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UNIVERSITY OF ALBERTA

EFFECTS OF FLOODING AND SUMMER FROSTS ON THE PHOTOSYNTHESIS OF SOME WOODY SPECIES IN ALBERTA PEATLANDS.

BY


QING LAI DANG

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

DEPARTMENT OF FOREST SCIENCE

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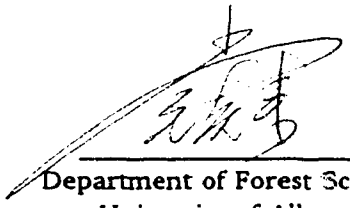

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ABSTRACT

The vast area of peatland forests in Canada is a potentially valuable and important resource for forestry and the environment. Trees on peatlands, however, grow slowly because of various environmental stresses. However, the stress physiology of these trees is poorly understood. In this thesis, I present results from several ecophysiological studies on two general projects: 1) The in situ physiological responses of peatland trees to flooding. 2) The impact of summer frosts on the in situ physiology of peatland trees, with special emphasis on photosynthesis.

Flooding stress was investigated by examining the diurnal variation and interrelationship of some physiological parameters in tamarack (Larix laricina (DuRoi) K. Koch), black spruce (Picea mariana (Mill.) B.S.P.), and swamp birch (Betula pumila L.) under naturally flooded and non-flooded conditions. It was found that flooding depressed photosynthesis and this depression was primarily attributed to decreases in mesophyll conductance. The mesophyll limitation to photosynthesis was similar for all three species. Swamp birch appeared to be better adapted to flooding than tamarack and black spruce.

In the study of summer frosts, a self-contained freezing chamber was developed. This chamber was proved to be a practical and economical means of summer frost simulation in the field. Using this apparatus, a series of freezing experiments were conducted in a 20 year-old stand of black spruce and tamarack. It was found that summer frosts of -3.5 ^{0}C or below significantly inhibited the photosynthetic capacity, photosynthetic quantum yield and the photochemistry of photosystem II. The response of photosynthesis to summer frosts consisted of two phases: 1) A depression phase where photosynthesis declined continuously; 2) A recovery phase where photosynthesis increased gradually. The length of the depression phase varied with species but not with the degree of freezing (>-6 ^{0}C). A full recovery of photosynthesis took more than 11 days. An integrated analysis of all data suggested the primary site(s) of summer frost damage to photosynthesis was located in the biochemical reactions. Results from a laboratory experiment showed the activity of Ribulose Bisphosphate carboxylase was inhibited by a -6 ^{0}C frost, but this enzyme did not appear to be the primary site of damage.

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INTRODUCTION

Peatlands are a valuable natural resource. There are about 422 million hectares of peatland in the world (Kivinen and Pakarinen 1981). The total area of peatland in Canada is about 118 million hectares, which is more than 1/4 of the world's total peatland resource. Eleven per cent of Canada's peatland is distributed in Alberta, covering about twenty per cent of Alberta's total land surface (Tarnocai 1984, Päivänen 1985). Such a vast area of peatlands represents a great potential for forestry. Nevertheless, because of various environmental stresses, trees on these sites grow extremely slowly (Payandeh 1973, Mäkitalo 1985). Forests in Alberta peatlands rarely grow to a commercial size (Lieffers and Rothwell 1986).

The growth of trees is a product of the interaction between their genetic make-up and their environment, which are expressed through physiological processes. During their life time trees experience various environmental stresses. Understanding the responses of physiological processes, particularly photosynthesis, to environmental stresses, is important in both tree improvement and silvicultural practice (Kramer 1986).

PEATLAND WATER TABLE

High water tables in peatlands usually limit tree growth. Roots of the trees growing on these sites are normally restricted to hummocks and above the upper limit of the water table fluctuation (Boggie and Miller 1976). Following heavy rain falls or snow-melt, however, the water table can rise above the hummocks (i.e. ground surface) and flood tree roots for extended periods of time. This situation is considered harmful to the physiology and growth of trees (Tang and Kozlowski 1982, Kozlowski and Pallardy 1984). Dang and Lieffers (1989) found tree rings of peatland black spruce showed slower growth in extremely wet years. Presumably tree roots were flooded for longer periods of time in those years than average or normal years. The physiological response and growth of tree seedlings to flooding have been studied in the greenhouse (e.g. Boggie 1972, Crawford 1982, Kozlowski 1984a, 1984b, 1986, Levan and Riha 1986). To the best of my knowledge, there are no field observations on the impact of soil flooding on the physiology of woody plants growing in natural peatlands.

SUMMER FROST

Freezing events during the growing season (summer frosts) are a severe environmental stress that limits plant growth. Dang and Lieffers (1989) found the annual ring growth of black spruce on Alberta peatland sites was less in years with low average minimum temperatures in the growing season. They hypothesized that the growth depression was related to the negative effects of summer frosts, which presumably occurred more frequently in those years. Studies have shown that summer frosts occur frequently at peatland sites in the boreal forest region (Hayter and Proudfoot 1978, Rothwell and Lieffers 1988). Previous studies have demonstrated that summer frosts can significantly limit forest productivity by depressing photosynthesis (DeLucia and Smith 1987, Lundmark and Hällgren 1987, Lundmark et al. 1988). Most of these studies, however, were conducted on seedlings. Effects of summer frosts on the productivity of large trees are poorly understood. In addition, the duration of the frost damage and subsequent recovery process of photosynthesis are unclear. In terms of annual forest productivity, the duration of frost damage to photosynthesis and the rate of recovery may be more important than the impact immediately following frost.

PHOTOSYNTHESIS

Photosynthesis is the process in which light energy is converted to chemical energy in the presence of chlorophyll. Photosynthesis is a complex process which consists of multiple

photochemical and biochemical reactions. Mesophyll resistance to photosynthetic CO_2 assimilation includes resistances to the diffusion of CO_2 (in aqueous phase) across the cell wall, membranes and cytoplasm to the carboxylation sites, and various biochemical and photochemical reactions (Bradford and Hsiao 1982; Edwards and Walker 1983). Although some studies have suggested the depression of photosynthesis resulting from summer frosts were mainly related to changes in mesophyll resistance (Pharis et al. 1970, DeLucia and Smith 1987, Lundmark and Hällgren 1987, Lundmark et al. 1988), understanding the mechanism of summer frost damage to photosynthesis requires further investigation of the responses of individual subprocesses of photosynthesis.

Chlorophyll fluorescence is the re-emission of the solar energy absorbed by photosynthetic pigments. It has been used widely in assessing stress damage to photosynthetic machinery, particularly photosystem II (e.g. Grafflage and Krause 1986, Strand and Lundmark 1987, Strand and Öquist 1988). Chlorophyll fluorescence is predominantly emitted from chlorophyll a associated with photosystem II reaction centres (Bolhàr-Nordenkampf et al. 1989). Chlorophyll fluorescence is generally measured after the green tissues are dark-adapted for a period of time. The difference between the initial level and the peak level of fluorescence is defined as the variable fluorescence. The variable fluorescence is a measure of the photochemical capacity of photosystem II (Horton 1985, Bolhàr-Nordenkampf et al. 1989).

Photosynthetic quantum yield is defined as the initial slope of the light response curve of net photosynthesis (Coombs et al. 1987, Walker 1989). It is normally calculated based upon the incident photon flux density and called apparent quantum yield of photosynthesis (Coombs et al. 1987). The photosynthetic quantum yield is a measure of the maximum overall efficiency of photosynthetic energy conversion (Grafflage and Krause 1986, Walker 1989). It is a particularly valuable indicator of stress inhibition of photosynthesis at a stand level where foliage is often shaded. When concomitantly measured with other photosynthetic parameters, such as variable fluorescence, the quantum yield can also help to identify sites of damage in photosynthesis.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the most important CO_2 fixing enzyme in all green plants. It is also the most abundant enzyme and protein in the biosphere (Gutteridge and Keys 1985, Ramage and Bohnert 1989). The concentration of Rubisco can reach 10 mg protein per gram fresh leaf (Coombs et al. 1987). Rubisco consists of more than 50% of the soluble protein in mature leaves (Getteridge and Keys 1985). Because of its importance in photosynthetic carbon reduction, Rubisco has attracted the attention of many plant physiologists and biochemists. Nevertheless, it was one of the most difficult enzymes to assay until an improved method was published by Lorimer et al. in 1977. Research has demonstrated the structure, configuration and activity of Rubisco were sensitive to changes in various environmental factors (Beadle and Jarvis 1977, Peaples and Dalling 1978, Huner and Macdowall 1979, Gezelius and Hallèn 1980, Seemann et al. 1985). OToole et al. (1976) reported changes in Rubisco activity are a good indicator of non-stomatal limitation to photosynthetic CO_2 fixation under water stress. Rubisco is believed to be a key enzyme responsible for physiological responses of plants to extreme temperatures (Graham and Patterson 1982).

Studying the effect of post-stress light regimes on photosynthesis can help to reveal primary sites of stress damage to photosynthesis. High intensity light has been reported to further depress photosynthesis after tree seedlings experience a freeze-thaw cycle (Lundmark and Hällgren 1987, Strand and Lundmark 1987). The mechanism for this phenomenon is believed to be related to damage of the photochemistry of photosynthesis caused by excess trapped solar energy when the CO_2 fixation apparatus is impaired (Greer et al. 1986). But to the best of my

knlowledge, no efforts have been made to examine the post-frost recovery processes of photosynthesis under different light regimes.

OBJECTIVES

The primary objectives of this thesis were:

1). To investigate the physiological response of peatland trees to flooding by examining the diurnal variation and interrelationships of ecophysiological parameters of three peatland woody species under flooded and non-flooded substrate conditions.
2). To investigate the extent and duration of summer frost damage to and the subsequent recovery process of photosynthetic capacity, photosynthetic quantum yield, chlorophyll fluorescence and the activity of ribulose bisphosphate carboxylase, in peatland trees.
3). To study the effect of post-frost light regime on the recovery process of the above photosynthetic parameters.

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II. DIURNAL VARIATION AND INTERRELATIONS OF ECOPHYSIOLOGICAL PARAMETERS IN THREE PEATLAND WOODY SPECIES UNDER FLOODED AND NON-FLOODED SOIL CONDITIONS

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INTRODUCTION

Peatlands in western Canada undergo significant variations in depth of water table during the growing season. The roots of trees on these sites are, however, generally confined to surface layers above the upper limit of water table fluctuation, with deeper roots being pruned off by anaerobic conditions (Boggie and Miller 1976; Mannerkoski 1985; Lieffers and Rothwell 1986). When the water table rises close to the peat surface, oxygen supply to tree roots will be reduced significantly (Kozlowski 1984b; Mannerkoski 1985). This can depress the photosynthesis and productivity of trees. Dang and Lieffers (1989) found black spruce on a natural peatland had lower annual tree ring growth in extremely wet years. Presumably, these were the years when tree roots experienced floodings for longer periods of time than average or normal years. Greenhouse experiments showed soil flooding depresses the photosynthesis of tree seedlings (Kozlowski 1984b). There are, however, no field observations on the impact of flooding stress on the physiology of woody plants in natural peatlands.

Studying diurnal patterns of water relations, photosynthesis and related parameters can provide fundamental information on plant responses and adaptations to natural environments (Schulze and Hall 1982). Diurnal patterns are extensively described for agricultural crops and some woody species (Leverenz 1981; Beadle et al. 1985; Kauhanen 1986) but none of these studies report on peatland trees.

In this paper, we examined the diurnal patterns of twig xylem water potential, photosynthesis, water use efficiency of photosynthesis, mesophyll and stomatal conductance to CO_2, and the interaction among these parameters, for tamarack (Larix laricina (DuRoi) K. Koch), black spruce (Picea mariana (Mill.) B.S.P.), and swamp birch (Betula pumila L.), under naturally flooded and non-flooded soil conditions in an Alberta peatland. We also discussed the mechanisms by which the photosynthesis in peatland trees is limited.

MATERIALS AND METHODS

The study site was a treed fen, located in the boreal foresteast of the Saulteaux River, about 36 km south east of Slave Lake, Alberta (55^0 8' N; 114^0 15' W). The forest canopy is open and dominated by black spruce (Picea mariana (Mill.) B.S.P.) and tamarack (Larix laricina (DuRoi) K. Koch). The average ages for black spruce and tamarack were 30 and 24 years, respectively. The common shrub species are Betula pumila L. (swamp birch) and Ledum groenlandicum Oeder. The average May-to-August precipitation for this general area totals 275 mm and the average July to August temperature is 15 ^{0}C (Monthly Record, Slave Lake Station, Climate Service, Environment Canada 1988).

Three woody species (black spruce, tamarack and swamp birch) were chosen for foliage gas exchange and twig xylem water potential (ψ_x) measurement. Three individuals of each species were selected. A branch at about breast height (tamarack and black spruce) or a terminal branch (birch) was chosen from each individual for measuring gas exchange. For black spruce, measurements were on 1-year-old needles only. For ψ_x measurement, a branch near the one for gas exchange measurement (tamarack and black spruce) or a terminal within the same clump as the one for gas exchange measurement (birch) was cut. The cut branch was inserted into a portable Scholander pressure chamber (PMS Instrument Company, Oregon, U.S.A.), with the cut end projecting through the rubber stopper. The pressure inside the chamber was increased gradually using compressed nitrogen gas. When xylem sap appeared on the cut surface of the cut branch, the reading of the pressure inside the chamber was taken immediately. This pressure is a good estimate of the xylem or foliage water potential of the twig (Ritchie and Hinckley 1975). The soil temperature in the root zone (at 15 cm depth) was also measured

using a dial-thermometer.

Gas exchange was measured using an open system consisting of a portable infra-red gas analyzer (LCA-2), a leaf cuvette (PLC), and an air supply unit (all from Analytical Development Corporation, Hoddeson, England). Ambient air was drawn from 4 m height using a tower. The air was passed through a desiccator before entry into the cuvette. The air flow through the cuvette was maintained at a rate of 10 mL s^{-1}. Air within the cuvette was made turbulent with a high speed fan. An infrared filter on the cuvette shield prevented heating inside the cuvette. In addition, gas exchange measurements were taken very quickly (< 1 min) to further reduce heating inside the cuvette. The cuvette contains sensors for measuring relative humidity, air temperature and photosynthetically active radiation. Because the leaf size was small and the air in the cuvette was highly turbulent (small boundary layer resistance), the temperature difference between leaf and air should be less than 0.7 ^{0}C (Nobel 1983). So the air temperature inside the cuvette was used as an estimate of leaf temperature.

Measurements were made on three days with different weather and soil moisture conditions: a cloudless day when the soil was flooded (July 8, 1988); a clear and hot day (July 26, 1988); and an overcast but warm day (August 4, 1988). These will be referred to as 'wet day', 'hot day', and 'cloudy day' respectively hereinafter. The ground water table on the wet day was 7 cm above the average peat surface level. This was the highest water table level observed at the study site from 1984 to 1990. The soil had been flooded for 3 days prior to the sampling day. The water tables on the hot and cloudy days (respectivley, 16 and 26 cm below the average peat surface) were in the normal range for that season, those two days were used as controls in assessing flooding effect on ecophysiological parameters. The maximum temperatures in the cuvette were 26, 35 and 27 ^{0}C respectively for the wet day, the hot day, and the cloudy day. The photosynthetically active photon flux densities (PAR) were above 1200 μmol m^{-2} s^{-1} during the period of gas exchange measurement on both the wet and the hot days. Since PAR was low (<800 μmol/m^2/s) on the cloudy day, natural light was supplemented by a Brinkman 'Q-beam' spot light which was positioned to supply light at 1600 μmol/m^2/s. The saturation PAR for photosynthesis of tamarack and black spruce was determined to be, respectively, 800 and 700 μmol/m^2/s.

The ambient water vapour pressure deficit (vpd) was recorded hourly on a CR21 data-logger (Campbell Scientific Corp., Canada). But vpd data were obtained only for the hot day because of equipment failures on the other two days.

