Relationship between temperature, respiratory loss of sugar and premature dehardening in dormant Scots pine seedlings

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Summary Increased intracellular sugar concentration is an important contributor to the increased cold tolerance of conifers in winter. This study examines the extent to which winter-time respiratory loss of sugars leads to premature dehardening. Two-year-old seedlings of Scots pine (Pinus sylvestris L.), grown and cold-hardened in the field, were exposed to different temperature regimes for 16 weeks while dormant. To minimize short-term carry-over effects, after the temperature treatments, all seedlings were conditioned to 5.5 °C and watered before the assessment of non-structural carbohydrates and cold tolerance. Needle sugar concentration was decreased by 54, 32, 21 and 9% following treatment at 5.5, 0, −1.5 and −8.5 °C, respectively. Sugar concentration did not decrease as much in root tissues as in needles because starch was mobilized in roots. Cold tolerance of needles was analyzed by controlled freezing, and the temperature causing an initial 10% damage (LT₁₀) was plotted as a function of needle sugar concentration, revealing a strong, linear relationship. When one-third of the initial sugars had been consumed, LT₁₀ had increased from −24.5 to −16.5 °C, and when one half had been consumed, LT₁₀ had increased to −10 °C. Consequences of these findings for the field performance of conifers are discussed in relation to climatic variation and change.

Keywords: carbohydrate, climate change, cold tolerance, Pinus sylvestris, respiration, winter damage.

Introduction

In autumn, soluble sugars accumulate to high concentrations in perennial plants, including conifers (e.g., Ericsson 1979). These sugars play a vital role in the cryoprotection of cells (Sakai and Yoshida 1968) and must be maintained as long as winter conditions prevail. Under certain conditions, however, a portion of the soluble sugars may be lost through respiratory activity. For example, substantial amounts are lost from seedlings during winter storage, a practice used in forestry to prevent seedlings from flushing until planting in spring. McCracken (1979) reported that 75% of the total carbohydrate reserve was lost when Pinus radiata D. Don seedlings were stored at 1 °C for four months. Winter storage at subfreezing temperatures may reduce sugar losses. Chomba et al. (1993) observed that starch reserves were reduced by 50% in Picea engelmannii Parry ex Engelm. seedlings stored at −5 °C for two months. It may be argued that cool storage differs from over-wintering in the field, because darkness during storage prevents compensatory photosynthetic gains. However, certain field situations are likely to impose larger restrictions on photosynthesis than on maintenance respiration. For example, photosynthesis ceases when needles begin to freeze at a temperature of −3 to −5 °C (Neilson et al. 1972), several degrees above the temperature at which respiration ceases (Ungerson and Scherdin 1965). Furthermore, after thawing, respiration reactivates much more rapidly than does photosynthesis (Pisek and Winkler 1958, Tranquillini 1964), and often peaks during repair of freeze-damaged components (Larcher 1981). The persistent depression of photosynthesis probably results from a combination of factors, including irreversible temperature-induced inhibition of enzymatic reactions (Strand and Öquist 1985), light-induced inhibition of electron transfer reactions (Öquist et al. 1987), and stomatal closure as roots are cooled or soil water freezes (Jurik et al. 1988). Although xylem water may occasionally melt, this supply is not adequate for sustained net photosynthesis (Troeng and Linder 1982), particularly in seedlings, which have a smaller proportion of vascular tissues compared to mature trees.

Thus, a winter climate characterized by frequent freeze-thaw cycles may impose larger restrictions on photosynthesis than on respiration. These climatic conditions seem to have become more prevalent in northern Scandinavia during the last decade (The Swedish Meteorological and Hydrological Institute (SMHI), personal communication). Mild winters may also be detrimental to tree carbon balance, because respiration is enhanced but photosynthesis is not because of lack of light. At the latitude of our laboratory (64° N), the daily solar irradiance in December is only 1% of that in June (SMHI, personal communication).

It is possible, therefore, that unusually mild or fluctuating winter temperatures, or a combination of both, may upset carbon balance, particularly in understory seedlings at high latitudes, resulting in decreased concentrations of soluble sugars, and therefore decreased cold tolerance. This study evaluates the effect of a worst case scenario in which photosynthesis is inactive while respiration proceeds at rates determined by prevailing temperatures. To this end, dormant seedlings of Scots pine (Pinus sylvestris L.) were exposed to different
temperature regimes for 16 weeks and subsequently analyzed for carbohydrate concentrations and cold tolerance.

