Responses of black spruce (*Picea mariana*) and tamarack (*Larix laricina*) to flooding and ethylene treatments.

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Summary

Black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) are the predominant tree species in boreal peatlands of Alberta, Canada where low nutrient availability, low soil temperature and high water table limit their growth. Effects of flooding for 28 days on morphological and physiological responses were investigated in greenhouse grown black spruce and tamarack seedlings in a growth chamber. Flooding reduced root hydraulic conductance, net assimilation rates and stomatal conductance and increased water use efficiency (WUE) and needle electrolyte leakage in both species. Flooded black spruce seedlings maintained higher net assimilation rates, stomatal conductance and WUE compared to flooded tamarack. However, flooded tamarack seedlings were able to maintain higher root hydraulic conductance compared to flooded black spruce seedlings. Needles of flooded black spruce developed tip necrosis and electrolyte leakage after 14 days of flooding and these symptoms were subsequently more prominent than in flooded tamarack seedlings. No visible injury symptoms were observed in flooded tamarack seedlings. Flooded tamarack seedlings also developed hypertrophied lenticels at their stem base. Application of exogenous ethylene resulted in a significant reduction of net assimilation, stomatal conductance, and root respiration only in black spruce while root hydraulic conductivity increased in both species. These results suggest that, although flooded black spruce seedlings maintained a higher rate of net assimilation and stomatal conductance than tamarack, black spruce failed to cope with the deleterious effects of prolonged soil flooding and exogenous ethylene as well as tamarack.

Keywords: electrolyte leakage, net assimilation, root hydraulic conductance, root respiration, stomatal conductance.

Introduction

Black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) are the dominant tree species in the boreal peatlands in Alberta, Canada. Natural stands of these species have very low productivity (Lieffers and Macdonald 1990). These organic sites are characterized by shallow water table and cold substrate temperatures (Van Cleve et al. 1983) and undergo significant variation in depth of water table during the growing season. The growth of roots of these species is generally confined to surface layers above the average water table depth, with deeper roots being pruned off by anaerobic conditions (Boggie and Miller 1976, Mannerkoski 1985, Lieffers and Rothwell 1986). Oxygen supply to tree roots decreases significantly when the water table rises close to the peat surface (Kozlowski 1984, Mannerkoski 1985).

Low oxygen (hypoxia-inducing) conditions, characteristic of flooded soils, result in a rapid reduction in transpiration, stomatal conductance and photosynthesis of many woody plants (Anderson et al. 1984, Levan and Riha 1986, Pereira and Kozlowski 1977, Pezeshki and Chambers 1986) resulting in decreased tree productivity. As an evergreen with sclerophyllous leaves, black spruce is considered a bog-adapted plant. Tamarack also has sclerophyllous leaves but is deciduous (Tyrell and Boerner 1987). Although black spruce and tamarack co-occur on moderately minerotropic peatlands, tamarack tends to dominate on wetter, minerotropic sites (Jeglum and He 1996, Tilton 1977, Kenkel 1987). A correlation between relative dominance and height growth along hydrologic and nutrient gradients suggests that performance differences underlie these species’ distributions among microsites within peatlands (Montague and Givnish 1996). Still the dominance of tamarack over black spruce on wetter sites is not well understood.

The ability of tamarack to transport oxygen to its roots and sustain limited respiration under anaerobic conditions (Conlin and Lieffers 1993) may play a role.

Flooding triggers ethylene accumulation in plants (Tang and Kozlowski 1984*a* and *b*, Blake and Reid 1981) and plant physiological processes such as net assimilation, transpiration, and stomatal conductance are inhibited by elevated levels of ethylene (Govindarajan and Poovaiah 1982, Kays and Pallas 1980, Pallas and Kays 1982). Ethylene produced during flooding (Kawase 1976) was the likely factor responsible for promoting the outgrowth of adventitious roots in tamarack (Hahn et al. 1920). Flood tolerance and emergence of adventitious root often occur simultaneously (Clemens et al. 1978, Sena Gomez and Kozlowski 1980a, 1980b).