ψ_x measurements were taken at 2-h intervals during daytime and at 4h intervals at night, for a period of 24 h on the wet and hot days and from 6:00 to 21:00 on the cloudy day. Gas exchange was measured at 2h intervals from 7:20 to 18:20 on the wet day, from 8:20 to 18:20 on the hot day, and from 6:20 to 21:20 on the cloudy day. One hour measurements were taken on the cloudy day for two time periods (i.e., 10:20 to 11:20, 19:20 to 21:20 h).

The foliage used in gas exchange measurements was collected at the end of each measurement period for leaf area determination. The leaf area for black spruce and tamarack was determined from dry mass using 'dry-mass vs leaf-area' equations (Macdonald and Lieffers 1990). The leaf area for birch was measured 4 times on a leaf area meter (LAMBDA Instruments Corporation LI 3100) and the average was used.

Net photosynthesis rate (P_{net}), leaf resistance to H_2O vapour (r), transpiration rate (E), and intercellular CO_2 concentration (C_i) were determined as described by Caemmerer and Farquhar (1981). Since the high speed fan in the cuvette and the design of the cuvette ensure a small boundary layer resistance (r_b) and r_b is generally very small for needles, the stomatal resistance to H_2O vapour (r_s) in tamarack and black spruce was assumed to be equal to r. The stomatal

conductance was calculated as: $g_s = 1/r_s$. The stomatal conductance for birch was calculated as: $g_s = 1/(r - r_b)$, where r_b is boundary layer resistance determined as described by Coombs et al. (1985). Stomatal conductance to CO_2 (g_c) was calculated as: $g_c = g_s/1.6$ (Coombs et al. 1985). Mesophyll conductance to CO_2 (g_m) was calculated as: $g_m = P_{net}/C_i$ (Fites and Teskey 1988). The water use efficiency of photosynthesis (WUE) was determined as: $WUE = P_{net}/E$ (Larcher 1983). P_{net}, E, g_c, and g_m were all expressed on a leaf area basis.

Regression analysis was conducted on the P_{net}-g_m relationships for each species-day combination. The homogeneity of the regression coefficients (slopes) was tested (Steel and Torrie 1980). The differences in P_{net} between different species, between different days, and species-day interactions, were examined after P_{net} was adjusted to the same g_m (covariance analysis). All statistical analyses were conducted using SAS statistics package for personal computers (SAS Institute Inc. 1987). The relationship of P_{net} to ψ_x, g_c, C_i, and leaf temperature, was analyzed by examining the plots of P_{net} versus these parameters. The relationship of WUE to ambient water vapour pressure deficit was also investigated.

RESULTS

1. Xylem water potentials (ψ_x)

In general, all three species had similar diurnal patterns of ψ_x on each of the three days (Figs.II.1a, 2a and 3a). On the wet and hot days, ψ_x decreased rapidly before 10:00 and increased after 18:00, fluctuating at low values between the two times. Both the daily average and minimum ψ_x on the wet day were higher than on the hot day and the cloudy day. On the cloudy day, the decrease in ψ_x started later, but the decline was faster and lasted longer than on the other two days (Fig.II.1a, 2a, 3a). The daily minimum ψ_x on the cloudy day was lower and delayed by 5 h compared to the wet day and the hot day. The range of the daily variation in ψ_x was the greatest on the cloudy day and smallest on the wet day. ψ_x on all sampling days generally did not return to the pre-dawn level before midnight. Among the three species, birch had the highest and tamarack had the lowest daily average and minimum ψ_x for all three days. On the wet day, the minimum ψ_x in birch occurred 4 hours later than tamarack and black spruce. The daily minimum water potentials for the wet day, the hot day, and the cloudy day, were respectively, -1.9, -2.4, and -2.7 Mpa for tamarack; -1.7, -1.9, and -2.2 Mpa for black spruce; and -1.2, -1.5, and -1.7 for birch.

2. Net photosynthesis (P_{net})

Tamarack and birch had similar diurnal patterns of P_{net} on each sample day, but the patterns were different on different days (Figs.II 1b & 3b). P_{net} for both species was relatively constant on the wet day; gererally decreased throughout the day with a small recovery after 16:20 on the hot day; and increased rapidly in the early morning and then decreased for the rest of the day on the cloudy day. The decrease in P_{net} for birch on the cloudy day started two hours later than tamarack. Black spruce, in contrast, had almost the same diurnal pattern for P_{net} on all three days (Fig.II.2b). Maximum P_{net} occurred in early morning and then decreased continuously throughout the day.

On average, P_{net} for birch was usually higher than tamarack and black spruce on all three days, whereas the difference between the latter two was small. The differences in P_{net} between species, however, were not always consistent among the sample days. Tamarack and black spruce had the lowest P_{net} on the wet day, while birch had similar values on the wet and hot days. Tamarack and birch had the highest P_{net} on the cloudy day, while black spruce had similar values on the hot and cloudy days. The range of daily fluctuations in P_{net} was the

smallest on the wet day and the largest on the cloudy day for all three species.

3. Mesophyll and stomatal conductance to CO_2

Mesophyll conductance generally had similar diurnal patterns to photosynthesis on all three days (Figs.II 1c, 2c, 3c). In general, all three species had similar diurnal patterns of stomatal conductance (g_c) on all three days. Stomatal conductance decreased continuously throughout the day except for an increasing period before 9:20 on the wet day (Figs.II 1d, 2d & 3d). Birch had greater variations on the wet and cloudy days than tamarack and black spruce. There were species-weather interactions that affected the magnitude of g_c. On average, tamarack and black spruce had the highest g_c on the cloudy day while g_c was similar on the wet and hot days (Figs.II 1d & 2d). In contrast, birch had the lowest average g_c on the hot day and the highest g_c on the cloudy day (Fig.II.3d).

4. Water use efficiency of photosynthesis (WUE)

The three species had similar diurnal patterns of WUE changes on the hot day: WUE decreased rapidly before 14:00 and recovered slightly afterwards (Fig.II.4b). On the wet day and cloudy day, however, there were obvious day-species interactions. On the wet day, WUE in tamarack and birch was relatively stable for most times of the day, whereas WUE in black spruce decreased continuously throughout the day (Fig.II.4a). On the cloudy day, the diurnal variation in WUE for tamarack was small while WUE in black spruce and birch generally deceased before 18:20 and recovered afterwards (Fig.II.4c). Interestingly, WUE in black spruce dropped almost to zero at 21:00 while WUE in the other two species was still very high (Fig.II.4c).

On average, WUE was the highest on the cloudy day for all three species (Figs.II 1e, 2e, 3e). In tamarack, WUE was the lowest on the hot day (Fig.II.1e) while for black spruce (Fig.II.e) and birch (Fig.II.3e) differences in WUE between the wet day and the hot day were generally small.

5. Interrelations among variables

P_{net} was positively and linearly related to g_m ($P < 0.05$) for all three species on all three days. Coefficients of determination for P_{net} regressed on g_m ranged from 0.75 to 0.97 (average = 0.90) (the plot of P_{net} on g_m for black spruce on the hot day is given in Fig.II.5 as an example). Homogeneity tests of regression coefficients showed no significant differences ($P > 0.05$) in slopes of P_{net}-g_m regressions for different day-species combinations. After P_{net} was adjusted to the same g_m, there were no significant differences in P_{net} between species or significant interactions between species and day ($P > 0.05$). However, P_{net} was significantly different ($P < 0.05$) among the 3 days (highest on the wet day, lowest on the hot day). In other words, for a given day, P_{net} for all the species responded similarly to changes in g_m, but the responses of individual species were affected by the weather and soil moisture conditions experienced on different days.

In general, g_c was 3.8 to 7.9 times greater than g_m. The g_c/g_m ratios were the highest on the wet day. There was no obvious difference in g_c/g_m between different species.

No clear relationship was found between P_{net} and ψ_x or leaf temperature for any species, or between P_{net} and Ci for black spruce or birch. In contrast, for tamarack, P_{net} was positively related to C_i on the cloudy day (data not presented).

For all three species, g_c and WUE both decreased as the ambient water vapour pressure deficit (vpd) increased on the hot day (Fig.II.6). The decrease was much faster before 10:20 than after. Stomatal conductance in birch appeared to be more sensitive (steeper slope) to changes in vpd

than in tamarack and black spruce (Fig.II.6a).

DISCUSSION

Our data showed that soil flooding had a negative impact on the photosynthesis of both black spruce and tamarack. P_{net} of these species on the wet day was lower than the other two days when the soil was not flooded. Depression of photosynthesis by soil flooding has also been observed in other tree species (Kozlowski 1984a; 1984b). In contrast to tamarack and black spruce, P_{net} in swamp birch on the wet day was as high as on the hot day. This indicates that swamp birch was probably better adapted to flooded soil conditions than tamarack and black spruce. Indeed, swamp birch occurs more frequently on wet peatlands than relatively drier peatland sites in central Alberta.

On the wet day, the substrate had been flooded for three days. The twig xylem water potential (ψ_x) on that day was higher and more stable throughout the day for all three species than the hot and cloudy days when the soil was not flooded, while the stomatal conductance (g_c) on the wet day was similar to the other two days in both diurnal variation and magnitude. This indicates that the roots of these species probably had the capacity to absorb water from the saturated peat soil at a relatively high rate. This capacity would be an advantage for species growing on peatland sites where soil flooding occurs frequently. Conlin and Lieffers (personal communication) found the root systems of tamarack and black spruce were able to acquire metabolic energy through anaerobic respiration when the soil was flooded. The fact that the daily minimum ψ_x on the wet day occurred four hours later in birch than tamarack and black spruce and that g_c in birch was much higher on the wet day than on the hot day could be taken as another line of evidence that swamp birch was better adapted to flooded soil conditions than the other two species.

The g_c results on the wet day were in contrast to the responses of stomata to flooding reported in the literature. Stomata generally close in response to flooding (Kozlowski 1982; 1984a; 1984b). The amplitude of diurnal fluctuations in stomatal conductance is much greater in flooded plants than non-flooded ones (Tang and Kozlowski 1982). We found the amplitudes of both stomatal conductance and its diurnal fluctuations were similar in flooded trees (on the wet day) and in non-flooded trees (on the hot and cloudy days). This discrepancy could simply reflect a difference in flood tolerance between seedings and saplings. However, since most of the reported flooding experiments were conducted in the greenhouse, it is also possible that the duration of flooding treatments in the greenhouse is not long enough or there are not enough flooding cycles to allow the seedlings to adapt.

For all species in this study, the ψ_x values early in the afternoons of the hot and cloudy days were below the recorded values for drought-stressed woody species: Buxton et al. (1985) observed that ψ_x in black spruce was -0.9 MPa after the seedlings were drought-stressed for 72 hours. Melzak et al. (1985) found that the photosynthesis in Pinus halepensis decreased dramatically when ψ_x was below -0.8 MPa. Similar results have been reported for birch (Osonubi and Davis 1980) and loblolly pine (Teskey et al. 1986). Most plant processes (protein synthesis, enzyme activities etc.) were depressed by foliage water potentials below -1.5 MPa (Kaufmann 1981). In this study, the daily minimum ψ_x for black spruce and tamarack was respectively -2.2 and -2.7 MPa. Taking into account the declining trend of photosynthesis and mesophyll conductance associated with the decline of ψ_x (Figs.II 1, 2 & 3), we concluded that these species were under water stress in the afternoons.

Peatlands generally have excess water in the substrate. Drought stress in peatlands is most likely due to an imbalance between the root capacity for absorbing water and the water loss

from transpiration. According to the resistor-capacitor theory (Passioura 1981), transpiration early in the day is dependent mainly upon the water stored in the canopy which is recharged over night, while later in the day water uptake by roots becomes more important. The root systems of peatland trees are shallow (Lieffers and Rothwell 1986) and therefore the effective absorbing surface may be small. In addition, the peat substrate is generally cold (4 to 7 ^{0}C at 15 cm for the three sampling days). Cold soil can significantly increase the root resistance to water uptake (Lopushinsky and Kaufmann 1984). Consequently the water uptake by roots was probably slow. The transpiration demand (i.e. water vapour pressure deficit), however, was generally higher in the afternoon than in the morning. Although stomatal conductance decreased in response to increases in vapour pressure deficit (Fig.II.6), this decrease might not be large enough to offset the imbalance between water loss and water input in the trees. These conditions were combined to expose the trees to water stress.

For all three peatland species, it seems that mesophyll conductance to CO_2 and its responses to weather and soil moisture conditions were primarily responsible for changes in photosynthesis, whereas stomatal conductance exerted little limitation on photosynthesis. The strong linear relationship between P_{net} and g_m provided good evidence to support this conclusion. The similarity of diurnal trends in P_{net} and g_c, however, suggests that stomatal conductance might also be a major factor limiting photosynthesis. If this were the case, P_{net} would be positively related to the CO_2 concentration in the intercellular space. Our data showed this relationship was poor. The fact that g_c was much greater than g_m suggests that changes in g_c would have a minor impact on photosynthesis. The similar diurnal trends in P_{net} and g_c could simply reflect the effect of mesophyll activities on the stomata, as suggested by Wong et al. (1979). The diurnal patterns of photosynthesis on different days showed that there were obvious species-day interactions. However, the adjustment of P_{net} for differences in g_m between species and between days eliminated these interactions. This clearly shows that the variation in photosynthesis was caused primarily by the variation in mesophyll conductance. In addition, decreases in g_c (e.g. in response to vpd increases) should result in an increase in water use efficiency of photosynthesis (WUE) if mesophyll conductance was not limiting (Osmonubi and Davies 1980). In our data, however, g_c and WUE changed in parallel to each other (Fig.II.6). This is another indication that low g_m was the major limiting factor to photosynthesis in the three peatland species. The primary role of mesophyll in limiting photosynthesis has also been observed in other species (Osonubi and Davies 1980; Farquhar and Sharkey 1982; Kozlowski 1984a; Melzack et al. 1985; Teskey et al. 1986).

The mechanisms of the mesophyll limitation to photosynthesis of the three peatland species are not well understood. Mesophyll conductance includes the diffusion of CO_2 (in aqueous phase) across the cell wall, membranes, and the cytoplasm to the carboxylation sites, and various biochemical and photochemical reactions (Bradford and Hsiao 1982; Edwards and Walker 1983). Any of these could contribute to the decrease in g_m. Decreases in g_m are often associated with mesophyll water status (Whiteman and Koller 1964). Water status change induced alteration in light harvesting, energy conversion (Bradford and Hsiao 1982), carboxylation (O'Toole 1976; Kaiser 1987), and the activity of fructose bisphosphatase (Berkowitz and Gibbs 1983) have been reported. Ögren and Öquist (1985) found that in severely drought stressed willow (leaf water potential < -1 MPa) reduction in P_{net} was solely attributed to decreased activity of ribulose bisphosphate carboxylase. Flooding reduces the activities of carboxylation enzymes and the chlorophyll content of some species (Kozlowski 1982). Flooding can also limit P_{net} through its limitation on carbohydrate translocation (Kramer and Kozlowski 1979). Also changes in plant hormones may be involved in the above or other ways (Bradford and Hsiao 1982).

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Figure II.1. Tamarack diurnal variations of: a) twig xylem water potential (ψ_x); b) net photosynthesis (P_{net}); c) mesophyll conductance to CO_2 (g_m); d) stomatal conductance to CO_2 (g_c); e) water use efficiency of photosynthesis (WUE) ($\bar{x} \pm$ se.; n=3). Data were collected on a clear day when soil was flooded (wet day), a clear and hot day (hot day), and a cloudy but warm day (cloudy day). The water table level on the wet day was 7 cm above the average peat surface level while it was 16 and 26 cm below the average peat surface respectively for the hot day and the cloudy day. g_m, g_c and WUE were not calculated for the first two measurements on the cloudy day because H_2O exchange measurements were not reliable due to heavy dew on the foliage early in the morning.

