440 m) located at Berthoud Pass, Colorado, and near Climax, Colorado, were -36 and -34°C, respectively (21).

DISCUSSION

This study shows that the -40°C winter hardiness limitation of deep undercooling is present in winter collected samples of numerous conifers and woody shrubs native to the Colorado Rocky Mountains, with an upper elevational limit at or below timberline. Furthermore, based on temperature lapse rates in mountains regions, predicted winter temperature minima are near -40°C in continental mountainous regions of western U.S. These results suggest the winter hardiness limitation of deep undercooling may be a factor determining timberline, the upper elevational limit of tree growth in this

region. This same explanation was used regarding the northern limit of the Eastern Deciduous Forest (9) and is consistent with numerous properties of timberline in the western U.S., e.g. the uniformity of timberline elevation over relatively wide areas, the latitudinal variation of timberline, and the visual winter damage observed in conifers at and above timberline. This explanation does not hold in more coastal mountain regions of Oregon and Washington, where predicted timberline temperature minima are higher than -40°C.

Thus, Colorado timberline woody plants are significantly less winter hardy than willow shrubs which grow above timberline or the Boreal Forest trees at polar timberline which do not deep undercool and survive much lower temperatures in winter (9). Therefore, from a winter hardiness point of view, the woody timberline plants of Colorado are more similar to the Eastern Deciduous Forest trees of the eastern part of the U.S. than to the Boreal Forest trees native to Canada and Alaska.

Recently, Sakai (20) found low temperature exotherms near -30°C in the winter buds of numerous subalpine (temperate zone) conifers native to Japan. The findings of low temperature exotherms in conifer stem and bud tissue strongly suggest that deep undercooling is an important frost avoidance mechanism of winter survival common to many conifer species. Previously, the deep undercooling frost avoidance mechanism had been found only in angiosperms (7).

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CHAPTER III

DEEP UNDERCOOLING AND WINTER HARDINESS IN PROVENANCE COLLECTIONS OF WHITE SPRUCE, BLACK SPRUCE, AND JACK PINE

ABSTRACT

Cuttings from trees grown in provenance collections of three boreal forest conifers, white spruce [*Picea glauca* (Moench) Voss], black spruce [*Picea mariana* (Mill.) B.S.P.], and jack pine (*Pinus banksiana* Lamb.), were collected at Rhinelander, Wisconsin in midwinter. Differential thermal analysis studies were conducted on leaf and stem tissue to detect the occurrence and variation in low temperature exotherms (LTE's) that result from the freezing of deep undercooled tissue water. LTE's occurred in the leaves of all 71 acquisitions of white and black spruce between -43 and -49°C, and in 6 out of 20 acquisitions of jack pine between -45 and -47°C. Leaf LTE's accounted for 0.15 to 1.00 percent of the freezable tissue water. LTE's were not found in the stem of any acquisition

of the three species. The four most northern acquisitions of white spruce showed little variability in the LTE temperature, having a range of only -47 to -48°C (mean = 47.8 $\pm$ 0.5°C). The four most southern acquisitions of white spruce showed greater variability, having LTE's between -43 and -48°C (mean = -45.8 $\pm$ 2.6°C). This suggests natural selection pressures in the north have favored trees with leaf LTE's at lower temperatures.

INTRODUCTION

An undercooled solution is one that remains in a liquid state at temperatures below its normal equilibrium freezing point (10). Deep undercooling refers to undercooling to temperatures approaching the homogeneous nucleation temperature of the solution, the low temperature limit for undercooling. The homogeneous nucleation limit is -38.1°C for pure water and between -40 and -47°C for in vitro plant solutions studied thus far (5, 18). Differential thermal analysis (DTA) has proved to be a valuable technique for detecting the presence of such deep undercooled fractions of plant tissue water (2, 7). DTA profiles or freezing curves have a low temperature exotherm (LTE) at the temperature where this deep undercooled fraction of water freezes. This is usually near -40°C (5).

Deep undercooling is a mechanism of frost avoidance in various tissues of numerous temperate zone hardwoods (7, 15, 16). In these species injury to various tissues is caused by the freezing of deep undercooled tissue water, and it is associated with the occurrence of a LTE. Injury has also been found to be associated with LTE's in the stem and leaf tissue of numerous conifer species native to the western U.S. (1) and in conifer buds (19).

Provenance collections of plants are useful in studying the influence of geographic origin on various aspects of winter hardiness. Species having wide latitudinal distributions may exhibit considerable variation in winter hardiness characteristics due to differences in natural selection pressures at different locations. Provenance collections have been used to study genetic variation in the rate of cold hardening and dehardening in red osier dogwood (20), red oak (4), and white pine (12). DeHayes (3) used provenance collections to study several aspects of cold hardiness in ponderosa pine. Recently, George, et al. (9) used provenance collections of four eastern deciduous hardwoods to study variation in deep undercooling and winter hardiness within species. This study is similar to that of George, et al.

In this study we report on the finding of LTE's in the leaves of three boreal forest conifer species. The objective of the study was to determine the variability of the LTE as a function of the geographic origin of the trees.

MATERIALS AND METHODS

Provenance collections of white spruce, black spruce, and jack pine grown at the North Central U.S. Forest Experiment Station in Rhinelander, Wisconsin were used in this study. Cuttings of approximately 20 cm in length were taken from lateral branches on January 10 and 11, 1979. Samples were placed in plastic bags, transported at 0°C to Colorado, and thereafter held at -10°C prior to hardiness evaluations.

DTA studies were conducted separately on stem and leaf samples of approximately 20 and 50 mg fresh weight, respectively, from the most recent years growth. DTA methods have been described in detail elsewhere (8), but in brief, the following method was used. A stem or leaf sample and an aluminum reference sample of approximately the same thermal mass were cooled at a constant rate of 1.0°C/min. Both the temperature difference between the living and reference sample and the reference sample temperature were monitored with thermocouples and recorded on a chart recorder. The freezing of tissue water warms the living sample relative to the aluminum reference and results in a temporary increase in the difference temperature and a corresponding peak or exotherm on the chart recorder.

Hardiness evaluations were conducted on all acquisitions of white spruce, and two acquisitions each

of black spruce and jack pine. Ten cm stem cuttings with intact leaves were used. Samples were stressed to temperatures between -5 and -70°C, at 5°C intervals, using a methanol bath refrigeration system with temperature control. The cooling rate was 10°C/hr. Samples contained in closed test tubes were removed from the bath at the desired test temperature, placed in a dewar containing methanol at the same temperature, and allowed to warm at 5°C/hr to above freezing. Thereafter, samples were held at 25°C and 100% relative humidity for six days and then examined visually for injury. The extent of oxidative browning of stem and leaf tissue was used as a criterion for rating injury. The injury temperature is the highest temperature at which greater than 50% injury occurred relative to the -5°C sample.

The Plant Hardiness Zone Map (13) was used to determine the average annual minimum temperature of the acquisition sites located in the contingent United States and southern Canada. The average annual minimum temperature of more northernly located acquisition sites in Canada (accession numbers 1658 and 1667 of white spruce), not located in the region covered by the plant hardiness zone map, were obtained from the Climatological Atlas of Canada (14). The average annual minimum temperature for three acquisition sites located in Alaska (accession numbers 1652, 1653, and

1654 of white spruce) were estimated by taking the ten year (1967 - 1976) average of the yearly extreme minimum temperature at the nearest weather station (21).

RESULTS AND DISCUSSION

DTA profiles of leaves of three boreal forest conifer species studied, their natural distributions, and the geographic origin of each acquisition are shown in Figure 1. Leaves from all acquisitions of white and black spruce and LTE's which were consistently found in repeated DTA runs on different samples from the same acquisition site. In jack pine, LTE's were found in the leaves of 6 out of 20 acquisitions (see Figure 1). Unlike white and black spruce LTE's found in jack pine leaves were not always found in repeated DTA runs. LTE's were not found in the stem tissue of any acquisition of the three species.

Tables I - III list geographical and climatological information, leaf LTE characteristics, and hardiness determinations (white spruce only) for the three conifer species studied. Including all sample acquisitions, 77 out of 91 DTA runs revealed LTE's in leaf

Figure 1. DTA profiles, natural distributions (11), and provenance collection acquisition sites of white spruce, black spruce, and jack pine. Closed circles on distribution maps indicate acqusitions having leaf LTE's. Open circles indicate jack pine acquisitions not having leaf LTE's. Arrows on distribution maps indicate the sample acquisition of the corresponding DTA profile shown for each species. The initiation temperature of the leaf LTE is shown for each DTA profile. The large high temperature exotherm in each DTA profile results from freezing that is not injurious to these tissues during winter. Map scale bar shown is 500 km.

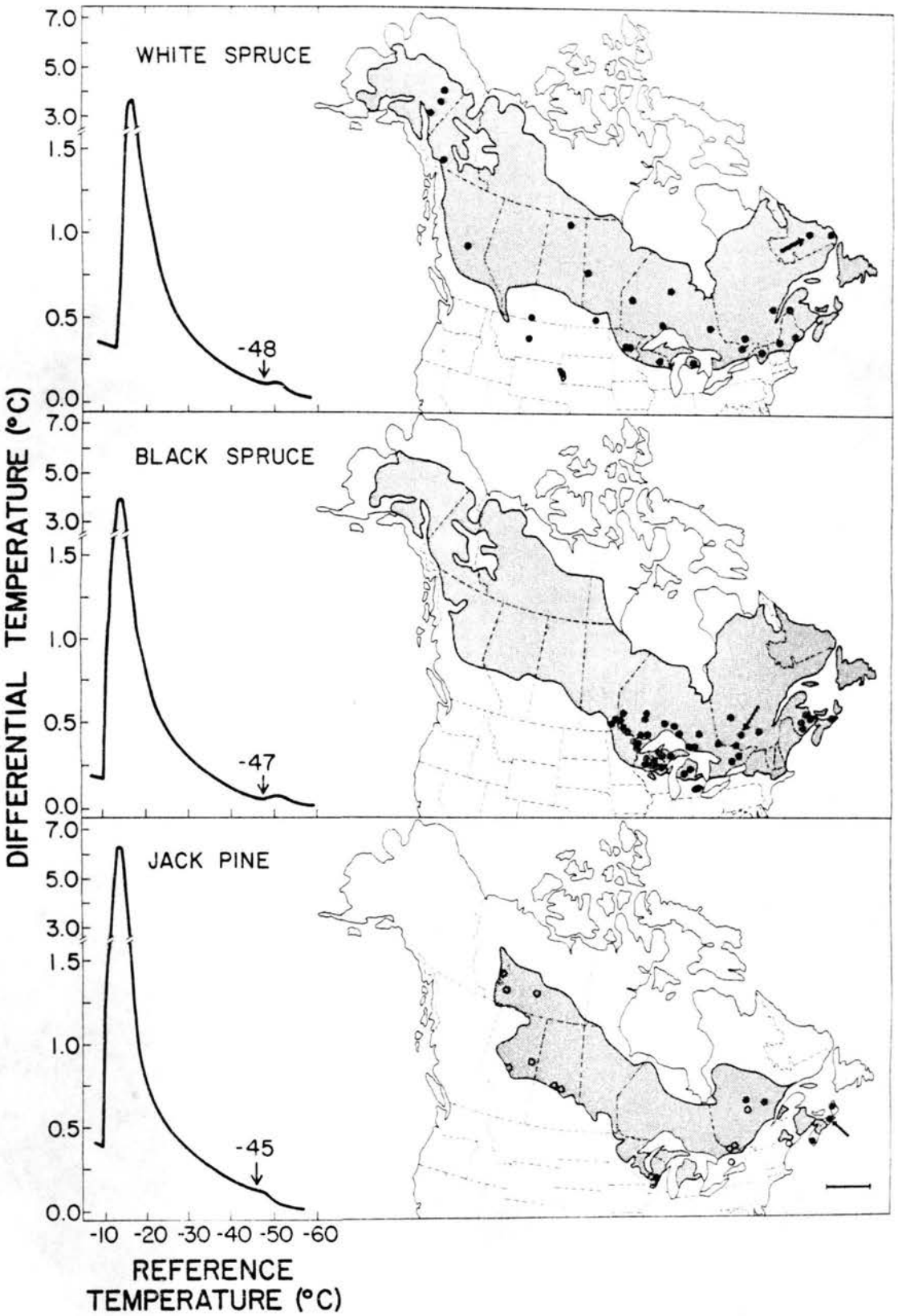

WHITE SPRUCE
-48
BLACK SPRUCE
-47
JACK PINE
-45
7.0
5.0
3.0
1.5
1.0
0.5
0.0
-10 -20 -30 -40 -50 -60
DIFFERENTIAL TEMPERATURE (°C)
REFERENCE TEMPERATURE (°C)

Table I. Geographic and winter hardiness data for provenance collection acquisitions of white spruce. The accession number, state or province, latitude (°N), longitude (°W), and average annual minimum temperature (AAMT) are listed for each acquisition site. The leaf LTE temperature listed is the initiation temperature of the exotherm. The value for the LTE size (% H_2O frozen) is based on determinations of the amount of heat released during the leaf LTE in comparison to the total amount of heat released during freezing of the leaf tissue water.