Although there have been several studies conducted to examine flooding tolerance of black spruce, no studies have compared flooding tolerance of black spruce and tamarack. In this paper, we examined the effect of four weeks of soil flooding on gas exchange, root hydraulic conductance, ethylene evolution and membrane damage in black spruce and tamarack. Subsequently, we examined the effect of exogenous ethylene on these physiological processes. We hypothesized that flood tolerance of tamarack is conferred by its greater tolerance to ethylene accumulation.

Materials and Methods:

Plant material and treatment application:

Six-month-old black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings were used in the flooding experiment. Seeds were obtained from the Alberta Tree Improvement and Seed Center (ATISC acc #4042). Seeds were stratified for three weeks at 4^{0}C and seeds were germinated in Petri-dishes. One week after germination, seedlings were transplanted to Spencer-Lemaire (300ml) containers (Spencer-Lemaire Industries Ltd. Edmonton, AB. Canada) containing (1:1:1; peat:moss:vermiculite) planting medium Pro-Mix BX (Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in the greenhouse under 21 ± 3^{o}C temperature and 16-h photoperiod. Seedlings were watered to run off every other day and fertilized once a week with 30-10-10 N-P-K fertilizer (Plant-prod®- Plant Products Co. Ltd., ON, Canada). Seedlings were transferred to 5” pots when they were three months old.

One week prior to the start of the experiment, seedlings were transferred to a controlled growth chamber with 21^{o}C/18^{o}C day/night temperature, 65% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent and incandescent lamps.

Treatment application and experimental design:

Seedlings from both black spruce and tamarack were randomly placed in buckets where flooding was imposed by submerging the seedlings to a root-collar level. Perforated buckets were used for control (non-flooded) seedlings to ensure adequate drainage. Data were collected on day 2, 5, 8, 11, 14, 18, 22 and 28 after treatment imposition. On each measurement day, the various physiological parameters were measured on five randomly selected seedlings from each species x treatment combination. Only one seedling of a species was withdrawn from a given bucket on a given measurement day. A total of 160 seedlings (N) were used in this experiment. The experiment was a split-split-plot design (between subjects: flooding, within-subjects: species and time) and buckets were considered nested within treatments.

The statistical model is as following:

$Y_{ijklm} = \mu + F_i + B_{j\,(i)} + S_k + FS_{ik} + B_{j(ik)} + T_l + TF_{il} + TS_{kl} + TFS_{ikl} + E_{ijkhm}$

Where, Y_{ijklm} = dependent variable (physiological parameter e.g., net assimilation, stomatal conductance, electrolyte leakage etc.), μ = overall mean, F = flooding treatment (i = 1,2), B = bucket within flooding treatment (j = 1,2,…..14); S = species (k = 1,2); T = time (l = 1,2, …8); E = error term (m = 1,2,…160). Flooding, species and time were considered fixed effects, bucket within treatment was a random effect.

Data were analyzed using analysis of variance (using version 8.1; SAS Institute Inc., Cary, NC. 1996) to determine the main and interactive effects of flooding, time and species. Means were compared for significant differences at $P < 0.05$ using Tukey's test.

a) Flooding experiment:

Measurements of net assimilation (NA), stomatal conductance (g_s) & water use efficiency (WUE)

Net assimilation (NA), Stomatal conductance (g_s) and water use efficiency (WUE; net carbon assimilation rate divided by transpiration rate) of the flooded and non-flooded black spruce and tamarack seedlings were measured using an open-system infrared gas analyzer (IRGA) (LCA-3: Analytical Development Company Ltd. Hoddesdon, U.K.) equipped with a conifer cuvette (Macdonald and Lieffers 1990). Net assimilation and stomatal conductance rate were calculated as described by Caemmerer and Farquhar (1981) and expressed on a leaf area basis. An artificial light source was used to supplement the PAR to 1000 μmol m^{-2} s^{-1}. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Gas exchange and all other physiological measurements were taken on days 2, 5, 8, 11, 14, 18, 22, and 28 after flooding imposition and expressed on a leaf area basis. Needles were carefully detached from the stem and their surface areas were measured after computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA).