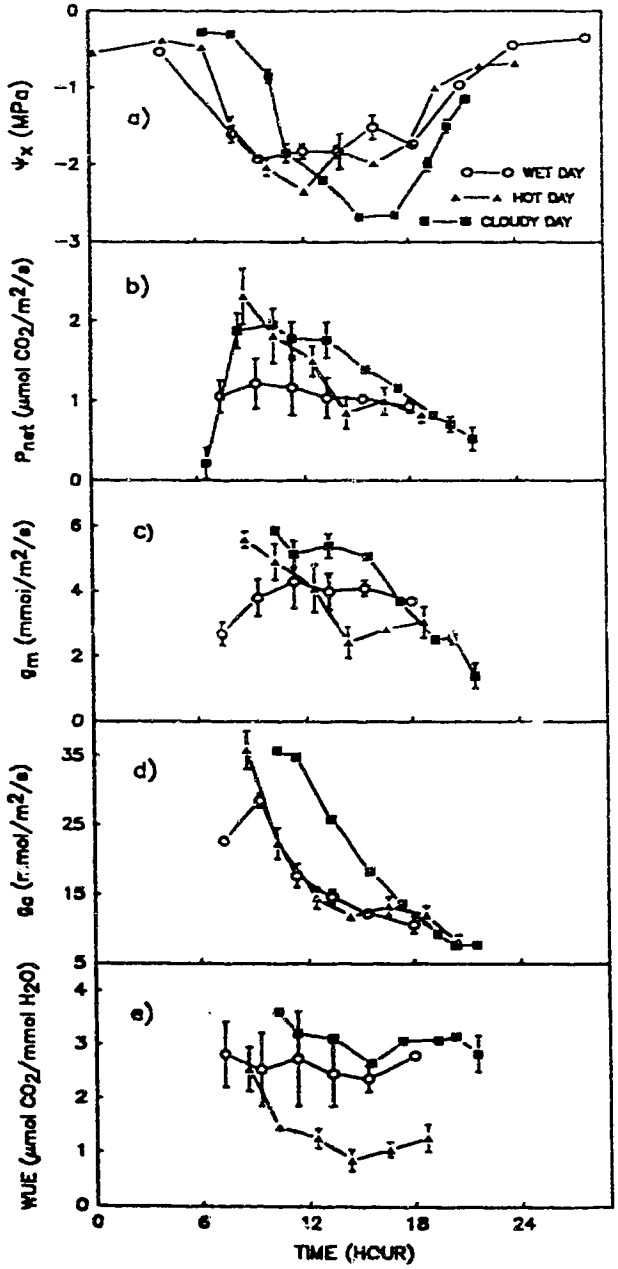


Figure II.2. Black spruce diurnal variations of: a) twig xylem water potential (Ψ_x); b) net photosynthesis (P_{net}); c) mesophyll conductance to CO_2 (g_m); d) stomatal conductance to CO_2 (g_c); e) water use efficiency of photosynthesis (WUE) ($\bar{x} \pm se.$; n=3). Explanations on the data are the same as Fig.II.1.

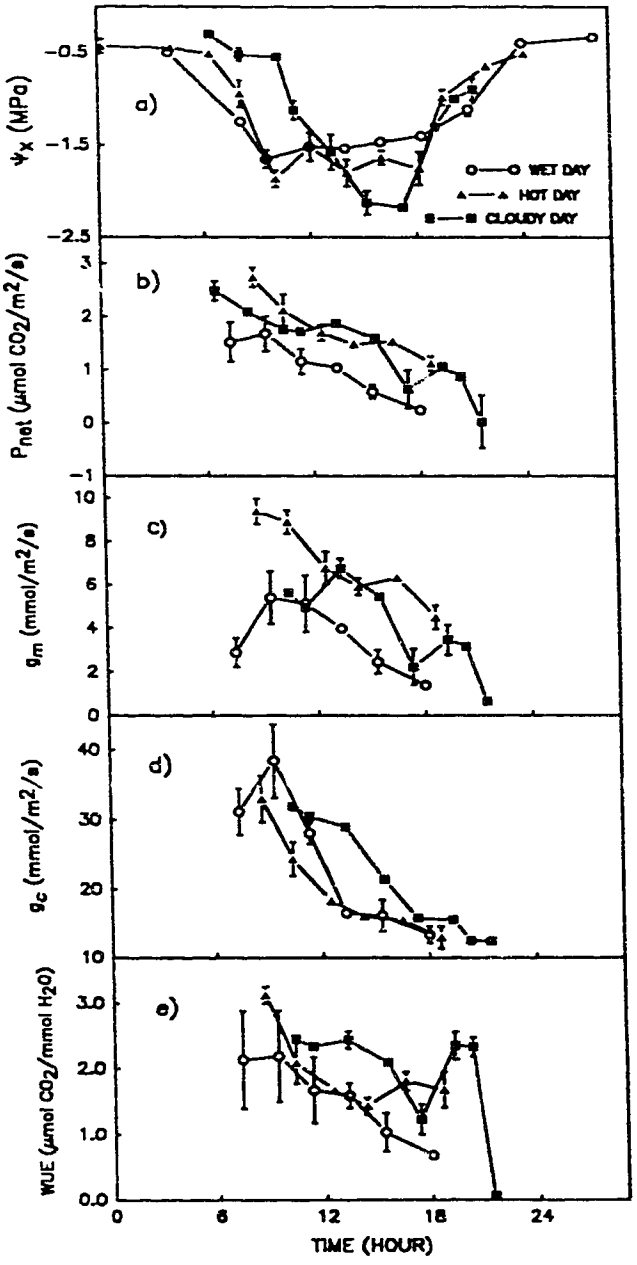


Figure II.3. Swamp birch diurnal variations of: a) twig xylem water potential (Ψ_x); b) net photosynthesis (P_{net}); c) mesophyll conductance to CO_2; d) stomatal conductance to CO_2 (g_c); d) water use efficiency of photosynthesis (WUE) ($\bar{x} \pm$ se.; n=3). Explanations on the data are the same as Fig.II.1.

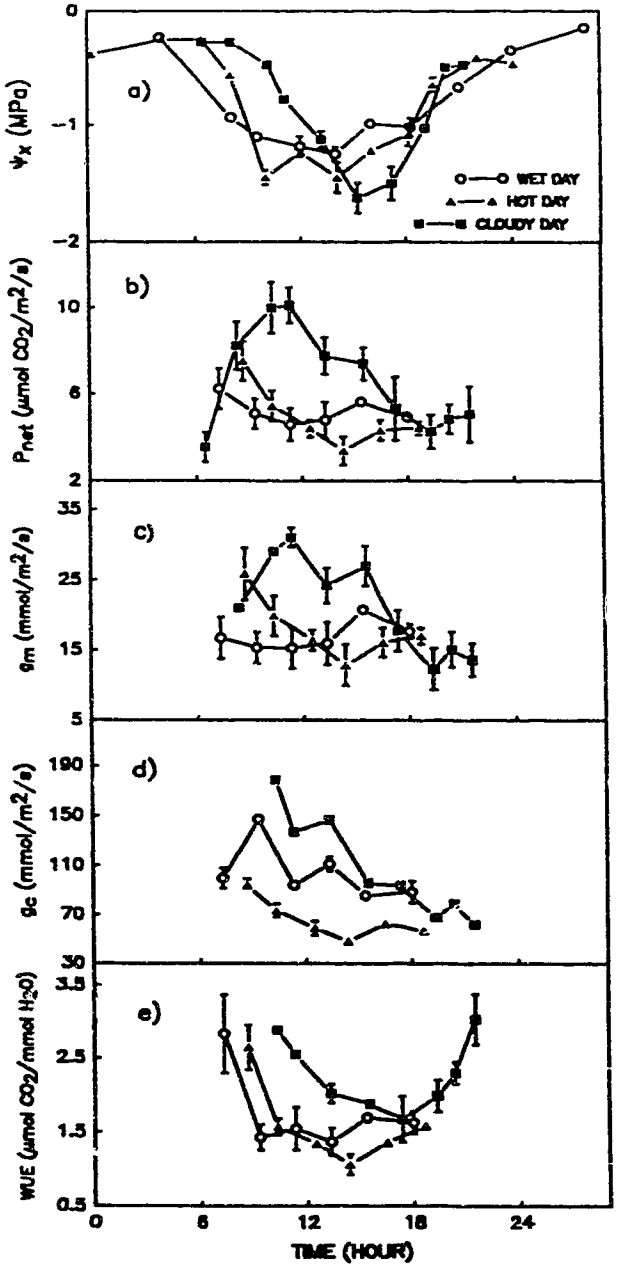


Figure II.4. Diurnal variations of water use efficiency of photosynthesis (WUE) in tamarack, black spruce and swamp birch on the wet day (a), the hot day (b), and the cloudy day (c). See Figure II.1 for more explanations.

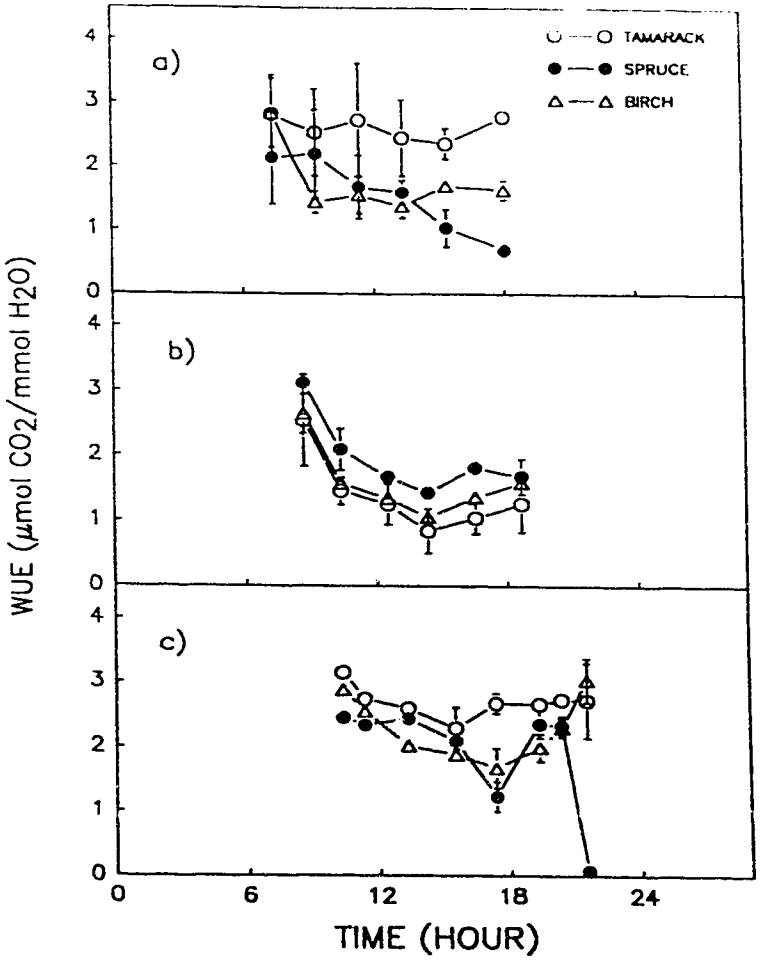


Figure II.5. Relationship between net photosynthesis (P_{net}) and mesophyll conductance to CO_2 (g_m) for black spruce on the hot day. $P_{net} = 0.31*g_m - 0.29$, $r^2 = 0.95$, n = 18. This graph is typical of P_{net}-g_m relationships for tamarack, black spruce and swamp birch on the three sampling days.

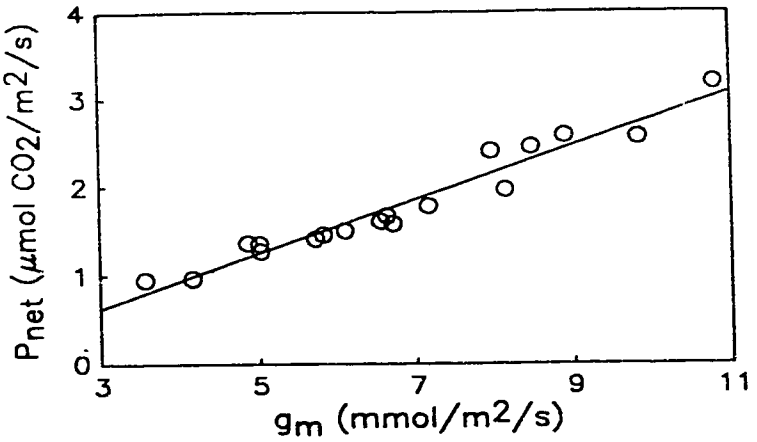
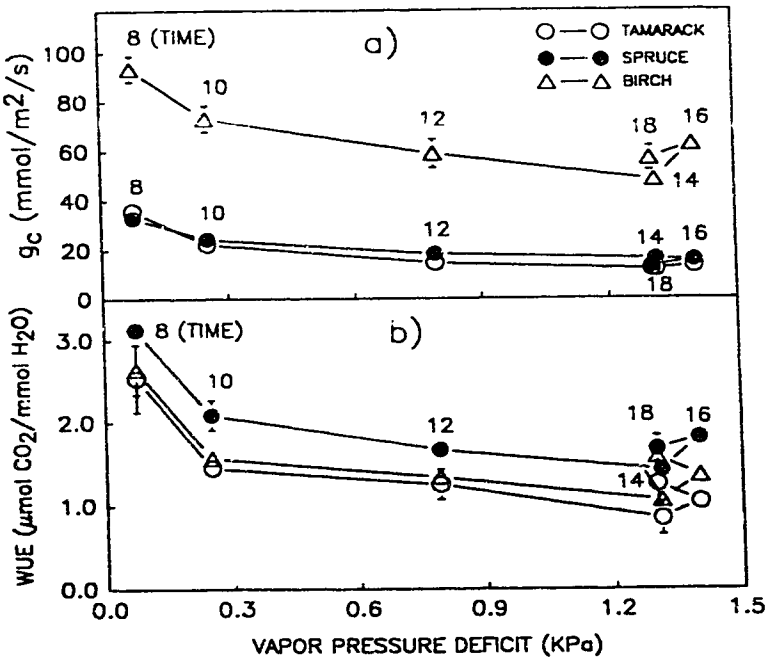


Figure II.6. Relationship of stomatal conductance to CO_2 (g_c) and water use efficiency of photosynthesis (g_m) ($\bar{x} \pm$ se.; n=3) to ambient water vapour pressure deficit for peatland tamarack, black spruce, and swamp birch on the hot day. The time of the day for each measurement is provided as numbers in the graphs.



III. A SELF CONTAINED FREEZING CHAMBER FOR TREE ECOPHYSIOLOGICAL STUDIES IN THE FIELD

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INTRODUCTION

Low temperature is one factor limiting the distribution and productivity of trees. Frosts, during the growing season, occur in some areas at high latitudes or high elevations (Christersson 1984; Christersson et al. 1987; Rothwell and Lieffers 1988). An understanding of the responses of trees to freezing events is critical for success in tree breeding programs and for frost protective measures in forestry. Equipment has been developed to conduct freezing experiments in the field (e.g. Holbert 1933; Johansson and Torssell 1956). However, most of this equipment is heavy, expensive, lacks the capacity for large sample sizes and requires electric power.

Due to the limitations of equipment, most freezing experiments are conducted in the laboratory on greenhouse grown seedlings (Pharis et al. 1970; Christersson et al. 1987; Lindström and Mattsson 1989) or on twigs transported from the field (Sakai and Weiser 1973; DeLucia and Smith 1987). Observations of frost damage in the field after the occurrence of natural frosts are also common (Strand and Lundmark 1987; Lundmark et al. 1988). While the above methods provide insight into the mechanisms of tree's responses to frosts, they have limitations. Laboratory experiments cannot provide information on the integrated effects of natural environments during and after frosts. Experiments using cut twigs also make it impossible to examine post freezing injury and recovery processes. Even though observations after natural frosts may be ideal, such events are sporadic and capricious and this makes it difficult to plan experiments.