Accession Number	Acquisition Site				Leaf LTE		Leaf Injury Temp. (°C)
	State or Province	Lat. (°N)	Long. (°W)	AAMT (°C)	Temp. (°C)	Size (% H_2O frozen)	
1628	South Dakota	44.2	103.8	-32	-48	0.65	<-70
1644	New York	44.4	74.1	-34	-44	0.30	<-70
1676	Michigan	44.5	83.8	-29	-48	0.20	-65
1655	Maine	44.8	68.6	-26	-43	0.40	-65
1649	New Hampshire	44.9	71.4	-37	-47	0.40	<-70
1645	Wisconsin	45.7	89.1	-34	-44	0.35	-65
1663	Ontario	45.7	76.9	-34	-47	0.35	<-70
1660	Quebec	46.5	76.5	-40	-45	0.35	<-70
1630	Montana	46.8	109.5	-32	-48	0.60	<-70
1669	Minnesota	47.6	94.1	-37	-47	0.40	-65

Table I. Continued.

Accession Number	Acquisition Site				Leaf LTE		Leaf Injury Temp. (°C)
	State or Province	Lat. (°N)	Long. (°W)	AAMT (°C)	Temp. (°C)	Size (% H_2O frozen)	
1647	Minnesota	47.6	94.2	-37	-46	0.30	<-70
1659	New Brunswick	47.8	68.4	-34	-49	0.70	<-70
1662	Ontario	48.0	81.0	-40	-48	0.50	<-70
1661	Quebec	48.3	71.4	-40	-48	0.55	<-70
1687	Ontario	48.5	89.5	-40	-46	0.65	<-70
1697	Saskatchewan	49.7	109.7	-43	-48	0.65	-65
1631	Manitoba	49.9	99.5	-37	-48	0.50	-65
1686	Ontario	52.3	81.7	-43	-46	0.50	-65
1657	Labrador	52.6	56.4	-32	-48	0.70	<-70
1658	Labrador	53.8	60.1	-34	-48	0.90	<-70
1677	British Columbia	54.0	123.0	-37	-47	0.30	<-70
1664	Manitoba	54.7	101.6	-43	-47	0.35	<-70
1678	Manitoba	56.9	92.9	-43	-46	0.40	<-70

Table I. Continued.

Accession Number	Acquisition Site				Leaf LTE		Leaf Injury Temp. (°C)
	State or Province	Lat. (°N)	Long. (°W)	AAMT (°C)	Temp. (°C)	Size (% H_2O frozen)	
1665	Saskatchewan	59.3	106.0	-46	-48	0.50	<-70
1667	Yukon	60.8	135.6	-46	-48	0.50	-65
1653	Alaska	63.8	144.9	-46	-48	0.35	<-70
1652	Alaska	65.4	144.5	-49	-47	0.30	<-70
1654	Alaska	66.6	145.2	-52	-48	0.30	<-70

Table II. Geographic and winter hardiness data for provenance collection acquisitions of black spruce. For explanation of terms and symbols used, see Table I.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (°N)	Long. (°W)	AAMT (°C)	Temp. (°C)	Size (% H_2O frozen)
4730	Michigan	42.6	83.5	-26	-46	0.50
4348	Michigan	44.2	85.6	-26	-47	0.30
4346	Michigan	44.6	84.3	-32	-47	0.50
4942	Ontario	44.8	78.1	-34	-47	0.55
4899	Ontario	45.2	77.2	-34	-46	0.55
4345	Wisconsin	45.3	88.5	-32	-46	0.45
5002	New Brunswick	45.6	66.5	-24	-47	0.35
4998	Nova Scotia	45.7	61.9	-26	-46	0.60
4997	New Brunswick	45.7	66.5	-34	-46	0.30
3267	Wisconsin	45.7	89.1	-34	-47	0.45
4277	Wisconsin	45.7	89.0	-34	-47	0.35
4296	Wisconsin	46.0	89.0	-34	-46	0.30
4946	Ontario	46.0	77.4	-37	-46	0.35

Table II. Continued.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (^{o}N)	Long. (^{o}W)	AATM (^{o}C)	Temp. (^{o}C)	Size (% H_2O frozen)
4349	Michigan	46.0	86.9	-29	-48	0.40
4344	Wisconsin	46.1	90.9	-37	-44	0.85
3293	Wisconsin	46.2	90.8	-37	-47	0.35
3274	Michigan	46.5	88.4	-32	-46	1.00
4347	Michigan	46.6	88.9	-32	-45	0.55
4959	Ontario	46.7	84.4	-32	-44	0.35
4945	Ontario	46.8	83.4	-34	-47	0.45
4993	New Brunswick	46.8	65.2	-32	-47	0.20
5004	New Brunswick	46.9	65.1	-32	-46	0.50
4979	Quebec	46.9	73.5	-40	-48	0.50
4911	Ontario	46.9	79.7	-37	-48	0.35
4353	Minnesota	47.0	91.3	-34	-49	0.50
4965	Quebec	47.1	76.0	-43	-47	0.75

Table II. Continued.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (^{o}N))	Long. (^{o}W)	AATM (^{o}C))	Temp. (^{o}C)	Size (% H_2O frozen)
4352	Minnesota	47.5	92.0	-37	-47	0.30
4355	Minnesota	47.7	92.5	-37	-47	0.40
4915	Ontario	48.2	82.4	-43	-47	0.70
4902	Ontario	48.6	85.3	-43	-47	0.40
4916	Ontario	48.7	90.2	-40	-45	0.50
4917	Ontario	48.7	91.7	-43	-47	0.40
4918	Ontario	48.8	93.7	-43	-48	0.70
4982	Quebec	49.1	77.0	-43	-46	0.45
4948	Ontario	49.1	85.8	-43	-46	0.40
4910	Manitoba	49.3	96.3	-40	-45	0.60
4921	Ontario	49.3	94.0	-43	-47	0.45
4914	Ontario	49.7	87.8	-43	-46	0.45
4919	Ontario	50.1	94.9	-43	-44	0.45

Table II. Continued.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (°N)	Long. (°W)	AATM (°C)	Temp. (°C)	Size (% H_2O frozen)
4908	Manitoba	50.1	95.5	-43	-46	0.45
4957	Ontario	50.3	90.7	-43	-46	0.40
4920	Ontario	50.7	90.6	-43	-47	0.35
4941	Ontario	50.8	94.3	-43	-46	0.45

Table III. Georgraphic and winter hardiness data for provenance collection acquisitions of jack pine. For explanation of terms and symbols used, see Table I.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (^{o}N)	Long. (^{o}W)	AATM (^{o}C)	Temp. (^{o}C)	Size (% H_2O frozen)
2228	Wisconsin	43.6	90.2	-32	none	none
2229	Wisconsin	43.8	89.8	-32	none	none
2172	Nova Scotia	44.0	65.4	-26	-47	0.15
2230	Wisconsin	44.3	89.7	-34	-47	0.15
2171	Maine	44.4	68.1	-26	none	none
2206	Ontario	44.7	77.1	-34	none	none
2173	Nova Scotia	45.3	61.0	-26	-45	0.15
2207	Ontario	45.5	76.9	-34	none	none
2208	Ontario	45.5	76.1	-34	none	none
2217	Nova Scotia	46.8	60.4	-23	-46	0.15
2201	Quebec	49.4	74.0	-46	none	none
2202	Quebec	49.6	72.2	-43	-47	0.20
2203	Quebec	50.4	73.9	-46	-46	0.30

Table III. Continued.

Accession Number	Acquisition Site				Leaf LTE	
	State or Province	Lat. (°N)	Long. (°W)	AATM (°C)	Temp. (°C)	Size (% H_2O frozen)
2250	Saskatchewan	53.1	106.1	-46	none	none
2251	Saskatchewan	53.8	107.0	-46	none	none
2256	Alberta	54.1	115.7	-43	none	none
2257	Alberta	55.2	111.9	-46	none	none
2261	Northwest Territory	61.8	121.3	-46	none	none
2260	Northwest Territory	62.4	114.8	-46	none	none
2262	Northwest Territory	63.2	123.5	-46	none	none

tissue ranging from -43 to -49°C (mean = -47 ± 1°C). George, et al. (9) found that LTE's in stem tissue ranged from -33 to -46 (mean = -42 ± 3°C) in provenance collections of four eastern deciduous hardwood trees. Thus, the boreal forest conifer species studied have a lower mean leaf LTE than the mean LTE of the hardwoods native to more southern latitudes (Figure 2).

Figure 3 shows that in white spruce there is more variation in the leaf LTE temperature of acquisitions from more southern latitudes than in those from northern latitudes. This pattern of increased variation in leaf LTE temperatures of more southern latitudes is emphasized in Figure 3 by the enclosure of all data points, except one, in a triangle. The four most northern acquisitions have leaf LTE temperatures ranging from only -47 to -48°C (mean = -47.8 ± 0.5°C). The four most southern acquisitions have leaf LTE temperatures ranging from -43 to -48 (mean = -45.8 ± 2.6°C). This suggests that natural selection pressures in the north have favored white spruce trees with leaf LTE's at lower temperatures.

No similar pattern of variation in leaf LTE temperatures as a function of latitude occurred in black spruce. The range of leaf LTE temperatures observed in black spruce, -44 to -49°C, was similar to the range observed in the southern acquisitions of white spruce (Tables I and II). This was likely due to the fact

Figure 2. Frequency of exotherms (LTE's) as a function of exotherm temperature in red oak stems and white spruce leaves. The red oak data are from a study by George, et al. (9) on a provenance collection of 34 trees grown at the Arnold Arboretum, Massachusetts. The white spruce leaves are from the Rhinelander, Wisconsin, provenance collection of 28 trees. Note that leaf LTE's in white spruce occur at lower temperatures than do stem LTE's of red oak. White spruce is native to the Boreal Forest of Canada and Alaska. Red oak is native to the Eastern Deciduous Forest of the U.S.