Root hydraulic conductance:

Root hydraulic conductance (K_r) is defined as water flow rate (kg s^{-1}) per unit pressure drop (MPa) driving flow through the entire root system whereas root hydraulic conductivity (L_p) is obtained by dividing the K_r value by root volume (measured by the water displacement method) and expressed in kg MPa^{-1} s^{-1} cm^{-3} root volume. Root hydraulic conductance (K_r) was measured in intact roots of the same seedlings used for the gas exchange measurements, using a High Pressure Flow Meter [HPFM] (Dynamax, Houston, TX, USA) as described by Tyree et al. (1995). The use of HPFM allows for measurements of intact roots since the water is applied under increasing pressure through an excised stem (around the root collar level) into the root system (Tyree et al. 1995). In this experiment, the stems of both species were cut at 2 cm above the root collar and flow rates of all the seedlings were measured at same temperature over a range of pressure from 0 to 2.75 MPa to obtain a linear pressure – flow relationship Tyree et al. (1995). Root hydraulic conductance of five root systems (from the same seedlings where all other gas exchange measurements were done) was measured from each species x treatment combination on each measurement day and was expressed in kg $MPa^{-1}s^{-1}$.

Electrolyte leakage:

Following measurements of hydraulic conductance electrolyte leakage, a measure of the cell integrity and of the loss of cell membrane leakiness, was measured on the same seedlings using a conductivity meter HI 8733 (Hanna Instruments Inc., Woonsocket, RI, USA) as described by Zwiazek and Blake (1990) and Renault et al. (1998). Needles (approximately 0.5 g) were taken from five seedlings per treatment combination (black spruce and tamarack, flooded and non-flooded), washed with deionized water three times and placed in separate tubes, each containing 20-ml of deionized water. After 5 hours of incubation on an orbital shaker at 50 rpm, initial measurements were taken for electrical conductivity of the solution. Total electrolytes of the samples were obtained by autoclaving the samples at 121^0C for 15 minutes followed by freezing overnight at $–85^0$C. The samples were thawed by placing them in a water bath at room temperature for about 5 hours. Total electrolytes of the sample solution were then measured and electrolyte leakage was calculated as a percentage of the total electrolytes.

Ethylene evolution:

Finally, ethylene evolution was determined for the same seedlings using a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard, Missisauga, ON, Canada) equipped with a flame ionization detector (FID) and an electronic integrator. The separation was carried out on a 30-m-long, 0.31-mm internal diameter GS-Q column (J & W Scientific, Folson, CA, USA) under the following conditions: oven temperature 60^0C, injector and detector temperatures 150^0C. The carrier gas was helium with linear flow rate of 30 cm s^{-1}. This GS-Q column is capable of distinguishing between ethylene and ethane.

To measure ethylene evolution from both flooded and non-flooded black spruce and tamarack seedlings, root samples and needles were enclosed in 9-ml septum vials and incubated for 10h at room temperature ($22±2^o$C) (modified after Tang and Kozlowski 1984*b*). A 0.025ml sample of the ethylene-containing air was injected into the gas chromatograph column. The concentration of ethylene was determined by comparing the peak area with pure ethylene standards. Released-ethylene was expressed as nL (g needle dry weight^{-1} h^{-1}.

b) Ethylene experiment:

Treatment application and Experimental design:

Six-month-old black spruce and tamarack seedlings, grown as described above, were used in the experiment. Roots of an intact seedling were placed in 500-ml brown non-reactive plastic containers with half of the volume filled with half-strength Hoagland's solution (Kamaluddin and Zwiazek 2002). Seedling root systems were supplied with 20 μL L^{-1} of ethylene into the plastic container from a pure ethylene gas cylinder through a 1-mm diameter tube. The tube attached and was stretched to the bottom of the container and then it was tightly closed. Control seedlings were not given any ethylene but were kept in sealed containers. Root systems were treated with entrapped ethylene for 12 hour. A syringe was used to withdraw a sampled of the entrapped ethylene for determination of its concentration by means of gas chromatography; the peak area of the sample was compared with that produced by standard (PRAXAIR, Danbury, CT, USA) pure ethylene. A 2X2 factorial design was used in the ethylene experiment with species (black spruce and tamarack) and ethylene addition (with or without) as main (fixed) effects. Twelve hours after application of the ethylene gas exchange, root hydraulic conductivity and needle electrolyte leakage were measured as described above. Data were analyzed using analysis of variance using SAS version 8.1 (SAS Institute Inc. 1987).