The freezing chamber described here provides an inexpensive, practical technique to achieve freezing conditions in the field.

MATERIALS AND METHODS

Chamber Construction and Assembly

The apparatus consists of a freezing chamber, two sets of wooden clamps, and a wooden post. The freezing chamber is held by the clamps and mounted on the post by a single bolt so that the post-chamber angle α can easily be adjusted to fit the twig angle to minimize the tension on and bending of the twig (Fig.III.1).

The freezing chamber has three parts: an outer insulation chamber, a freezing medium package, and an inner protection chamber. The outer chamber consists of two half-chambers, each cut from a 30 x 28 x 6 cm polystyrene board. The central portion of each board was hollowed out to produce a cavity of 20 x 18 x 2.5 cm and a twig channel was cut at one end of the chamber (Fig.III.2). The apparent thermal conductivity, material density and specific heat of the polystyrene board are 0.0361 W/m/^{o}C, 24 kg/m^{3}, and 1.14 kJ/kg/^{o}C, respectively (Croy and Dougherty 1984). A NaCl-ice mixture was used as the freezing medium. Ice chips were obtained from an ice-making machine (ICE-O-MATIC, A Welbilt company, NSF Testing Laboratory, Ann Arbor. Mich.). "Sifto" coarse salt (NaCl) (Domtar Inc., Sifto NaCl Division, Mississauga, Canada) was used. Salt and ice mixtures were used as a freezing medium in orchards (West and Edlefsen 1917), but the associated apparatus is neither easily replicated nor suitable for use in the forest.

The inner protective chamber, to protect the twig from mechanical damage caused by the pressure of the freezing medium, was made from a petri dish (90 x 90 x 9 mm). Holes (about 1 cm apart) were drilled through the petri dish walls to facilitate heat exchange during the cooling process and a twig channel was also cut in one end.

A sponge gasket was used around the perimeter of the two half outer chambers and around the twig channel. The two half chambers were pressed and held together by the wooden clamps held parallel to the twig.

The procedures for applying the apparatus in situ were as follows:

1) Install the wooden post about 15 cm from the tip of an experimental branch and mount one set of the wooden clamps onto the post by a single bolt (Fig.III.1).
2) Weigh NaCl and ice chips (ice chips can also be measured by volume).
3) Mix ice chips and NaCl by shaking them together vigorously in a large covered container.
4) Quickly put the mixture into two separate 18 X 20 cm zip-lock plastic bags and seal the bags.
5) Hold one of the half outer chambers horizontally and place in sequence in the hollow space: a bag of freezing medium, the inner chamber containing the tip of an experimental branch and the second bag of the freezing medium.
6) Insert the sponge gasket and cover with the second half of the outer chamber.
7) Press and loosely clamp the two half chambers together using the wooden clamps on the post.
8) Slide the chamber along the clamps to fit the twig angle, put on the second set of clamps and tighten the clamps.

Chamber Testing

The following tests were conducted on the apparatus:

1) Temperature calibration

Temperature calibration experiments were conducted at 5 ^{0}C ambient temperature in a growth chamber for the following NaCl:ice mass ratios: 0:600, 10:600, 20:600, 40:600, 60:600, 80:600 and 100:600. Each NaCl:ice mixture had 3 replicates. The temperature in the freezing chamber was sampled at 10 second intervals using teflon coated copper-constantan thermocouples (the diameter of the sensor wire was 0.12 mm) attached to a CR21X data-logger (Campbell Scientific Canada Corp.). A temperature calibration curve was developed using minimum cooling temperatures (average of 3 replicates) in the freezing chamber.

2) Cooling rate, temperature sustainability and the effects of ambient temperatures

The freezing experiments for the NaCl:ice ratios noted above were also conducted at 20 ^{0}C ambient temperature. The differences in minimum cooling temperatures which were obtained at the two different ambient temperatures were tested by ANOVA. The cooling rate and temperature sustainability over 2 hours for the two different ambient temperatures were examined.

3) Spatial temperature variation in the chamber

The NaCl:ice ratio for this test was 16(g):1.65(l). The test was conducted at 20 ^{0}C. A black spruce twig was inserted along the diagonal of the inner chamber. Three copper-constantan thermocouples were attached to needles on the main stem. Four thermocouples were attached to separate lateral shoots. The 3 probes on the main stem were located about 5 cm apart at the tip, the mid-point, and at the base of the stem. The lateral shoots were selected on both sides of the main stem and thermocouples were positioned about 3 cm from the main stem. The minimum temperatures over a 2-hour period from the seven thermocouples were recorded on the data-logger.

4) Moisture loss from foliage

This test was to examine whether the low osmotic potential of the ice-salt mixture inside the zip-

lock bag would cause any excessive moisture loss from the foliage. Ten small branch tips of tamarack were cut and weighed on an analytic balance. Five of them were cooled to -6 ^{0}C, held at that temperature for two hours in the freezing chamber, and weighed again. The other five twigs were also treated as above except ice chips were substituted for the ice-salt mixture. The difference in moisture loss between the -6 and 0 ^{0}C treatments was tested using a t-test.

5) Protective efficiency of the inner chamber

Mature black spruce and tamarack trees (6 of each species) were selected for assessing the protective efficiency of the inner chamber on the foliage. Photosynthesis, transpiration rate and stomatal resistance of foliage on the selected twigs from these trees were measured using a portable gas exchange system (The Analytical Development Co. Ltd., England) at 10:00 AM on the day prior to placement in the chambers (temperature = 24 ^{0}C, photosynthetically active radiation (PAR) irradiance = 1400 - 1800 $\mu E/m^2/s$). The same trees and the same twigs were re-used for the following experiment. At 7:00 AM on the next day, freezing chambers, containing a freezing medium substitute (a similar volume of polystyrene beads to simulate the pressure from the NaCl:ice mixture) were put on branch tips of 3 trees of each species. Branch tips of the remaining trees were placed into freezing chambers without either freezing medium (substitute) or inner chambers (i.e., no pressure on the foliage), and were used as controls. The freezing chambers were left on the trees for 2 hours. The branch tips were inspected visually for mechanical damage immediately after the freezing chambers were removed. At 10:00 AM, photosynthesis, transpiration, and stomatal resistance on the same twigs were remeasured. The weather conditions on that day were similar to those of the previous day (temperature = 25 ^{0}C, photosynthetic radiation flux density = 1460 - 1850 $\mu E/m^2/s$).

The differences in these physiological parameters between the two treatments (ie. with and without pressure) were tested by ANCOVA using the measurement of the previous day as a covariant. A paired t-test was used to test if the same trees performed similarly before and after the chamber (with pressure) was applied.

6) Field trials

The technique was tested in the field on 9 mature black spruce and 9 tamarack. The temperature of the foliage in the chamber was monitored as noted above.

RESULTS

1) Temperature calibration

Minimum cooling temperatures between 0 and -20.3 ^{0}C were obtained at 5 ^{0}C ambient temperature as NaCl:ice ratios increased from 0:600 to 100:600 (gram:gram) (Fig.III.3).

2) Cooling rate, temperature sustainability and the effects of ambient temperatures

The temperature in the chamber generally reached the minimum value within 4 to 28 minutes depending on the NaCl:ice ratios and ambient temperatures. The higher the ambient temperature or the greater the NaCl:ice ratio, the longer time it took for the temperature in the chamber to reach the minimum. For example, it took 4 and 12 minutes for a 10:600 NaCl:ice mixture to cool the chamber to a minimum temperature of -3.4 ^{0}C at ambient temperatures of 5 and 20 ^{0}C, respectively. Salt and ice mixtures of 10:600 and 100:600 at an ambient temperature of 20 ^{0}C required 12 and 28 minutes, respectively, to produce minimum temperatures. The cooling rate was the greatest during the first 8 minutes for all the NaCl:ice mixtures.

There was no significant difference between the minimum cooling temperatures at 5 and 20 ^{0}C

ambient temperatures ($P > 0.05$). The temperature in the chamber increased gradually after the minimum was reached. A higher ambient temperature produced a faster temperature increase. For example, the temperature increments over a 2-hr period for a NaCl:ice mixture of 10:600 was 0.4 ^{0}C at 5 ^{0}C and 0.6 ^{0}C at 20 ^{0}C.

3) The spatial variation in temperature in the chamber

The minimum cooling temperatures in the chamber from the seven probes ranged from -3.7 to -4.6 ^{0}C and had a mean of -4.1 ^{0}C and a standard deviation of 0.3 ^{0}C.

4) Moisture loss from foliage

The moisture loss from foliage was 2.76 and 2.65% respectively for the 0 and -6 ^{0}C treatments. The t-test showed there was no significant difference in moisture loss between the two treatments ($P > 0.25$).

5) Protective efficiency of the inner chamber

No visual mechanical damage to the foliage was observed from the pressure of the freezing medium substitute. No significant difference ($P > 0.05$) in photosynthesis, transpiration and stomatal resistance was detected between the two groups of trees treated by freezing chambers with and without the freezing medium substitute (i.e., polystyrene beads). There was also no significant difference ($P > 0.05$) in photosynthesis, transpiration, and stomatal resistance of the branch tips after treatment (i.e., freezing chamber containing polystyrene beads) compared to pre-treatment (i.e., the previous day).

6) Field trials

In field trials, it generally took 2 people about 3 minutes to set up the apparatus on a twig. The course of temperature changes in the chamber was similar to our laboratory results.

DISCUSSION

The freezing chamber described here is efficient, economical and practical under field conditions. The technique can be applied to branches of trees, above ground portions of seedlings or other plants. Depending on the NaCl:ice ratio of the freezing mixture, relatively stable temperatures of 0 to -20.3 ^{0}C can be obtained inside the chamber. The technique itself is non-destructive to foliage being studied. Therefore, the response of the trees and recovery processes can be monitored continuously for as long as desired.

Ice chips and NaCl form an imbalance system in which solid water transforms into liquid phase quickly. This phase change draws thermal energy from the nearest environment, lowering its temperature until equilibrium is reached (provided the system is reasonably adiabatic). Within a certain range, the greater the amount of NaCl added to a fixed mass of ice, the lower is the final temperature. The minimum temperature which can be obtained equals the eutectic point (-21 ^{0}C) of the freezing mixture (Lewis 1932). The process is also dependent on the amount of plant tissue in the chamber and the insulating properties of the outer chamber. The insulating material and the ambient temperature are especially important in determining the sustainability of the chamber temperature as both of them affect the heat flux into the chamber.

Several modifications to the chamber to accommodate different freezing experiments can be envisioned. First, fast cooling rates may be inappropriate for many physiological studies, particularly when incipient injury is of interest (Steffen and Palta 1987). Slower cooling rates of

foliage can be achieved by using an insulated inner chamber and/or coarser salt. For example, a cooling rate of 4.5 ^{0}C per hour (0.075 ^{0}C /min) for the final 10 ^{0}C drop in temperature was obtained by insulating the inner chamber with a 2 cm thick armaflex sheet insulator (Armstrong, Canada). Still slower cooling rates could be achieved by adding more insulation. Second, larger foliage samples may be handled by increasing the size of the chamber and the volume of the freezing medium. In our experiments the foliage was less than 1% of the mass of the ice. If the mass of the foliage is large relative to the mass of the freezing medium, however, the thermal energy from the foliage may slow the cooling process. Finally, if longer periods of stable low temperature are required, more insulation could be added to the outer chamber.

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Figure III.1. A cutaway view of the field set up of the freezing chamber. The chamber is mounted to the pole with a single bolt. The chamber can be slided forward or backward within the wooden clamps so that the angle α is adjusted to fit the twig angle and minimize the physical stretch on the twig (1-inner chamber, 2- freezing medium, 3- outer chamber, 4-bolt, 5-wooden clamp, 6- mounting bolt).

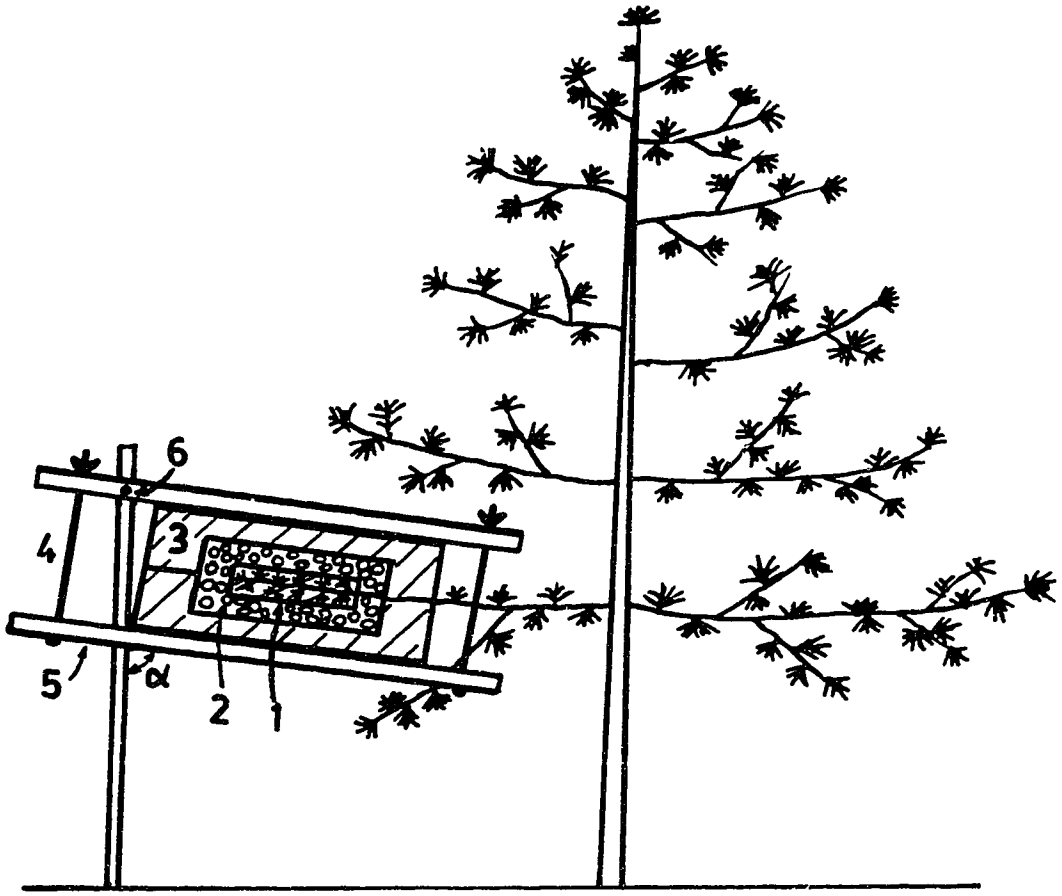


Figure III.2. One half of the outer freezing chamber. The material is polystyrene board.