Materials and methods

Two-year-old seedlings of Scots pine (seed orchard progeny Östteg, average clonal origin 65° N, interior of Scandinavia) were raised in an outdoor nursery (64° N, 20.5° E) according to standard procedures. During the first and second year, seedlings were grown in 50-cm³ and 2-dm³ Styrofoam pots, respectively. In early January, seedlings were randomized into sets of 24–48 individuals to be used for treatments, with one set reserved as a control.

Treatments, lasting 16 weeks, were carried out in freezers with temperatures set at 0.3 ± 0.3 °C, −1.5 ± 0.2 °C and −8.5 ± 0.3 °C, and in a cold room at 5.5 ± 0.5 °C. Temperatures were measured with thermocouples (0.05 mm in diameter) connected to data loggers (CR10, Campbell Scientific Inc., Logan, UT). Forced air circulation ensured that temperature gradients were small. Plants in freezers received no light, but plants in the cold room received a photon flux density of 10–30 µmol m⁻² s⁻¹ (8-h photoperiod), as measured by a quantum sensor (Li-189; Li-Cor Inc., Lincoln, NE). On average, water content of needles had decreased by less than 5% following treatment at −8.5 °C and 5.5 °C, and by 20% following treatment at −1.5 °C and 0 °C. The larger water deficit at the latter temperatures was probably related to increased fluctuation in temperature in the cooling system. Before sampling, all seedlings, including controls, were preconditioned to 5.5 °C for 12–14 days, during which time they were watered to regain full water status. Needle water potentials were found to be high (−0.42 ± 0.03 MPa) when measured with a Scholander pressure chamber (Scholander et al. 1964).

Needles for freeze tests were sampled from leading shoots within 2–8 cm of the apical buds, with the same amount sampled from each seedling belonging to a replicate. In a cold room, needles were cut into segments of 1 cm, washed in distilled water (2 × 60 s), and transferred to test tubes (0.4 g per tube) containing distilled water (0.2 ml). Tubes were kept in the cold room until the next morning, when all tubes, except controls, were transferred to an ethanol bath (Hetofrig CB 10 with thermostat 04 PG 623, Birkröd, Denmark), where they were cooled at a rate of 4.8 °C h⁻¹, beginning at 0 °C. When a test temperature was reached, as indicated by a subsmerged thermocouple, tubes were rapidly transferred to the cold room to thaw. Sixty minutes after the last tubes had been transferred, 4 ml of incubation medium containing 0.002% Triton X-100 and 10 mM boric acid was added to all tubes. Tubes were shaken at 250 rpm (KS250 shaker, IKA Labortechnik, Staufen i. Br., Germany) for 18 h. Conductivity of the medium was measured at a constant temperature of 25.0 °C, maintained by keeping the measuring cell (Model 6.0907.110, Metrohm, Switzerland) and the tubes in a temperature-controlled water bath. Measurements were repeated after the tubes had been heated for 10 min in boiling water and shaken for an additional 18 h. The proportion of damaged cells was calculated after linear scaling, such that unfrozen controls represented zero damage, and heated samples represented 100% damage. The electrolyte leakage value of unfrozen controls did not significantly differ among categories.

Needles (sampled as for freeze tests) and roots < 2 mm in diameter were analyzed for carbohydrate content. Samples were frozen in liquid nitrogen, stored at −60 °C for one month, and then freeze-dried and ground to powder in a ball mill. Samples of 50 mg were extracted four times with 80% ethanol in the presence of 20 mg ml⁻¹ polyvinylpolypyrrolidone (PVP). The total amount of soluble sugars was determined according to the anthrone method, as developed by Jermy (1975), to yield identical colour reactions for sucrose, fructose and glucose. Glucose standards were used for calibration. The residue from the extraction was analyzed for released glucose using an enzymatic method (510-DA, Sigma Diagnostics). Amounts of carbohydrates were expressed on a dry weight basis.

Results

Scots pine seedlings with the same history of growth and cold hardening in the field were exposed to four temperature regimes beginning in mid-winter and lasting 16 weeks. Long-term effects of winter temperatures were specifically examined because, after treatments, all seedlings, including controls, were conditioned to 5.5 °C for two weeks before analysis. Figure 1 shows the initial and final concentrations of non-structural carbohydrates in needles and roots. The total carbohydrate concentration decreased more in roots than in needles. For example, the total carbohydrate concentration decreased by 37% in roots and by 21% in needles, following treatment at −1.5 °C. Starch degradation accounted for much of the carbohydrate decrease in roots: following the −1.5 °C treatment, there was 61% starch degradation. Virtually no starch was found in any category of needles. The decline in needle sugar concentration was strongly dependent on temperature and amounted to 54, 32, 21 and 9% after treatment at 5.5, 0, −1.5 and −8.5 °C, respectively (Figure 1). Hence, sugar consumption proceeded well into the subfreezing range, albeit at a reduced rate.