Root Respiration:

Root respiration of the whole root system was also measured by recording oxygen uptake with a Clark-type electrode (Yellow Springs Instruments, Yellow Springs, OH, USA). Root systems of the ethylene-treated and un-treated black spruce and tamarack seedlings (n = 5) were placed in an airtight cylinder containing half-strength Hoagland's solution. Oxygen uptake rates were recorded every two minutes for 20 minutes. Respiration rate was expressed as average of oxygen uptake over time expressed in mg L^{-1}.

Results:

a) Flooding experiment:

Root morphology:

Visible damage of roots was observed in flooded seedlings of both species. Flooding reduced the root mass in both flooded black spruce and tamarack seedlings compared to their non-flooded controls (not quantified, easily visible). No adventitious roots were observed in any flooded seedlings while stem hypertrophy (swelling at the flood-water line) was observed in flooded tamarack seedlings.

Physiological responses:

Flooding significantly reduced net assimilation, stomatal conductance and root hydraulic conductance while increasing water use efficiency and needle electrolyte leakage in both species. A significant (P <0.0001) reduction in net assimilation (NA) rate was observed in both black spruce and tamarack seedlings under flooding compared to their non-flooded controls (Figure 1A, Table 1a). Non-flooded black spruce seedlings maintained a higher net assimilation rate per unit leaf area than non-flooded tamarack seedlings throughout the experiment. The same trend was observed in flooded seedlings until 14 days of flooding after which net assimilation rate of black spruce declined sharply. There were no significant effects of bucket within flooding treatment on the measured response variables.

A significant ($P < 0.0001$) reduction in stomatal conductance was also observed from 2 d under flooded conditions when compared to non-flooded seedlings of both species (Figure 1B, Table 1a). No significant species differences were observed in stomatal conductance under flooding. Flooding significantly ($P < 0.0083$) increased the water use efficiency in flooded seedlings of both species compared to their non-flooded controls (Figure. 1C, Table 1a). Flooded tamarack seedlings had significantly higher WUE on day 2, 5, and 18) WUE when compared with flooded black spruce seedlings.

Flooding significantly ($P < 0.0001$) reduced root hydraulic conductance in both black spruce and tamarack seedlings compared to their non-flooded controls (Figure 1D, Table 1a). However, on day 14, flooded tamarack seedlings were able to maintain a higher root hydraulic conductance compared with flooded black spruce seedlings.

Flooding also significantly increased ($P < 0.0001$) electrolyte leakage in the foliage of both black spruce and tamarack seedlings (after 14 days of flooding) compared to their non-flooded controls (Figure 1E, Table 1a). However, on day 22, electrolyte leakage was significantly higher in flooded black spruce seedlings compared to flooded tamarack seedlings. Flooded black spruce seedlings appeared to develop needle necrosis on day 14 and continued to increase over time and about 40% of the total needles showed signs of necrosis in flooded black spruce seedlings by the end of the experiment. On the contrary, no necrosis was observed in flooded tamarack seedlings. The severity of needle necrosis was consistent with the degree of electrolyte leakage over time.

Both flooded black spruce and tamarack seedlings (root and needle samples) produced ethylene. However, no significant trends in relation to species or flooding treatment were observed.

b) Ethylene treatment:

Application of exogenous ethylene resulted in a significant reduction in net assimilation ($P < 0.0021$) in black spruce seedlings while there were no significant effects on tamarack (Figure 2A, Table 1b). Stomatal conductance significantly ($P<0.0004$) declined in both species after ethylene treatment (Figure 2B, Table 1b). Ethylene treatment also resulted in a significant reduction in root respiration ($P < 0.0001$) in both species (Figure 3A, Table 1b). However, the magnitude of these reductions was higher in black spruce than in tamarack (Figures 2B and 3A). Root hydraulic conductivity increased significantly ($P < 0.0005$) in tamarack after treatment with ethylene but there was no significant effect for black spruce (Figure 3B, Table 1b). Although electrolyte leakage increased with ethylene treatment for both species, it was statistically not significant.

Discussion:

Although the general patterns of response were similar for both species there were some key differences in timing and magnitude of responses. Overall, our results suggest that tamarack seedlings were more resistant to four weeks of flooding than black spruce. Flooding reduced net assimilation and stomatal conductance from day two through day 28 for both species. Stomata began to close within two days of flooding (Figure1B). The functional significance of stomatal closure seems to be to restrict water loss from the shoots during the time of lowered root hydraulic conductance (K_r), an effect observed simultaneously in both black spruce and tamarack from day two of flooding imposition (Figure 1D).