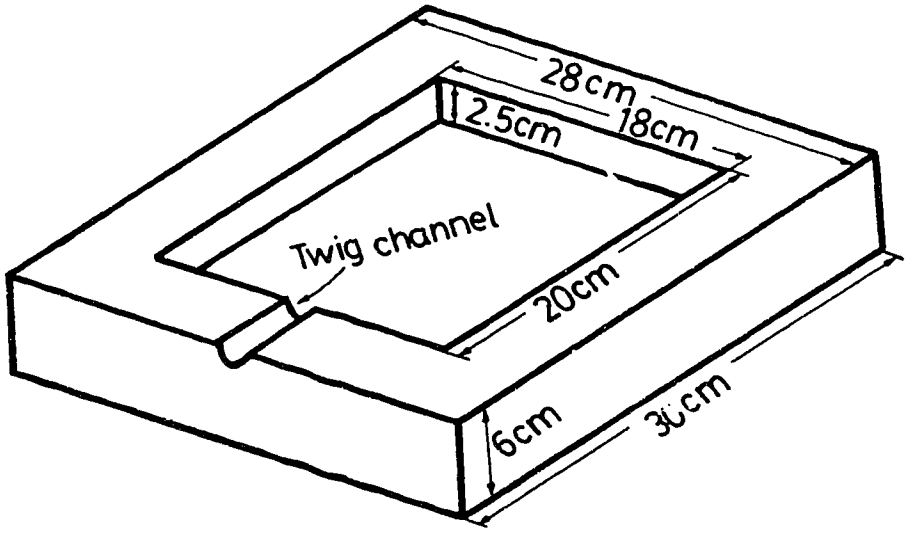
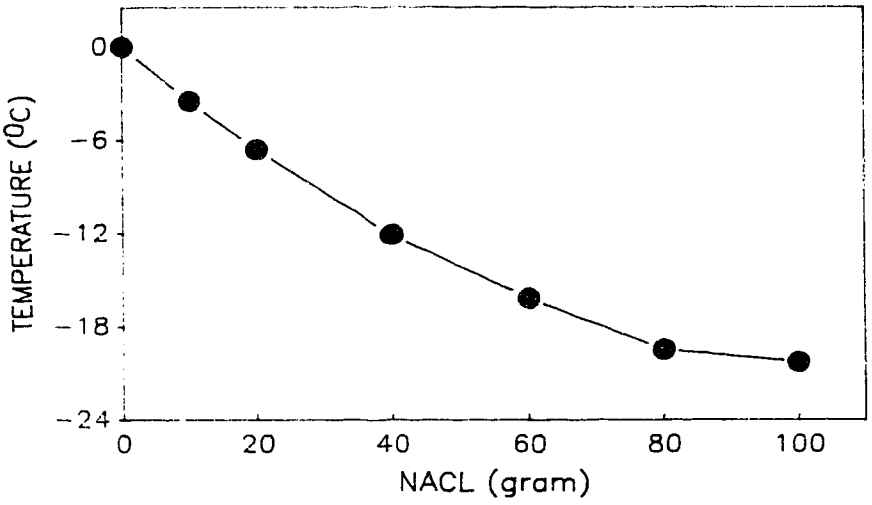


Figure III.3. Calibration curve for the relationship between minimum cooling temperature and NaCl:ice mass ratio. The amount of ice is 600 gram.



IV. EFFECTS OF SUMMER FROSTS AND SUBSEQUENT SHADE ON PHOTOSYNTHESIS IN PEATLAND TAMARACK AND BLACK SPRUCE

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INTRODUCTION

Summer frosts, freezing events during the growing season, are common at high latitudes or high elevations (Christersson 1984; Christersson et al. 1987). Summer frosts can occur every month during the summer in boreal peatlands (Hayter and Proudfoot 1978; Rothwell and Lieffers 1988). Morphological damage or death of conifer seedlings due to frosts is common in these areas (Christersson 1984; Christersson et al. 1987; Landmark and Hällgren 1987; Lundmark et al. 1988). Damage from moderate to light summer frosts can be limited to a temporary depression of photosynthesis (DeLucia and Smith 1987; Lundmark and Hällgren 1987; Lundmark et al. 1988). Indeed, the reduction in annual ring growth of peatland black spruce (Picea mariana) in years with lower average minimum temperatures in the growing season may be related to summer frosts (Dang and Lieffers 1989). However, the extent and duration of the depression in photosynthesis and subsequent recovery processes in conifer saplings after summer frosts are not well understood.

Intense light following a frost is reported to further increase the frost damage to photosynthesis in conifer seedlings (Lundmark and Hällgren 1987; Strand and Lundmark 1987). Effects of post-frost light regime on large trees in the forest, however, are poorly understood.

The objectives of this study were to examine: 1) effects of summer frosts on light saturated net photosynthesis (P_{net}), mesophyll conductance to CO_2 (g_m), stomatal conductance to CO_2 (g_c), and water use efficiency (WUE), and the recovery of these parameters after frosts, in 20 year-old peatland tamarack (Larix laricina (DuRoi) K. Koch) and black spruce (Picea mariana (Mill.) B.S.P.); 2) effects of light regime on post-frost damage and recovery of the above parameters.

MATERIALS AND METHODS

The study site was a treed fen located 26 km west of Edmonton ($53^0$34'N; $113^0$31'W). The site had an average slope <1%, was poorly drained and forested by an open stand of black spruce and tamarack (20 years old, 2 m high (average), 3000/ha stem density).

Selected branches (at about 1.4 m from the ground) of black spruce and tamarack were frozen to a specified temperature by a polystyrene freezing chamber using an NaCl-ice mixture as the freezing medium (Dang et al. 1991a). The control trees of black spruce were treated similarly as frozen trees except ice chips (0^0C) were substituted for the salt-ice mixture. This duplicated the possible crushing damage from the freezing medium on the foliage of frozen trees. For controls of tamarack, polystyrene beads were substituted for the salt-ice mixture because an inner chamber was used to protect foliage from the crushing damage of the freezing medium (Dang et al. 1991a). All freezing treatments started at about 4:00 AM and lasted for 2 hours.

Foliage gas exchange was measured using an open system consisting of a portable infra-red gas analyzer (LCA-2), a leaf cuvette (PLC), and an air supply unit (all from Analytical Development Corporation, Hoddeson, England). Ambient air was drawn from 4 m height using a tower. The air passed through a desiccator before entry into the cuvette. The air flow through the cuvette was maintained at a rate of 10 mL s^{-1}. Air within the cuvette was made turbulent with a high speed fan. An infrared filter on the cuvette shield prevented heating inside the cuvette. In addition, gas exchange measurements were taken very quickly (< 1 min) to further reduce heating inside the cuvette. The cuvette contains sensors for measuring relative humidity, air temperature and photosynthetically active radiation. Measurements started at about 10:00 AM in all experiments. To minimize the effect of the time of the day on gas exchange (Dang et al. 1991b), measurements were made in pairs of a treatment and a control. Visual damage was also recorded.

When there was insufficient sunlight during gas exchange measurement, light levels were

boosted to 1600 μmole m^{-2} s^{-1} using a Brinkman 'Q-beam' spot light.

The foliage on the experimental twigs was collected at the end of each experiment. Leaf area of the foliage was determined as described by Macdonald and Lieffers (1990).

Black Spruce Experiment 1.

Nine typical black spruce trees were selected, three of which were used as control (0 °C), three cooled to -4 °C, and another three cooled to -8 °C. Freezing was conducted July 1st, 1989. Gas exchange of current year and one year old needles was measured on the day before freezing, on the freezing day, and on the 1st, 2nd, 4th, and 11th days after freezing.

The weather was sunny on most days following freezing, but cloudy on the 2nd and 8th days after freezing. The ambient temperature was 3.5 °C when the freezing started.

Black Spruce Experiment 2

On July 25, 1989, freezing chambers were applied to 12 typical black spruce, six as control (0 °C) and six cooled to -3 °C. After the freezing chambers were removed, the treated branches from half of the control and half of the -3 °C trees were shaded from direct sunlight. The shade lasted for two days. Gas exchange of the current year and one year old needles was measured on the day prior to freezing and two days after.

On the freezing day, it was sunny in the morning and partially and intermittently cloudy in the afternoon. On the following two days, it was mainly sunny. The ambient temperature was 4 °C when freezing started.

Experiments on Tamarack.

Two separate freezing experiments were conducted on tamarack. The design of these experiments was the same as in black spruce experiment 2, but the number of replicates for each light-freezing combination was increased from three to six trees. Experiment 1 (-3.5 °C) and experiment 2 (-6 °C) were conducted respectively on July 14 and 20 of 1990. The ambient temperatures at the time of freezing were 4 and 0.4 °C respectively for experiment 1 and 2. The shading period was 4 days for both experiments.

Gas exchange for experiment 1 was measured on the day before freezing, the freezing day, and 1, 4, 6, and 8 days after freezing. Measurements for experiment 2 were made on the day prior to freezing, the freezing day, and 1, 2, 3, 4, 6, and 11 days after freezing. The shaded foliage was generally exposed to full sunlight for less than 3 minutes during gas exchange measurements, then put back into shade again.

Data Analyses

Net photosynthesis rate (P_{net}), leaf resistance to H_2O vapour (r), transpiration rate (E), and intercellular CO_2 concentration (C_i) were determined as described by Caemmerer and Farquhar (1981). Since the high speed fan in the cuvette and the design of the cuvette ensure a small boundary layer resistance (r_b) and r_b is generally very small for needles, the stomatal resistance to H_2O vapour (r_s) was assumed to be equal to r. The stomatal conductance was calculated as: $g_s = 1/r_s$. Stomatal conductance to CO_2 (g_c) was calculated as: $g_c = g_s/1.6$ (Coombs et al. 1987). Mesophyll conductance to CO_2 (g_m) was calculated as: $g_m = P_{net}/C_i$ (Fites and Teskey 1988). The water use efficiency of photosynthesis (WUE) was determined as: $WUE = P_{net}/E$ (Larcher 1983). P_{net}, g_c, and g_m were all expressed on a leaf area basis. These are called parameters hereafter.

The response to frost of a parameter on a given day was calculated by dividing the parameter

of a treatment tree by its paired control on the same day, and expressed as a percentage. Controls for the shaded and frozen trees are the shaded but unfrozen trees, while unshaded and unfrozen trees served as controls for unshaded and frozen trees. The ratinal for this approach is that control and treatment trees were exposed to the same environment all the time except during the freezing treatment. Under the assumption that control and treatment trees responded in parallel to changes in the environment, this process should have eliminated the effect of day-to-day variation in weather. A further standardization was made by adjusting the response-time curve up or down so that the response for the pre-freezing day was equal to 100%. The standardized response reflected the net response of a parameter to the frost (e.g. 60% indicated the parameter was reduced by 40%). Pre-treatment measurements have been used in a similar way in evaluating effects of fertilizers on tree growth (Salonius et al. 1982, Ballard & Majid 1985).

When the response of a parameter for a given day was greater than 80% of the control, the freezing effect was tested using one-way analysis of covariance (ANCOVA) (covariate = pre-treatment measurement) on the original parameter. Effects of shade were tested by comparing the parameters of the shaded trees on a given day with the unshaded trees using one-way ANCOVA (covariate = pre-treatment measurement).

RESULTS

Black Spruce

In experiment 1, the damage caused by the -8 ^{0}C frost was severe, resulting in mortality and shedding of both current and one year old needles. For the first two days following freezing, small amounts of P_{net} were detectable in both current and one year old needles. Current needles started turning brown at the tip on the first day after freezing. By the 2nd day, current needles were almost totally brown and only respiration was detected. For one year old needles, weak P_{net} was still detected on the 2nd day, but they also started turning brown. By the 4th day, both current and 1 year old needles were senescent and abscising.

For the -4 ^{0}C frost, current needles of black spruce responded similarly as to the -8 ^{0}C frost (became senescent) while one year old needles showed immediate decreases in P_{net} followed by recovery. The tips of one year old needles turned brown 4 days after freezing. For the first 4 days after freezing, P_{net} and g_m of one year old needles decreased continuously (Fig.IV.1a & 1b). On the 4th day, no P_{net} was detected. Significant recovery of P_{net} and g_m occurred 11 days after freezing, however, they were still less than 60% of their controls (Fig.IV.1a & 1b). In contrast, the -4 ^{0}C frost had no significant impact on g_c four days after freezing (Fig.IV.1c, P=0.19). On the freezing day and two days after freezing, however, g_c in frozen trees was significantly lower than the controls (P = 0.045 and 0.002 respectively). WUE generally responded to the -4 ^{0}C frost similarly as P_{net} and g_m, with low values in the first three days after freezing followed by recovery (Fig.IV.1d).

In the second experiment on black spruce, the -3 ^{0}C frost depressed P_{net}, g_m, g_c and WUE of current needles, respectively, by 69, 68, 45, and 47% two days after freezing. The decreases of P_{net}, g_m, g_c, and WUE in one year old needles were, respectively, 67, 68, 18, and 62%. No colour change was observed in either current or one year old needles. Post-frost shade did not show any significant effects on P_{net}, g_m, g_c, or WUE (P > 0.22) two days after freezing in either current or one year old needles.

Tamarack

For - 3.5 ^{0}C frost, no colour change in foliage was observed for the first 3 days after freezing.

On the fourth day, however, both shaded and unshaded needles had brown tips.

On the day of freezing, P_{net}, g_m and WUE dropped by more than 40% (Fig.IV.2a, 2b, & 2d) while g_c decreased by 22% for the unshaded and 35% for the shaded needles (Fig.IV.2c). Stomatal conductance in unshaded trees recovered fully the first day after freezing but was still strongly depressed in the shaded trees. WUE of shaded trees recovered completely 8 days after freezing. At that time, P_{net} and g_m of both shaded and unshaded trees, g_c in shaded and WUE in unshaded trees, were still less than 86% of the control trees ($P < 0.05$).

P_{net} of shaded tamarack was higher than the unshaded trees ($P=0.03$) on 8th day following freezing. g_m and WUE in shaded trees were higher than the unshaded trees ($P < 0.05$) 4 days following freezing (Fig.IV.2b & 2d). In contrast, g_c of shaded trees was lower than unshaded ($P < 0.02$, Fig.IV.2c) on the 1st, 6th, and 8th days following freezing. After the -6 ^{0}C frost, all parameters measured in tamarack decreased immediately (Fig.IV.3a-3d). Gradual recovery in all parameters started 2 days after freezing. Eleven days after freezing, P_{net}, g_m, and WUE in the frozen trees were still less than 80% of the controls. The effect of frost was the largest on g_m and the least on g_c. Mesophyll conductance was 67% of control trees 11 days after freezing while g_c in unshaded trees recovered completely 2 days after freezing.

Following the -6 ^{0}C frost, P_{net}, g_m, and WUE in shaded trees was similar to the unshaded trees for all post-freezing days except on the 1st day after freezing when the net respiration (negative P_{net}) of and g_m shaded trees was lower than the unshaded ($P=0.03$, Fig.IV.3). In contrast, g_c in shaded trees was lower than unshaded trees on the first and second days after freezing (Fig.IV.3c, $P = 0.002$ and 0.026 respectively).

DISCUSSION

My data show that the response of photosynthesis to summer frosts consisted of two phases, i.e. a depression phase and a recovery phase. In the depression phase, light saturated net photosynthesis declined continuously. The length of this phase seemed to vary with species but not with the degree of freezing. For black spruce, the depression phase lasted for at least 4 days (there were no measurements between 5th and 10th days after freezing). In contrast, the depression phase in tamarack lasted for only two days at both the -3.5 and -6 ^{0}C frosts. In the recovery phase, light saturated net photosynthesis increased gradually. The recovery was generally fast for the first few days, then slowed down. Apparently, a full recovery of photosynthesis required more than 11 days. Pharis et al. (1970) found full recovery of photosynthesis in cold-acclimated Pinus ponderosa (Laws.) and Pseudotsuga menziesii var. menziegii (Mirb.) required several weeks.

Frosts can result in various damage to plant cells, e.g. cytoplasmic dehydration, membrane lipid phase separation and consequent impairment of membrane bound enzymes (Burke and Stushnoff 1979; Levitt 1980), ion leakage and consequent environment changes around the chloroplast (e.g. altered ion concentration and electrical potential gradients) (Steffen and Palta 1987). These changes generally result in an immediate drop in photosynthesis as observed for both species. Frosts can also cause metabolic imbalances in the cell and these imbalances take a longer time to be revealed (Burke and Stushnoff 1979). It is possible that metabolic imbalances were more severe in black spruce, resulting in a longer depression phase. Palta et al. (1977) proposed the post-thaw progressive damage in onion bulbs was due to the inability to reabsorb the solution from intercellular spaces and continued loss of solutes after thawing. Rapid and slow injuries from freezing were also observed in spinach (Hincha et al. 1988). The most likely reason for the difference in the length of depression phase between the two species, however, might be related to differences in their rates of frost damage repair.