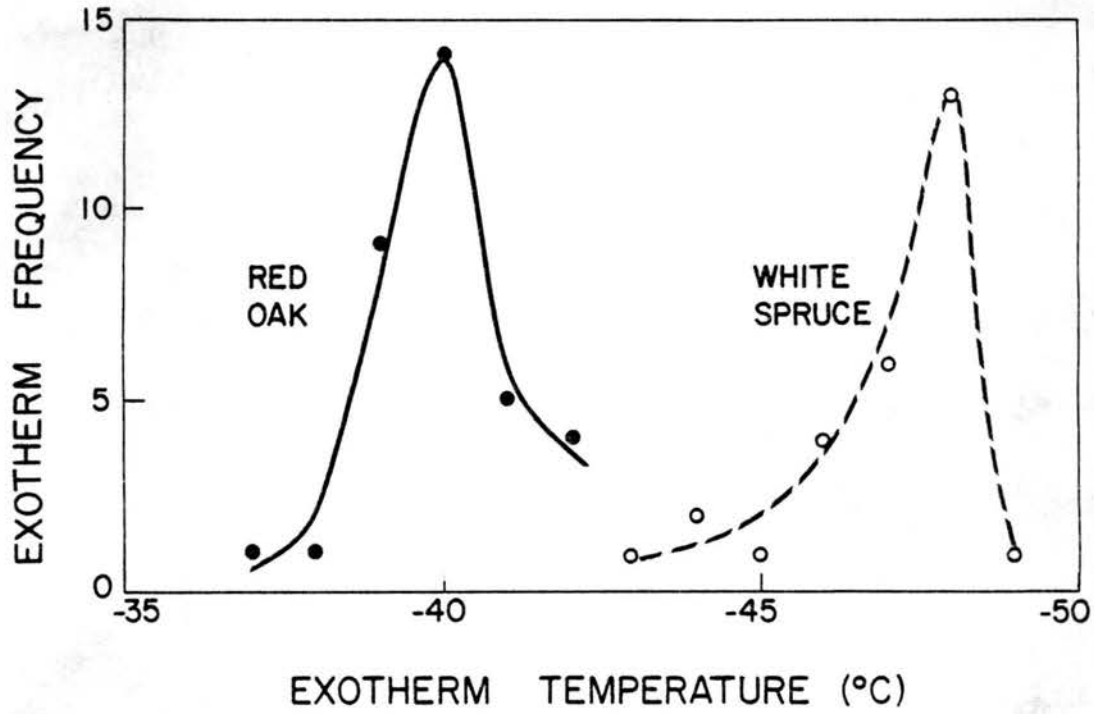
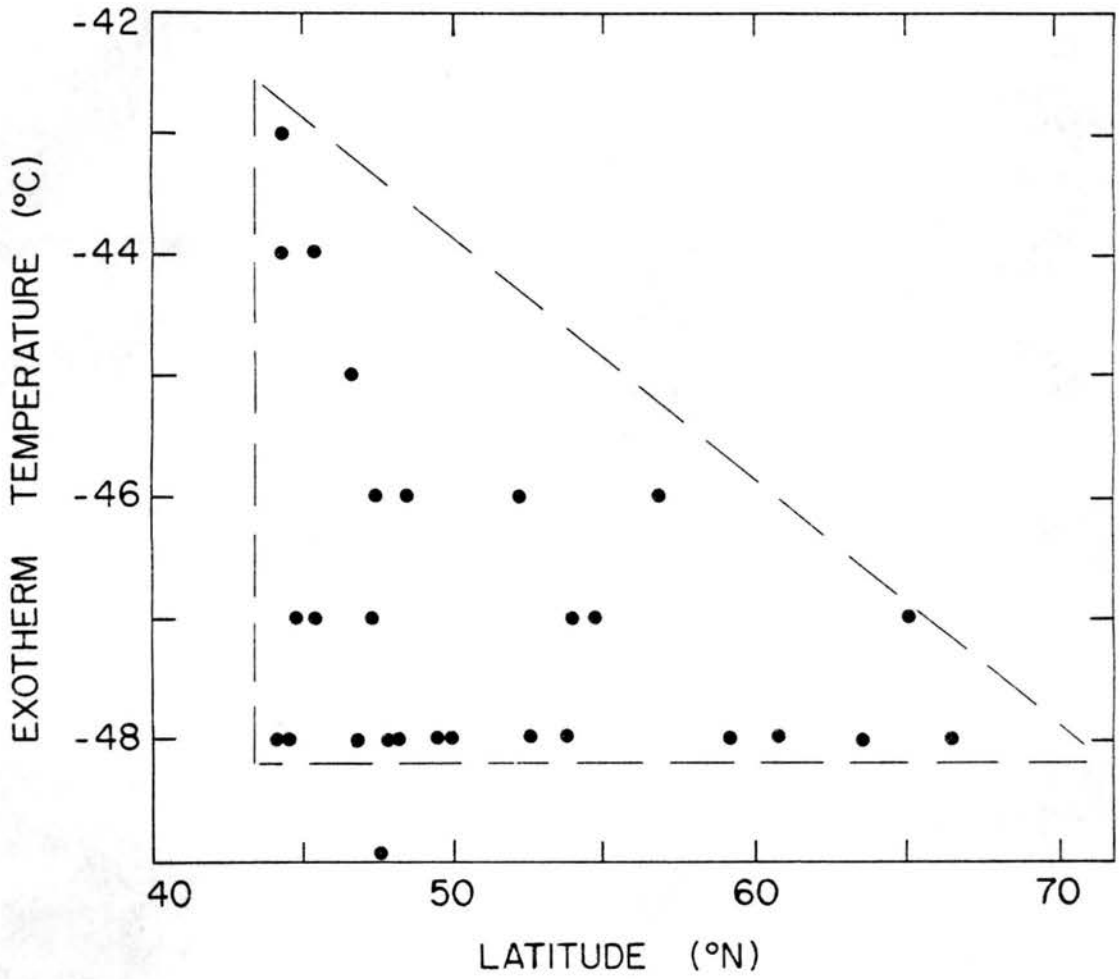

15
10
5
0
EXOTHERM FREQUENCY
RED
OAK
WHITE
SPRUCE
-35
-40
-45
-50
EXOTHERM TEMPERATURE (°C)

Figure 3. Leaf exotherm (LTE) temperature as a function of latitude for acquisitions of white spruce. All data points, except one, are enclosed in a triangle to emphasize the increased variation of LTE temperatures in samples from southern acquisitions as compared to samples from northern acquisitions.


-42
-44
-46
-48
EXOTHERM TEMPERATURE (°C)
40
50
60
70
LATITUDE (°N)

that all acquisitions of black spruce were from the southern part of its natural distribution (Figure 1). In jack pine, there was variation in the occurrence of leaf LTE's in the acquisitions studied. Leaf LTE's were found in 6 out of 13 acqusitions from south of 51°N latitude, but were not found in seven acquisitions from north of 51°N latitude (Figure 1, Table III). Similar to white spruce, this suggests that natural selection pressures in the north have favored jack pine trees with no LTE in the leaf tissue.

Tables I - III list the results of calculations to determine the relative amount of water freezing during the LTE, e.g. LTE size. These values are based on the amount of heat released during the LTE in relation to the total amount of heat released during the freezing of the leaf tissue water. The mean value for percent water freezing during the leaf LTE of white spruce, black spruce, and jack pine is 0.45 $\pm$ 0.15%, 0.45 $\pm$ 0.15%, and 0.20 $\pm$ 0.05%, respectively. The percentages given may be under estimated by approximately 25% (18) because the variation in the heat of fusion of water freezing at different temperatures could not be estimated.

Simple linear regression analysis was also conducted to compare the leaf LTE temperature and size with the latitude and average annual minimum temperature of the acquisition site. In all comparisons made

between the above parameters, the linear correlation coefficient (r) was always less than 0.41. Thus, with the exception of increased variation of the leaf LTE temperature in white spruce samples from southern acquisitions, there was little or no linear relation between the parameters studied.

LTE's in hardwood stems vary in size between species (7) and result from the freezing of deep undercooled tissue water in the living wood ray parenchyma cells (6). In the hardwoods, injury is generally associated with the occurrence of LTE's (7). Large LTE's cause considerable cell damage, and such injury is easy to detect in viability tests. Freezing injury in species which have small LTE's is generally more difficult to detect. The results of hardiness determinations on all white spruce acquisitions (Table I) and on two acquisitions each of black spruce and jack pine did not indicate injury associated with the leaf LTE. Most acquisitions of white spruce tested survived the lowest test temperature of -70°C (Table I). Acquisitions 2262 and 2172 of jack pine also survived the lowest test temperature. Acquisitions 4941 and 4998 of black spruce were injured at -65 and -60°C, respectively. It is possible that injury symptoms were not detected in association with the LTE's because of the small amount of the tissue affected by the freezing of the deep

undercooled tissue water. The mean percent tissue water freezing in leaf LTE's ranged from only 0.20 to 0.45 for the species studied. It is also possible that these tissues tolerate the small amount of injury associated with the leaf LTE.

The temperature or size of the leaf LTE in white spruce did not change when a sample was held at -19°C for 48 hours. This suggests that white spruce leaves do not lose the LTE when exposed to subfreezing temperatures for extended periods of time. Such prolonged exposure to subfreezing temperatures occurs routinely within the native distribution of white spruce.

CONCLUSION

The results of our studies on three boreal forest conifer species show that all acquisitions of white and black spruce and some acquisitions of jack pine have LTE's in leaf tissue that account for less than one percent of the freezable tissue water. Comparisons of north vs. south acquisitions revealed that LTE's of white spruce from the extreme northern part of its distribution occur over a smaller and lower temperature

range than do LTE's from the extreme southern part of its distribution. In jack pine the extreme northern acquisitions did not have LTE's; in comparison, some central and southern acquisitions did have LTE's. These findings suggest that natural selection pressures in the north have favored conifer trees with no LTE in leaf tissue or a LTE at lower temperatures. Furthermore, comparisons of our results with those of George, et al. (9), shows that the LTE's of leaf tissue of boreal conifers are, in general, smaller and occur at lower temperatures than do the LTE's of stem tissue found in hardwoods native to more southern latitudes.

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CHAPTER IV

FREEZING AVOIDANCE AND WINTER HARDINESS IN SEEDS OF CONIFER DWARF MISTLETOE (*ARCEUTHOBIUM*)

ABSTRACT

Winter collected seeds of two dwarf mistletoe species, *Arceuthobium americanum* and *A. cyanocarpum*, native to the Colorado Rocky Mountains, avoided freezing to near -33°C by deep undercooling their tissue water. The principal hosts of these mistletoes are lodgepole pine and limber pine, respectively. Differential thermal analysis freezing profiles revealed that all freezable tissue water of each seed froze in one low temperature exotherm near -33°C. Freezing tests showed that seeds of *A. americanum* cooled to -40°C, below the low temperature exotherm point, had 0% germination. In contrast, seeds cooled to -30°C had 52% germination, similar to 54% for control seeds held at -2°C. This winter hardiness limitation may be a factor in limiting the elevational or latitundinal distribution of these dwarf mistletoes.

INTRODUCTION

Many mature seeds which contain little water are very resistant to freezing stress, and will even survive immersion in liquid air, -183°C (15). In general, freezing resistance of mature seeds is inversely related to water content (14). Recently, freezing avoidance by undercooling to about -20°C, was found in hydrated seeds of lettuce, rice, and wheat (12, 13). Many annual seeds overwinter protected by snow cover, organic matter, or soil. Thus, freezing avoidance to -20°C may provide sufficient resistance for winter survival of partially hydrated seeds. Freezing avoidance by deep undercooling has been most extensively studied in various woody plant tissues (3, 5, 7).

Dwarf mistletoe (*Arceuthobium*) is a common parasite of conifers in the temperate zones (11). The seeds of *Arceuthobium* overwinter attached to the leaves or stems of the host tree and are more likely to be exposed to ambient winter temperature extremes than seeds which overwinter on the soil. The level or mechanism of freezing resistance of the seed or vegetative tissue of mistletoes has not been studied.

This communication reports on the finding of freezing avoidance by deep undercooling to near -33°C in the seeds of two dwarf mistletoe species native to North America.

MATERIALS AND METHODS

Cuttings of ponderosa pine (*Pinus ponderosa*), lodgepole pine (*P. contorta*), and limber pine (*P. flexilis*) parasitized with *Arceuthobium vaginatum A. americanum* and *A. cyanocarpum*, respectively, were collected between 2400 and 2600 m elevation in the Roosevelt National Forest, Colorado, on March 5, 1979. Cuttings were placed in plastic bags and returned to the laboratory where they were held at -2°C until used. Overwintering seeds were removed from the host tissue for use in differential thermal analysis (DTA) studies. A similar collection of 300 *A. americanum* seeds was made at the same location later in March for use in freezing tests. DTA studies, as described in detail by George, et al., (8), were conducted on seeds of each species. Unless otherwise noted in the text, all DTA studies were conducted on seeds that were not moistened to initiate external ice nucleation. In brief, the DTA method used was as follows. From one to ten seeds and an aluminum reference sample of approximately the same thermal mass were cooled (or warmed) at a constant rate of 1.0°C/min. Both the temperature difference between the living and reference sample and the reference sample temperature were monitored with thermocouples and recorded on a chart recorder. The freezing of tissue

water warms the living sample relative to the aluminum reference and results in a temporary increase in the difference temperature and a corresponding peak or exotherm on the chart recorder.

Freezing tests were conducted on seeds of *A. americanum* to determine at what temperature injury occurred. Sample lots of 50 seeds were wrapped in moist cotton gauze, placed in test tubes, and sealed. One sample tube was returned to -2°C to serve as a control. Two sample tubes were cooled at 5°C/hr to the desired test temperature in a temperature controlled methanol refrigeration bath. Sample tubes were removed at -30 and -40°C and warmed in dewars to above freezing. The -2°C sample tube was also warmed. Each sample lot of seeds was removed from the gauze and immersed in distilled water for 24 hours at ambient laboratory temperature and light conditions. Seeds were subsequently immersed in a 3% solution of H_2O_2 for five days under the same laboratory conditions. Hydrogen peroxide hastens seed germination (6, 16). Seeds were then rinsed in distilled water and percent germination of each treatment was determined. Seeds were considered germinated if protrusion of the radical and swelling due to imbibition of water occurred.