The effect of decreased sugar concentration on cold tolerance was examined by subjecting needles to controlled freezing, and then analyzing them for electrolyte leakage as an indicator of cell damage. Figure 2 shows the percentage of cells damaged as a function of test temperature for the different categories. The temperature causing an initial 10% damage (LT₁₀) was extracted from these curves and plotted as a function of needle sugar concentration (Figure 3). As shown by the strong, linear correlation in Figure 3, cold hardiness decreased in direct proportion to the decline in sugar concentration. When one-third of the initial sugars had been consumed, LT₁₀ had increased from −24.5 °C to −16.5 °C, and when one half had been consumed, LT₁₀ had increased to −10 °C.
Discussion

Cold tolerance decreased in direct proportion to the respiratory loss of soluble sugar in dormant Scots pine seedlings. Although the importance of soluble sugars for cold hardening is well established (Sakai and Larcher 1987), the strength of the relationship shown in Figure 3 is novel. Most studies in the past have followed the seasonal trend in cold tolerance (e.g., Sutinen et al. 1992), allowing sugar concentration to covary with other factors of importance, such as cellular water content and membrane properties. In the present study, this source of variation was minimized by using seedlings that had completed physiological cold hardening before treatment, and had regained full water status before measurement.

Figure 1. Concentrations of soluble sugars (filled bars) and starch (open bars) in needles (N) and roots (R) of Scots pine seedlings, before and after exposure to temperatures of −8.5, −1.5, 0, and 5.5 °C for 16 weeks. Starch concentration was < 0.2% of dry weight in all needles. Mean ± SE values are given for 4–6 replicate groups, each consisting of 6–8 individuals.

Figure 2. Cold tolerance of needles of Scots pine seedlings analyzed before (A) and after exposure to temperatures of −8.5 °C (B), −1.5 °C (C, ●), 0 °C (C, ○) and 5.5 °C (D) for 16 weeks. Mean ± SE values are given for 4–6 replicate groups, each consisting of 6–12 individuals.
Carbohydrate consumption did not stop at −8.5 °C (Figure 1). In agreement with this is the observation that Scots pine shoots are capable of respiration down to at least −9 °C, as measured by gas exchange in winter (Ungerson and Scherdin 1965). Long-term mean winter temperature is −7 °C at our laboratory in northern Sweden, but recent winters have been up to 5 °C warmer (SMHI, personal communication); therefore, the respiratory consumption of cryoprotective sugars may be increased.

The extent to which the results reported here can be extrapolated to the field depends in part on the degree to which photosynthesis is exceeded by respiration in winter, which in turn depends on the prevailing conditions of temperature and light. During frost-free winters in southern England, coniferous seedlings are capable of photosynthesis (Fry and Phillips 1977) and solar irradiance in early and late winter is sufficient to sustain dry weight gains (Rutter 1957). However, during an exceptionally mild winter in western Norway—where winter solar irradiances are much lower than in England—seedlings were reported to lose dry weight (Prinz 1933). In a recent experiment with temperatures raised by 4 °C, significant carbohydrate losses were again reported for seedlings over-wintering in this area (Skre 1997), demonstrating that, during exceptionally mild winters at high latitudes, carbohydrate reserves can be consumed to the extent that partial dehardening occurs.

Sugar depletion may not be restricted to frost-free conditions. Milder than average winters could increase the frequency of freeze–thaw cycles. This may cause direct injury to over-wintering foliage, as observed in red spruce (Perkins and Adams 1995), and may also sensitize plants to freezing by enhancing respiration (Larcher 1981) at a time when photosynthesis has not recovered (Tranquillini 1964). During the 1991–92 winter in north-eastern Sweden, temperatures 5 °C higher than average coincided with the highest frequency of freeze-thaw cycles recorded in at least 35 years (SMHI, personal communication), as well as with the dieback of whole stands of Vaccinium myrtillus L., a major understory dwarf shrub in this region (unpublished observations). A likely cause of this dieback was discovered during the following, equally mild winter: sugars were progressively lost during the course of the winter, leading to premature dehardening (Ögren 1996).

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References