Recovery from freezing damage is an active process, requiring energy (Steffen and Palta 1987). In this study, the repairing energy could come from three sources: 1) photosynthetic products of the injured foliage, 2) breakdown of cell reserves, and 3) carbohydrates translocated from uninjured foliage. Contribution from the first source was probably small, particularly for the first few days. It could be possible that tamarack needles had more internal reserves or faster translocation of sugars from uninjured tissues to the injured foliage than black spruce, which resulted in a shorter depression phase.

In our experiments, light saturated photosynthesis decreased and recovered in parallel with mesophyll conductance in both species (Figs.IV.1 to 3). This is in agreement with earlier findings that depressed mesophyll conductance was primarily responsible for the low photosynthesis after summer frosts (DeLucia and Smith 1987; Lundmark et al 1988). The depression in mesophyll conductance may have resulted from frost damage to photosystem II (Klosson and Krause 1981; Grafflage and Krause 1986; Strand and Lundmark 1987), changes in its environment around the chloroplast (Steffen and Palta 1987), membrane lipid phase transition (Levitt 1980) and subsequent impairment of membrane bound enzymes (Burke and Stushnoff 1979).

Photosynthesis was not closely related to stomatal conductance, particularly in unshaded trees in this study (Figs.IV.1, 2, 3). For example, the -3 ^{0}C frost depressed g_c in current needles of black spruce by 45%; this is more than twice as much as one year old needles (19%). The depression of net photosynthesis, however, was similar in current and one year old needles. This is in contrast to the finding that g_c decreased in parallel with photosynthesis following summer frosts in seedlings of Picea engelmannii (Parry) (DeLucia and Smith 1987) and Pinus sylvestris L. (Lundmark et al. 1988). This was probably another indication that g_c was not the primary factor limiting photosynthesis.

My data suggest that the degree of apparent photoinhibition varied with the degree of freezing. In tamarack, photoinhibition was a factor after the -3.5 ^{0}C frost whereas no photoinhibition was detected after the -6 ^{0}C frost. The same phenomenon was observed in cold-acclimated Scots pine (Strand and Öquist 1985). This difference might be related to the sites of frost damage to photosynthetic apparatus. Photoinhibition is caused by excess trapped light energy when CO_2 fixation processes are impaired. It is possible that the -6 frost damaged photochemical apparatus to a similar degree to or more severely than biochemical processes, whereas the -3.5 ^{0}C frost may have primarily damaged the biochemical machinery. It is also possible that the -6 ^{0}C frost turned on the repair and/or protection mechanisms of photosystems to a larger extent than the -3.5 ^{0}C frost; this resulted in more severe photoinhibition in the -3.5 ^{0}C frost. Photoinhibition damage and repair occur concomitantly (Greer et al. 1986). The measured photoinhibition is the net difference between damage and repair. Photosystems can be protected by various mechanisms (Krause 1988), e.g. the carotenoid zeaxanthin (Demmig-Adams et al. 1989) and photosystem II cycling (Horton 1989).

My data suggest that summer frosts are a severe environmental stress and can depress photosynthesis for a long period of time. For example, on average, the photosynthesis in tamarack wad depressed by 35% for more than 9 days following the -3.5 ^{0}C frost. Earlier studies (Hayter and Proudfoot 1978; Rothwell and Lieffers 1988) have shown that frosts can occur every month in the growing season in peatland areas. The resistance of trees to summer frosts, therefore, should be given serious consideration. Further research is warranted to test the response of key elements of the photosynthetic apparatus to summer frosts.

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Figure IV.1. Response of current and one year old needles of black spruce to a simulated summer frost of -4 °C in: a) light saturated net photosynthesis rate (P_{net}), b) mesophyll conductance (g_m), c) stomatal conductance to CO_2 (g_c), and d) water use efficiency (WUE). Values ($\bar{x} \pm$ se.; n=3) in the graph are expressed as the percentage of the control. On the axis, time -1 indicates the pre-freezing day, 0 indicates the freezing day, and positive values the days after freezing. Note: negative g_m and WUE were calculated from net respiration (negative P_{net}); this is just for the continuity of the response line.

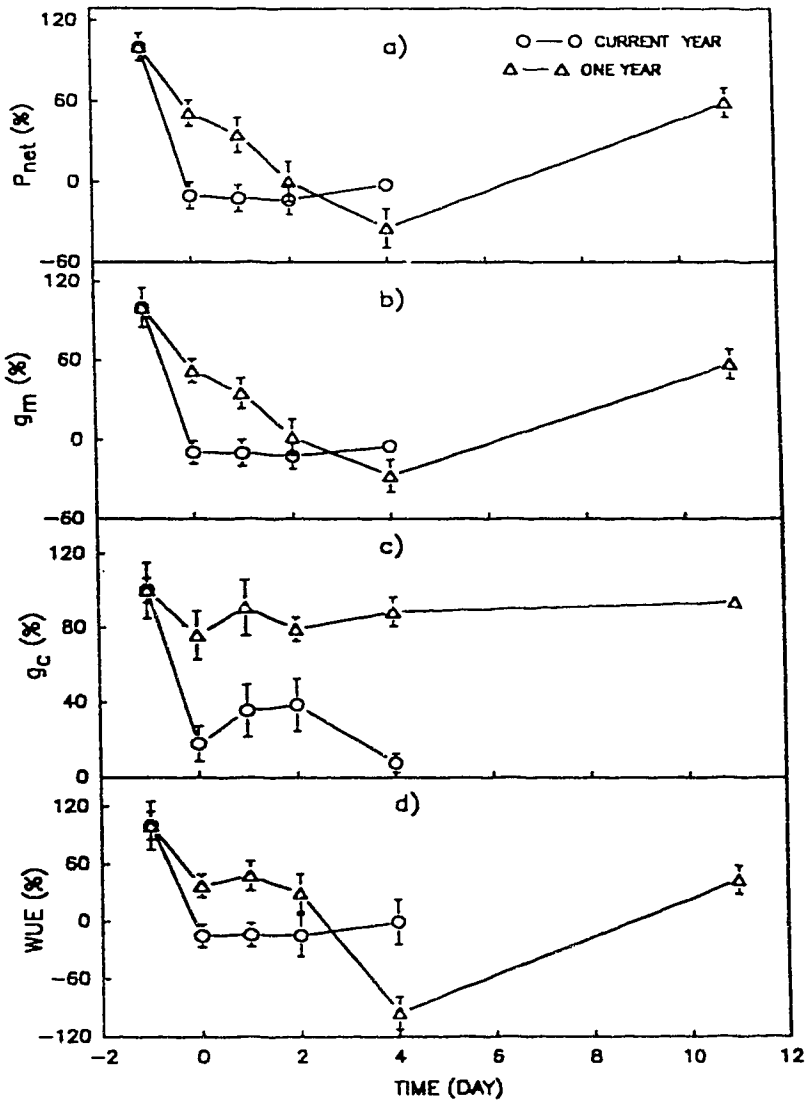


Figure IV.2. Response of tamarack to a simulated summer frost of -3.5 ^{0}C. Some of the trees (shaded) were shaded from direct sunlight for four days immediately following freezing. Other explanations are as in Fig.IV.1.

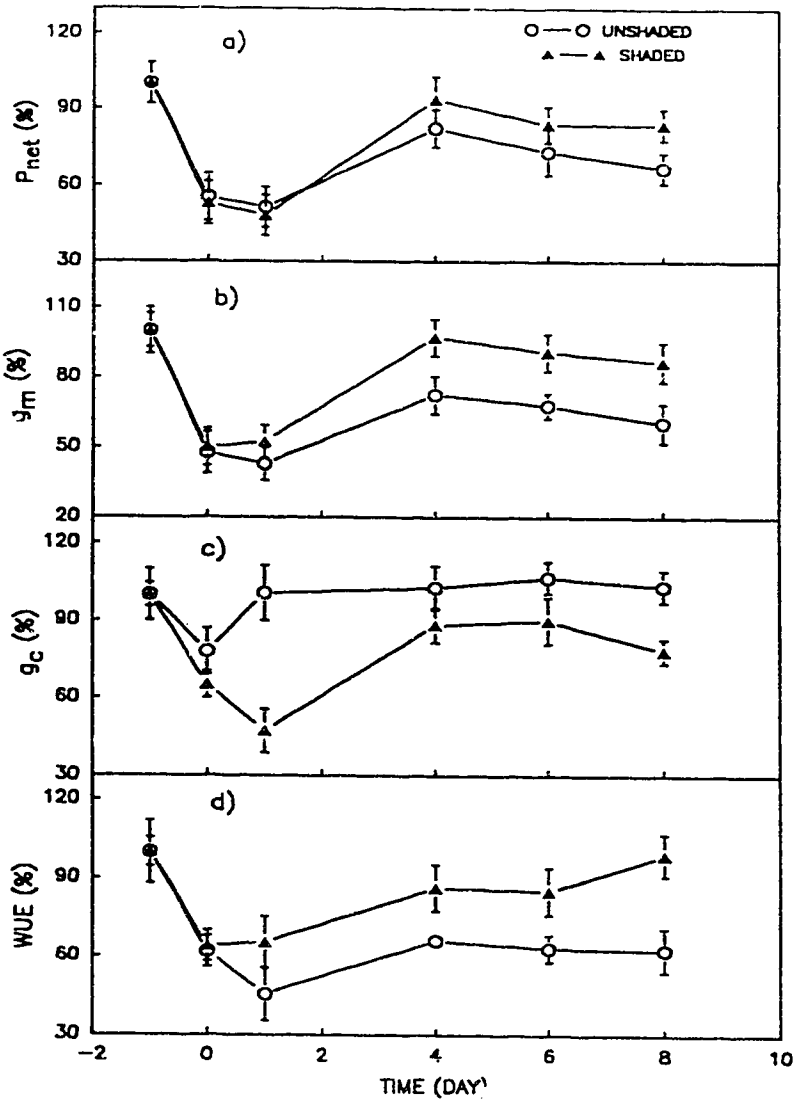
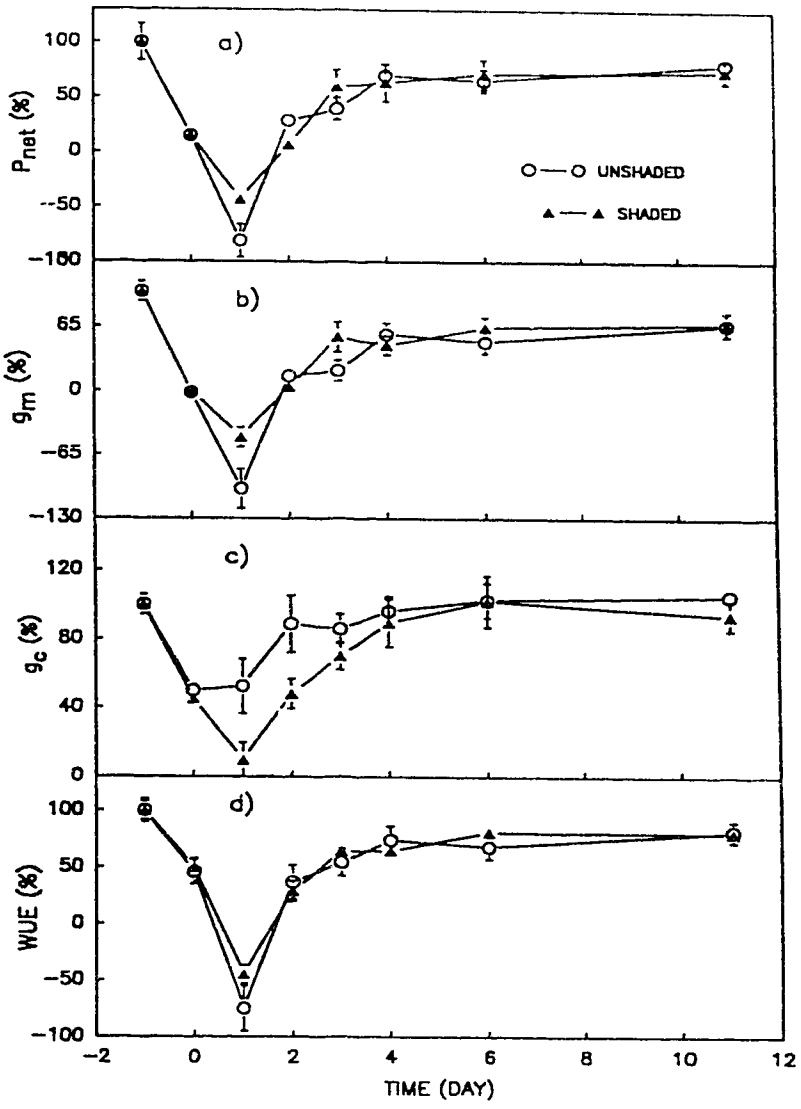


Figure IV.3. Response of tamarack to a simulated summer frost of -6 ^{0}C. Explanations are as in Figs.IV.1 & 2.



V. EFFECTS OF SUMMER FROSTS AND SUBSEQUENT SHADE ON PHOTOSYNTHETIC QUANTUM YIELD AND CHLOROPHYLL FLUORESCENCE IN PEATLAND TAMARACK

INTRODUCTION

Frosts during the growing season are common at high elevations or high latitudes, particularly in boreal peatland sites (Bayter 1978, Christersson 1984, Christersson et al. 1987, Rothwell and Lieffers 1988). Morphological damage or death of conifer seedlings in those areas is common (Christersson 1984, Christersson et al. 1987, Landmark and Hällgren 1987, Lundmark et al. 1988). Effects of the summer frost on conifers can also be limited to a temporary depression of photosynthesis and the depression is not related to stomatal factors (Pharis et al. 1970, DeLucia and Smith 1987, Lundmark and Hällgren 1987, Lundmark et al. 1988). Reduction in annual ring growth of peatland black spruce (Picea mariana) in years with lower than average minimum temperatures in the growing season may be related to summer frosts (Dang and Lieffers 1989). Intense light following a frost is reported to further depress photosynthesis in conifer seedlings (Lundmark and Hällgren 1987, Strand and Lundmark 1987).

In the current study, we froze 20 year-old peatland tamarack (Larix laricina (DuRoi) K. Koch) in the field using a self-contained freezing chamber. We investigated the mechanisms of frost damage to photosynthesis and effects of light regime on the post-frost recovery process of photosynthesis by concomitantly examining changes in the apparent quantum yield of photosynthesis and variable fluorescence of photosystem II for up to 17 days following simulated frosts.

MATERIALS AND METHODS

Study Site

The study site was a treed fen which was located 26 km west of Edmonton ($53^0$34'N, $113^0$31'W). The site had an average slope <1%, was poorly drained, and forested by an open stand of black spruce (Picea mariana (Mill.) B.S.P.) and tamarack (Larix laricina (DuRoi) K. Koch) (averaged 20 years old, 2 m high, 3000/ha stem density).

Frost Simulation

Summer frosts were simulated using a self-contained freezing chamber which used an NaCl-ice mixture as the freezing medium (Dang et al. 1991). Control trees were treated similarly to the frozen trees except that polystyrene beads were substituted for the salt-ice mixture. An inner chamber was used to protect foliage from the crushing damage of the freezing medium.

At 4:00 AM July 14, 1990, freezing chambers were applied to selected branches (at 1.4 m height) of twenty-four typical tamarack, twelve as control and twelve cooled to -3.5 ^{0}C. The freezing lasted for two hours. Immediately after the freezing chambers were removed, the treated branches from half of the control and half of the frozen trees were shaded from direct sunlight, using polystyrene board mounted on a wooden post. The shade was maintained for four days. The apparent quantum yield of photosynthesis (ϕ) and chlorophyll fluorescence were measured on the day before freezing, on the freezing day and for up to 17 days following freezing (for details see the next section).