RESULTS

Figure 1 shows a typical freezing/thawing profile of one lodgepole pine dwarf mistletoe seed (*A. americanum*). The difference in temperature between the seed and an aluminum reference sample is shown on the ordinate vs. the reference temperature on the abscissa. When the seed is cooled at a constant rate, a single low temperature exotherm occurs at -36°C on the freezing profile. Thus, all of the freezable tissue water in the seed avoids freezing by deep undercooling above -36°C. Warming the same seed at a constant rate results in an endotherm peak at -3°C on the thawing profile. The area under each exotherm or endotherm is proportional to the amount of water that freezes or thaws at that temperature. The area of the two peaks in Figure 1 are the same within $\pm$ 10%, and account for the freezing and thawing of the seed tissue water. This large difference in freeze/thaw temperatures is an inherent property of deep undercooled tissue water (7). Low temperature exotherms of the seeds studied ranged from -29 to -37°C (mean of -33°C for 19 seeds). The results are summarized in Table I.

When DTA studies were conducted on more than one seed at a time, the number of low temperaure exotherms corresponded to the number of seeds used. This

Figure 1. Freezing and thawing profiles of one *A. americanum* seed. The low temperature exotherm at -36°C in the freezing profile and the endotherm at -3°C in the thawing profile result, respectively, from the freezing and thawing of deep undercooled tissue water in the seed.

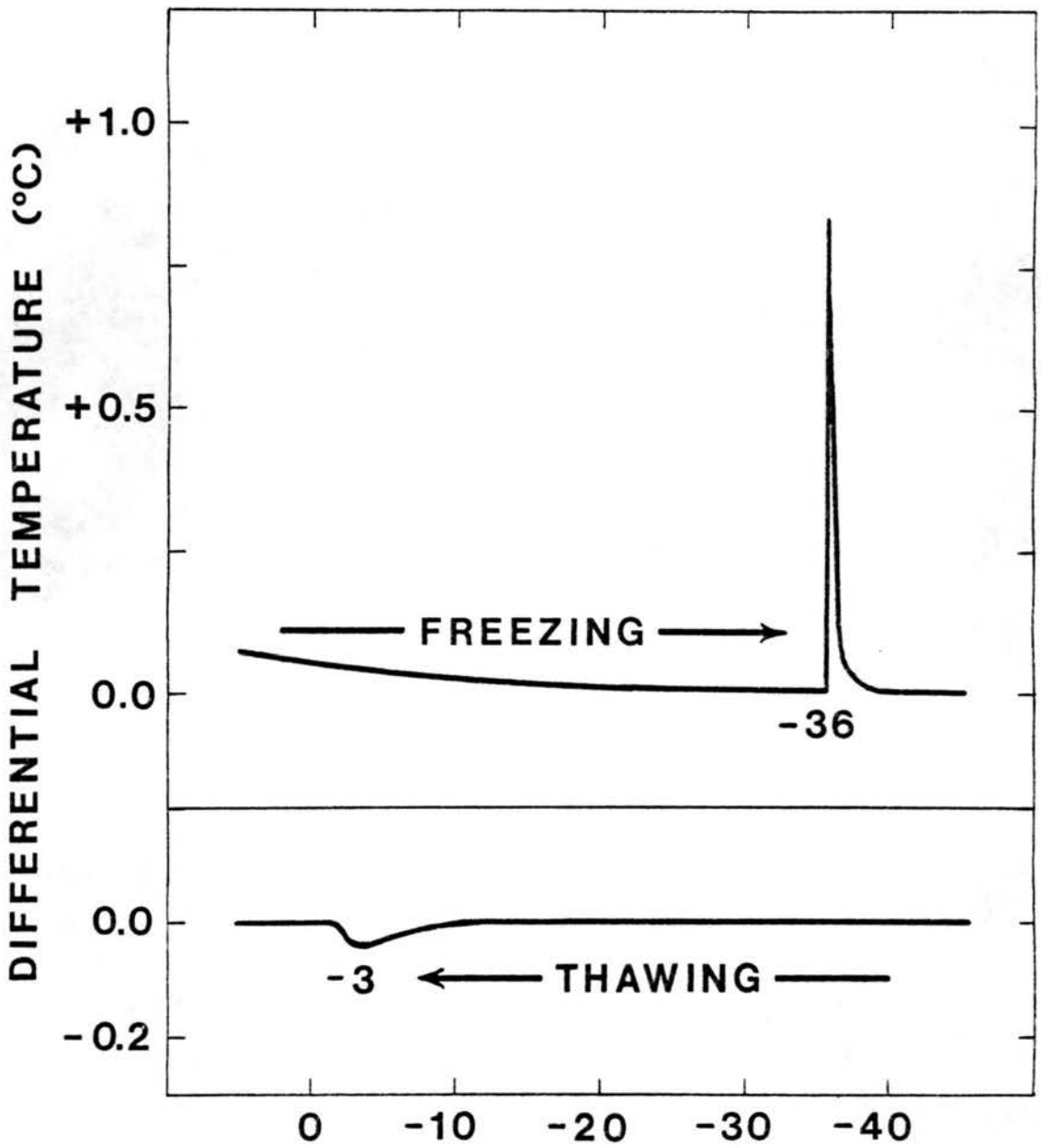

DIFFERENTIAL TEMPERATURE (°C)
+1.0
+0.5
0.0
FREEZING
-36
0.0
-0.2
-3
THAWING
0
-10
-20
-30
-40

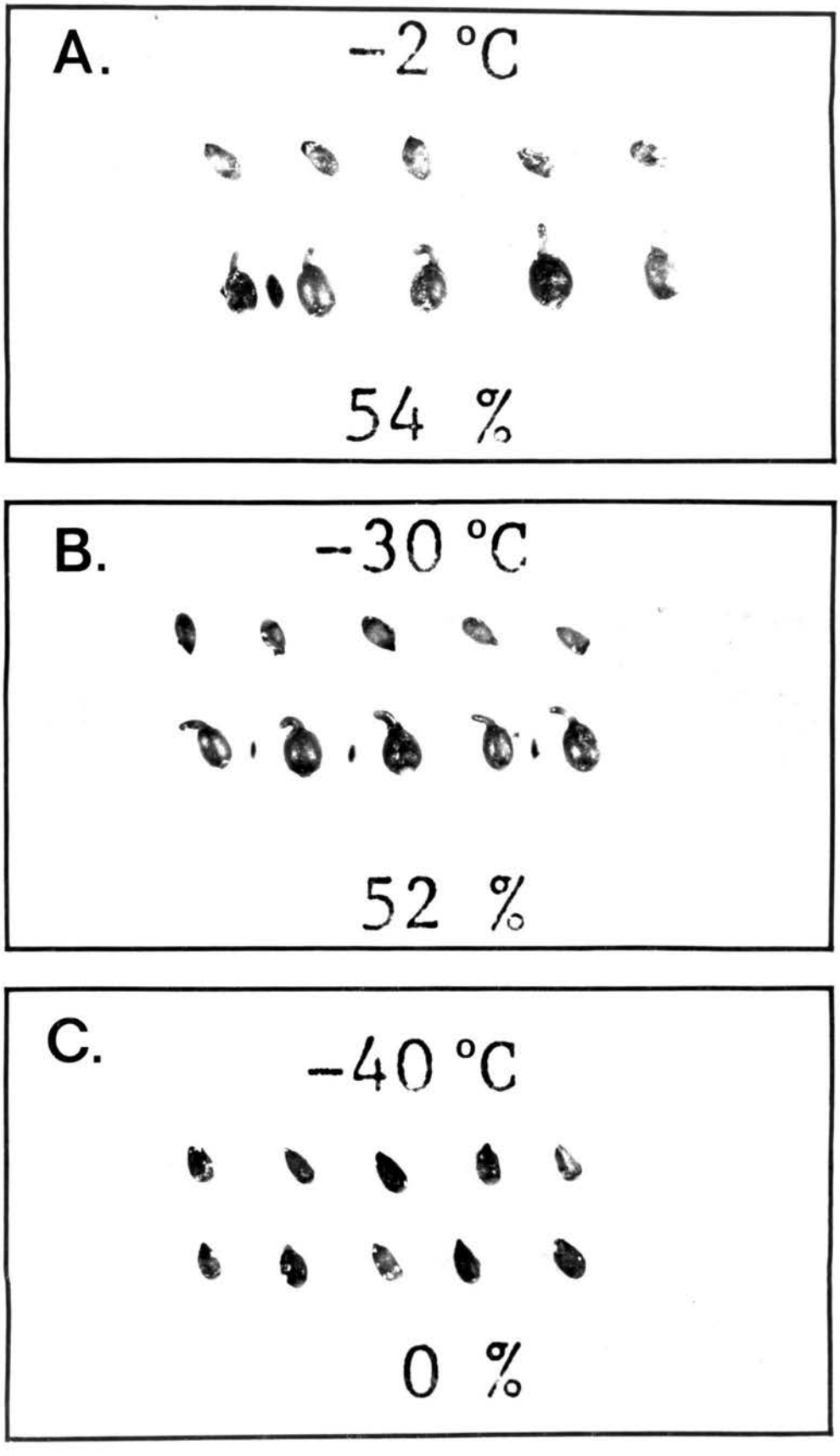
Table I. Results of DTA studies on seeds of three dwarf mistletoe species.

Species	Low Temperature Exotherm (^{o}C)		Number of Seeds Frozen
	Range	Mean ± St. Dev.	
A. americanum	-32 to -37	-35.1 ± 1.9	11
A. cyanocarpum	-29 to -34	-32.3 ± 1.6	8

suggests that the deep undercooled tissue water in each seed is isolated from external nucleation. To further validate this, DTA studies were conducted on seeds wrapped in moist cotton gauze to initiate freezing near 0°C. Such external inoculation with ice did not affect the size or position of the seed low temperature exotherm relative to that of non-inoculated seeds. Seeds wrapped in moist cotton gauze were also cooled to -19°C, held at that temperature for 24 hours, and subsequently cooled to lower temperatures. This prolonged prefreezing treatment did not appreciably affect the low temperature exotherm position or size relative to seeds frozen without the prefreezing treatment.

Figure 2 shows results of germination tests on *A. americanum* seeds to determine if injury is associated with the occurrence of the low temperature exotherm. Seeds cooled to -40°C, below the temperature range of low temperature exotherms for this species (see Table I), did not germination (Figure 2C). In contrast seeds cooled to -30°C, slightly above the temperature range of the low temperature exotherms, had a germination percentage of 52% (Figure 2B). This is not significantly different from the value for control seeds (54%) held at -2°C (Figure 2A).

Figure 2. Results of germination tests on winter collected *A. americanum* seeds subjected to three temperatures. A: 54% germination at -2°C. B: 52% germination at -30°C. C: 0% germination at -40°C. The ten seeds shown, taken from a lot of 50 seeds of the corresponding temperature treatment, approximate the percent germination of that treatment.


A.
-2 °C
54 %
B.
-30 °C
52 %
C.
-40 °C
0 %

DISCUSSION

The results of this study show that overwintering seeds of two dwarf mistletoe species avoid freezing because their cellular water deep undercools to near -33°C. Seeds exposed to lower temperatures do not germinate. This winter survival mechanism may be of ecological significance, because the seeds overwinter attached to the leaf or stem of the host tree and are exposed to ambient winter temperature extremes.

In the Colorado Rocky Mountains (40°N latitude), the upper elevational limits of *A. vaginatum*, *A. americanum* and *A. cyanocarpum* are 2800, 3050, and 2990 m, respectively (9, 11, 17). In this region, *A. vaginatum* extends to the upper elevational limit of its principal host, but *A. americanum* and *A. cyanocarpum* do not (9, 17). Over a nine-year period, at the same region as sited above, Barry (2) found the average yearly minimum temperature to be -34°C and the extreme minimum temperature to be -38°C at 3050 m. In general, minimum temperatures decrease with increasing elevation in mountainous regions (1). In the above study, Barry (2) found the average yearly minimum temperature decreased 3°C per 1000 m increase in elevation. Thus, average yearly minimum temperatures at 3050 m are -34°C and lower at higher elevations. This fact and a -33°C winter hardiness limit in the

seeds of *A. americanum* and *A. cyanocarpum* suggest that freezing avoidance by deep undercooling in the seeds of these species may be a factor limiting their elevational distribution in this region. Previous studies (10) suggested that shortening of growing season at higher elevations may limit the elevational distribution of *A. americanum*.