Early stomatal closure during flooding can occur with and without leaf dehydration (Kramer and Jackson 1954, Pereira and Kozlowski 1977). Several experiments have shown that a reduction in soil oxygen can also induce stomatal closure without a change in leaf water potential (Sojka and Stolzy 1980, Stolzy et al. 1961). However, Else et al. (2001) reported that stomatal closure and slower leaf expansion in flooded *Ricinus communis* were due to a decrease in root hydraulic conductivity that reduced leaf water potential. Stomatal control mechanisms in flooded plants may vary with duration of flooding, condition of the root system, and growth stage (Coutts 1981). Stomata of *Picea sitchensis* seedlings closed gradually as shoots dehydrated over a period of flooding (Coutts 1981). We saw a similar trend of gradual stomatal closure coupled with a reduction in root hydraulic conductance presumably due to the impact of oxygen deficits on membranes in black spruce and tamarack in this study. Our results contrast with those of Reece and Riha (1991) in which root hydraulic conductance of tamarack and white spruce appeared to be unaffected after 10 days of flooding.

Although root hydraulic conductance and stomatal conductance were significantly reduced in flooded seedlings, there was an increase in WUE on day 14 of flooding. This increase in WUE corresponds to a gradual decline in stomatal conductance, which is likely attributable to the fact that the flow of water vapor through the stomata is more sensitive to partial closure than is the flow of carbon dioxide. An increase in electrolyte leakages in flooded black spruce seedlings is likely a consequence of membrane damage. As a consequence of membrane damage, the net assimilation rate declined in flooded black spruce seedlings. Although flooded black spruce seedlings were able to maintain higher net assimilation and stomatal conductance compared with flooded tamarack seedlings during the initial phase of flooding, they showed reduced root hydraulic conductance. Black spruce needles exhibited discoloration and necrosis, which could possibly be explained by high membrane damage. In flooding stress, ultra-structural symptoms appeared to be related to the development of nutrient imbalance in needles of Norway spruce and Scots pine (Palomaki et al. 1994). In contrast to black spruce, flooded tamarack seedlings had reduced net assimilation and stomatal conductance but were able to maintain higher root hydraulic conductance. Consequently flooded tamarack had a lesser degree of membrane damage and showed no visual symptoms of damage.

It is interesting to note that while flooded black spruce seedlings failed to demonstrate any morphological acclimation to flooding, tamarack showed stem hypertrophy (swelling at the flood-water line). Stem hypertrophy has been reported to facilitate the aeration of submerged roots (Vartapetian and Jackson 1997). Blake and Reid (1981) reported that hypertrophy, especially the production of spongy aerenchyma tissue above the water level, may allow the plant to eliminate ethylene as well as aerate the roots, therefore, this mechanism could be helpful with avoiding flooding stress. In our study, no adventitious roots were observed in any flooded seedlings.

In terms of morphological responses, tamarack had a higher root mass with many new roots, than black spruce seedlings and maintained that even during flooding. This is of particular importance since young roots and root tips have the highest respiration rate per unit volume of tissue (Nilsen and Orcutt 1996). It has been already reported that tamarack can sustain root respiration during anaerobiosis (Conlin and Lieffers 1993). It should also be noted that during flooding soil nitrogen is dominated by ammonium ions while nitrate decreases (Armstrong et al.

1994). Uptake of ammonium requires root respiration (Chapin et al. 1987). This might also contribute to tamarack's ability to withstand prolonged flooding.

Increased ethylene synthesis has been shown to be associated in many plant species with oxygen deficiency and flooding (Kawase 1976, Wample and Reid 1979, Drew et al. 1981, Metraux and Kende 1983, Raskin and Kende 1984). Ethylene concentration in shoots is often higher when roots are submerged in water with low oxygen content (Kawase 1972, Jackson and Campbell 1976). Membrane damage and leaf epinasty in stressed seedlings are often attributed to increased ethylene production in plants during stress (Blake and Reid 1981). In our study, electrolyte leakage increased significantly in flooded seedlings of both black spruce and tamarack (Figure 1E). However, we did not observe a consistent trend in ethylene accumulation in flooded black spruce and tamarack seedlings.