The ambient temperature was 4 ^{0}C when the freezing started. The weather was cloudy or raining for part of the day on July 15, 16, 17, 19, 25, and 26. It was sunny and warm on all other days during the experiment.

On July 20 1990, the experiment was repeated at -6 ^{0}C. The ambient temperature at the time of freezing was 0.4 ^{0}C for this experiment. The weather was sunny and warm for all days during this experiment except when it rained for part of the day on July 25 and 26. The needle temperature was monitored during the treatment using thermo-couples.

Gas exchange measurement and calculation of ϕ

Foliage gas exchange was measured using an open system consisting of a portable infra-red gas analyzer (LCA-2), a leaf cuvette (PLC), and an air supply unit (all from Analytical Development Corporation, Hoddeson, England). Ambient air was drawn from 4 m height using a tower. The air passed through a desiccator before entry into the cuvette. Air within the cuvette was made turbulent with a high speed fan. For each tree, gas exchange measurements were made at three low photon flux densities (between 40 and 175 $\mu mol/m^2/s$). The light levels were controlled as follows: The foliage was shaded from natural light using a cloth bag consisting of two layers of white cloth with one layer of black cloth in between. Inside the bag, light was supplied by a 20W Halogen coolspot lamp (OSRAM Canada Ltd., Mississauga, Ontario), a neutral density light filter, and a sliding mechanism. Different photon flux densities were obtained by varying the distance between the leaf chamber and the lamp through the sliding mechanism, in combination with different filters. The light supply system was screwed onto the leaf chamber which was mounted on a sturdy tripod.

Gas exchange for experiment 1 (-3.5 ^{0}C frost) was measured on the day before freezing, the freezing day, and 1, 4, 6, and 8 days after freezing. On the 4th day, however, only the shaded trees were measured. Measurements for experiment 2 (-6 ^{0}C frost) were made on the day prior to freezing, the freezing day, and 1, 2, 3, 4, 6, and 11 days after freezing.

The foliage of the experimental twigs was collected at the end of each experiment. Leaf area of the foliage was determined as described by Macdonald and Lieffers (1990).

Net CO_2 assimilation rate (A) was determined as described by Caemmerer and Farquhar (1981). The apparent quantum yield of photosynthesis (ϕ) was defined as the initial slope of the photosynthesis to incident-light response curve (Coombs et al. 1987, Walker 1989) and calculated from the three measured net CO_2 assimilation rates and corresponding photon flux densities. The quantum yield reflects the maximum overall efficiency of photosynthetic energy conversion (Coombs et al. 1987, Walker 1989).

Measurement of chlorophyll fluorescence

Four needles were sampled from each tree and dark-adapted for 5 min. The initial level (F_o) and peak level (F_p) of chlorophyll fluorescence were measured at monochromatic illumination (670 nm) of 700 $\mu mol/m^2/s$, using a SF-30 fluorometer (Richard Brancher Research Ltd, Ottawa). Variable fluorescence (F_v) was calculated as: $F_v = F_p - F_o$. To eliminate differences in the area of fluorescence emitting surface among samples, F_v was standardized using F_o, i.e. F_v/F_o. Chlorophyll fluorescence is predominantly emitted from chlorophyll-a associated with photosystem II (Bolhàr-Nordenkampf et al. 1989). The variable fluorescence exclusively reflects the photochemical capacity of photosystem II (Horton 1985, Bolhàr-Nordenkampf et al. 1989) and has been used widely to assess stress damage to photosystem II (e.g. Grafflage and Krause 1986, Strand and Öquist 1987, 1988). Both quantum yield and fluorescence of frost-treated trees were expressed a percentage of control trees (see Chapt. IV for details).

RESULTS

Apparent quantum yield of photosynthesis (ϕ)

The -3.5 and -6 ^{0}C frosts caused similar initial damage to ϕ (61 to 63 % reduction on the freezing day) in shaded and unshaded trees (Figs.V.1a & 2a). Following both the -3.5 and -6 ^{0}C frosts, ϕ in shaded trees showed a further decrease in response to the removal of shade on the 4th day after freezing followed by a recovery on subsequent sample days. There were, however, differences in the recovery process between shaded and unshaded trees and between the two

frosts. There was no significant recovery in either shaded or unshaded trees eight days following the -3.5 frost, whereas there was a rapid recovery in ϕ four to six days following the -6 ^{0}C frost and the recovery started two days earlier in the unshaded trees than the shaded trees (Fig.V.2a). The recovery of the apparent quantum yield in unshaded trees was consistently greater than the shaded trees after 4 days following the -6 ^{0}C frost (Fig.V.2a).

Variable fluorescence (F_v/F_o)

The variable fluorescence in shaded trees responded similarly to the -3.5 and -6 ^{0}C frosts, i.e. declined rapidly and then followed by a rapid recovery (Figs. 1b & 2b). F_v/F_o in unshaded trees, in contrast, showed different response patterns to different frosts. Following the -3.5 ^{0}C frost, F_v/F_o in unshaded trees declined rapidly, stayed at low values for eight days, then increased to the pre-freezing level (Fig.V.1b). In response to the -6 ^{0}C frost, F_v/F_o in unshaded trees decreased gradually and continuously for four days (freezing day inclusive) followed by a rapid recovery (Fig.V.2b).

DISCUSSION

My data show the efficiency of the overall photosynthetic energy conversion was severely inhibited by summer frosts and this inhibition lasted more than four days. The frosts of -3.5 and -6 ^{0}C inhibited the apparent quantum yield of photosynthesis (ϕ) to a similar degree on the day of freezing in both the shaded and the unshaded trees. Frost inhibition on ϕ was also observed in other studies (Strand and Öquist 1985a, DeLucia and Smith 1987).

A high ϕ requires a close and balanced association among different subprocesses of photosynthesis, including the stoichiometric production and efficient utilization of ATP and NADPH. Summer frosts could depress ϕ at different sites. The photochemical capacity of photosystem II, as reflected by variable fluorescence (F_v/F_o), was reported to be the primary site of frost damage to the photosynthetic electron transport chain (Grafflage and Krause 1986, Strand and Lundmark 1987, Strand and Öquist 1988). Concomitant measurements of F_v/F_o and ϕ in this study showed F_v/F_o and ϕ responded to summer frosts differently, indicating photosystem II was not the primary site of summer frost damage to photosynthetic electron transport in tamarack saplings. While this was in contrast to the above studies, Strand and Öquist (1985b) and Somersalo and Krause (1990) also found similar results in their studies. The discrepancy might be related to different plant materials and experimental conditions used in each experiment.

Photosystem II is considered to be more vulnerable to environmental stresses than photosystem I, the light harvesting pigment complexes, or the cytochrome b/f complex (Barber 1985). The fact that Photosystem II was not the primary site of summer frost damage suggested that the primary damage site was located in the biochemical processes. Rumich-Bayer and Krause (1986) found CO_2 fixation was the most sensitive photosynthetic process to freeze-thaw stress and its inhibition was independent of the inactivation of thylakoid membranes. Somersalo and Krause (1990) attributed the frost induced reduction in photosynthesis to the impairment of the light activation of Calvin cycle. Strand and Öquist (1988) also hypothesized that the frost induced inactivation of some enzymes was the main cause of photosynthetic depression. It has been reported that the activities of several enzymes in the Calvin cycle were inhibited by frosts (Rumich-Bayer et al. 1987, Krause et al. 1988). Response analysis of photosynthesis to CO_2 concentration indicated both the activity of Rubisco carboxylase and RuBP regeneration was impaired by freezing (Strand and Öquist 1985a). ATP synthesis was reported to be strongly inhibited by frosts due to membrane leakage of protons (H^+) and frost effects on the coupling-

factors (Grafflage and Krause 1986). Inhibition of ATP synthesis can substantially reduce the quantum yield of photosynthesis (Baker et al. 1988).

Comparison of the responses of F_v/F_o and ϕ in the shaded and unshaded trees to the two different frosts showed there was a light-freezing interaction. Following the -3.5 ^{0}C frost, there was generally no difference in ϕ response between the shaded and unshaded trees, whereas the variable fluorescence in the shaded trees recovered much faster than in the unshaded trees (Fig.V.1), indicating shading was beneficial for the recovery of PSII function. This phenomenon may be related to post-freezing photodamage as observed in other studies (e.g. Lundmark and Hällgren 1987). After the -6 ^{0}C frost the apparent quantum yield of photosynthesis in the unshaded trees recovered faster and more completely than in the shaded trees, while there was generally no difference in the recovery process of F_v/F_o between the shaded and unshaded trees (Fig.V.2). The slower recovery of ϕ in the shaded trees indicated that the recovery of quantum yield was probably a light-mediated process. Studies have shown the synthesis of some chloroplastic proteins required light and light could also prevent some proteins from turning over (Baker and Markwell 1985). It is possible that the amount of light received by the shaded trees (about 20% of that received by the unshaded trees at midday) in those trees was not enough to keep the repair processes, which required light, in full operation. The similar recovery rate of F_v/F_o in the shaded and unshaded trees might indicate a stimulation of a protection mechanism against photodamage by the -6 ^{0}C frost. In conclusion, the mechanisms of light-freezing interaction found in this study may be related to different sites of damage resulting from frosts of different severity and the stimulation of repairing and/or protecting mechanisms by certain degrees of freezing.

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Figure V.1. Response of the apparent quantum yield of photosynthesis (a) and variable fluorescence of photosystem II (b) to simulated summer frost of -3.5 ^{0}C. Some of the trees (SHADED) were shaded from direct sunlight immediately after freezing. Values ($\bar{x}$ ± se., n=6) in the graph are expressed as the percentage of the control. On the axis, time -1 indicates the pre-freezing day, 0 indicates the freezing day, and positive values the days after freezing.

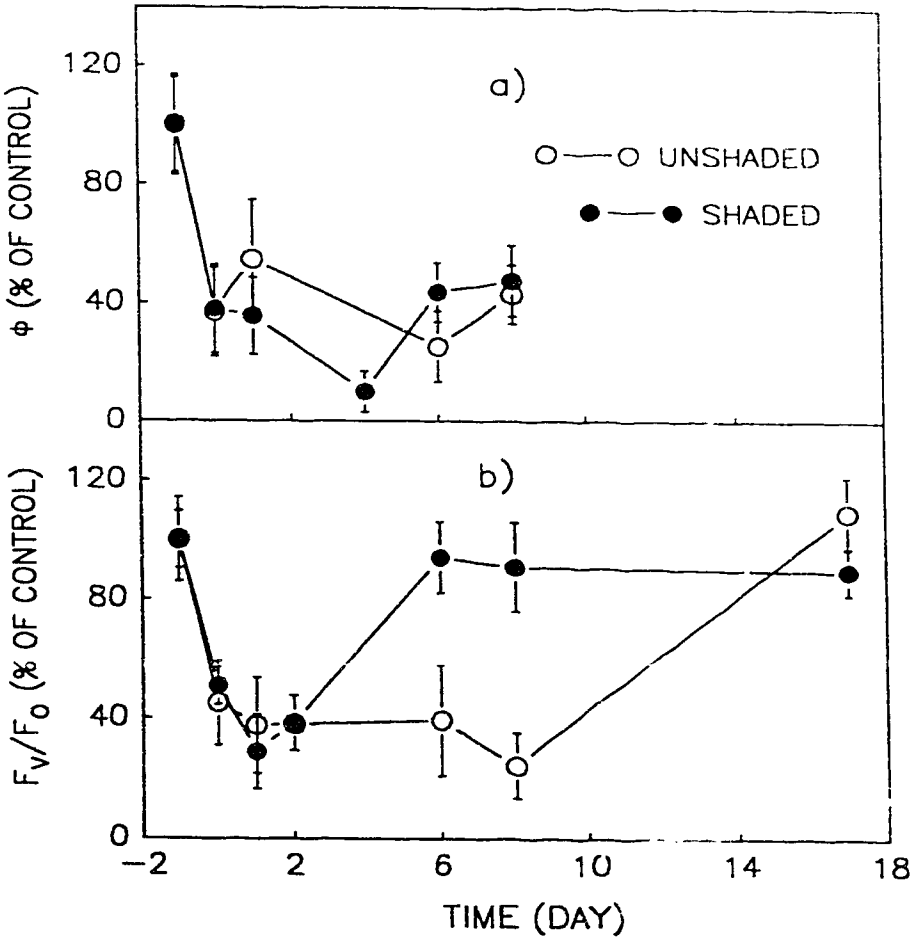
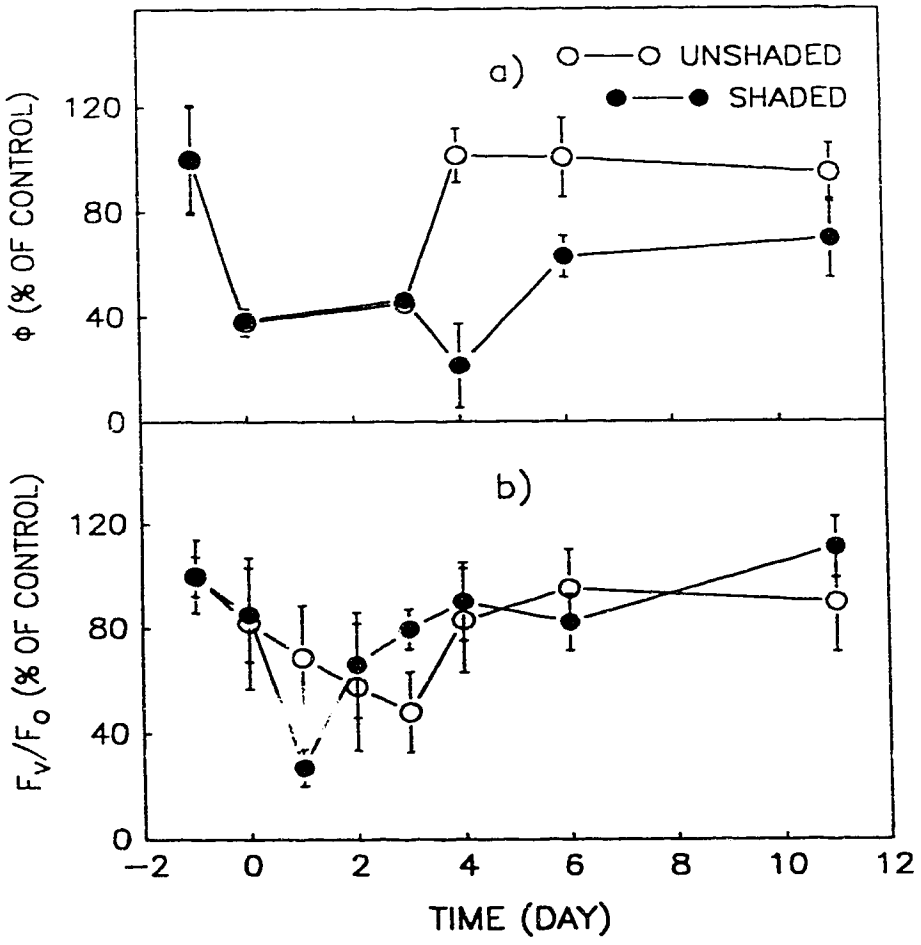


Figure V.2. Response of photosynthetic quantum yield (a) and variable fluorescence (b) to a simulated summer frost of -6 °C. Explanations are the same as in Figure V.1.