The northern distribution of *A. americanum* extends to 58°N latitude in Canada (11). Since temperatures drop below -40°C at this latitude, further studies are needed to determine how seeds of this species survive in this northern region. The low temperature exotherm in winter-collected flower buds of some *Prunus* species native to Canada will move to lower temperatures decrease in size, and eventually disappear by prolonged exposure to freezing temperatures (4). By this prolonged prefreezing treatment, such *Prunus* buds survived -80°C, whereas thawed and refrozen buds were injured in association with a low temperature exotherm. Possibly, the same phenomemon occurs in mistletoe seeds native to Canada. Our results show that *A. americanum* seeds collected in Colorado do not lose the low temperature exotherm when held at -19°C for 24 hours.

The finding of freezing avoidance by deep undercooling in dwarf mistletoe and annual seeds (12, 13) suggests this winter survival mechanism may be common

to seeds of many other temperate zone plants. Furthermore, this winter hardiness limitation may be of ecological significance in limiting the distribution of tree species which have seeds that overwinter exposed to winter temperature extremes. This may be the case in conifer seeds present in overwintering cones.

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CHAPTER V

WINTER HARDINESS IN VEGETATIVE TISSUES OF CONIFER MISTLETOES, *ARCEUTHOBIUM* AND *PHORADENDRON*

ABSTRACT

Winter injury temperatures were determined for the vegetative tissue of six conifer dwarf mistletoe parasites (*Arceuthobium* spp.) and one American conifer mistletoe parasite (*Phoradendron juniperinum*) collected in Colorado and Arizona. Injury temperatures were determined by three viability tests; relative electrolyte loss, visual rating, and TTC dye reduction. *A. cyanocarpum* and *A. americanum* had the lowest injury temperatures, $\leq$-60°C. *A. douglassi* and *P. juniperinum* had the highest injury temperatures, -25 to -35°C. The vegetative tissue of the mistletoe species studied do not avoid freezing by deep undercooling. Therefore, the tissues studied may have maximum winter hardiness levels even lower than those determined in this study.

Arceuthobium (dwarf mistletoe) and *Phoradendron* (mistletoe) species parasitize a wide variety of conifers in North America (8, 13). Their distribution is more restricted than that of the host tree, suggesting a difference in response to environmental factors. Low temperatures, in particular low temperature extremes, have been implicated as a causal factor in limiting the northern and elevational distributions of some species of *Arceuthobium* and *Phoradendron* (7, 12). Although field observations of low temperature injury to mistletoes have been reported, studies have not been conducted to determine subfreezing injury temperature in controlled freezing tests. This communication reports the results of such freezing tests on winter collected vegetative tissue of six *Arceuthobium* and one *Phoradendron* species. The relevance of these findings to distributional limits of mistletoe species is also discussed.

Stem cuttings containing the mistletoe parasites were collected from trees in the Arapaho-Roosevelt and Gunnison National Forests in Colorado and the Apache-Sitgreaves National Forest in Arizona from March 1 to 5, 1979. Cuttings were placed in plastic bags on ice and returned to the laboratory where they were held at -2°C until use. Freezing tests, as described in detail elsewhere (1), were conducted on vegetative tissue of each species. Samples of *A. cyanocarpum*, *A. americanum*, and

A. vaginatum were stressed to temperatures between -15 and -70°C. Samples of *A. microcarpum*, *A. apacheum*, *A. douglassi* and *P. juniperinum* were stressed to temperatures between -15 and -55°C. Samples were cooled to the desired temperature at 5°C/hr, removed immediately, and rewarmed to room temperature at 5°C/hr. The injury temperature for each species was determined by three viability tests. Briefly, these tests on stressed tissue were a visual rating of injury, measurement of relative electrolyte loss, and triphenol tetrazolium chloride (TTC dye) reduction. The methods used in these viability tests are described in detail elsewhere (1, 5, 10). Differential thermal analysis (6) freezing studies were also conducted on the vegetative tissue to determine at what temperatures freezing occurred.

The injury temperatures for winter collected vegetative tissue of the conifer mistletoe species studied are listed in Table I. These results serve as a relative indication of winter hardiness between species for the samples collected at the given locations. The results do not necessarily indicate the maximum attainable winter hardiness within each species. In extensive studies of winter hardiness in *Salix* spp., Sakai (9) has shown that twigs which only resist freezing to -15°C when collected from a native site can resist freezing to -50°C if hardened at -3°C for 14 days. He also showed that the level of hardiness within a species varies greatly as a function

Table I. Injury temperatures for vegetative tissue of mistletoe species as determined by three viability tests.[1] The relative electrolyte loss injury temperature is the temperature at which the transition from low to high electrolyte loss occurred. The visual rating injury temperature is the highest temperature at which at least 50% of the sample appeared injured relative to the -15°C sample. The TTC dye reduction injury temperature is the highest temperature at which at least 50% of the sample failed to reduce the dye.

Mistletoe species, conifer host	Source	Injury Temperature (°C) as determined by:		
		Relative Electrolyte loss	Visual Rating	TTC Dye Reduction[2]
A. cyanocarpum, limber pine	Colo.	<-70	<-70	-60
A. americanum, lodgepole pine	Colo.	-60	-60	-65
A. vaginatum, ponderosa pine	Colo.	-40	-40	-45
A. microcarpum, Engelmann and blue spruce	Ariz.	-35	-35	-40
A. apachecum, white pine	Ariz.	-30	-45	-35
A. douglasii, Douglas fir	Ariz.	-25	-30	-35
P. juniperinum, Utah juniper	Colo.	-30	-30	-35

[1] After stressing the tissue, samples were held at 25°C for five days before evaluating injury. Cuttings of stressed tissue, 2 mm long, were cut into two longitudinal sections and used in each viability test.

[2] The concentration of TTC used was 1 mg per 100 ml H_2O. Stressed samples were evaluated for injury 24 hrs after immersion in the TTC solution.

of geographic origin and minimum winter temperatures at the native site. Twigs collected from more northern locations survived lower temperatures than twigs of the same species from southern locations. Thus, *A. americanum* collected in midwinter from near its northern limit (approximately 60° north latitude) may survive much lower temperatures than -60 to -65°C determined for samples collected in Colorado.

Differential thermal analysis freezing curves of the vegetative tissue of the mistletoe species studied did not reveal low temperature exotherms associated with the freezing of deep undercooled tissue water. Such low temperature freezing imposes a -40°C winter hardiness limit (3). Since the vegetative tissue of *A. americanum* and the other mistletoes studied did not have this mechanism of freezing avoidance to near -40°C, it is possible that the tissues may survive extremely low temperatures. They may even survive immersion in liquid nitrogen (196°C) if properly acclimated.

Therefore, these findings do not support the contention that low temperature injury to the vegetative tissue of the mistletoe species studied limits their distribution. This does not preclude the possibility that low temperature injury to other specific tissues or at other times during the year, e.g. late spring or early fall, limit their distribution. Studies by

Baranyay and Smith (4) indicate that early fall frosts reduce seed dispersal of the current season in hemlock dwarf mistletoe (*A. tsugense*). They suggest that such injury to seeds may be a significant factor in limiting the northern and upper elevational distribution of dwarf mistletoes. There are other recent findings that suggest midwinter low temperature injury to some dwarf mistletoe seeds may limit their distribution. Freezing avoidance by deep undercooling all freezable tissue water to -35°C has been found to be the winter survival mechanism of seeds of *Arceuthobium americanum* and *A. cyanocarpum* (2). Thus, the seeds have a midwinter hardiness limit near -35°C. The results are in agreement with studies by Smith and Wass (11). They showed that seeds of *A. americanum* inoculated onto lodgepole pine in the fall of the year in British Columbia, Canada, had 0% germination and infection after the 1971-1972 winter, when the lowest recorded temperature in the region was -39°C. In comparison there was 38% germination and infection of inoculated seeds after the following 1972-1973 winter, when the lowest recorded temperature reached only -32°C.

In conclusion, these results indicate a relative level of winter hardiness of vegetative tissue between the mistletoe species collected in Colorado and Arizona.

Since winter hardiness within a species can vary greatly with geographic origin, these results do not necessarily indicate the maximum attainable winter hardiness within each species. Furthermore, since the mechanism of winter survival of these tissues is not freezing avoidance, it is unlikely that midwinter low temperature injury to vegetative tissue limits the distribution of the species studied.

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APPENDIX I

LOW TEMPERATURE EXTREMES; THEIR EFFECT ON PLANTS AT TIMBERLINE

ABSTRACT

In Rocky Mountain National Park of Colorado, cessation of tree growth occurs at 3339 $\pm$ 113 m independent of exposure. The cause may be due to a -40°C winter hardiness limit of the dominant timberline trees and other woody flora. Low temperature extremes at timberline are near -40°C and lower at higher elevations.

INTRODUCTION

At timberline a prominent transition occurs in the flora of mountainous regions; it is cessation of upright tree growth. Above this elevation only low growing woody shrubs and herbaceous alpine plants survive. Timberline, as used here, refers to the 'alpine' timberline of mountainous regions in the temperature zone and not to the northern limit of tree growth, 'boreal' timberline. In Rocky Mountain National Park, this transition is abrupt and occurs at an extremely uniform elevation. In this region timberline is at the same ele-elevation on north, south, east, and west facing slopes (4).

There have been numerous studies attempting to determine the environmental factor responsible for this cessation of tree growth. The findings are summarized in reviews by Arno (1), Daubenmire (7), and Tranquillini (12). The environment at timberline is indeed severe, and increases in severity at higher elevations (3). Severe wind, low temperature, and shortness of growing season are but a few of the environmental stresses at timberline.

There have not been studies conducted to determine the winter hardiness of the timberline flora of this region in relation to the minimum temperatures that occur at the elevation of timberline. In general

plant tissues survive winter temperature extremes by either tolerating or avoiding ice formation (10). Plant cells that survive by tolerance of ice do so by dehydration in winter. The freezing of water occurs outside of the cell, extracellularly, without injury to the living cellular constituents. Such plant tissue in midwinter is extremely hardy and can even survive immersion in liquid nitrogen, -196°C, without injury (11). Another mechanism of winter survival is for cellular water to remain in a liquid state without ice formation at temperatures well below its normal freezing point. This occurs within hydrated cells, in constrast to extracellular freezing which is accompanied by cellular dehydration. This mechanism of freezing avoidance, deep undercooling (synonomous with deep supercooling), has a low temperature limit of about -40°C (6). This survival mechanism is effective if temperatures remain above -40°C, but lethal if temperatures drop below -40°C. Thus, it is an abrupt 'all or nothing' type of plant response to low temperatures extremes.

The sharpness of timberline transition in the Colorado Rocky Mountains suggests that such an abrupt plant stress response may be the cause. In this paper, our recent winter hardiness studies of the timberline flora of this region are summarized. Numerous conifers and hardwoods of this flora have an abrupt -40°C winter hardiness limit. Furthermore, climatological data show

that low temperature extremes are near -40°C at timberline elevation and lower at higher elevations.

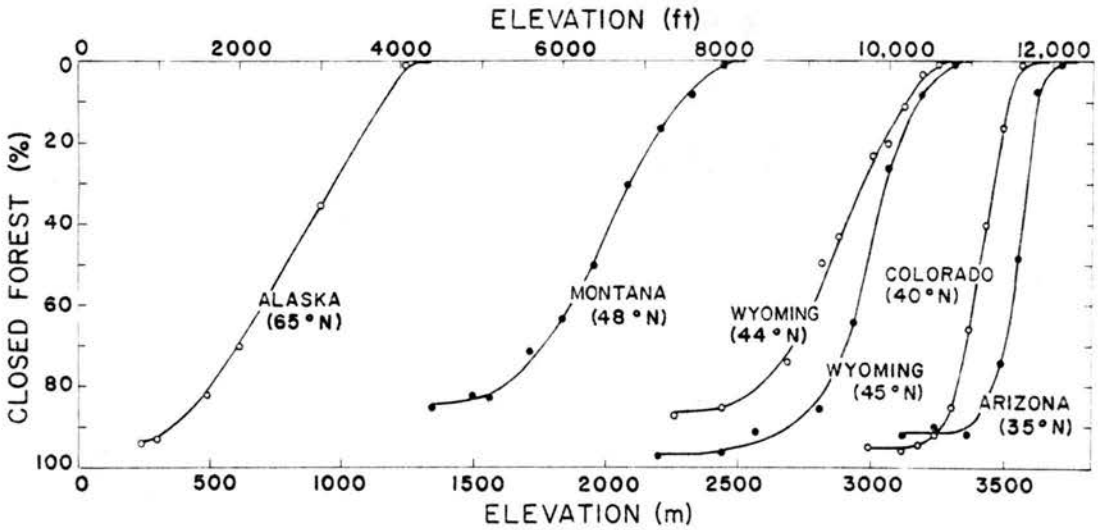
TIMBERLINE

The discussion here will be restricted to alpine timberline in the temperate region of North America, and in particular to timberline of the Colorado Rocky Mountains. The timberline transition as a function of elevation is shown for the following regions in Figure 1.