Since exogenous ethylene produces symptoms typical of flooding in plants (e.g., formation of adventitious roots, leaf epinasty, and stem hypertrophy Kawase 1972, Wample and Reid 1979), seedlings of both black spruce and tamarack were treated with exogenous ethylene in another experiment. Since root containers were kept sealed during the ethylene treatment the observed response may be due to the combined effect of ethylene, reduced oxygen and high carbon dioxide.

Reductions in net assimilation and stomatal conductance as a result of exogenous ethylene treatment in this study were consistent with earlier findings (Govindrajan and Poovaiah 1982, Pallas and Kays 1980). There is variability between species in their response to ethylene. Changes in stomatal conductance generally exceed that of net assimilation although for some species the ethylene-induced changes in stomatal conductance and net assimilation are not necessarily well coupled (Taylor and Gunderson 1986). In our study black spruce and tamarack differed in their responses to ethylene application.

Root hydraulic conductivity was measured to take into account the variability of root volume in different seedlings since the duration of the ethylene treatment was short. Increased hydraulic conductivity in ethylene treated seedlings of both black spruce and tamarack might be explained by the increased activity of aquaporins. In plants, aquaporins are thought to regulate the water flow through membranes during growth, development and stress responses (Cheng et al. 1997). Evidence suggests that ethylene enhances protein phosphorylation (Raz and Fluhr 1993), which in turn enhances aquaporin activity and increased hydraulic conductance. Exogenous ethylene also increased root hydraulic conductivity in hypoxic aspen (Kamaluddin and Zwiazek 2002). Increased root hydraulic conductance appeared to be a key factor in the better growth performance and higher net photosynthesis of *Coffea canephora* plants under conditions of water excess (Fahl et al. 2001).

Continued water uptake by roots is oxygen dependent because both the active ion uptake needed to maintain an osmotic gradient across the root (Rowe and Beardsell 1973) as well as membrane hydraulic conductivity (Glinka and Reinhold 1962) are known to require oxygen. Although root respiration was reduced with ethylene treatment in both species, the magnitude of reduction was greater in black spruce possibly explaining the resulting lower root hydraulic conductivity.

In conclusion, our study demonstrated that tamarack exhibits greater flooding resistance compared with black spruce. Black spruce was more responsive to exogenous ethylene. We suggest that morphological acclimations, maintaining high rates of root hydraulic conductance and the ability to withstand ethylene induced by flooding are the key factors conferring greater flooding tolerance in tamarack than black spruce.

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Table 1. Analysis of variance (ANOVA) results for different dependant variables in response to flooding showing the levels of significance (*P*) for the main fixed effect flooding, flooding x time and flooding x species (a), and for the main effect of ethylene and ethylene x species (b).

(a) Flooding treatment:

Variables	Flooding	Flooding x Time	Flooding x Species
Net assimilation	0.0001	0.0001	0.0001
Stomatal conductance	0.0001	0.0001	0.3300
Water use efficiency	0.0075	0.3677	0.7026
Root hydraulic conductance	0.0001	0.3893	0.0014
Electrolyte leakage	0.0001	0.0001	0.7431

(b) Ethylene treatment:

Variables	Ethylene	Ethylene x Species
Net assimilation	0.0021	0.0645
Stomatal conductance	0.0004	0.8098
Root respiration	<0.0001	0.0221
Root hydraulic conductivity	0.0005	0.0507

Figure captions:

1. Effects of flooding for 28 days on net assimilation (A), stomatal conductance (B), water use efficiency (C), root hydraulic conductance (D), and electrolyte leakage (E) in black spruce and tamarack seedlings. Vertical bars indicate S.E.M. (n=5).
2. Effect of exogenous ethylene on net assimilation (A), and stomatal conductance (B) in black spruce and tamarack seedlings. Vertical bars indicate S.E.M. (n=5). Bars with the same letter are not significantly different at $P < 0.05$.
3. Effect of exogenous ethylene on root respiration (A), and root hydraulic conductivity (B) in black spruce and tamarack seedlings. Vertical bars indicate S.E.M. (n=5). Bars with the same letter are not significantly different at $P < 0.05$.