VI. EFFECTS OF SUMMER FROSTS ON THE ACTIVITY OF RIBULOSE-1, 5-BISPHOSPHATE CARBOXYLASE IN TAMARACK

INTRODUCTION

Freezing events during the growth season (summer frosts) are a common environmental stress for plant growth at high latitude or high elevations, particularly at boreal peatland sites (Hayter 1978, Christersson 1984; Christersson et al. 1987; Rothwell and Lieffers 1988). Summer frosts can significantly depress photosynthesis in conifers and the depression is not related to stomatal factors (DeLucia and Smith 1987, Lundmark and Hällgren 1987, Lundmark et al. 1988, Dang et al. 1991). The finding that summer frosts predispose coniferous seedlings to photodamage (Lundmark and Hällgren 1987, Strand and Lundmark 1987) suggests the primary site of summer frost damage was probably located in the biochemical processes of photosynthesis. Rumich-Bayer and Krause (1986) found the frost-induced inhibition of photosynthetic CO_2 assimilation in isolated protoplasts was independent of the inactivation of thylakoid membranes.

Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), the most important CO_2 fixation enzyme for all green plants, is believed to be a key enzyme responsible for responses of plants to extreme temperatures (Graham and Patterson 1982). The in vivo activity of Rubisco has been found to change in parallel with environmentally induced alterations in photosynthetic capacity (Seemann et al. 1985). Changes in Rubisco activity were studied for some conifers under water stress (O'Toole et al. 1976, Beadle and Jarvis 1977) and during cold hardening (Gezelius and Hallén 1980, Öquist et al. 1980). In the current paper, we investigated the response of the activity of Rubisco carboxylase in tamarack (Larix laricina (DuRoi) K. Koch) to a simulated summer frost of -6 ^{0}C.

MATERIALS AND METHODS

Seedlings:

Seedlings of tamarack (Larix laricina) were grown from seeds of the same mother tree in the greenhouse in May 1989. Seedlings were moved outdoors in July of the same year and kept outside until July 1990 when they were moved back to the greenhouse under summer conditions (16 hour light, day/night temperate 24/17 ^{0}C). Sunlight in the greenhouse was supplemented by high pressure sodium light on cloudy days and early mornings and late evenings.

Frost treatment:

The summer frost was simulated in a growth chamber where air and soil temperatures were controlled separately. Four seedlings were cooled at night and in darkness from room temperature to -6 ^{0}C at a rate of 4 ^{0}C/h, held at -6 ^{0}C for two hours and then thawed in darkness at a rate of about 6 ^{0}C/hour. Soil temperature was maintained at 6 ^{0}C during the frost treatment. The seedlings were put back to the greenhouse after the frost treatment. Another four seedlings were kept in the greenhouse all the time and served as controls.

Determination of Rubisco activity:

The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in both frost-treated and control seedlings was determined on the day prior to freezing, and 1, 3, 5, and 8 days following freezing.

Most chemicals used in this experiment were from Sigma Chemical Company (U.S.A.), but Folin-Ciocalteau for protein analysis and liquid scintillation cocktail were from DBH Inc. (Canada). Micro syringes were from Hamilton Company (Reno, Nevada). All other measuring devices were from Fisher Scientific (Canada).

1. Preparation of leaf extract:

Procedures for leaf extract preparation were based on methods of chloroplast extraction for conifers developed in the Forest Genetics Laboratory at University of Alberta (Aleksiuk, personal communication), with some modifications. Half gram samples of needles were taken from different branches of each tree and soaked for 2 hour in 5 ml wash buffer containing 349 mM sorbital, 65 mM Tris, 2.16 mM EDTA, 1% Tween 80, pH 8.0. The needles were cut into small pieces and homogenized using a polytron in 5 ml extraction buffer containing 346 mM sorbitol, 50 mM Tris, 5 mM EDTA, 0.1% BSA, 1% Tween 80, pH 8.0. The homogenate was filtered through 1 layer of Miracloth and 2 layers of cheese cloth, twice. All the above procedures were performed on ice. The filtrate was centrifuged at 5927g, 4 ^{0}C. The pellet was suspended in 5 ml extraction buffer and centrifuged as above. The pellet was resuspended in 5 ml wash buffer and centrifuged at 5110g at 4 ^{0}C. The pellet was then resuspended in 1 ml wash buffer and kept on ice for Rubisco assay.

2. Assay of Rubisco carboxylase

The procedures for the activation and assay of Rubisco carboxylase were based on Lorimer et al. (1977) and Coombs et al. (1987). A 0.5 ml aliquot of the leaf extract was applied to a small column of Sephadex G-25 which was equilibrated with 100 mM Tris-HCl, pH 8.6, 10 mM $NaHCO_2$, 1 mM dithiothreitol, 20 mM $MgCl_2$, at room temperature. The eluate was collected in a volume of 1 ml. This process activates the enzyme and removes low-molecular-weight compounds and metabolites which otherwise interfere with the assay (Lorimer et al. 1977).

The assay was conducted under a fumehood in 1.5 ml vials at 25 ^{0}C. The vial had a screw cap with an open top and TFE-faced silicon septum (from Wheaton, Millville, New Jersey, U.S.A.). The vials were held in a specially designed plastic water jacket which was connected to a water bath. The water jacket can accommodate 12 vials at a time. Each vial initially contained 430 µl of CO_2-free 100 mM Tris-HCl-NaOH carboxylase buffer containing 20 mM $MgCl_2$, 5 mM dithiothreitol, pH 8.2. The buffer was purged with N_2 before use. 50 µl of 0.2 M [^{14}C] $NaHCO_3$ (specific activity = 0.4 Ci/mol) and 10 µl of 20 mM D-Ribulose-1,5-Diphosphate (Sodium salt: Hydrate, purity 98%, from Sigma Chemical Company, U.S.A.) were then added into the vial. The vial was temperature-equilibrated before the reaction was initiated by the injection of 10 µl of the activated enzyme. The reaction was stopped 60 s after its initiation by the injection of 100 µl 2N HCl. The contents of the vial were transferred into a liquid scintillation vial and dried at 95 ^{0}C. The acid-stable radioactive products of the reaction was determined on a liquid scintillation counter.

The protein content of the enzyme suspension was determined using Lowry method as described by Coombs et al. (1987).

RESULTS

The total specific activity of Rubisco carboxylase in the control trees ranged from 0.35 to 0.40 µmol CO_2/min/mg protein, which was similar to the values reported for conifers growing in summer conditions (Gezelius and Hallén 1980). Surprisingly, the Rubisco activity of the frost treated seedlings in this study increased the next day following the frost treatment, but decreased three days after freezing. There was no sign of recovery in Rubisco activity in the frost treated trees eight days after freezing (Fig.VI.1).

DISCUSSION

A surprising finding of the current study is that the activity of Rubisco carboxylase tended to increase the next day after the summer frost treatment. This result is in a clear contrast to the

finding that summer frosts significantly depressed photosynthesis next day following frost (Pharis et al. 1970, DeLucia and Smith 1987, Lundmark and Hällgren 1987, Lundmark et al. 1988, Chapt. IV). The subsequent decreasing trend of Rubisco activity in this paper is also in contrast to the post-frost damage and recovery processes of photosynthesis in the same species, as reported by in Chapter IV. These contrasting trends suggest changes in Rubisco activity contributed little to the summer frost depression of photosynthesis.

The mechanism controlling the changes in Rubisco activity following the summer frost is unclear. The activation state, conformation change and concentration of the enzyme and the concentration of tight-binding inhibitors are important factors affecting the enzyme activity. The activation state was not a factor in this study since the enzyme was fully activated in the experiment. Although freeze-thaw cycles can change the conformation of Rubisco (Peoples and Dalling 1978, Huner and Macdowall 1979), it is unlikely that the conformation change would have increased the enzyme activity the next day after freezing. It is also unlikely that the concentration of tight-binding inhibitors would have decreased following freezing stress. The most likely reason for the increase of Rubisco activity might be that the frost preferentially sped up the breakdown of non-Rubisco proteins. This could have increased the value of Rubisco activity since the enzyme activity was expressed on a total protein basis. Freeze-thaw treatments generally cause conformation changes of proteins which in turn influence the susceptibility of the proteins to degradation, but the degradation of Rubisco is insensitive to conformation changes (Peoples and Dalling 1978).

One possible explanation for the decrease of Rubisco activity three days after freezing might be that the freezing event probably damaged the system of Rubisco synthesis or assembly. Rubisco is a holoenzyme which consists of eight small subunits (SSU) and eight large subunits (LSU). The LSU is encoded by chloroplast DNA and synthesized in the chloroplast. The SSU is encoded by nuclear DNA and synthesized as precursors. The SSU precursors have to be transported into the chloroplast and assembled with the LSU into the holoenzyme (Gutteridge and Keys 1985, Coombs et al. 1987, Ramage and Bohnert 1989). It would not be surprising if such a complicated system was disturbed by the significant changes of cellular environments induced by the freezing-thawing treatment. It might also be possible, however, that the frost induced the synthesis of isoenzymes of Rubisco, which had a lower activity. Shomer-Ilan and Weisel (1975) found keeping cabbage at 5 ^{0}C for 24 hours produced Rubisco with greater electrophoretic mobility. In addition, CO_2 and O_2 are mutual competitors for the same enzyme and the same substrate (RuBp). My data could not excluded the possibility that frost-induced changes in Rubisco might have increased its affinity to O_2, which would have increased the consumption of fixed CO_2 by photorespiration.

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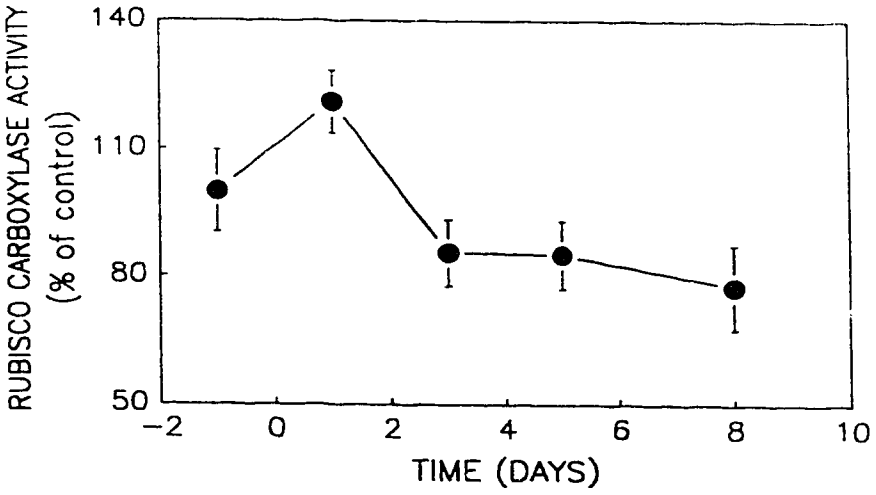
Figure VI.1. Response of the activity of Ribulose-1,5-bisphosphate carboxylase in tamarack to a simulated summer frost of -6 ^{0}C. Values ($\bar{x}$ ± se., n=4) in the graph are expressed as the percentage of the control. On the axis, time -1 indicates the pre-freezing day, 0 indicates the freezing day, and positive values the days after freezing.



VII. GENERAL DISCUSSION

PHOTOSYNTHESIS AND FLOODING STRESS

A significant finding of this thesis is that soil flooding depressed photosynthesis of peatland trees through the mesophyll and the mesophyll limitation appeared not to be related to water stress. It is generally believed that flooding-induced stomatal closure is the primary limiting factor to photosynthesis of plants under flooding (Phung and Knipling 1976, Kozlowski 1984a, 1986). The analysis of the relationship of photosynthesis to mesophyll conductance, stomatal conductance and intercellular space CO_2 concentration, and comparison of mesophyll and stomatal conductances in this thesis demonstrated stomatal resistance exerted little limitation to photosynthesis under flooded soil conditions. Previous studies have shown water stress developed in foliage when the roots were flooded (Kramer 1951, Kramer and Jackson 1954, Gill 1975, Kozlowski and Pallardy 1984). Water stress can directly limit the mesophyll capacity for photosynthesis (Osonubi and Davies 1980, Farquhar and Sharkey 1982, Melzack et al. 1985, Teskey et al. 1986). The foliage water potentials in this study, however, were higher under flooded than non-flooded soil conditions (Chap.II), indicating the trees were at least not water-stressed as severely under flooded as under non-flooded soil conditions if there was water stress at all.

Results in this thesis suggested a direct communication between roots and mesophyll cells when the roots were flooded, but the mechanism is unknown. One of the stresses flooded roots experience is the shortage of oxygen supply (Mannerkoski 1985). The metabolism in the roots changes correspondingly (Kozlowski and Pallardy 1984). In peatland tamarack and black spruce, for example, the aerobic respiration in roots was replaced by fermentation under flooded soil conditions (Conlin T.C. and Lieffers V.J., personal communication). It is possible that some products of the changed metabolism in the roots, e.g. ethanol (Crawford and Baines 1977), were transported into the mesophyll cells and these products directly or indirectly interfered with photosynthetic processes. Soil toxins produced under flooding, such as reduced ions (Crawford 1982), might have played a similar role. Nutrient deficiency in the foliage is also likely to be a contributing factor to the depressed mesophyll capacity for photosynthesis under flooding. Deficiency of mineral nutrients develops in the foliage when soil is flooded (Kramer 1969, Lee 1978).

PHOTOSYNTHESIS AND SUMMER FROSTS

A major finding of this thesis was that summer frosts inhibited the photosynthesis of peatland trees at multiple sites. Chlorophyll fluorescence measurements showed the photochemistry of photosystem II was inhibited by summer frosts (Chapter V). The quantum yield data indicated the efficiency of electron transport chain of photosynthesis was significantly reduced by summer frosts (Chapter V). The activity of the CO_2 fixation enzyme (Rubisco) was also inhibited (Chapter VI).

It was also demonstrated that the primary site of summer frost damage to photosynthesis was located in the biochemical reactions. Although both the overall efficiency of photosynthetic energy conversion and the photochemical activity of photosystem II (F_v/F_o) were inhibited by summer frosts, comparison of patterns of the recovery of these two parameters indicated the damage of photosystem II was not primarily responsible for the depressed photosynthesis (Chapt.V). Photosystem II is believed to be more sensitive to environmental stresses than other processes or complexes of the photochemistry of photosynthesis (Barber 1985). It was, therefore, not unreasonable to hypothesize that the primary site of summer frost damage to photosynthesis was located in the biochemical reactions. The fact that the photosynthetic capacity (Figs.IV.2 to 3) was depressed to a larger degree than the photosynthetic quantum yield

(Figs.V.1 and 2) can be considered as direct evidence to support this hypothesis. Data on Rubisco carboxylase activity suggested this enzyme was not a primary factor limiting the dark reactions of photosynthesis. Although other researchers have found activities of several other enzymes in the carbon reduction cycle of photosynthesis were also inhibited by freezing-thawing cycles (Rumich-Bayer et al. 1987), it is difficult to identify the primary site(s) of summer frost damage to photosynthesis by comparing their results to those in this thesis because of differences in plant materials (e.g., different species, in vitro vs. in vivo studies) and freezing protocols used in different studies. The data in this thesis and in the literature, however, can not rule out the possibility that the leakage of thylakoid membranes to protons (H^+) and/or inhibition to the coupling factors caused by summer frosts might be primary limiting factor(s) to photosynthesis. Either of them will directly limit ATP production. ATP is a direct driving force for carbon reduction in photosynthesis. In addition, membrane leakage to H^+ will prevent the alkalization of the chloroplastic stroma, which is necessary for the activation of several enzymes in the carbon reduction cycle (Enser and Heber 1980).

Results of this thesis clearly demonstrate to the forest managers and administrators that the summer frost can be a severe environmental stress limiting forest productivity in peatlands. For example, on average the -3.5 ^{0}C frost depressed the net photosynthesis of tamarack by 35% for more than 9 days. Previous studies (Hayter and Proudfoot 1978; Rothwell and Lieffers 1988) have shown that frosts can occur every month in the growing season in Alberta peatlands. Peatland drainage tends to increase the frequency and severity of summer frosts (Pessi 1958). Obviously more attention and financial support are necessary in research on summer frosts and tree's resistance to the frost. Application of results from such research in tree improvement programs has the potential for increasing forest productivity in peatlands, particularly in drained peatlands.

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