Humphreys Peak, AR (35°N)
Rocky Mountain National Park, CO (40°N)
Teton National Park, WY (44°N)
Yellowstone National Park, WY (45°N)
Glacier National Park, MT (48°N)
McKinley National Park, AK (65°N)

Figure 1 shows two important features of timberline in these regions. First, the transition from closed forest to open alpine tundra is very abrupt in southern regions, Arizona and Colorado, and more gradual in northern regions, Montana and Alaska. In Colorado 80% of the transition occurs over a 200 m change in elevation. In contrast, in Alaska 80% of

Figure 1. The timberline transition from closed forest to open alpine tundra of six mountainous regions in the western U.S. Percent closed forest values (open and closed circles) were calculated from contour lines on U.S. Geological Survey topographical maps at various elevations.


ELEVATION (ft)
0
2000
4000
6000
8000
10,000
12,000
CLOSED FOREST (%)
0
20
40
60
80
100
ALASKA
(65°N)
MONTANA
(48°N)
WYOMING
(44°N)
WYOMING
(45°N)
COLORADO
(40°N)
ARIZONA
(35°N)
0
500
1000
1500
2000
2500
3000
3500
ELEVATION (m)

the transition occurs over a 800 m change in elevation (Figure 1). Figure 1 also shows that timberline occurs at higher elevations in southern regions than in northern regions. In general the elevation of timberline decreases about 125 m per degree latitude in moving from south to north.

The elevation at which timberline occurs in Colorado is also independent of exposure. To determine this, measurements were made on U.S.G.S. topographical maps of Rocky Mountain National Park. The elevation of timberline was recorded at 1.6 km (one mile) intervals along the line forming the upper limit of the closed forest. The total distance covered in this manner was 177 km. These data were averaged to give the following results:

3339 $\pm$ 113 m, all exposures included
3327 $\pm$ 94 m, north exposures included
3366 $\pm$ 85 m, south exposures included
3328 $\pm$ 120 m, east exposures included
3349 $\pm$ 135 m, west exposures included

It is unlikely that one environmental factor would cause timberline to be at 3328 m on east facing slopes and that a different factor would cause it to be at a nearly identical elevation, 3327 m, on north facing slopes. The abruptness of the timberline transition in Colorado (Figure 1) and its uniformity of elevation

over a large region independent of exposure suggest that one environmental factor is ultimately limiting tree growth.

TIMBERLINE LOW TEMPERATURE EXTREMES

There are few weather stations at the elevation of timberline in Colorado. Those near this elevation have recorded low temperature extremes near -40°C (13). An extensive climatological study of the Niwot Ridge of the Colorado Rocky Mountains by Barry (3) showed the extreme minimum temperature at 3050 m was -37°C over a eighteen-year period. In general there is a 3°C/1000 m lapse rate in average yearly extreme temperatures with increasing elevation in this region. This is in close agreement with studies of Baker (3) on temperature lapse rates in mountainous regions. His results suggest a 5°C decrease in average minimum temperature per 1000 m gain in elevation.

Although duration of low temperature, e.g. shortness of growing season, has been implicated as a cause of timberline (12) low temperature extremes have not. This may be due to the lack of a systematic study of the winter hardiness of timberline flora.

TIMBERLINE FLORA WINTER HARDINESS

Winter hardiness studies were conducted on numerous conifers and woody shrubs native to the Colorado Rocky Mountains. Differential thermal analysis, as described in detail elsewhere (8), was used to detect low temperature freezing near -40°C. Such low temperature freezing in plant tissue limits its hardiness to -40°C (6). Hardiness determinations were also made on all species studied to determine at what temperature injury occurred.

Figure 2 shows the results of differential thermal analysis studies on tree and shrub species native to Colorado and having an elevational limit at or just below timberline. Peaks in the freezing profiles indicate the freezing of water. All species have a large peak near -10°C which is not injurious to the tissue in midwinter. The presence of freezing peaks near -40°C indicate the freezing of deep undercooled tissue water. Such peaks were found in the stem and leaf tissue of numerous conifers studied and in the stem tissue of the woody shrubs studied. Note the close association of the injury temperature (solid circle) with the low temperature freezing peak in all species (Figure 2). The results of our winter hardiness studies (5) are summarized in Table I.

Figure 2. Freezing profiles for timberline flora native to Colorado Rocky Mountains. Peaks on freezing profiles indicate the freezing of tissue water. Solid circles on the temperature scale indicate the injury temperature (Becwar et al., 1979).

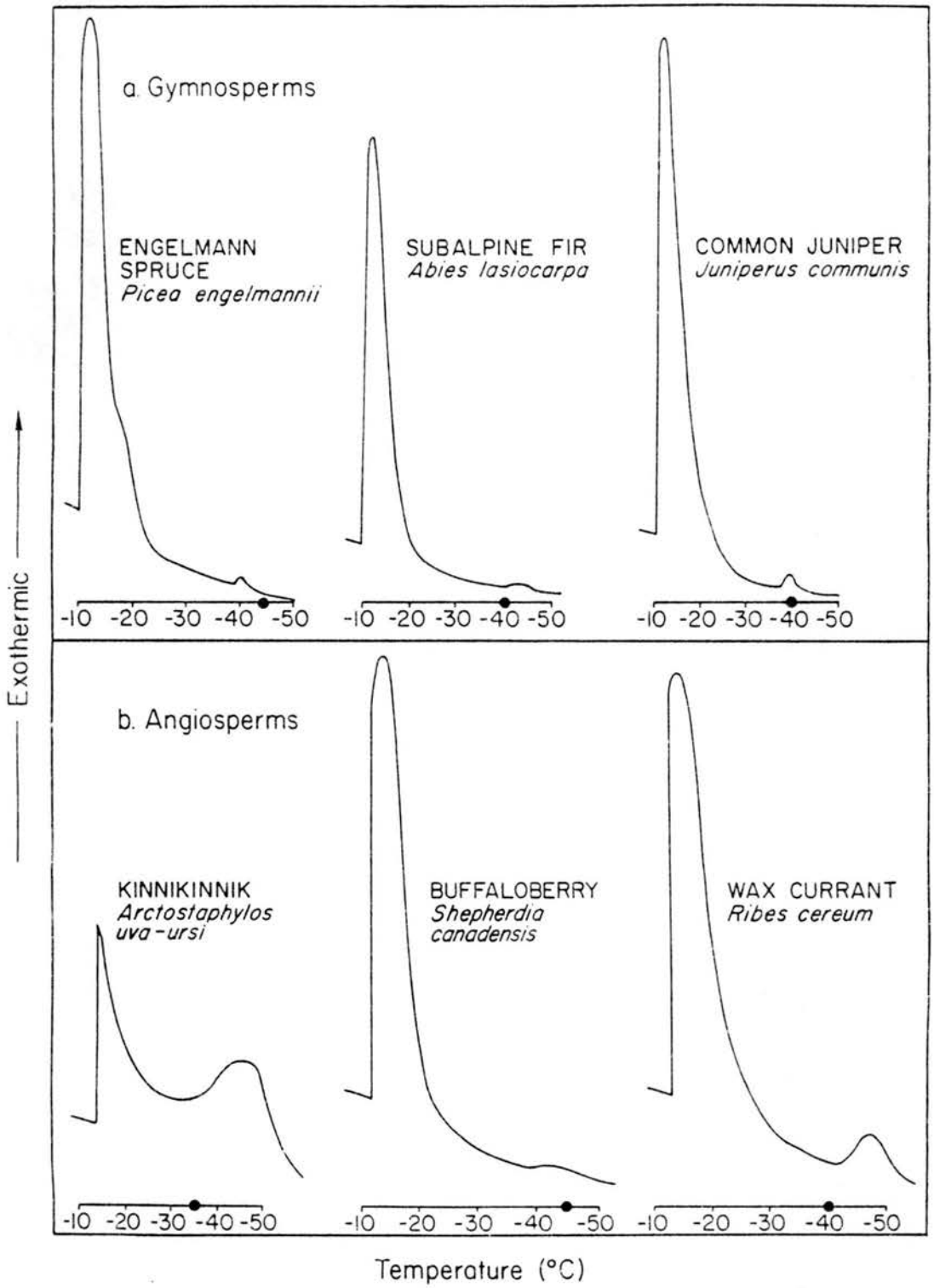

a. Gymnosperms
ENGELMANN SPRUCE
Picea engelmannii
SUBALPINE FIR
Abies lasiocarpa
COMMON JUNIPER
Juniperus communis
b. Angiosperms
KINNIKINNIK
Arctostaphylos uva-ursi
BUFFALOBERRY
Shepherdia canadensis
WAX CURRANT
Ribes cereum
-10 -20 -30 -40 -50
Exothermic
Temperature (°C)

Table I. Winter hardiness limits of timberline flora native to the Colorado Rocky Mountains. The elevation limits given are based on herbarium collections thorughout the entire state of Colorado (9). Asterisks indicate not found

Species	Colorado Elevation Limit (m)	Low Temperature Freezing (°C)	
		Peak	Injury
CONIFERS			
Engelmann spruce	3660	-39	-45
Subalpine fir	3660	-40	-40
Limber pine	3660	-46	-45
Common juniper	3440	-38	-40
Rocky Mtn. juniper	3350	-41	-45
Douglas fir	3350	-40	-40
Lodgepole pine	3350	*	*
Blue spruce	2900	*	*
Ponderosa pine	2830	*	-45
WOODY SHRUBS			
Willow	4270	*	*
Kinnikinnik	3570	-37	-35
Rubus spp.	3570	-39	-40
Buffaloberry	3510	-40	-45
Wax currant	3470	-40	-40
Mtn. maple	3200	-39	-40
Snowberry	3200	-39	-40
Sagebrush	3080	-34	-35
Mtn. mahogany	3050	-38	-40
Ninebark	3050	-38	-40
Snakeweed	3050	-38	-35
Bitterbrush	2740	-38	-40
Skunkbrush	2740	-39	-40
Wild grape	2130	-40	-40

SUMMARY

Our results show that in Colorado the timberline transition is abrupt and at an extremely uniform elevation in Rocky Mountain National Park. This suggests that there is one limiting factor causing the cessation of tree growth in this region. The sharpness of this transition suggests that an abrupt plant stress response to the gradually worsening environment at higher elevations may be the cause. Numerous winter hardiness studies have shown that freezing avoidance to -40°C is a common characteristic in many temperate zone woody plants. This is an abrupt plant stress response; injury occurs below -40°C but not above. The occurrence of this -40°C winter hardiness limit in the timberline flora and the presence of -40°C temperatures at that elevation suggests that freezing avoidance may limit tree growth at timberline. To obtain a more complete understanding of the role of this winter hardiness limit at timberline, more precise winter temperature measurements need to be made at various timberline locations in mountainous regions.

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APPENDIX II

EFFECTS OF SOLAR AND SUPPLEMENTAL 280 TO 320 nm UV IRRADIANCE ON HERBACEOUS CROP PLANTS

ABSTRACT

This study was conducted to determine the effect of increased levels of 280-320 nm UV radiation on plant dry weight and height of four herbaceous crop species; wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*), and pea (*Pisum sativum*). Plants were grown at a 3000 m elevation field site in Colorado (39°N latitude, 107°W longitude) to make use of the increase in 280-320 nm UV radiation relative to sea level. At the high elevation field site there was a reduction in wheat plant height of plants grown under cellulose acetate and aclar filters which transmit 280-320 nm solar UV radiation relative to plants grown under mylar filters which exclude 280-320 nm solar UV radiation. There were no differences in dry weight of the four species studied between the above treatments. There were also no differences in plant dry weight between treatments that received natural solar radiation at the field site and

those that received the same and a supplemental increase in 280-320 nm UV radiation from FS40 sunlamps. The maximum level of supplemental 280-320 nm UV irradiance at the field site was 478 mW/m^2. This corresponds to an increase that could result from a 20% reduction of stratospheric ozone. In a separate study, conducted in a greenhouse, there was a reduction of plant height of wheat plants exposed to 740 mW/m^2 of supplemental 280-320 nm UV irradiance relative to control plants. There was no signficiant reduction of wheat or pea dry weight in the greenhouse study. These results suggest that the species studied can tolerate a significant increase in 280-320 nm UV radiation with little or no adverse effect on plant dry weight.

INTRODUCTION

Destruction of stratospheric ozone due to increased concentrations of halocarbons and nitrogen oxides in the atmosphere (4, 6, 11) could have an adverse ecological impact. Increased levels of solar UV radiation, resulting from ozone depletion (13), could affect food crop production. This study attempts to measure the magnitude of the effect of an increase in 280-320 nm UV irradiance on four herbaceous crop species.

The UV spectrum is often divided into three regions as follows: 200-280 nm, 280-320 nm, and 320-400 nm. In the 200-280 nm region, sometimes referred to as the far ultraviolet region or UV-C, DNA and aromatic amino acids have absorption peaks at 260 and 280 nm, respectively (5, 9). Although these two molecules are likely involved in UV radiation injury, the precise mechanism of injury is not known. At present the natural solar spectrum cuts off at 290 nm (7). An increase in UV radiation due to ozone depletion would result in shorter wavelengths reaching the earth's surface. Based on calculations of Shettle and Green (13), even a 50% ozone depletion would not allow significant transmission of UV radiation below 280 nm. Therefore, the possible deleterious effects of an increase in 280-320 UV radiation are of ecological significance. This region of the UV spectrum is sometimes

referred to the middle ultraviolet or UV-B region of the spectrum. The 320-400 nm region of the UV spectrum, near ultraviolet or UV-A radiation, is known to induce photo-repair of injury caused by shorter wavelengths of UV radiation in plants (12).

Part of this study took advantage of the natural high levels of solar UV radiation at high elevations as a primary method for increasing UV radiation levels. The experimental site was located 3000 m above sea level in the Colorado Rocky Mountains. Caldwell's (1) work suggests an increase in biologically effective UV irradiance (280-320 nm) of 2.5 to 12.6% at this elevation relative to sea level. Koller (7) demonstrated the presence of shorter wavelengths of UV radiation at high elevations in the Alps. Thus, the anticipated high UV radiant flux density and shorter wavelengths of UV radiation at high elevations were seen as a natural way to simulate the effect of ozone depletion.

Plant species were chosen for the study based on their known sensitivities to UV radiation, short growing season requirements at the experimental site, and their importance as food crops. Cline and Salisbury (3) investigated the UV (254 nm) sensitivity of radish and peas and found them to be sensitive and very sensitive, respectively. They were included in the study as UV sensitive plants. Potato was chosen because its origin is in the high elevation equatorial regions of South

America and therefore may be more resistant to high levels of UV radiation. Wheat was chosen because it has been reported to be resistant to high levels of UV radiation (8).

MATERIALS AND METHODS

Crop species and cultivars tested were: pea, *Pisum sativum* 'Alaska'; wheat, *Triticum aestivum* 'Inia 66'; potato, *Solanum tuberosum*, 'Kennebec'; and radish, *Raphanus sativus*, 'Cherry Belle'. Plants were grown in containers in a peat, vermiculite, and sand medium (40:30:30% by volume). For a complete description of chemical and hydrological properties of this medium see Moore, et al. (10).

The field experimental site was at 3000 m elevation in the Colorado Rocky Mountains, 39°11' north latitude and 106°56' west longitude. Two separate experiments were conducted at this site.

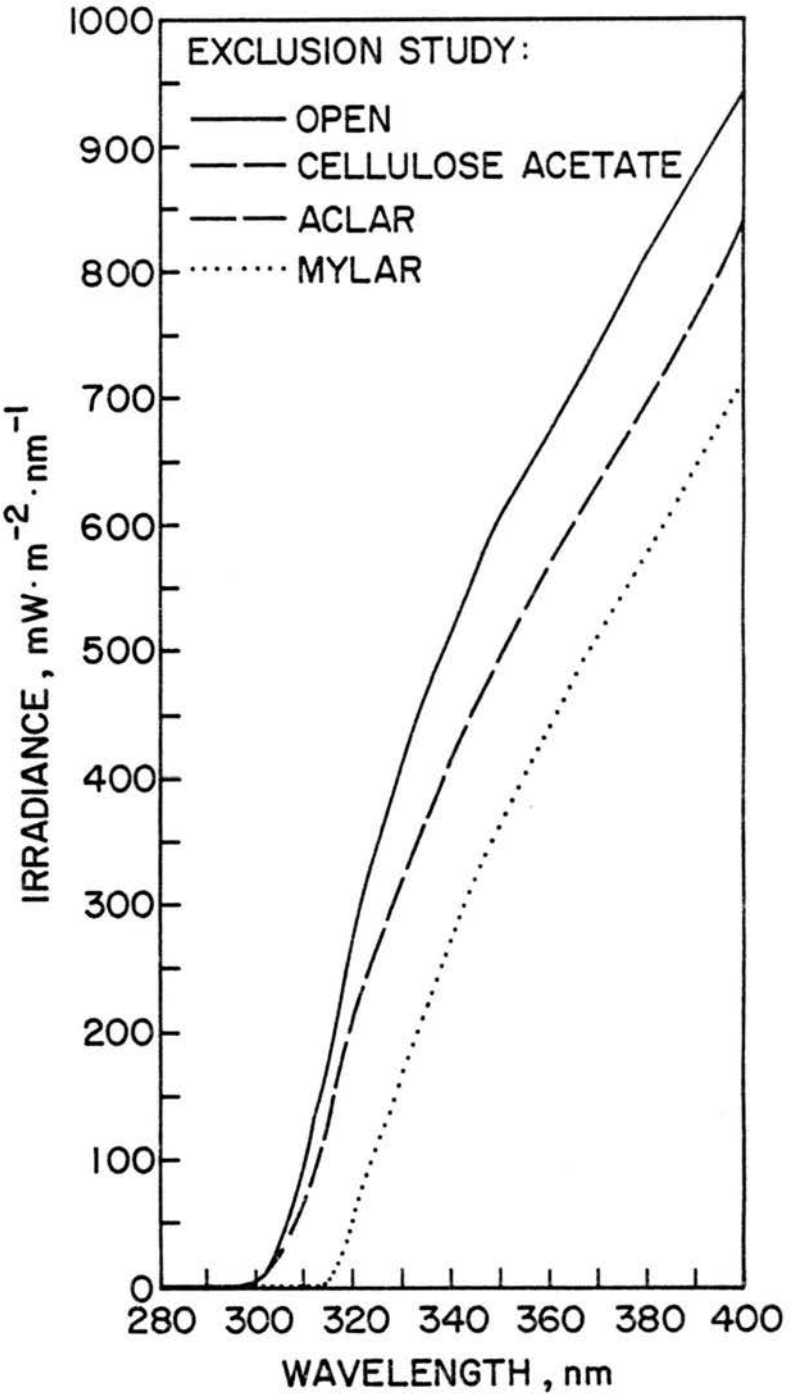
EXCLUSION STUDY

This part of the field experiment involved the use of filters to exclude specific regions of the natural UV spectrum and shading to reduce total insolation. Five treatments were used in this experiment. Cellulose acetate (5 mil thickness) and aclar (1.5 mil thickness) excluded UV radiation below 280 and only slightly reduced insolation above 280 nm. Mylar (5 mil thickness) excluded UV radiation below 320 nm and only slightly reduced insolation above 320 nm. Thus, plants grown under cellulose acetate and aclar received 280 to 320 nm UV radiation, whereas those under mylar did not. The fourth treatment was an open control, without filters. Spectral evaluation of the filters at the experimental site are compared to the open control in Figure 1. The fifth shaded treatment reduced total insolation by 26% to simulate sea level insolation. The experimental design was a randomized complete block with three replications per plant species. Treatment differences were tested by using the F test and if differences were significant, means were then separated using LSD procedures at the 5% level.

FIELD LAMP STUDY

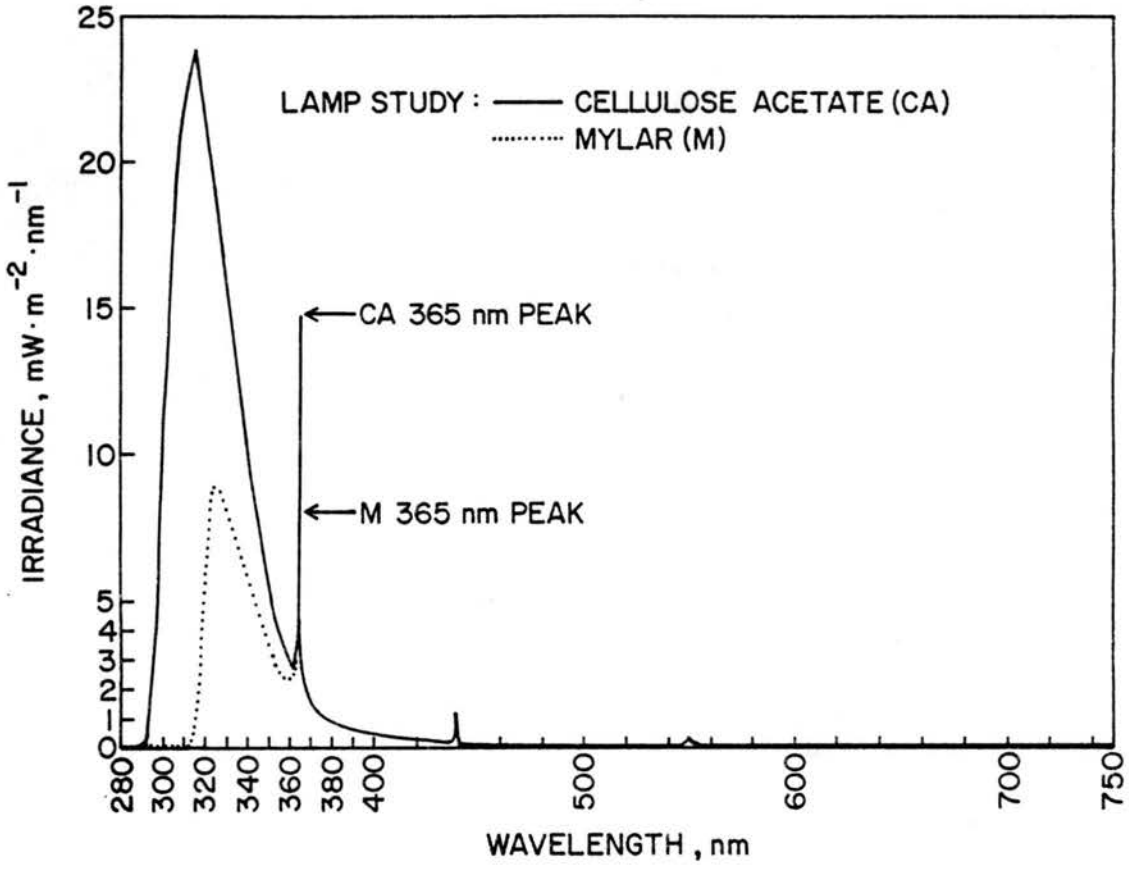
In this part of the experiment plants received natural solar radiation and supplemental UV radiation from Westinghouse FS40 UV lamps. Plants were exposed to the supplemental radiation for 6 hours each day (3 hours before and

Figure 1. Solar spectra in open and under exclusion study filters at field experimental site. Measurements were taken with a UV spectraradiometer at 11 am (Mountain Standard Time) on August 11, 1977. Note that cellulose acetate and aclar filters transmit essentially all 280-320 nm solar UV radiation, while the mylar filter excludes essentially all 280-320 nm UV radiation.


EXCLUSION STUDY:
OPEN
CELLULOSE ACETATE
ACLAR
MYLAR
IRRADIANCE, mW·m⁻²·nm⁻¹
WAVELENGTH, nm
0
100
200
300
400
500
600
700
800
900
1000
280
300
320
340
360
380
400

after solar noon). There were three treatments in the lamp study. The first was lamps filtered with cellulose acetate which allowed transmission of UV radiation to as low as 280 nm. The second was lamps filtered with mylar which excludes UV radiation below 320 nm. See Figure 2 for a comparison of cellulose acetate and mylar filtered FS40 lamp spectra. Lamp filters were replaced every third day. The third treatment was a non-lamp control with only lamp reflectors to reproduce the microclimate of the lamp fixtures without adding radiation. The experimental design used in the lamp study was a split plot with whole plots consisting of the above three treatments. Each plot was split into ten subplots of position under the filtered lamps or reflectors. Plants in the center position under the cellulose acetate filtered lamp treatment received the highest level of supplemental UV radiation and those in the outer position the lowest. Supplemental irradiance levels at the plant canopy under the cellulose acetate filtered lamps ranged as follows from the center to the outer position: 478, 385, 273, 204, 170, 123, 94, 85, 47, and 25 mW/m^2. There were two replications of the three treatments for each of the four species. Container grown plants were placed under the lamp and exclusion study fixtures just prior to emergence. Filtered lamps and reflectors were maintained at 110 cm above the plant canopy throughout the duration of the lamp study.

Figure 2. Cellulose acetate and mylar filtered FS40 lamp spectra. See Figure 1 caption for details.


LAMP STUDY : —— CELLULOSE ACETATE (CA)
····· MYLAR (M)
CA 365 nm PEAK
M 365 nm PEAK
IRRADIANCE, mW·m⁻²·nm⁻¹
WAVELENGTH, nm
0
1
2
3
4
5
10
15
20
25
280
300
320
340
360
380
400
500
600
700
750

GREENHOUSE LAMP STUDY

In addition to the field study, pea and wheat plants were grown under filtered FS40 UV lamps in a glass greenhouse during the 1977-1978 winter at Fort Collins, Colorado (1526 m elevation, 40°35' north latitude, 105°04' west longitude). The two treatments used were cellulose acetate and mylar filtered lamps. The experimental procedures and design used were identical to the field lamp study with the following exception. Lamp fixtures were maintained at a distance such that the 280-320 nm UV irradiance level at the plant canopy under the cellulose acetate filtered lamps ranged as follows from the center to the outer position: 740, 640, 575, 330, 200, and 95 mW/m^2.

Parameters measured in both the field and greenhouse study included pea, wheat, potato, and radish dry weight and pea and wheat plant height. Duration of plant exposure to the treatments is cited in the results.

RESULTS AND DISCUSSION

EXCLUSION STUDY

Comparisons of the parameters measured between plants grown under cellulose acetate, aclar, and mylar filters are given in Tables I through III. In such an exclusion experiment, effects which are to be attributed to 280-320 nm UV radiation must be evident under cellulose acetate and aclar filters where solar 280-320 nm radiation is present. Such UV radiation effects should not appear under mylar filters where 280-320 nm UV radiation is not present. The only parameter measured showing a difference between the two types of filters which could be attributed to 280-320 nm UV radiation was wheat plant height (Table I). All other measurements between filter treatments on wheat and the other species showed no significant differences attributable to 280-320 nm UV radiation. Note that wheat exposed to 280-320 nm UV radiation under cellulose acetate and aclar filters tended to be shorter than plants which did not receive 280-320 nm UV radiation under the mylar filters. This effect was observed only in the wheat measured at 14 and 31 days. Mixed results, not wholly attributable to 280-320 nm UV radiation, were obtained at 50 days exposure. Comparison of open and 26% shade treatment also showed a relative stunting of wheat in the open. This latter effect was clear throughout the exposure period (Table I).

Table I. Exclusion study wheat plant height and dry weight. Mylar filters exclude 280-320 nm solar UV radiation. Cellulose acetate and aclar filters transmitt 280-320 nm solar UV radiation. Measurements were taken 14, 31, and 50 days from emergence. Data listed are mean values of three replications of 32 plants within each treatment. Unlike letters within each column of data indicate significant differences at the 95% level of confidence. Valid comparisons are between filter treatments or between the open and shaded treatment.

Treatment	Plant Height			Dry Weight	
	Days from Emergence				
	14	31	50	31	50
	- - - - - cm - - - - -			- g/plant -	
Mylar	18.6a	28.9b	46.3b	0.72	2.42
Cellulose Acetate	17.0b	27.9c	46.4b	0.79	2.46
Aclar	17.0b	28.0c	47.3a	0.66	2.42
Open	15.1c	26.6d	42.8d	0.71	2.49
Shade (26%)	17.2b	29.9a	44.6c	0.67	2.39

Table II. Exclusion study radish foliage and root dry weight. Measurements were taken 24 and 36 days from emergence. See Table 1 heading for further details.

Treatment	Folage Dry Weight		Root Dry Weight	
	Days from Emergence			
	24	36	24	36
	- - - - - - - - - g/plant - - - - - - - - -			
Mylar	0.35	0.73	0.29	0.89ab
Cellulose Acetate	0.33	0.79	0.25	0.95a
Aclar	0.31	0.76	0.23	0.90a
Open	0.35	0.62	0.29	0.76b
Shade (26%)	0.28	0.54	0.19	0.62c

Table III. Exclusion study potato foliage and tuber dry weight. Measurements were taken 49 days after emergence. Data listed are mean values of three replications of 16 plants within each treatment. See Table 1 for further details.

Treatment	Dry Weight	
	Foliage	Tubers
	- - - - - - - - g/plant - - - - - - - -	
Mylar	7.93	14.52
Cellulose Acetate	7.72	12.12
Aclar	7.62	13.98
Open	7.35	14.21
Shade (26%)	7.79	14.30

A factor that could affect the above results is temperature differences between treatments. Such temperature differences could cause differences in the parameters measured which might be misinterpreted to be 280-320 nm UV radiation effects. Therefore, temperature measurements were made and the results are summarized in Table IV. The results show that there was little difference in mean hourly air temperature between the treatments. (Although not listed, even a more detailed analysis of maximum and minimum air temperatures also showed differences between treatments to be negligible.) Mean hourly plant temperatures under the filter treatments were about 1.5°C higher than under the open treatment, but there was essentially no difference between the filter treatments. Measurements of total solar radiation (Table IV) also indicate that the total insolation under the filters was similar. Spectral responses under the three filters (Figure 1) indicate that cellulose acetate and aclar are essentially identical and that mylar excludes UV radiation below 320 nm and slightly reduces radiation above 320 nm. Therefore, based on these measurements (Table IV, Figure 1), it appears safe to conclude that observed differences between the filtered treatments can be attributed to differences in UV radiation. As indicated, the only observed difference in the exclusion study that could be attributed to UV-B radiation effects was a reduction in wheat plant height.

Table IV. Exclusion study temperature and total solar radiation measurements. Air temperatures were measured with thermocouples. Plant temperature were measured with an IR thermometer. Temperature data listed are mean values recorded over a 24-hour period. Solar radiation was measured with a pyranometer. Data values in parenthesis indicate magnitude of the deviation from the measured value in the open.

Treatment	Mean Hourly Temperature (^{o}C)		Solar Radiation (W/m^2)
	Air	Plant	
Open	12.9	12.7	803
Cellulose Acetate	(+0.7)	(+1.4)	(-63)
Aclar	(0.0)	(+1.5)	(-21)
Mylar	(+0.2)	(+1.6)	(-84)
Shade (26%)	(-0.2)	(0.0)	(-208)

FIELD LAMP STUDY

In this experiment there was no response between the three treatments that could be attributed to 280-320 nm UV radiation (Tables V and VI). There was also no difference in plant dry weight within the cellulose acetate filtered lamp treatment as a function of position that could be attributed to 280-320 nm UV radiation. Therefore, the results listed in Table 5 are for the plants receiving the maximum level of supplemental 280-320 nm radiation under the cellulose acetate treatment (478 mW/m^2) and for the corresponding center positions under the mylar and non-lamp treatments.

It is known that the longer wavelengths of UV radiation (320 to 400 nm) and photosynthetically active radiation (400 to 550 nm) can repair or nullify the adverse effects of shorter wavelength UV radiation in plants (2, 12). Although the cellulose acetate and mylar filtered lamp treatments did not supplement the 400-550 nm region of the spectrum, the cellulose acetate filtered lamp treatment did supplement the 320 to 400 nm region of the spectrum (Figure 2). Therefore, the possibility exists that any detrimental effect to the plants caused by the supplemental 280-320 nm UV radiation was repaired by the supplemental 320-400 nm radiation or by natural solar radiation above 320 nm.

Another complicating factor was observed in the wheat plant height results of the field lamp study.

Table V. Field lamp study plant dry weight measurements. Dry weight data are for the center position in each treatment. This position corresponds to the maximum level of supplemental 280-320 nm UV irradiance (478 mW/m^2) in the cellulose acetate treatment. Data are mean values of two replications of four wheat, radish, and pea plants and two potato plants.

Treatment	Days Exposure	Dry Weight		
		Cellulose Acetate (+280 to 320 nm UV)	Mylar (Control)	Non-lamp (Control)
		- - - - - - - - - g/plant - - - - - - - - -		
Wheat, foliage	32	0.91	0.76	0.92
Wheat, foliage	50	2.50	2.50	2.80
Potato, foliage	49	7.40	7.00	5.50
Potato, tuber	49	14.00	15.50	12.90
Radish, foliage	28	0.35	0.28	0.36
Radish, root	28	0.44	0.42	0.50
Pea, foliage	23	0.51	0.52	0.68
Pea, foliage	28	0.96	1.01	1.26

Table VI. Field lamp study plant height measurements. Plant height data are for the center and outer positions in each treatment. Values in parentheses are mean plant heights for the ten positions within each treatment. The center and outer positions in the cellulose acetate treatment correspond to the maximum and minimum levels of supplemental 280-320 nm UV irradiance, 478 and 25 mW/m^2, respectively. Unlike letters after data indicate a significant difference at the 95% level of confidence within a treatment at each exposure data. Data for the center and outer position of each treatment are mean values of two replications of four plants.

Species	Days Exposure	Position Under Lamps	Plant Height		
			Cellulose Acetate (+280 to 320 nm UV)	Mylar (Control)	Non-lamp (Control)
			- - - - - - - - - - cm - - - - - - - - - -		
Wheat	14	Center	14.2a [13.7]	14.3a [13.8]	14.7a [13.7]
		Outer	13.0b	13.5b	13.4b
	32	Center	27.9 [25.8]	26.6 [26.1]	26.6 [25.8]
		Outer	24.9	26.2	24.9
	50	Center	42.9c [39.7]	40.9c [40.0]	42.9c [41.0]
		Outer	36.7d	38.9d	39.6d
Pea	23	Center	23.0 [22.9]	23.4 [22.4]	25.4 [23.8]
		Outer	22.1	24.3	25.6

Differences in wheat plant height were observed as a function of position regardless of treatment (Table VI). In general, wheat plants in the center positions were taller than those grown in the outer positions. In the case of wheat plants measured after 14 and 50 days exposure, there were significant differences in plant height as a function of position. Since this complicating effect was observed regardless of treatment it may be due to the fixture shading of the centrally located plants from the primary UV source, the sun.

GREENHOUSE LAMP STUDY

Wheat plants exposed to the highest level of 280-320 nm UV radiation, 740 mW/m^2, under cellulose acetate filtered lamps showed a reduction of plant height relative to control plants under mylar filtered lamps. The following wheat plant height data are mean values of two replications of eight plants per treatment.

Position under lamps	Wheat Plant Height	
	Cellulose Acetate (+280 to 320 nm UV)	Mylar (control)
	- - - - - - - cm - - - - - - -	
Center	37.1	41.2

This reduction in wheat plant height is in agreement with similar results obtained in the field exclusion study. Since wheat plant height measurements were not taken over a range of 280-320 nm UV irradiance levels, the threshold level of irradiance required to cause a reduction in plant height was not determined.

There was no significant dry weight reduction of pea and wheat plants exposed to supplemental 280-320 nm UV irradiance relative to control plants in the greenhouse lamp study (Table VII). Slight differences in plant dry weight were observed between cellulose acetate and mylar filtered lamp treatments and between levels of 280-320 nm UV irradiance within the cellulose acetate treatment. These differences were not significant or consistent and therefore could not conclusively be attributed to 280-320 nm UV irradiance.

In summary, the results of this study suggest that wheat, potato, radish, and pea plants can tolerate an appreciable increase in 280-320 nm UV irradiance without an adverse affect on dry weight accumulation. Both the field exclusion and lamp study and the greenhouse lamp study results support this conclusion. The only adverse response attributable to 280-320 nm UV irradiance was a reduction in wheat plant height. Based on these results, it seems safe to conclude that a 20% reduction of stratospheric ozone would not have an adverse effect on the plants studied.

Table VII. Greenhouse lamp study pea and wheat plants dry weight measurements. Plant dry weight data are for the center and outer positions in each treatment. Values in parenthesis are mean dry weights for the six positions within each treatment. The center and outer positions in the cellulose acetate treatment correspond to the maximum and minimum levels of supplemental 280-320 nm UV irradiance, 740 and 95 mW/m^2, respectively. Center and outer position data of each treatment are mean values of two replications of eight plants.

Species, Tissue	Position Under Lamps	Dry Weight	
		Cellulose Acetate (+280 to 320 nm UV)	Mylar (Control)
		- - - - - - - mg/plant - - - - - - -	
Peas, foliage	Center	172 [186]	188 [182]
	Outer	194	186
Wheat, foliage	Center	192 [198]	222 [211]
	Outer	208	221

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